Department of Biotechnology
Government of India

Water4Crops

European Commission

Annual Report

Integrating Bio-treated Wastewater Reuse with Enhanced Water Use Efficiency to Support the Green Economy in EU and India (India side)

ICRISAT INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS

teri MSSRF NEERI SAB India Eurofins The Byke Sugar Works Ltd.

Annual Report

Integrating Bio-treated Wastewater Reuse with Enhanced Water Use Efficiency to Support the Green Economy in EU and India (India side)

Submitted to

Department of Biotechnology (DBT)
Ministry of Science and Technology
New Delhi - 110 003, India

Submitted by

ICRISAT
INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS
IDC ICRISAT DEVELOPMENT CENTER

Contents

List of Figures

List of Tables

Summary

The simultaneous effects of agricultural growth, industrialization and urbanization are further increasing pressure on limited water resources. Water resources are depleting at faster rate than the rate of recharge, thus the world is experiencing moderate to severe water shortages. In fact, one third of global population will face water scarcity by 2025. Current and future fresh water demand could be met by enhancing water productivity. Three basic principles for improving water productivity are reduce the water use, substitute the good quality water with marginal quality water, and recycle the wastewater. In order to address the issues of water scarcity for food production as well as to dispose of domestic and industrial wastewater safely, Water4Crops project through India-EU collaboration under "FP7-KBBE-2012-6-Singlestage" has been approved. The Indian consortium consists of 15 research partners including private companies along with the research institutions and similarly EU consortium consists of 22 partners including private companies and the research institutions. The main objective of the project is to enhance the safe use of treated wastewater in agriculture through valorization and improved water use efficiency through genetic enhancement as well as management practices including irrigation practices.

In work package 1, work has been conducted at four locations: SAB Miller (Sangareddy, Telangana), Jain Irrigation System Ltd (Jalgaon, Maharashtra), Ugar Sugar Works (Belgavi, Karnataka), and K.C.P. Sugar and Industries Corporation Ltd (Laksmhipuram). The physic-chemical characteristics of the wastewaters from these sites suggested that these wastewaters may not be suitable for direct reuse in agriculture. Moreover, the impact of long term application of wastewater for irrigation on soil health and crop productivity is also assessed. At SAB Miller and Laksmhipuram site of K.C.P. Sugar and Industries Corporation Ltd, constructed wetland were prepared to treat the effluent coming from effluent treatment plant of the factories. At Lakshmipuram site, agro-aqua system was demonstrated using treated water coming from constructed wetland. The constructed wetlands at both site were able to reduce Chemical Oxygen Demand (COD) by 30-92%. Dark colour of the industrial effluent is also one limiting factor for reuse of wastewater. In this package, an indigenous bacterial consortium is developed that reduced color by 32%. The total removal of 58% at the site is due to the cumulative treatment effect of adapted bacteria and algal consortium followed by activated charcoal, whereas in the lab study, only 8.8% of colour was reduced by 2nd generation adapted bacterial consortium. Algal treatment was also studied to remove the COD from distillery wastewater. Four studies of bacterial treated distillery effluent carried out using free cells of algae, growing algal cells, *Strychnos potatorum seed, moringa seeds* observed reduction in COD by 4000, 4666, 933, and 15066 mg/L respectively. The bacterial and subsequent algal treatment of DSW couldn't reduce the saline content to meet the irrigation standard (EC: 0 to 3 mS/cm). Hence, one of the research leads is to use the halophytes for phyto-remediation in constructed wetland. The study has to be carried out to evaluate the uptake of salinity and other organic contaminants by different sp. of halophytes.

The research in implementation work related to domestic wastewater (work package 2) is being conducted at multiple locations: NEERI and Pandherkawad (Nagpur), ICRISAT and Kothapally (Telangana), UAS, Dharwad (Karnataka), and Mavanur, Katnur and Gabbur in Dharwad (Karnataka). The wastewaters at all of these sites are not suitable for direct reuse

in agriculture. However, farmers are using these wastewaters as it is. The wastewater samples were also characterized for different microbial groups *viz.* Bacteria, Fungi, *Actinomycetes*, *Azotobacter* and *Rhizobium*. These microbial isolates were tested to treat the wastewater. The water4crops teams have constructed wetland as a decentralized wastewater treatment system at these locations. The regular monitoring of the performance of constructed wetland has indicated the high treatment efficiency of contaminants. For example, COD removal efficiency from field scale wetlands constructed at ICRISAT and Kothapally is about 30- 60% and from pilot scale constructed wetland at NEERI is highest 90-95%. Apart from wastewater treatment, remediation of degraded soil due to long term application of wastewater is also being studied under this work package. Microbial consortium of *Enterobacter aerogenes, Azospirillum irakense, Enterobacter cloacae,* and *Pseudomonas sp*. was used to reclaim the degraded land as Ugar Sugar site.

Efficient use of treated wastewater is major goal of third work package. In this package, impact of wastewater reuse in agriculture on crop and soil will be assessed through laboratory and field experiments. The experimental sites for the impact assessments are SAB Miller, Ugar Sugar, KCP Sugar Industry, ICRISAT, Jain Irrigation, and UAS Dharwad. At each site, farms are selected for conducting filed experiments. One of the tasks in this package is developing efficient irrigation system. The experiments were conducted by Jain Irrigation System Ltd to assess the feasibility of treated wastewater from food processing plant in agriculture through drip irrigation system. Different configurations of emitters for micro irrigation system were tested and suitable emmiters were identified. Laboratory and field experiments were conducted to assess the effect of wastewater irrigation on crop and soil. Apart from irrigation, the scope of this package is also extended to agro-aqua farming system. One of the expected outcomes of the integrated approach of reusing bio-treated distillary effluent first in aquaculture and then in agriculture is for fertilizer savings.

Comparative physiological studies on pearl millet, sorghum and maize provided useful information on common and crop specific mechanisms of drought tolerance in these crops. The studies on chickpea confirmed introgression of the genomic region controlling drought tolerance traits. Screening of tomato germplasm for stress tolerance based on fruit yield and physiological characters was found effective. The studies led to better understanding of genetic mechanisms and interrelationships of these traits. These traits were found useful surrogates in breeding for water use efficient (WUE) genotypes. Root studies in different tomato species observed the positive and significant association of root traits (root length, root dry weight) and root to shoot ratio with fruit yield.

The fourth work package is about improving crop cultivars that use water effectively. The genetic material of crop exchanged between Indian and EU consortium. crop specific mechanisms of drought tolerance in pearl millet, sorghum and maize were assessed through comparative physiological studies in lysimeters. The studies on chickpea confirmed introgression of the genomic region controlling drought tolerance traits. Screening of tomato germplasm for stress tolerance based on fruit yield and physiological characters was found effective. The studies led to better understanding of genetic mechanisms and interrelationships of these traits. These traits were found useful surrogates in breeding for water use efficient (WUE) genotypes. In tomato, high yielding and drought tolerant genotypes were identified and hybridization was under taken to introgress drought

tolerance traits from two wild species (S. pennellii and S. galapagenes) into the cultivated species.

A common online platform prepared for both the EU and the Indian consortium to exchange and share their experiences about project activities they are undertaking. It is designed to host discussion on upcoming factsheets especially on the topics like legislation and cost-benefits of waste water treatment and reuse technologies. This discussion will provide inputs to the innovation process in WP5. The external stakeholders from Innova Platforms were also invited to the group. Euro-India Research Center is coordinating Work Package 5 and 6. On 28th May 2014, first Indian INNOVA meeting was organized. The meeting brought together the Industry experts from CII (Confederation of Indian Industry), EBTC (European Business Technology Center), Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) Germany and EnviroTech Water Management Pvt. Ltd. in the field of wastewater treatment and water use efficiency. These experts were challenged to explore business opportunities for the new technologies in the domains of wastewater reuse and valorization, and water use efficiency that are being developed in Water4Crops project. The EU-India Joint water4crops website is the main dissemination tool to showcase significant results and outcomes and project events. The website is regularly updated with information from both EU and Indian side. Apart from project activities, the news, events and related articles are also posted in the website. This conveys to outsiders that W4Cs is a joint project between India & EU and both sides are working together.

The final joint meeting was conducted during 15–17 June 2016 at Casuarina Hall, India Habitat Center (IHC), New Delhi. Hon'ble Minister of State, Ministry of Science & Technology and Ministry of Earth Sciences, Government of India Mr YS Chowdary, Mr. Vijay Raghavan, Secretary, Department of Bio-Technology, Government of India, and H.E. Tomasz Kozlowski, the Ambassador of the European Union to India and participants from EU and India side consortiums attended this meeting. Important recommendation as highlighted by Hon'ble Minister in that focus should be on translation of scientific and technological findings into solutions for common people (Scaling-up). Water4crops consortium have piloted decentralized wastewater system at 28 locations. This project need to be extended to next phase for scaling-up of technologies develop under this project. All the technologies and achievement from this project need to be compiled with detailed documentation and made available for Government and other stakeholders as some of these technologies may be supported by government initiatives such as Swatch Bharat Mission.

The water4Crops-India consortium partners are working to develop and implement the technologies to tackle the wastewaters from industries as well as small communities and provide solutions for emerging water, energy and related problems for achieving sustainable development in the country. Wastewater treatment and its reuse in agriculture is also complementing the Swachha Bharat Mission by govt. of India. In brief, the consortium project Water4Crops has made good progress upto second year and works under each deliverable is on track. Number of manuscript are in pipe line and partners are taking initiative to take the project learning's to policy maker for scaling-up of key activities of project. The consortium team is working as one team and substantial progress has been achieved and plans for strengthening and expanding the work during the third year are already in place.

Background

Ensuring global food security for the ever growing population that will cross nine billion by 2050 and reducing poverty is a challenging task. The increased food production has to come from the available and limited water and land resources which are finite. Neither the quantity of available water nor land has increased since 1950, but the availability of water and land per capita has declined significantly due to increase in global human population. For example, in India per capita water availability has decreased from 5177 m^3 in 1951 to 1820 m^3 in 2001 due to increase in population from 361 million in 1951 to 1.02 billion in 2001 which is expected to rise to 1.39 billion by 2025 and 1.64 billion by 2050 with associated decrease in per capita water availability of 1341 m^3 in 2025 and 1140 m^3 by 2050 respectively. There is an urgent need to manage water resource efficiently through enhancing water use efficiency and demand management. Water availability for food production is not only restricted to fresh water but wastewater re-use is also emerging as an integral part of demand management (Al-Jayyousi 2003; Al-Hamaiedeh and Bino 2010).

With rapid expansion of cities and domestic water supply, quantity of gray/wastewater is increasing in the same proportion. Almost 90% of total water supplied for domestic use was generated as wastewater which would be diverted for agriculture purpose. Grey water use in agriculture contributes significantly to the supply of fresh fruits and vegetables to urban markets. However, there is higher risk associated with human health and the environment on the use of wastewater especially in developing countries, where rarely the wastewater is treated and large volumes of untreated wastewater are being used in agriculture (Buechler and Scott 2006).

In the above context, the water4crops project explores the possible opportunities of wastewater use in agriculture both in Indian and in European context. Since, the project is involving both research and industries as consortium partners, this will help in identifying efficient treatment methodologies. This co-creation process will boost the business development in the field of bio-treatment, wastewater re-use, and agricultural innovations to reduce the water footprint. This process would integrate the role of co-learning, links between traditional and industrial agri-production systems, better utilization of market opportunities. This project would facilitate researchers and project partners to conduct science based research on wastewater treatment and its management would open-up various avenues for up-scaling process. This project aims at twinning leading examples from cases in Europe with cases in India for exploiting agricultural water use in better ways.

Objectives of the Project

1. Develop and demonstrate integrated treatment processes for agro-food industry effluents targeted at recovery of economically useful components and recycling of water suitable for irrigation
2. Selection and optimization of microbial consortium to reclaim degraded lands and bio-treatment of municipal wastewater for re-use in agriculture
3. Enhancing water use efficiency through improved irrigation systems, agronomic practices and using validated simulation models
4. Assess impacts of treated wastewater on soil, crop produce and groundwater quality

5. Increasing saline wastewater use efficiency through Integrated Mangrove-Fishery Farming System
6. Mapping and characterization of quantitative trait loci (QTL) for drought tolerance related traits in maize, sorghum, pearl millet, chickpea and tomato
7. Improving drought adaptation using marker-assisted breeding and trait-based selection approaches in maize, sorghum, pearl millet, chickpea and tomato
8. Evaluate and optimize the proposed combinations of bio-treatment and wastewater reuse from a perspective of supporting green growth and to boost interaction between knowledge organizations and industries of the European and Indian parties.

Strategy

The Water4Crops consortium partners have a common mandate to find solutions for emerging water and related problems for achieving sustainable development in Europe and India. The consortium is designated to satisfy all the project objectives, permitting to treat and reuse wastewaters for non-potable uses. The consortium is a conglomeration of public research institutes, private non-government research institutes, universities, private industries both large and small, and consulting firms from Europe and India thus forming a perfect example for international public private partnership. The list of consortium member is given in Table 0-1. This include premier research institute from the countries in the field of environmental and agricultural sciences. ICRISAT is lead institute for Indian consortium and IRSA-CNR is lead institute for EU consortium.

National research institutes like The Energy and Resources Institute (TERI) and National Environmental Engineering Research Institute (NEERI), who are the pioneer institutes of industrial wastewater research, will be engaged in finding solutions for reusing wastewater in different sectors. On field research institutes, along with the strategic research on water use efficiency, International Crops research Institute for the Semi-Arid Tropics (ICRISAT), University of Agricultural Sciences Dharwad (UASD), and Bangalore (UASB) are involved for conducting the research on water and crop management aspects. For dissemination, coordination and management, Euro-India Research Center (EIRC) and ICRISAT have vast experience. Industry partners of Water4Crops India consortium include – SAB Miller, Ugar Sugar who will work towards developing and demonstrating integrated treatment processes for bio-refinery effluents. Another industry JISL will be involved in agricultural and water management activities including bioremediation of degraded wasteland (due to untreated wastewater irrigation) and bio-treatment of municipal wastewater for reuse in agriculture. MSSRF will develop water efficient crop variety for selected crops and on integrated mangrove-fishery farming system to optimise use of saline wastewater.

Besides consortium approach other important part of strategy are mirror case approach, innovative modular biotechnological approach, co-learning, co-creation of new products leading to be business opportunities. The miror cases are at the Emilia Romagna region (Italy) and at ICRISAT (Telangana State, INDIA). Both regions offer potential for excellent application of technology development research in increasing/diversifying agricultural production. Water4Crops is aimed at providing for the first time an innovative combination of individual technical improvements to bridge bio-treatment of wastewater and increased water efficiency with a

trans-disciplinary identification of agri-business opportunities and the related requirements for tailoring technological innovations. Water4Crops is based on three Pillars: P1: Biotechnological wastewater treatment, P2: Improved water use efficiency, P3: Enabling Green Economy. Each of them is structured into Work Packages (Table 0-2) (P1-WP1: Valorization, treatment and reuse of agrofood industry wastewater; P1-WP2: Innovative municipal wastewater bio-treatment for agricultural reuse; P2-WP3: Agricultural water management; P2-WP4: Improving water use efficiency and drought tolerance via genomic approaches and modelling; P3-WP5: Methodology for trans-disciplinary approach; P3-WP6: Dissemination and technology transfer. WP7: Coordination and Management covers the whole project.

Table 0-1 List of consortium members from India and EU.

Indian consortium	EU consortium
• International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) • The Energy and Resources Institute (TERI) • University of Agricultural Sciences Dharwad (UASD) • MS Swaminathan Research Foundation (MSSRF) • National Environmental Engineering Research Institute (NEERI) • Jain Irrigation Systems Limited (JISL) • Euro India Research Centre (EIRC) • SABMiller (SABM) • University of Agricultural Sciences Bangalore (UASB) • Ugar Sugar (UGSG) • KCP Sugar Industries	• Istituto di Ricerca Sulle Acque - Consiglio Nazionale delle Ricerche (IRSA-CNR), Dept. of Bari, Italy • Natural Environment Research Council, NERC - Centre for Ecology and Hydrology, (NERC-CEH), Wallingford, UK • University of Applied Sciences Northwestern Switzerland (FHNW), Muttenz, CH • Alma Mater Studiorum – University of Bologna (UNIBO), Bologna, Italy • VITO - Flemish Institute for Technological Research, Brussels, Belgium • Technical University of Crete (TUC), Crete, Greece • Helmholtz Centre for Environmental Research (UFZ), Germany • University of Catania (UNICT) – Department of Agri-food and Environmental Systems Management [GESA], Catania, Italy • Unité Mixte de Recherche Gestion Eau Acteurs Usages (GEAU-Cemagref), France • Institut National de la Recherche Agronomique (INRA), France • Stichting Dienst Landbouwkundig Onderzoek (ALTERRA), Wageningen, NL • Consorzio di Bonifica di Secondo Grado per il Canale Emiliano Romagnolo (CER), Bologna, Italy • Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), Eschborn, Germany • INOFEA GmbH, Basel, Switzerland. • SIMA-tec GmbH, Germany • BionActis International Group SA (Bionactis), Valais, Switzerland • PHYTOREM S.A., Miramas, France • BioPlanta GmbH, Leipzig, Germany • Environmental Nutritional and Health Services S.A.(Envinhealth), Greece • Horta srl (HORTA), Piacenza, Italy • S.T.E.P. Consulting GmbH (STEP), Germany

Table 0-2 List of work packages and work package leader.

WP No.	Work package title	Co-ordinator
1	Agro-food industry wastewater valorization and reuse	Dr. Malini Balkrishnan
2	Bio-treatment of municipal wastewater for reuse and bioremediation of degraded lands	Mr. Prashant Thawale
3	Agricultural water management	Dr. Suhas Wani
4	Development of water efficient crop varieties	Dr. Pooran Gaur
5	Enabling green growth using water treatment and reuse innovations	ICRISAT/ERIC/GIZ
6	Dissemination and technology exchange	Ms. Surbhi Sharma
7	Coordination and management	Dr. Suhas Wani Ms. Surbhi Sharma

Table 0-3 List of deliverable during project period.

Delivera-ble No.	Deliverable name	WP No.	Delivery date	Status
1.1	Detailed characterization of selected wastewaters	WP1	Month 12	Complete
1.2	Demonstration of CW and HRTS systems	WP1	Month 30	Ongoing
1.3	Demonstration of fungal decolourization system	WP1	Month 42	Complete
1.4	Demonstration of algal treatment system	WP1	Month 48	Complete
1.5	Carbons and membranes for the recovery of phenolics / pigments	WP1	Month 48	Ongoing
1.6	Impact of treated and untreated wastewater use on soil, crop and groundwater quality	WP1	Month 48	Ongoing
2.1	Report on microbial consortium formed using available strains	WP2	Month 12	Complete
2.2	Optimized microbial consortium for remediation of degraded land	WP2	Month 24	Ongoing
2.3	Demonstration of CWs and HRTS systems	WP2	Month 30	Complete
2.4	Bio-remedial measures tested to improve degraded lands due to use of wastewater	WP2	Month 36	Ongoing
2.5	Report on impact assessment of wastewater use in agriculture	WP2	Month 48	Ongoing
3.1	Benchmark sites characterized	WP3	Month 12	Complete
3.2	Efficient irrigation system evaluated	WP3	Month 36	Ongoing
3.3	Impact assessment of wastewater on crops, soil and groundwater documented	WP3	Month 48	Ongoing
3.4	Validated models for enhancing WUE at field and micro-watershed level	WP3	Month 48	Onoing
3.5	Increased land and saline wastewater productivity in 20 ha	WP 3	Month 48	Ongoing

Delivera-ble No.	Deliverable name	WP No.	Delivery date	Status
3.6	Replicable model demonstrated for integrated saline wastewater use and livelihood options	WP 3	Month 48	Ongoing
3.7	Package of agro-aqua farming system available for replication	WP 3	Month 48	Ongoing
3.8	Enhanced capacity of community, other stakeholders and MSSRF staff on saline wastewater farming	WP 3	Month 48	Ongoing
3.9	Availability of tool kit on agro-aqua farming system in print and multimedia format	WP 3	Month 48	Ongoing
4.1	Information on the most adequate combinations of species/genotypes x environment x management for different drought scenarios in India and EU	WP4	Month 36	
4.2	Information on QTL (QTL combination) underlying the drought adaptation traits in maize, sweet sorghum, pearl millet and tomato at particular drought stress environments	WP4	Month 36	
4.3	Mechanisms for improved water use efficiency and salinity tolerance characterized across crop species	WP4	Month 48	
4.4	Chickpea breeding lines with improved drought adaptation	WP4	Month 48	
4.5	Trained human resources in research on drought adaptation of crops and integrated breeding for drought adaptation	WP4	Month 48	
5.1	Database of stakeholders	WP5	Month 12	Complete
5.2	Report of agribusiness opportunities	WP5	Month 24	Complete
5.3	Position papers on wastewater topics	WP5	Month 48	Ongoing
6.1	Internal report on customer / entrepreneur demands and technological offer	WP6	Month 12	Complete
6.2	Webpage and Public Dissemination material	WP6	Months 6,12,24,36, 42	Complete
6.3	Report on training course including online curricula	WP6	Month 36	Ongoing
7.1	Workshop to workout common protocols to be adopted by the partners in the project	WP7	Month 12	Complete
7.2	First year annual report to DBT	WP7	Month 12	Complete
7.3	Second year annual report to DBT	WP7	Month 24	Complete
7.4	Third year annual report to DBT	WP7	Month 36	Ongoing
7.5	Fourth year annual report to DBT	WP7	Month 48	-

1 Work Package: Agro-Food Industry Wastewater Valorization and Reuse

1.1 Objectives

To develop and demonstrate integrated treatment processes for agro-food industry (biorefinery) effluents targeted at (a) recovery (direct or after conversion) of economically useful components from agro-food industry/biorefinery wastewater and (b) production of treated water suitable for irrigation purposes.

1.2 Detailed characterization of selected wastewaters

Four sites were selected in WP1 to study potential of industrial wastewater recycling in agriculture. Profile of the wastewater and degraded soil collected from these sites were presented in the first annual report.

Table 1-1 Selected sites for reuse of industrial wastewater

Sl No.	Site	Industry type
1	SAB Miller, Sangareddy, Telangana	Brewery
2	Ugar Sugar Works, Belgavi, Karnataka	Distillery
3	K.C.P. Sugar and Industries Corporation Ltd, Chennai, Tamilnadu (Lakshmipuram and Vuyyuru)	Distillery
4	Jain Irrigation System Ltd, Jalgaon, Maharashtra	Food processing

1.3 Demonstration of CW and HRTS systems

1.3.1 SAB Miller Sangareddy

The performance of the CW was observed during the study period. Due to frequent problem due to the leakage of the inlet pipe that supplies ETP effluent to the CW inlet, the CW suffered periods of absolute dry spell and water logged condition. The constructed wetland the vegetation covers of *Napier* nor could Bamboo survive.

Figure 1-1 The condition of CW in Aug 2015, SAB Miller, Sanga Reddy, Telangana, India.

Taking the learnings from ICRISAT constructed wetland site about the robustness of *Cana indica* replanting was carried during the month of October 2015.

Figure 1-2 Planting of Cana indica in CW, SAB Miller, Sanga Reddy, Telangana, India.

Figure 1-3 Gradual stabilization and growth of Cana indica in CW, SAB Miller, Sanga Reddy, Telangana, India.

The plant could establish in a short time and the CW was stabilized by the month of December, 2015. In order to increase the per square meter plant density re-distribution of suckers was carried out during Dec 2015. The average inlet and outlet wastewater characterises during Dec 2015 and March 2016 is given in Table 1-2 In order to study the survival rate and growth halophyte species (*Sesuvium portulacastrum*) was introduced.

Figure 1-4 Introduction of Sesuvium portulacastrumin the CW, SAB Miller, Sanga Reddy, Telangana, India.

Table 1-2 The average inlet and outlet wastewater characterizes during Dec 2015 and March 2016

Sl. No	Parameters	Unit	Inlet	Outlet	Efficiency (%)
1	Calcium	mg/L	75.69	68.45	9.57
2	Chemical oxygen demand (COD)	mg/L	96	64	33.33
3	Chloride	mg/L	174	166	4.60
4	Electrical conductivity	mS/cm	3.39	3.12	-
5	Potassium	mg/L	58.89	58.72	0.29
6	Magnesium	mg/L	25.23	22.82	9.57
7	Sodium	mg/L	439	397	9.57
8	Inorganic nitrogen	mg/L	18	12	33.33
9	pH at 25 ° C	-	7.98	8.78	-
10	Phosphate	mg/L	1.88	1.17	37.77
11	Sulfate	mg/L	2.83	2.48	12.37
12	Total dissolved solids (TDS)	mg/L	2019.50	1432	29.09
13	Total Alkalinity	(mg/L as CaCO3)	606	515	15.02
14	Total hardness	(mg/L as CaCO3)	610	540	11.48

The treated water was utilized for sugar cane cultivation in the adjacent fields (4 acres). The problem of inlet water supply re-surfaced in the month of May 2016 and as a result availability of treated water for sugar cane cultivation got affected (Figure 1-5). In absence of any other available source of water the crop suffered from nutrient deficiency. As nutrient deficiency at a critical growth stage of sugar cane cannot be rectified with the application of fertilizer later on, stunted growth and a below average yield (1.8 quintal) was observed (Figure 30).

Figure 1-5 Water starvation of sugarcane during critical growth period

Figure 1-6 Different Phases of Sugarcane cultivation at SAB Miller, Sanga Reddy, Telangana, India

It is worth mentioning during these dry spells *Cana indica* growth in the CW too suffered however the growth of the halophytes species peaked-up significantly.

Figure 1-7 Growth of halophyte in the CW during water starvation phase at SAB Miller, Sanga Reddy, Telangana, India

During the last few months it was observed that the distribution of inlet wastewater was not uniform in the entire bed of the constructed wetland living corners and margin areas dry. In order to rectify this problem the top sand layer from the first 1 meter of the inlet side was removed and the gravel layer was exposed for the inlet water. The sand thus removed was utilized to create a bund structure at the inlet side to maintain sub-surface flow. The modification was found to be very effective to achieve the desired objective.

1.3.2 Lakshmipuram site

1.3.2.1 Water mass balance in constructed wetland

The overall water mass balance in the constructed wetland is shown in table 1-3 and it is calculated based on the below equation. In general there are various factors taken into account for computing the gain and loss of water from constructed wetland like precipitation, infiltration, runoff, evaporation and transpiration, however the wetland at Lakshmipuram is a concrete sealed construction and hence factors like infiltration and runoff are neglected.

$$Qi - Qo + (P * A) - (ET * A) = dv/dt \quad \text{------- (2)}$$

where Qi Rate of inflow (m^3/day)
Qo Rate of outflow (m^3/day)
P Precipitation rate (m/day)
ET Evapotranspiration rate (m/day)
A Surface area of wetland (m^2)
V Water storage in wetland (m^3)
t Time (day)

Table 1-3 Hydrology balancing in constructed wetland

CWL Beds	Length of CWL beds (m)	Width of CWL (m)	Coarse aggregate volume (m^3)	Porosity	Volume of water in the void (m^3)	Volume of water above aggregate level (m^3)	Total volume of water in the CWL	Precipitation (m^3)	Evaporation (cm/day)	Transpiration (cm/day)	ET losses (m3)	Net volume of water in CWL (m^3)
C1	3.24	11.10	21.59	0.53	11.36	7.20	18.55	0.54	0.747		0.269	18.821
C2	2.11	11.10	14.04	0.49	6.94	4.68	11.62	0.35	0.758		0.177	11.786
C3	11.18	11.10	74.46	0.50	37.08	24.82	61.90	1.85	0.733	0.212	1.173	62.574
C4	3.24	11.10	21.59	0.53	11.36	7.20	18.55	0.54	0.747		0.269	18.821
C5	2.11	11.10	14.04	0.49	6.94	4.68	11.62	0.35	0.758		0.177	11.786
Total	**21.88**	**------**	**145.72**	**-------**	**73.67**	**48.57**	**122.24**	**3.61**	**3.74**		**2.07**	**123.79**

The inflow of water from filtration tank to constructed wetland is 19.44m^3/day. The porosity of different substrate is measured and the average porosity of CWL is 49.8%. Coarse aggregate volume multiplied by the actual porosity of each bed gives the volume of water in the void i.e. 73.67m^3. Volume of water above the aggregate was calculated simply by multiplying length x width x 0.2m depth which constitutes 48.57 m^3. Summation of volume of water in the void and volume of water above the aggregate gives the total volume of water (122.24 m^3) in the CWL. The net volume of water in the wetland is estimated by adding precipitation gain and subtracting evaporation and transpiration losses with respect to each bed. The net volume of water in the CWL is 123.79 m^3 which slightly lesser (1.76m^3) than the previous year. Major reasons are due to climatic variables like reduced precipitation and increased temperature. The hydraulic retention time of constructed wetland is 6.4 days and the hydraulic loading rate is 2.91 m/day.

1.3.2.2 Modeling the performance of constructed wetlands using Subwet 2.0

The SubWet 2.0 model, a horizontal subsurface flow modelling program is designed to predict the treatment efficiency of the constructed wetland.

- It is based on 16 rate constants specific to a variety of processes involved in the treatment of BOD, Ammonia, Nitrate, Org-N and Total Phosphorus uses an integrated approach and performance based data to calibrate the model to site conditions.
- It is a software program used in designing of constructed wetlands for treatment and water quality improvement which was originally developed by UNEP-DTIE-IETC.
- This model can consider the influence of several factors at one time while empirical equations are generally not able to consider more than two factors at one time and usually in isolation of the other influential parameters.

- SubWet allow managers to predict the impact of treatment efficiency based on an alteration to the aerial loading rates, hydraulic retention time (HRT) and the desired level of influent treatment.
- The model can also be used as a predictive tool to help managers determine the size of wetland needed to meet treatment objectives. This will assist managers in determining if the current wetland size can accommodate projected growth in population and anticipated effluent volumes.
- The model can be used to predict treatment performance of the constructed wetland with anticipated alterations in the size of the treatment area.
- Eventually, SubWet can be used by resource managers to demonstrate the treatment benefit acquired from the use of designated treatment wetlands and can also be used as a predictive tool to forecast the potential areas that could provide from the application of selected management operations. This will help resource managers in cost benefit analysis when planning for future needs.

The main objective of this modelling effort with Sub wet2.0 in the present study is to assess the performance of constructed wetland demonstrated by MSSRF in KCP sugar factory at Lakshmipuram, Andhra Pradesh which treats the primary treated sugar effluent.

The physico chemical parameters of the water samples collected before and after treatment in hybrid model constructed wetlands are used to run the SubWet model. The input data are the physical dimensions for the selected constructed wetland like slope, hydraulic conductivity, the precipitation factor (1.0 means that the precipitation does not dilute the wastewater, while the precipitation factor 2.0 means that the wastewater is diluted by a factor 2) are entered in the design window. In the forcing functions window, the length of simulation, temperature, concentrations of BOD, nitrate-N, ammonium-N, total phosphorus etc. of untreated wastewater is entered. Porosity as a fraction is given as input. Oxygen concentration in each compartments of the constructed wetland are entered in 5 boxes – A, B,C, D and E. Water volume and retention time in days per box is calculated when the respective buttons are pressed. “Initial Values” window allows us to enter the concentration of BOD, nitrate-N, ammonium-N, total phosphorus and organic nitrogen in five compartments. In the parameters window, default values chosen for warm climate can be given as input. Simulation window allows us to enter the treated physico-chemical parameters. Y axis maximum value, time step 50 or 70 can be entered and model can be run for simulation. Printed simulated values can be taken using file print option.

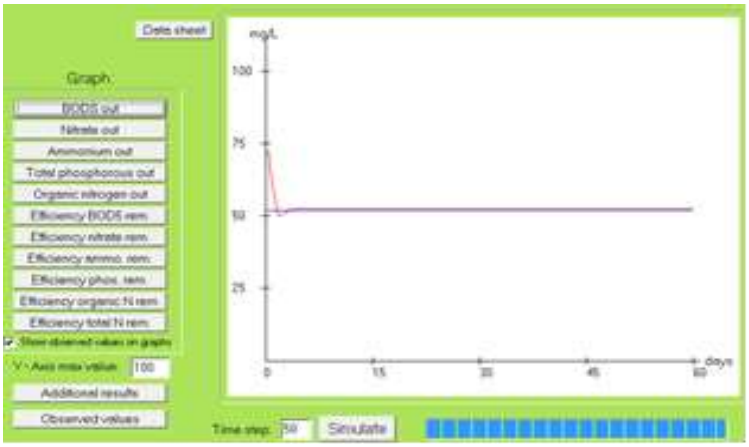


Figure 1-8 Observed BOD Concentration with Nitrate.

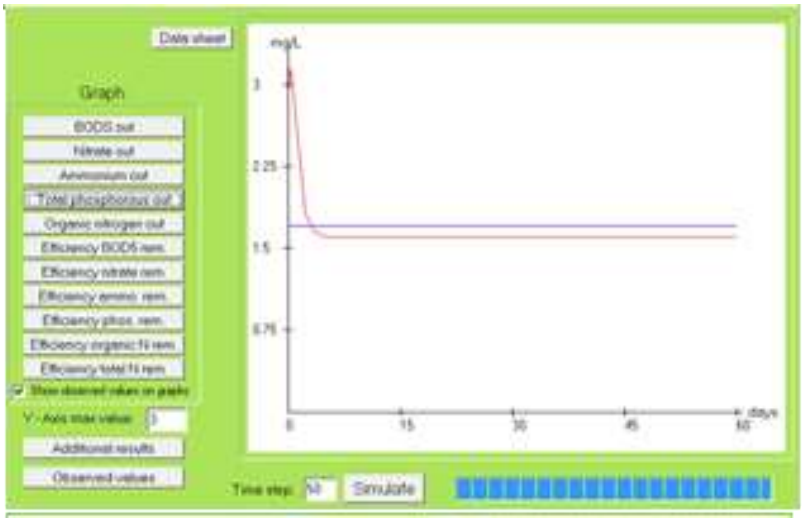


Figure 1-9 Observed BOD Concentration with Nitrate and Ammonium

The graph (Fig.1-8&1-9) shows that the blue line (observed) and red line (simulated) both are very close which indicate that the model prediction is good with observed BOD concentration. Although the simulated results for BOD_5 and phosphorous is close to the actual observed concentrations measured in the sugar effluent exiting the constructed wetland, the nitrogen compounds show less agreement between simulated and observed results suggesting that SubWet requires calibration for these compounds. For example, the simulated value for nitrate is approx. 0.18 mg/L and yet the observed value is 0.7 mg/L.

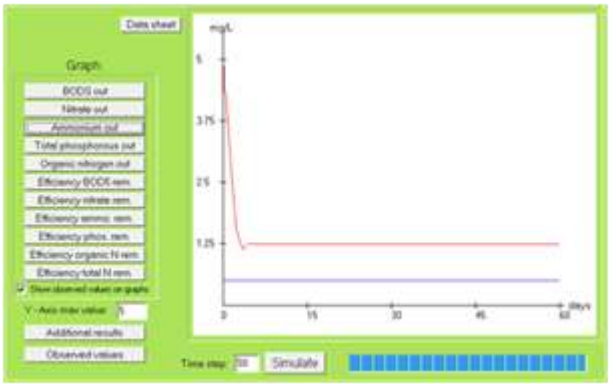


Figure 1-10 Nitrate observed and simulated values.

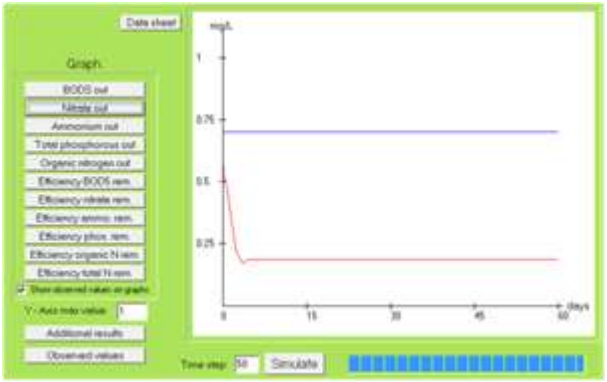


Figure 1-11 Ammonium observed and simulated values

The above graphs (Fig. 1-10 & 1-11) indicate that simulated nitrate and ammonium results vary much with the observed results. The difference is unacceptable and it could be due to overestimation of the denitrification rate or an underestimation of the nitrification rate. The simulated nitrate concentration is a product of both denitrification (conversion to nitrogen gas) which removes nitrate from the effluent stream, and nitrification (conversion of ammonium to nitrate) which produces nitrate. Likewise the model results for ammonium once again shows a minor discrepancy between the simulated ammonium concentrations (approx. 1.25 mg/L) in comparison to the observed value which is closer to 0.5 mg/L. With the simulated value and the observed value percentage deviation can be calculated for each of the parameters. If the difference is below 15%, the simulated values can be accepted without calibration. If it is greater than 15% the model should be calibrated with specific default values.

Table 1-4 Comparison of simulated and observed concentrations for the constructed wetland data

Parameters	Units	Simulated results	observed results	% Deviation
BOD	mg/l	52.4	5	0.8
Nitrate -N	mg/l	0.18	0.7	74
Ammonium -N	mg/l	1.2	0.5	140
Phosphorus	mg/l	1.6	1.7	5.9

Table 1-4 summarizes the difference between the observed and simulated results for BOD, nitrate-N, ammonium-N and total phosphorus. The simulated BOD and the total phosphorus values are close to the observed values. However, the values for nitrate-N and ammonium-N, are not acceptable but can be improved if the SubWet is calibrated for this specific location.

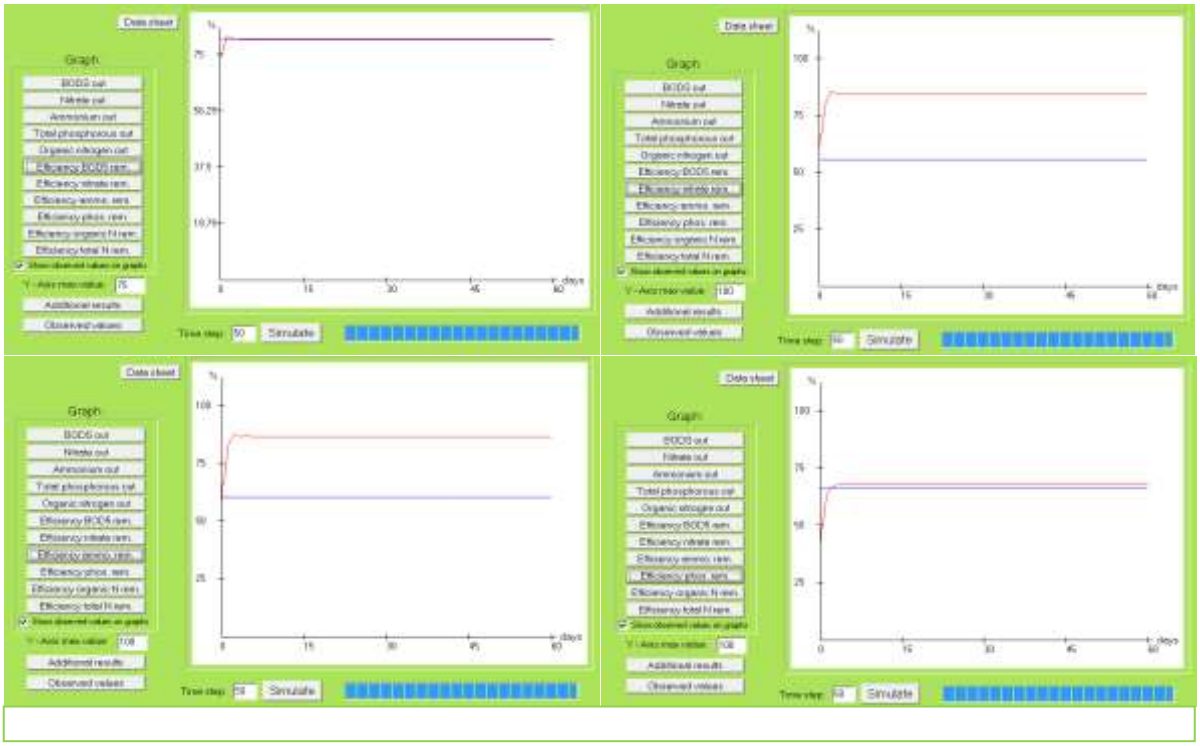


Figure 1-12 Efficiency in removing the physico-chiemical parameters

Figure 1-12 illustrates the removal efficiencies of BOD, nitrate, ammonium and phosphorus. Figure 1-13 explains the treatment efficiency of the constructed wetland derived from the SubWet Model 2.0. The overall treatment efficiency of the constructed wetland is evaluated as very efficient. The simulated output indicates that the BOD concentration have been reduced from 260 mg/l to 52 mg/l with the treatment efficiency being 79.8%. Similarly for the nitrate-N, ammonium-N and total phosphorus the treatment efficiencies are 84.2%, 86.5% and 68% respectively.

Thus it can be concluded from the above study that the treatment efficiency of the constructed wetland is very much appreciable and the SubWet model can be used for similar constructed wetlands in assessing its performance. However, it can be recognized that factors such as inter-year variability in climate, loading rates and the composition of the wastewater coming out from the industry outlet can still introduce variability in the predicted year to year values. Hence, it is anticipated that increased monitoring and the generation of additional measured data will help in assessing the treatment performance of the constructed wetland.

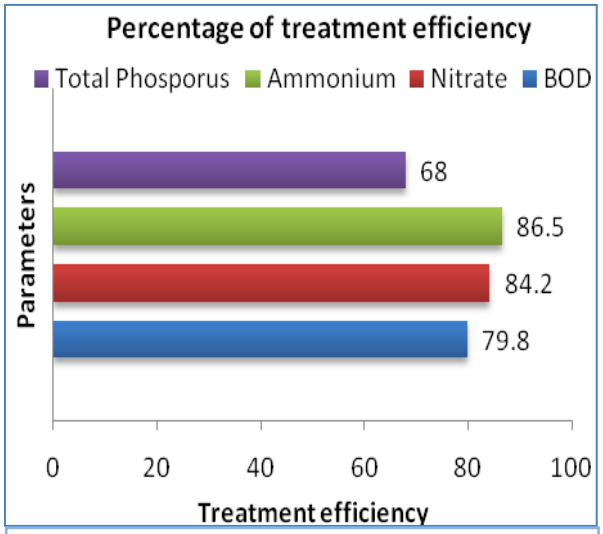


Figure 1-13 Treatment efficiency of CWL

1.3.2.3 Role of algal consortium in treating SE and agribusiness potential

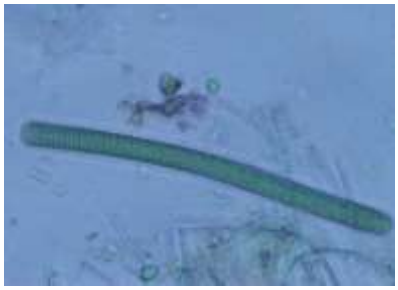
Type of algal consortium
BED 1 - *Spirogyra* sp., *Phormidium* sp.
BED 2 - *Spirogyra* sp., *Chroococcus* sp.
BED 3 - *Spirogyra* sp., *Phormidium* sp., *Cladospora* sp., *Amphora* sp.,
BED 4 - *Gomphospaeria* sp., *Anabaena* sp., *Phormidium* sp., *Amphora* sp., *Cladospora* sp.
BED 5 - *Chlorococcum* sp.

***Spirogyra* sp.**

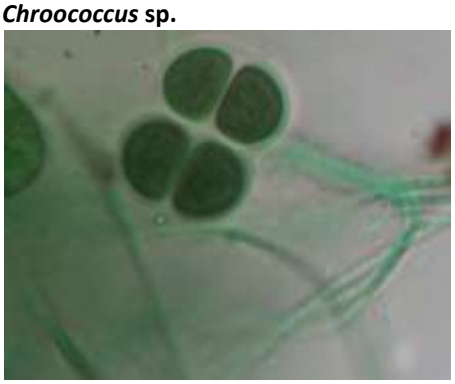
Spirogyra is also known as water cell, silk weed. It is filamentous green algae. The Plastids in form of one or more marginal spiral ribbons is the main characteristic of the genus and it also contains pyrenoid and nucleus. Cell walls are parallel sided and straight. Cells with 10-100 µm in width are joined end to end in un-branched.

***Phormidium* sp.**

The phase contrast microscopic observations compared with APHA standards revealed filaments arranged in irregular fashion. The cells were rectangular and have un-constricted or slightly constricted cross walls. Trichome lacked heterocyst and not tapered, and has a sheath extending beyond the end of the trichomes. The filaments were long, cylindrical and at times curved or spiralled.



***Chroococcus* sp.**



Cells are blue-green in color and macroscopic colony mounded. Within the outside sheath, microscopic colonies are found with indistinct trichomes. *Chroococcus* are usually found in colonies of two, four, or eight cells with a transparent protective covering sheath containing photosynthetic pigments. It is a prokaryote and therefore lacks any of the membranous organelles of eukaryotes. *Chroococcus* is commonly present in the sludge of lake and river bottoms etc.

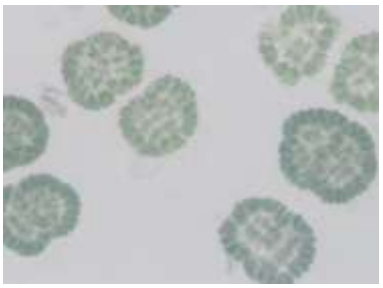
***Cladospora* sp.**

The phase contrast microscopic observations compared with APHA standards revealed that all cells were essentially alike, light to medium green, cylindrical. Filaments embedded in gelatinous matrix. Branches long with cross walls.

***Amphora* sp.**

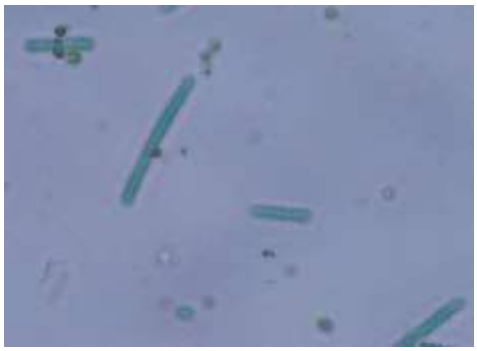
The microscopic observations compared with APHA standards showed that the *Ampora* sp. cells had elliptical, flat truncate ends the valves had no transverse septa. The valves are asymmetrical and are sometimes smaller or constricted at each end of the cell. Both raphes (ridge) lie on the same side of the valve.

Gomphospaeria aponina



In Greek gomphos –a bolt, spharia –ball. The Cell are longitudinally unsymmetrical (two sides unequal in shape), at least in valve view. The Cells ovate to heart-shaped connected to centre of bead by colour less stalks. The colonies are within a mucilaginous envelope.

***Anabaena* sp.**



Anabaena is blue green algae. It has two or more distinct layers of gelatinous sheath around each cell. It has uniseriate, straight, curved or coiled trichrome that may be constricted at the cell walls. The blue green to yellow green colored cells may be spherical, ellipsoidal, cylindrical, or bent, but over all look like a *string of beads*. Heterocyst are intercalary or terminal or both. The terminal cells may be rounded, tapered or conical in shape. It has thick walled resting cells called akinetes are found adjacent to the heterocyst.

***Chlorococcum* sp.**

Chlorococcum sp. is a genus of green algae. It is unicellular with spherical or slightly oblong cells of varied size. The cells may be solitary or in irregular clumps sometimes forming films on moist or submerged surfaces. Each cell has a single cup shaped, parietal chloroplast with a pyrenoid. Plastid fills ¾ or more of the cell.

1.3.2.4 Biomass and treatment mechanisms

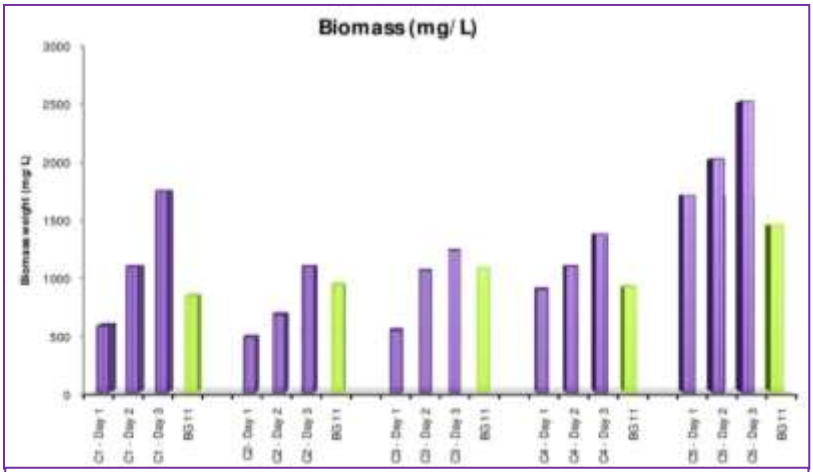


Figure 1-14 Algal biomass from different compartments of CWL

Biomass, in ecology is the mass of living biological organisms in a given area or ecosystem at a given time. It can include microorganisms, plants or animals. Algal biomass is the amount of algae in a water body at a given time. The biomass of C1 bed has reached the maximum growth of 1760 mg/l in day 3 whereas the algal consortium in C2 bed showed maximum growth of 1100 mg/l on day 3. And in C3 the highest growth of biomass can be seen in day3 of 1240 mg/l. In C4 and C5 bed shows the increased level of biomass in day3. In every bed [C1, C2, C3, C4, C5] the minimum growth was observed in day1. The biomass for micro algal culture was measured at lab scale in mg/ml. With increase in time, the biomass of algal inoculants increased from the date of inoculation. For the estimation of biomass the medium used was BG11. In BG11 the biomass attained the maximum growth in C5 bed with 1460 mg/l. However, the biomass of algal consortium in the sugar effluent in constructed wetland is higher than that grown in BG11 medium which may be due to the presence of more available organic compounds in the effluent. The biomass growth in C3 bed is low compared to C1, C4 and C5 which may be due to the lack of sunlight which is covered by *Typha* sp. Also, the algal biomass in C2 and C4 beds were less compared to C1 and C5. This may also due to the reason that the shadow Typha is on C2 in the forenoon and on C4 in the afternoon. Although the biomass concentration is low in these beds, the treatment of sugar effluent depends on various factors like hydrology, influent concentration, pH, temperature, synergism within the consortium and etc.

The sugar effluent interacted cells of algal consortium from the C3 bed of CWL were centrifuged at 3000 rpm for 15 minutes. The smear of algal biomass was made on the glass slide and air dried. The dried smear was washed by ethanol and then air dried. The glass slide was fixed on specimen mount with carbon tape. The gold was sputtered on the sample in argon atmosphere. The surface morphology of algae was observed under Scanning Electron Microscopy (S-400, HITACHI, and Tokyo, Japan) (Samuel et al 2012). In Fig 10a the cells of algal consortium were observed. This is an evidence that the algal consortium synergistically grew together and helped in the treatment process.

Nitzschia amphibian

In Fig. 10b, the structure of diatom *Nitzschia amphibian* was observed. Valves taper to bluntly rounded apices. Fibulae are distinct, 7-9 in 10 µm. The central nodule, evident as a wider space between two central fibulae, is present. Striae are characteristically prominent and distinctly punctate, and may be irregularly spaced.

Achnanthes subhudsonis var. kraeuselii

In Fig. 10c, the structure of diatom *Achnanthes subhudsonis var. kraeuselii* was observed. The.Valves are narrow and lanceolate. The raphe valve has a lanceolate axial area becoming narrow at the apices. Striae are radiate and more broadly spaced at the central valve than near the ends. The rapheless valve has a more narrow lanceolate axial area than the raphe valve. The axial area of the rapheless valve is asymmetrically bent near the apices in many specimens. Striae are radiate on the rapheless valve, but less so than on the raphe valve. The striae are composed of punctate areolae.

Achnanthidium exiguum

The structure of diatom *Achnanthidium exiguum* was observed in Fig. 1-15d of scanning electron micrograph. The Valves are linear-elliptical to elliptical-lanceolate with narrowly capitate, subcapitate, rostrate, or subrostrate apices. Larger valves are sometimes slightly constricted in the middle. Both raphe and rapheless valves have a narrow slightly sigmoid axial area. The raphe valve has a distinct fascia that is often slightly wider on one side. The rapheless valve has a small, transapically rectangular, often asymmetric, central area. The raphe is straight, but deflected to opposite sides near the apices, with terminal raphe fissures strongly curved to opposite sides. The external proximal raphe ends are simple, located in slight “pin-hole” depressions, giving the appearance that they are expanded. Internally, the central raphe ends curve toward opposite sides. The striae are radiate on both valves, but almost parallel at the apices. A few very small areolae may be present on the mantle of both valves. Areolae are round or transapically elongated externally, apically elongated internally within the striae.

***Cladospora* sp.**

In fig. 1-15e, the structure of algae *Cladospora* sp was observed. A cylindrical filament was with long branches were present. The cross walls were present along the branches. It looked like a bone with joints.

***Spirogyra* sp.**

In fig. 1-15f, the structure of algae *Spirogyra* sp. was observed. Cell walls are parallel sided and straight. The Plastids in form of one or more marginal spiral ribbons is the main characteristic of the genus and it is present inside the cells which can’t be seen in HRSEM. Cells with 10-100 µm in width are joined end to end in un-branched which also a typical feature of *Spirogyra* sp..

***Phormidium* sp.**

In figure 1-15g, the cells of blue green algae *Phormidium* sp. was observed. The filaments were arranged in irregular fashion. The cells were rectangular and have un-constricted or slightly constricted cross walls. Trichome lacked heterocyst and not tapered, and has a sheath extending beyond the end of the trichomes. The filaments were long, cylindrical and times curved or spiraled.

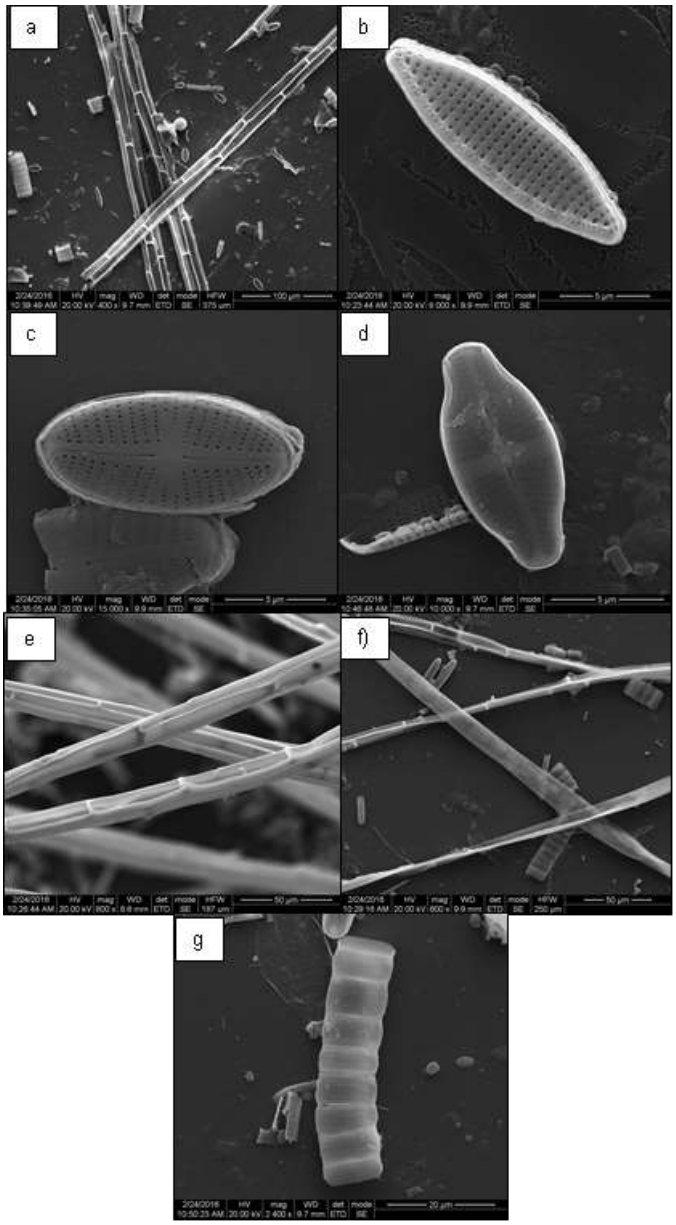


Figure 1-15 High resolution Scanning electron microscopic images of a) consortium b) Nitzschia amphibian; c) Achnanthes subhudsonis var. kraeuselii; d) Achnanthidium exiguum; e) Cladospora sp. f) Spirogyra sp. g) Phormidium sp.

The surface elemental analysis of un-interacted and sugar effluent interacted adapted algal consortium was carried out by Energy Dispersive X-ray spectroscopy. The gold sputtered samples were analyzed and the spectra were recorded using JEOL JSM-5510 equipment.

The EDX spectra of algal consortium a) un-interacted b) interacted with sugar effluent in C3 bed of CWL is shown in fig. 1-16. The carbon and oxygen peaks correspond to the surface of algal biomass. In the algal consortium interacted with sugar effluent, decrease in oxygen wt% and carbon wt% shows that adsorption of contaminants from distillery effluent has masked the algal surface. A significant increase in calcium, sodium, and magnesium ions on the surface of sugar effluent interacted cells of algal consortium compared to the un-interacted cells confirm that the cationic contaminants present in the effluent are

effectively adsorbed on to the surface of algal cells. The intensity of the peak for silica in the EDX data is due to the glass slide on which the sample is studied.

Energy Dispersive X-Ray Spectroscopy

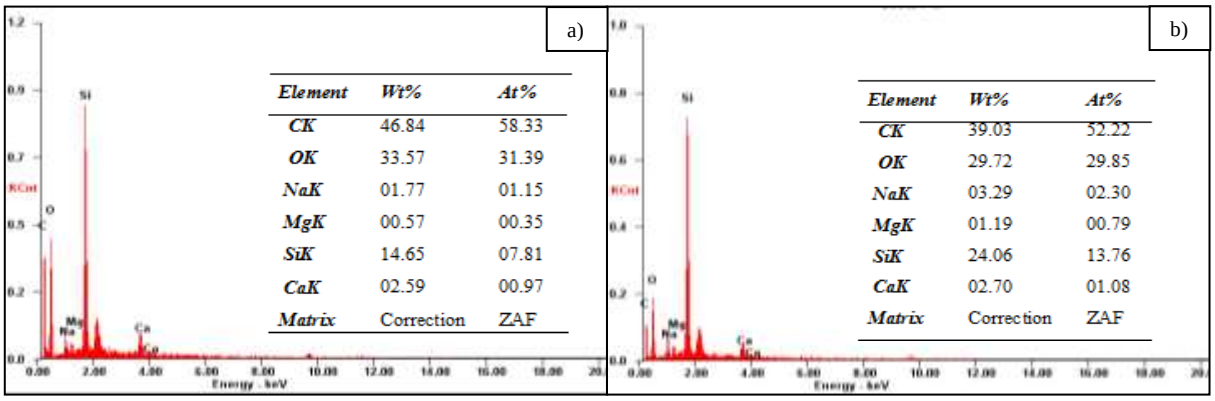


Figure 1-16 EDX spectra of algal consortium a) un-interacted b) interacted with sugar effluent

1.3.2.3.1 Mechanism of algal treatment

Fourier Transform Infra Red spectroscopy (FT-IR)

The FT-IR of algal consortium from C3 bed in the constructed wetland was carried out as the treatment in C3 bed was more efficient than the other beds. The surface chemical characteristics of the adapted algal consortium were characterized by Fourier Transform-Infra Red Spectrometer (Nicolet 6700, Thermo Scientific instruments groups, U.S.A). One mg of each lyophilized algal sample (interacted, un-interacted with distillery effluent) was mixed with 100 mg KBr and the fine powdered mixture was then pressed in a mechanical die press to form a pellet by applying a pressure of 1200 psi for about 5 min. The transparent tablets were inserted in the instrument and the spectra were recorded from 4000 - 500 cm^{-1}.

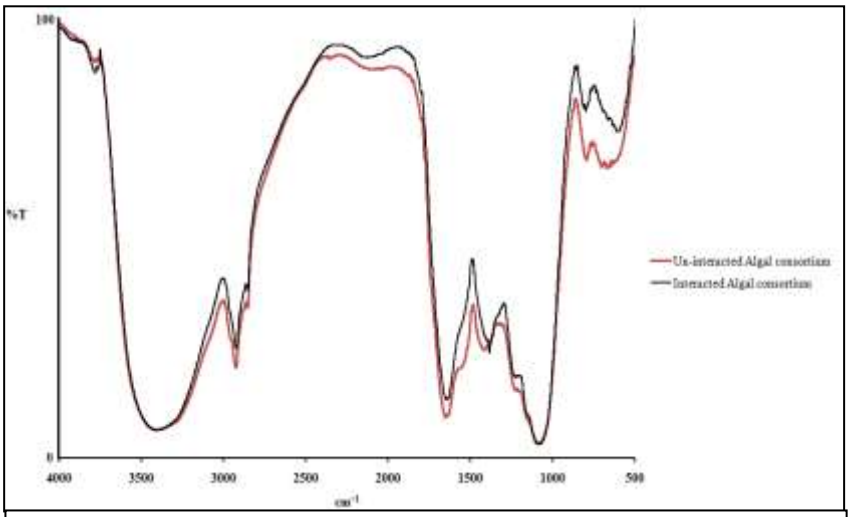


Figure 1-17 FT-IR spectrum of adapted algal consortium interacted & un-interacted with sugar effluent

Table 1-5 Assignment of bands found in FTIR spectra of adapted Algal consortium un-interacted and interacted with sugar effluent

Main peak in un-interacted	Main peak in interacted	Assignment	Probable site for functional group
3408	3408	N–H and O–H stretching vibrations from polysaccharides and proteins	Cell wall – direct interaction of OH with
2924	2919	CH_3 asymmetric stretching from lipids, proteins, polysaccharides and nucleic acids	Proteins and carbohydrates in the cell wall
2853	2851		
1653	1653	Amide I (protein C=O stretching)	Peptides – amino acids/amides
1542	–	Amide II (protein N–H bending and C–N stretching)	Peptides – cell wall
1413	1384	carboxylate ion group (COO−)	cellular
1087	1084	Carbohydrate (-O-C) of polysaccharides. Nucleic acid (other	Polysaccharides – extra cellular
796, 702, 669	800, 600	CH_2 Vibrations of Polysaccharide	Polysaccharide - cell wall

The IR spectra of control and treated algal consortium from the C3 bed are shown respectively in fig.1-17. The spectrum of the un-interacted algae displays absorption bands near 1653 and 1413 cm^{-1}, respectively due to the C=O stretching bands of the carboxylate ion group and COO− of terminal amino acid (Bellamy 1978; Silverstein et al 1991). After the interaction with sugar effluent, the algal consortium exhibited the spectrum with clear decrease of the asymmetrical C=O stretching bands at 1653 cm^{-1} and its symmetrical stretching band at 1384 cm^{-1} with a peak shift. These changes are due to the binding of the carboxylate anion functional group with cations such as Ca^{2+}, Na^{2+}, Mg^{2+} and etc by coordination (Zhou and Wang 1994). This corroborates to the observation discussed in EDX spectra. In this case, most of the carboxylate ion had complexed or chelated with the cations because the symmetrical stretching band at 1413 cm^{-1} moved to a lower frequency at 1384 cm^{-1} (Zhou and Wang 1994), which may be overlaid by other lower frequency bands, resulting in deepening the peak valley between 1653 and 1413 cm^{-1}. Also, the Amide II protein's N–H bending and C–N stretching (cell wall) at 1542 cm^{-1} decreased and weekend which also further deepened the peak valley between asymmetrical and symmetrical stretching of carboxylate ions. This clearly shows that the disappearance of band is due to the binding of anions (organic contaminants) to its surface. A peak shift from 1087 to 1084 cm^{-1}, corresponds to the interaction of very few cations in effluent with the oxygen of the hydroxyl group (C–O–H) from saccharides. This is not an adsorption process as there was no significant change in peak intensity. The peak at 3408 cm^{-1} had a peak shift after interaction with sugar effluent without decrease in peak was due to the interaction of phenol and amine groups (NH) in algal biomass and exopolysaccharide with ions present in effluent. A peak shift with decrease in intensity from 2924, 2853 to 2919, 2851 cm^{-1} is due to the interaction of salts present in effluent with the CH_3 asymmetric stretching from lipids, proteins, polysaccharides of algal cell wall. A moderately intense peak was visible at the range of 796, 702, and 669 cm^{-1} representing CH_2 Vibrations of polysaccharide present in

cell wall which decreased significantly and shifted to 800, and 600 cm^{-1} which is due to the binding of cations to the algal cell wall.

1.3.2.3.2 Agribusiness potential

Spirogyra sp.

- Used as an bio fertilizer
- Produces large amount of oxygen
- Important source of natural bioactive compounds for antibiotics, antiviral, antioxidants, anti-inflammatory, and cytotoxin purposes
- *S. porticalis* has 13 known bioactive chemo types with phyto–pharmaceutical including fatty acid, esters, sterols, unsaturated alcohols and alkynes.
- Lens paper is manufactured in Japan used for cleaning optical instruments

***Phormidium* sp.**

- Used as growth promoting substances. P.tenus has been reported to induce greater height and yield in rice. It has also been seen to increase protein content of rice grains.
- Used as a natural dye and it is named as phycocyanin.

***Chroococcus* sp.**

Chroococcus uses an extensive quantity of atmospheric carbon for photosynthetic processes, creating free oxygen in the atmosphere. In addition, *Chroococcus* is part of the first genus to use water to access electrons and hydrogen for photosynthesis, which also produces more free oxygen to be used by other organisms. This makes it an interesting member for water treatment process. Detailed studies on their genome have not yet been carried out.

***Cladospora* sp.**

- Eaten as a delicacy and commonly known as Mekong weed.
- Extracts from Cladophora, can kill pathogenic Pseudomonas and Mycobacterium

1.3.2.4 Water quality and treatment mechanism at Lakshmipuram site

Physico-chemical properties

The quality of industrial treated sugar effluent was improved by passing the effluent through filtration tank and then to the CWL. Following interpretation is from the annual study for the period of March 2015 to February 2016 as presented in Table 4 to 10. The raw water in this year had a dominating alkaline pH which despite change in the seasons never came below 8.2. No significant change in Ec was observed. But, TDS had decreased by 200 mg/L from pre-monsoon to post monsoon. This decrease is evidently observed during and after NE monsoon which had a decent rainfall that could have diluted the dissolved solids. The total hardness, Chloride and all the parameters remained high in the first two seasons and NE monsoon helped in the decrease of these parameters in raw water. Whereas, phosphate increased a little during the NE monsoon and post monsoon which may be due to the addition of organic content though leaves falling from trees around the tank during wind, rain and autumn. This shows that the water quality of raw water had the

contaminants above the permissible level throughout the year despite seasonal changes and there were hardly any treatment happening naturally.

The filtration tank has a significant role in the removal of TDS, TSS, Total hardness, Chloride, total alkalinity, COD and BOD compared to the wetland beds except C3. The removal of other cations is significant yet less compared to the wetland beds. The treatment in this tank is unaltered by the seasonal variations yet it may have an indirect effect. It is affected by the influent load of contaminants from the raw water which is affected by the NE monsoon. The filtration tank helps in removing coarse particles like algae before entering the wetland. Along with algae, contaminants are too removed from the effluent in the FT.

The constructed wetland receives water from the FT and the initial load varied in different seasons. The treatment efficiency of each bed in the CWL didn't vary even though the initial concentration of pollutants entering each bed varied in different seasons. This may be because the conversion of contaminants into organic products (plant, microbes) has been at a rate for which the available influent load is still above the required load. This can be understood with a detailed long term study with decreasing influent load. So, it is evident that the decrease in initial load during NE monsoon and post monsoon seasons, the quality of effluent improved significantly.

It is evident that the reduction in pH was facilitated by both biological and chemical processes in the wetland. In reduction of conductivity, the key biological process is that the salts (sodium, nitrate, chloride, calcium etc) assimilated by microbes are adhered to the plant roots and up-taken by the emergent macrophytes. TDS removal is due to physical and biological processes such as sedimentation, filtration, bacterial decomposition and adsorption in the wetland. In C3 tank, the bacterial decomposition is favored by the aerobic bacteria present on the soil surface and the adsorption and uptake of various ions by both floating and emergent macrophytes play a vital role in reducing TDS. A large quantity of phosphate is removed by the wetland treatment. The removal is due to the uptake by algal biomass formed on the surface of the substrates which is a biological process. Also, physical process like sedimentation and filtration would have played an important role. The phosphate removal was notably high in the C3 bed which is due to the chemical processes such as phosphate adsorption, complexation and precipitation. Phosphate uptake by both emergent and floating macrophytes and biotic assimilation by the microbes present in the wetland soil and *Typha* roots played an important role in the enhanced removal of phosphate from sugar effluent (Watson, et al., 1989). The reduction of total hardness observed in the C1, C2, C4 & C5 beds can be corroborated to the decrease in dissolved solids like salts due to physical process such as sedimentation and filtration. The reduction of total hardness was relatively high in C3 bed than the other beds which is due to the biological mediated chemical processes. As attributed for EC reduction similar biological process was observed in the reduction of total hardness by uptake microbial degraded salts by plant roots. Reduction in total alkalinity is due to the adsorption by microbes present on the surface of substrates in wetland beds. Also, the exopolysaccharide produced by these microbes help in adsorption and sedimentation of the carbonate salts present in the effluent which is a combined effect of biological and physical process.

Cumulative effect of physical, biological and chemical processes reduced a considerable amount of the total alkalinity in the C3 bed. The biological process was facilitated by the uptake of carbonates by the emergent and floating macrophytes. The microbes helped in the absorption and sedimentation which are chemical and physical processes. The sediments on reaction with the enzymes in the rhizosphere region helped in further reduction of total alkalinity. The reduction of COD and BOD in different beds is due to degradation of organic pollutants by the algal biomass on substrate surfaces. On the other hand rapid growth of algae and saturation will hinder the removal of COD hence algal biomass from wetland is taken away. This continuous removal of algae helps to maintain the reduction of COD and BOD in these beds. In C3 bed, enhanced removal of COD and BOD can be attributed to the enhanced supply of oxygen by the diatoms and emergent macrophytes. Though the floating macrophytes like algae and duckweeds are removing organic compounds it largely depends on the diatoms and emergent macrophytes for its oxygen demand in water. Here, the removal of COD and BOD is facilitated by biological and chemical process. This process helps to increase the dissolved oxygen concentration in water.

Table 1-6 On water quality status of raw water in different seasons

Parameters	Pre-monsoon				SW Monsoon				NE Monsoon				Post-Monsoon			
	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD
pH	8.42	8.49	8.44	0.04	8.2	8.46	8.36	0.14	8.43	8.53	8.49	0.05	8.43	8.68	8.56	0.1264
Ec (mS)	1.79	1.79	1.79	0	1.79	1.79	1.79	0	1.8	1.88	1.83	0.04	1.8	1.85	1.82	0.027
Temp	30.2	37.7	32.7	4.33	30.2	30.8	30.4	0.35	28	31.5	29.4	1.86	28.5	29.4	28.9	0.4276
TDS (mg/L)	658	658	658	0	658	658	658	0	471	658	596	108	458	472	465	6.87
Total hardness (mg/L)	400	400	400	0	400	400	400	0	310	440	385	66.8	303	312	308	4.55
Chloride (mg/L)	310	310	310	0	310	310	310	0	219	308	271	46.4	208	214	211	3.1213
Phosphate (mg/L)	5	5	5	0	5	5	5	0	5.03	5.7	5.34	0.34	5.03	5.18	5.1	0.0754
Sulphate (mg/L)	2	2	2	0	2	2	2	0	2	2.2	2.07	0.12	2	2.2	2.09	0.1026
Total alkalinity (mg/L)	520	520	520	0	520	520	520	0	523	554	539	15.6	523	539	531	7.8425
COD (mg/L)	5000	5000	5000	0	5000	5500	5250	250	2500	6000	4586	1844	1026	1057	1041	15.387
Nitrate (me/L)					1.7	1.7	1.7		1.45	1.8	1.61	0.18	1.58	1.7	1.63	0.0631
Magnesium (me/L)					2.9	2.9	2.9		2.7	2.9	2.8	0.1	2.8	3	2.9	0.1
Calcium (me/L)					2.33	2.33	2.33		2.12	2.3	2.22	0.09	2.23	2.29	2.26	0.0334
DO (mg/L)					2.3	2.3	2.3		2.45	2.6	2.52	0.08	2.4	2.52	2.46	0.0621
TSS (mg/L					126	126	126		117	136	125	9.52	122	125	123	1.8225
BOD (mg/L)									300	300	300		260	268	264	3.9

Table 1-7 water quality status of FT in different seasons

Parameters	Pre-monsoon				SW Monsoon				NE Monsoon				Post-Monsoon			
	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD
pH	8.36	8.38	8.37	0.01	8.12	8.36	8.28	0.14	8.35	8.43	8.39	0.04	8.35	8.6	8.47	0.1252
Ec (mS)	1.79	1.79	1.79	0	1.79	1.79	1.79	0	1.79	1.83	1.8	0.02	1.79	1.85	1.82	0.0269
Temp	30.1	37.9	32.7	4.47	30.1	30.1	30.1	0	27.8	31.2	29.4	1.72	28.2	29	28.6	0.423
TDS (mg/L)	646	646	646	0	646	646	646	0	462	646	585	106	446	459	453	6.69
Total hardness (mg/L)	390	390	390	0	390	390	390	0	298	400	363	56.7	291	300	295	4.3625
Chloride (mg/L)	300	300	300	0	300	300	300	0	206	298	258	47	198	204	201	2.9637
Phosphate (mg/L)	5	5	5	0	5	5	5	0	5	5.4	5.13	0.23	5	5.15	5.08	0.075
Sulphate (mg/L)	2	2	2	0	2	2	2	0	2	2.1	2.03	0.06	2	2.2	2.09	0.1026
Total alkalinity (mg/L)	495	495	495	0	495	495	495	0	496	512	505	7.81	496	511	504	7.4463
COD (mg/L)	4000	4000	4000	0	4000	5100	4500	557	2372	5000	3893	1362	881	907	894	13.213
Nitrate (me/L)					1.7	1.7	1.7		1.42	1.7	1.56	0.14	1.56	1.61	1.59	0.0253
Magnesium (me/L)					2.8	2.8	2.8		2.7	2.8	2.75	0.05	2.75	2.9	2.81	0.0775
Calcium (me/L)					2.2	2.2	2.2		2.03	2.2	2.12	0.09	2.12	2.18	2.15	0.0317
DO (mg/L)					2.3	2.3	2.3		2.5	2.7	2.57	0.12	2.5	2.58	2.53	0.0433
TSS (mg/L					110	110	110		102	113	108	5.65	106	109	108	1.59
BOD (mg/L)									265	265	265		241	248	245	3.615

Table 1-8 On water quality status of C1 bed in different seasons

Parameters	Pre-monsoon				SW Monsoon				NE Monsoon				Post-Monsoon			
	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD
pH	8.31	8.32	8.32	0.01	7.98	8.32	8.21	0.2	8.29	8.37	8.32	0.04	8.3	8.55	8.42	0.1245
Ec (mS)	1.78	1.78	1.78	0	1.78	1.78	1.78	0	1.78	1.8	1.79	0.01	1.79	1.84	1.82	0.0269
Temp	30.6	37.7	33	4.1	30.6	30.8	30.7	0.12	28.4	31.6	29.5	1.87	28.7	29.6	29.1	0.4305
TDS (mg/L)	620	620	620	0	620	620	620	0	434	620	558	107	429	442	435	6.435
Total hardness (mg/L)	385	385	385	0	385	385	385	0	287	390	354	58	286	294	290	4.2855
Chloride (mg/L)	295	295	295	0	295	295	295	0	198	293	252	48.6	195	201	198	2.9205
Phosphate (mg/L)	4.5	4.5	4.5	0	4.5	4.5	4.5	0	4.52	4.8	4.67	0.14	4.6	4.74	4.67	0.069
Sulphate (mg/L)	2	2	2	0	2	2	2	0	2	2	2	0	2	2.1	2.05	0.0503
Total alkalinity (mg/L)	475	475	475	0	475	475	475	0	485	589	521	58.7	485	499	492	7.2723
COD (mg/L)	3500	3500	3500	0	3500	4000	3750	250	1704	4100	3154	1275	684	704	694	10.253
Nitrate (me/L)					1.53	1.53	1.53		1.31	1.5	1.41	0.1	1.3	1.5	1.4	0.1
Magnesium (me/L)					2.7	2.7	2.7		2.53	2.7	2.62	0.09	2.7	2.8	2.73	0.0577
Calcium (me/L)					2.1	2.1	2.1		1.95	2.03	1.99	0.04	2	2.06	2.03	0.03
DO (mg/L)					2.8	2.8	2.8		2.5	3.1	2.85	0.31	2.7	2.8	2.76	0.0531
TSS (mg/L					104	104	104		96	105	100	4.62	104	107	106	1.5598
BOD (mg/L)									228	228	228		215	221	218	3.225

Table 1-9 On water quality status of C2 bed in different seasons

Parameters	Pre-monsoon				SW Monsoon				NE Monsoon				Post-Monsoon			
	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD
pH	8.23	8.36	8.32	0.08	7.89	8.36	8.2	0.27	8.2	8.31	8.27	0.06	8.15	8.39	8.27	0.1223
Ec (mS)	1.6	1.6	1.6	0	1.6	1.6	1.6	0	1.61	1.72	1.68	0.06	1.65	1.7	1.67	0.0248
Temp	30.2	38	32.8	4.47	30.2	30.5	30.3	0.17	28.3	31.4	29.5	1.68	28.3	29.1	28.7	0.4245
TDS (mg/L)	603	603	603	0	603	603	603	0	418	603	541	107	409	421	415	6.135
Total hardness (mg/L)	378	378	378	0	378	378	378	0	282	382	348	56.6	280	288	284	4.2
Chloride (mg/L)	285	285	285	0	285	285	285	0	190	283	244	48.6	185	190	188	2.7722
Phosphate (mg/L)	3.8	3.8	3.8	0	3.8	3.8	3.8	0	3.81	4.4	4.04	0.32	4.1	4.22	4.16	0.0615
Sulphate (mg/L)	1.9	1.9	1.9	0	1.9	1.9	1.9	0	1.9	1.9	1.9	0	2	2.06	2.03	0.03
Total alkalinity (mg/L)	460	460	460	0	460	460	460	0	461	474	467	6.8	466	480	473	6.9884
COD (mg/L)	3000	3000	3000	0	3000	3200	3133	115	1547	3200	2616	927	515	530	523	7.7223
Nitrate (me/L)					1.47	1.47	1.47		1.26	1.37	1.31	0.05	1.2	1.42	1.34	0.122
Magnesium (me/L)					2.3	2.3	2.3		2.39	2.79	2.55	0.21	2.5	2.6	2.56	0.052
Calcium (me/L)					2.1	2.1	2.1		1.9	2	1.97	0.06	1.9	2.06	1.99	0.0808
DO (mg/L)					3	3	3		3.1	3.3	3.18	0.1	3.1	3.3	3.2	0.098
TSS (mg/L					92	92	92		87	97.7	91.4	5.57	95.2	98.1	96.7	1.4285
BOD (mg/L)									198	198	198		162	167	164	2.43

Table 1-10 On water quality status of C3 bed in different seasons

Parameters	Pre-monsoon				SW Monsoon				NE Monsoon				Post-Monsoon			
	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD
pH	7.3	7.33	7.28	0.04	7.26	7.41	7.31	0.09	7.31	7.52	7.4	0.11	7.41	7.63	7.52	0.111
Ec (mS)	1.4	1.42	1.42	0	1.42	1.42	1.42	0	1.43	1.54	1.48	0.06	1.42	1.46	1.44	0.021
Temp	30	37.9	32.8	4.36	30.3	30.3	30.3	0	28.4	31.4	29.5	1.69	27.8	28.6	28.2	0.417
TDS (mg/L)	587	587	587	0	587	587	587	0	390	587	521	114	385	397	391	5.775
Total hardness (mg/L)	320	320	320	0	320	320	320	0	246	320	294	41.1	231	238	235	3.471
Chloride (mg/L)	270	270	270	0	270	270	270	0	164	268	226	54.9	153	157	155	2.292
Phosphate (mg/L)	2.4	2.4	2.4	0	2.4	2.4	2.4	0	2.43	2.9	2.68	0.24	2.5	2.6	2.56	0.052
Sulphate (mg/L)	1.5	1.5	1.5	0	1.5	1.5	1.5	0	1.5	1.6	1.53	0.06	1.5	1.6	1.55	0.05
Total alkalinity (mg/L)	420	420	420	0	420	420	420	0	407	432	419	12.6	420	433	427	6.303
COD (mg/L)	800	800	800	0	800	900	867	57.7	700	817	766	59.8	303	312	307	4.542
Nitrate (me/L)					0.84	0.84	0.84		0.73	1.1	0.87	0.2	0.8	1	0.9	0.1
Magnesium (me/L)					2.1	2.1	2.1		1.95	2.21	2.06	0.13	2	2.1	2.05	0.05
Calcium (me/L)					1.65	1.65	1.65		1.52	1.7	1.6	0.09	1.6	1.65	1.62	0.024
DO (mg/L)					3.5	3.5	3.5		3.5	3.7	3.6	0.1	3.5	3.61	3.57	0.059
TSS (mg/L					71	71	71		68.3	72.1	70	1.93	70.2	72.3	71.3	1.053
BOD (mg/L)									146	146	146		85	87.6	86.3	1.275

Table 1-11On water quality status of C4 bed in different seasons

Parameters	Pre-monsoon				SW Monsoon				NE Monsoon				Post-Monsoon			
	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD
pH	7	7.18	7.1	0.07	7.04	7.1	7.06	0.03	7.04	7.2	7.11	0.08	7.18	7.4	7.29	0.108
Ec (mS)	1.4	1.37	1.37	0	1.37	1.37	1.37	0	1.36	1.37	1.36	0.01	1.37	1.41	1.39	0.021
Temp	30	37.9	32.6	4.62	29.9	29.9	29.9	0	28.4	31.1	29.3	1.54	28.3	29.1	28.7	0.424
TDS (mg/L)	574	574	574	0	574	574	574	0	383	574	510	110	378	389	384	5.67
Total hardness (mg/L)	305	305	305	0	305	305	305	0	207	304	270	54	203	209	206	3.04
Chloride (mg/L)	264	264	264	0	264	264	264	0	137	262	212	66.1	128	132	130	1.921
Phosphate (mg/L)	1.8	1.8	1.8	0	1.8	1.8	1.8	0	1.82	2.2	2.01	0.19	1.7	1.75	1.73	0.026
Sulphate (mg/L)	1.4	1.4	1.4	0	1.4	1.4	1.4	0	1.3	1.4	1.37	0.06	1.4	1.44	1.42	0.021
Total alkalinity (mg/L)	395	395	395	0	395	395	395	0	387	400	394	6.43	397	409	403	5.951
COD (mg/L)	500	500	500	0	500	600	540	52.9	423	518	474	47.4	122	126	124	1.837
Nitrate (me/L)					0.8	0.8	0.8		0.62	0.71	0.68	0.05	0.7	0.8	0.74	0.053
Magnesium (me/L)					1.7	1.7	1.7		1.7	1.74	1.72	0.02	1.6	1.9	1.73	0.153
Calcium (me/L)					1.42	1.42	1.42		1.37	1.4	1.39	0.02	1.4	1.44	1.42	0.021
DO (mg/L)					4.4	4.4	4.4		4.5	4.7	4.58	0.1	4.4	4.64	4.51	0.118
TSS (mg/L					58	58	58		59	60	59.6	0.55	58.9	60.6	59.8	0.883
BOD (mg/L)									81	81	81		52	53.6	52.8	0.78

Table 1-12 On water quality status of C5 bed in different seasons

Parameters	Pre-monsoon				SW Monsoon				NE Monsoon				Post-Monsoon			
	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD	Min	Max	Ave	SD
pH	7.2	7.23	7.18	0.05	7.15	7.17	7.16	0.01	7.1	7.24	7.17	0.07	7.3	7.52	7.41	0.11
Ec (mS)	1.4	1.4	1.4	0	1.4	1.4	1.4	0	1.41	1.49	1.45	0.04	1.42	1.46	1.44	0.021
Temp	30	37.7	32.7	4.33	30.2	30.2	30.2	0	28.1	31.3	29.4	1.72	28.2	29	28.6	0.423
TDS (mg/L)	580	580	580	0	580	580	580	0	386	580	515	112	386	398	392	5.79
Total hardness (mg/L)	310	310	310	0	310	310	310	0	215	309	276	52.3	210	216	213	3.153
Chloride (mg/L)	268	268	268	0	268	268	268	0	141	266	217	66.7	136	140	138	2.042
Phosphate (mg/L)	2.2	2.2	2.2	0	2.2	2.2	2.2	0	2.21	2.6	2.37	0.2	2.3	2.4	2.36	0.051
Sulphate (mg/L)	1.4	1.4	1.4	0	1.4	1.4	1.4	0	1.4	1.5	1.43	0.06	1.4	1.5	1.45	0.05
Total alkalinity (mg/L)	400	400	400	0	400	400	400	0	400	413	405	7.47	404	416	410	6.064
COD (mg/L)	700	700	700	0	700	750	717	28.9	524	713	632	97.6	176	181	178	2.635
Nitrate (me/L)					0.81	0.81	0.81		0.65	0.9	0.76	0.13	0.7	0.9	0.8	0.1
Magnesium (me/L)					1.8	1.8	1.8		1.81	2	1.88	0.11	1.9	2.06	1.99	0.081
Calcium (me/L)					1.48	1.48	1.48		1.43	1.6	1.5	0.09	1.5	1.65	1.58	0.076
DO (mg/L)					4	4	4		3.8	4.4	4.13	0.31	4	4.22	4.11	0.112
TSS (mg/L					64	64	64		63	66.6	64.4	1.95	63.7	65.6	64.7	0.956
BOD (mg/L)									104	104	104		67	69	68	1.005

Table 1-13 Water quality comparison				
Parameters	**RW**	**C3**	**C5**	**Fish Tank**
pH	8.4	7.3	7.1	7.1
Eh	-76.9	-8.5	4.0	-1.7
Ec (mS)	1.8	1.4	1.4	0.8
Temp	30.8	30.7	30.5	29.1
TDS (mg/L)	623.8	552.2	540.3	374.3
Total hardness (mg/L)	381.1	302.4	284.0	198.7
Chloride (mg/L)	284.1	241.3	231.1	139.6
Phosphate (mg/L)	4.9	2.4	1.8	1.4
Sulphate (mg/L)	2.0	1.5	1.4	1.1
Total alkalinity (mg/L)	524.6	418.4	393.2	403.4
COD (mg/L)	4630.6	743.2	453.9	251.3
Caustic alkalinity (mg/L)	BDL	BDL	BDL	BDL
Nitrate (me/L)	1.6	0.9	0.7	0.5
Magnesium (me/L)	2.9	2.1	1.7	1.4
Calcium (me/L)	2.3	1.6	1.4	1.0
DO (mg/L)	2.5	3.6	4.5	5.4
TSS (mg/L	124.5	70.7	59.4	48.1
BOD (mg/L)	272.9	101.2	59.8	45.5

Treated water from C5 is collected in settling tank, after 14 days of retention time the water is pumped to fish tank. The fish tank plays a significant role in altering the water quality which is evident from the percentage reduction observed in water quality parameters measured in C5 (i.e. outlet of constructed wetland) and fish tank. The highest percentage reduction of 44.6% is noted in COD which may be attributed to the sedimentation process happening in the settling tank. While 42.9% and 30.7% reduction of Ec and TDS is observed this may be due to the aggregation and sedimentation of the dissolved solids. The decrease in total hardness from 284 to 199 mg/L (30% reduction) is due to the settling of carbonates and bicarbonates while the 39.6% reduction of chloride is observed which is due to the biological process and reduction of salts confirming the enhancement of water quality. Percentage reduction of 28.6% in nitrate and calcium, 23.9% in BOD, 22.2% in phosphate, 21.4% in sulphate, 19% in TSS and 17.6% in magnesium was observed respectively. This enhancement of water quality from fish tank enables the suitability for reuse in agriculture meeting the irrigation standards.

Influence of climate variability on wastewater quality parameters

It is very clear that climate change has a great impact on the quality, quantity and availability of water.

The main climate change impacts projected on water quality, availability and treatment of the constructed wetland are rise in water temperature especially during summer months which is most vulnerable to change in dissolved oxygen level. Such a change will require additional treatment of water for growing fishes.

Other impacts are reduced summer precipitation, leading to a reduction of stored water in reservoirs fed by seasonal rivers; precipitation variability and seasonal shifts in stream flow; reduction in inland groundwater levels; increase in evapotranspiration as a result of higher air temperatures; the lengthening of the growing season; and increased irrigation water usage.

Dissolved Oxygen is very low during summer because: the solubility of oxygen decreases significantly with an increase in temperature, and re-aeration decreases due to low inflow of water in the constructed wetland. Besides variationin DO, ammonia levels in a wetland compartment may be affected by climate variability.

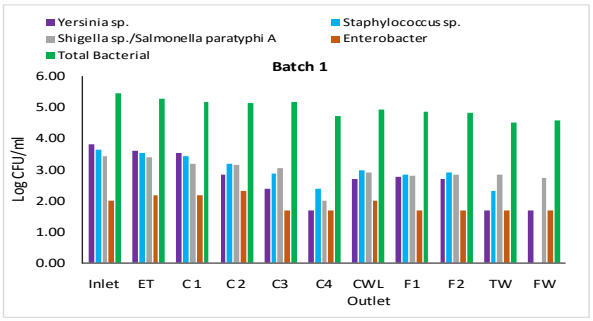


Figure 1-18 18 Total microbial & pathogen population July 2015

1.3.2.5 Biological properties

The initial microbial load in the source water was log 5.46 CFU/ml which after treatment in CWL reduced to log 4.93 CFU/ml a slight reduction was observed in the treatment tanks (Fig. 1-18). Similar to the previous batch the total microbial load was maintained at 5 log CFU/ml throughout the treatment plant in the different column (1-5) of the CWL. Determination of the pathogenic population detected *Staphylococcus* sp., at log 5 CFU/ml and *Enterobacter* sp. at log 3 CFU/ml, while *Shigella* sp./*Salmonella paratyphi* A were detected at low concentration. *Yersinia* sp. was detected from settling tank to Fish tank but the CFU/ml varied in the different treatment tanks. (Fig 1-19). The Bacterial pathogenic population in the different treatment tanks determined with specific media revealed the presence of *Yersinia* sp., *Staphlylococcus* sp., *Shigella* sp/*Salmonella paratyphi* A. and *Enterobacter* sp. in all the treatment columns from the SE to FCT. The population of *Yersinia* sp. was reduced to ≤ 2 log CFU/ml in CW4 compared to other sampling points (Fig 1-18).

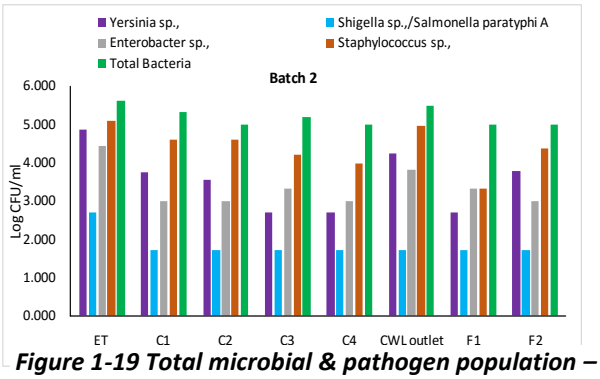


Figure 1-19 Total microbial & pathogen population – Jan 2016

1.3.3 Vuyyuru site

1.3.3.1 Performance of sequential treatment of distillery effluent

The sequential integration of the individual bio-treatment systems like bacterial, phyco, bio-char, phyto plays a significant role in increasing the efficiency of treating the distillery effluent. The change in the sequence can highly affect the change in improved water quality. A high removal of COD and BOD is due to adapted bacterial and algal consortium. Removal of calcium and chloride shows that treatment is efficient in reducing the salt concentration. Assimilation by microbial consortium played a significant role in the reduction of magnesium, nitrate, sulphate and phosphate in distillery effluent. However the salinity level found to be the same hence phytoremediation with was used to reduce salinity which described below.

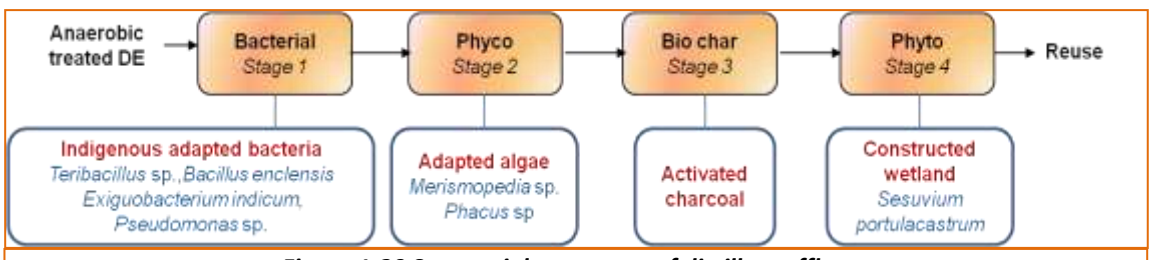


Figure 1-20 Sequential treatment of distillery effluent

Halophytes in Constructed Wetland

Halophyte in CWL

Observations from the field trials on irrigating with bio-treated distillery effluent and anaerobic treated distillery effluent for halophytes showed a luxuriant growth and hence *Sesuvium portulacastrum* was planted inside CWL to study the phytoremediation potential of halophytes, especially for reduction in salinity which is a big challenge in distillery effluent. Vegetative fragments were planted inside CWL and slowly it stabilized its survival and stated growing well within the CWL. The water quality of the CWL treated effluent was analyzed and compared with anaerobic treated distillery effluent with an average salinity of 8.7 PPT and in bio-treated distillery effluent it is 7.9 PPT which shows an evidence of reduction in salinity which pertains to the role of halophytes in the CWL. Systematic observations are being done to study the phytoremediation potential of halophytes, however further studies on chlorophyll content, flowering, yield, and plant uptake will substantiate the actual potential of halophytes in phytoremediation will be carried out.

1.3.3.2 Role of Phyco-remediation and status of adapted algal consortium

Type of algal consortium

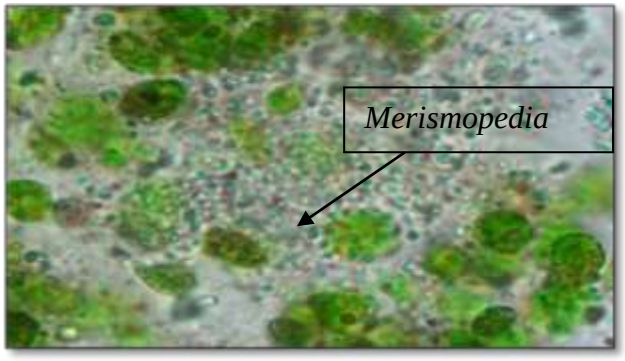
The algal consortium used in the current treatment process is a binary consortium consisting of *Phacus* sp., and *Merismopedia* sp.

Morphology and Identification

As per the APHA guidelines, the microalgae present in the ST tank of treated distillery effluent was confirmed as *Phacus* sp., and *Merismopedia* sp. under phase contrast microscope

Phacus sp. algae from treated distillery effluent under phase contrast microscope at 100x magnification Light green oval shaped or spherical cells were observed which were flattened and leaf like in appearance. The pellicle was quite rigid. The cells possessed contractile vacuoles and had red pigmented stigma to sense light**.**

Phacus sp.



Merismopedia sp. algae observed in treated distillery effluent under phast conrast microscope at 100x magnification.

Spherical to oval densely arranged cells forming flat colonies in mucilage not extending outside a colony's margin. No distinct sheath around individual cells.

Biomass, EPS and treatment mechanisms

The effects of physico-chemical parameters on growth of algal biomass and EPS were investigated. It was observed that there were very significant correlation between the increase in algal growth, EPS production and the reduction of different parameters. The increase in biomass not only depends on EPS content but also associated with the different pollutants bound on the surface of algal cells. High production of EPS under high polluted conditions indicates the involvement of EPS in protecting the algal cells against highly adverse conditions. The EPS production of the adapted algal consortium in distillery effluent is significantly high compared to that of the un-interacted cells.

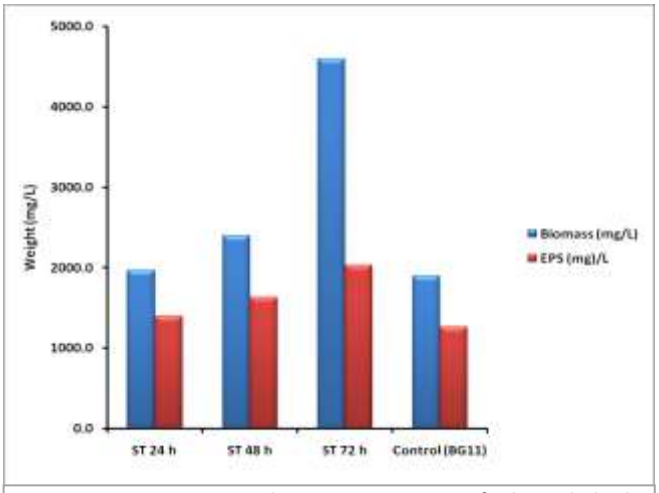


Figure 1-21 Biomass and EPS concentration of adapted algal consortium at 24, 48 and 72 h interval compared to control (un-interacted)

High Resolution Scanning Electron Microscopy

The scanning electron micrographs of *Phacus* sp. a) un-interacted b) interacted with distillery effluent are shown in Fig. 1-22 The cells un-interacted with distillery effluent were 7.2 µm length and 3 µm diameter. The cells of adapted *Phacus* sp. were measured to be 7.01 µm length and 4.35 µm diameter. Significant increases in the diameter of cells were observed after interaction with the effluent which may be due to the adsorption of contaminants on the surface of algae. The morphology of algal surface was changed from smooth to rough surface with shrinkages on interaction with distillery effluent (Fig. 1-22 a, b). Also, presence of exopolysaccharide can be seen around the cells interacted with distillery effluent compared to the un-interacted ones. This clearly shows that the algal cells undergo a very harsh environment and they have adapted themselves to survive and thrive in the distillery effluent.

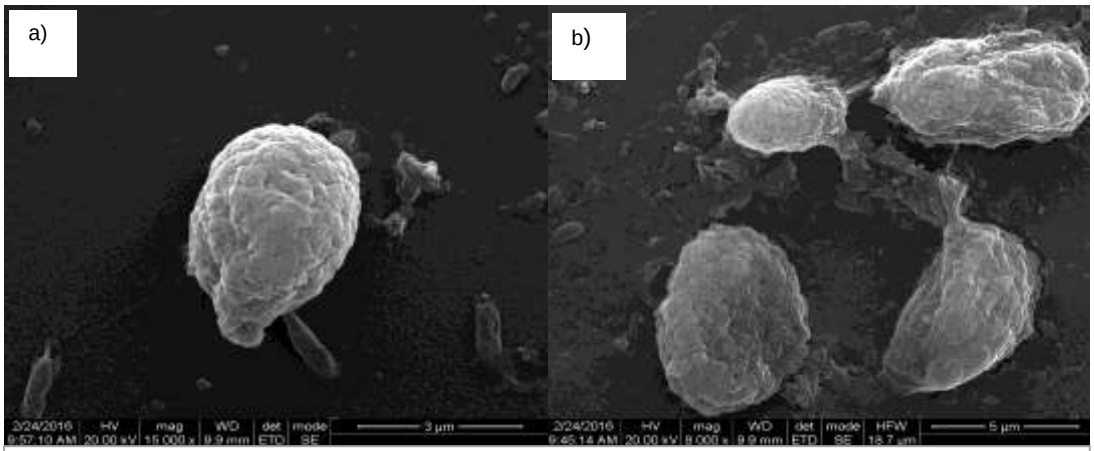


Figure 1-22 HR-SEM images of Phacus sp. a) un-interacted b) interacted with distillery effluent cells

The scanning electron micrographs of algae *Merismopedia* sp. a) un-interacted b) interacted with distillery effluent are shown in Fig 1-23. No significant change in the size was observed in distillery effluent interacted cells compared to the un-interacted ones. The morphology of algal surface was changed from smooth to rough on interaction with distillery effluent which is clearly evident from the micrographs (Fig. 1-23 a, b). Also, a smooth slimy layer of exopolysaccharide is prominently visible around the distillery effluent treated *Merismopedia* sp. cells compared to the un-interacted ones. These attributes clearly show that the algal cells have adapted to distillery effluent by producing EPS to protect them and carry out treatment process.

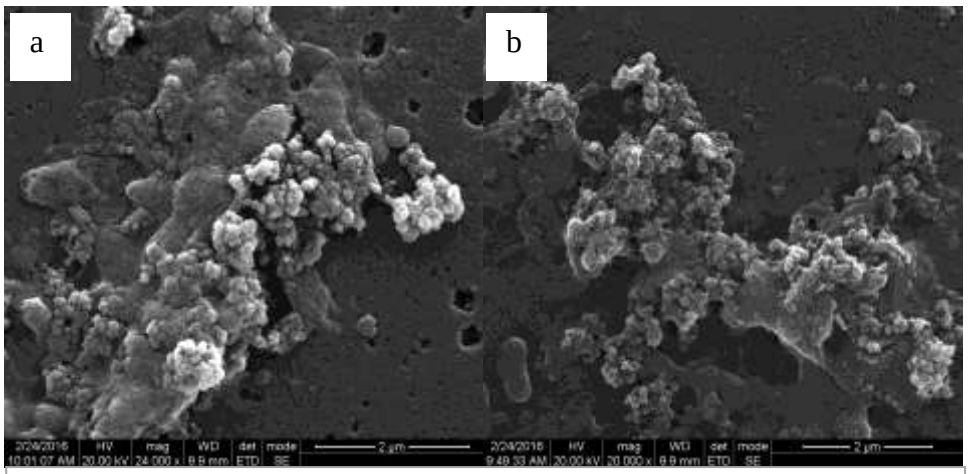


Figure 1-23 HR-SEM images of algae Merismopedia sp. a) un-interacted b) interacted with distillery effluent

Energy Dispersive X-Ray Spectroscopy

The EDX spectra of algal consortium a) un-interacted b) interacted with distillery effluent is shown in Fig. 1-24. The carbon and oxygen peaks correspond to the surface of algal biomass. In the algal consortium interacted with distillery effluent, increase in oxygen wt% with a decrease in carbon wt% shows that adsorption of contaminants from distillery effluent has masked the algal surface accompanied by pumping out oxygen through cellular diffusion. A significant increase in calcium, sodium, and magnesium ions on the surface of distillery effluent interacted cells of algal consortium compared to the un-interacted cells confirm that the cationic contaminants present in the effluent are effectively adsorbed on to the surface of algal cells. The intensity of the peak for silica in the EDX data is due to the glass slide on which the sample is studied.

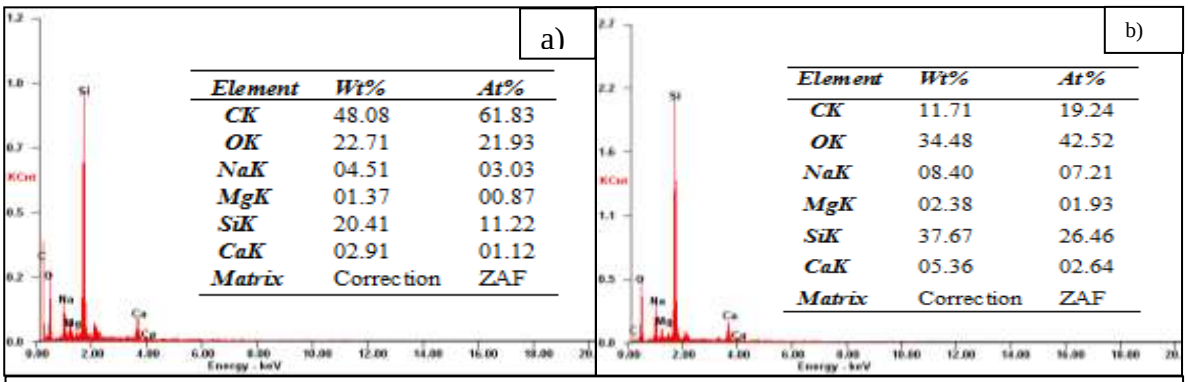


Figure 1-24 EDX spectra of algal consortium a) un-interacted b) interacted with distillery effluent

Mechanism of algal treatment

The interaction of distillery effluent with the algal consortium was investigated by the Fourier transform infra red spectroscopy (ft-ir). The IR spectra of control and treated algal consortium are shown respectively in Fig.1-25. The spectrum of the control algae displays absorption bands near 1648 and 1416 cm^{-1}, respectively due to the asymmetrical and symmetrical C(--- $O)_2$ stretching bands of the carboxylate ion group (COO^-) of terminal amino acid (Bellamy 1978; Silverstein et al 1991). After the interaction with distillery effluent, the algal consortium exhibited the spectrum with clear changes of the asymmetrical C(--- $O)_2$ stretching band at 1644 cm^{-1} decreasing and its symmetrical stretching band at 1416 cm^{-1}. These changes are typical of the complexation of the carboxylate anion functional group by coordination with cations such as Ca^{2+}, Na^{2+}, Mg^{2+} and etc. (Zhou and Wang 1994). In this case, most of the carboxylate ion had complexed or chelated with the cations because the asymmetrical stretching band at 1648 cm^{-1} moved to a lower frequency (Zhou and Wang 1994), which may be overlaid by other lower frequency bands, resulting in deepening the peak valley between 1644 and 1416 cm^{-1}; A significant decrease in peak intensity accompanied by a shift in the frequency from 1230 to 1226 cm^{-1} is due to the binding of hydroxyl groups of melanoidin with P=0 stretching of phosphodiesters in algae treated with distillery effluent. The peak at 1153 cm^{-1} corresponding to cellulose which forms the skeleton of algal cell wall present in untreated algal cells has weakened and disappeared due to the adsorption of contaminants to the cell wall which is clearly evident. A shift can be observed from 1085 to 1068 cm^{-1} with the decrease in peak intensity, corresponding to the interaction of cations with the oxygen of the hydroxyl group (C–O–H) from saccharides. The decrease in intensity at 3286 to 3370 cm^{-1} with peak shift after interaction with distillery effluent was due to the phenol and amine groups (NH) in algal biomass and exopolysaccharide. A very considerable decrease in the intensity with peak shift of both the absorption bands at 3286 to 3370 cm^{-1} and 1085 to 1068 cm^{-1}, respectively due to the νO–H and δO–H + νC–O of the hydroxyl group from saccharides, was observed in the graph, which shows that the free hydroxyl group decreased after adsorption of cations. The result made clear that some polysaccharides on the peptidoglycan layer of the algal cell wall had hydrolyzed to shorter saccharides such as oligosaccharides, dioses, and monoses and a further layer of adsorption of organic compound has took place which in this case probably would be the melanoidin polymer. A moderately intense peak was visible at the range of 701 and 585 cm^{-1} representing CH_2 Vibrations of Polysaccharide present in cell wall which shifted to 702 and 580 cm^{-1} which is due to the binding of cations to the cell wall.

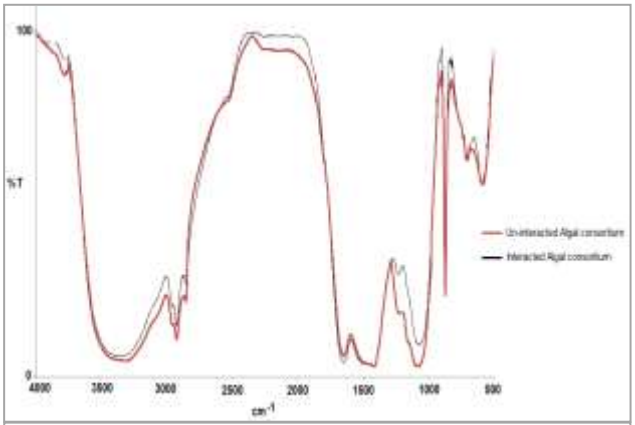


Figure 1-25 FT-IR spectrum of adapted algal consortium interacted and un-interacted with distillery effluent

Hence it is understood that the algal cell wall and exopolysaccharide have different functional groups N–H, O–H, CH_3, C=O, COO^-, CH_2 and P=0 which on interaction with cations (Ca^{2+}, Na^{2+}, Mg^{2+} and etc.) present in distillery effluent are able to remove the contaminants. This decreases the

salinity and hardness of the effluent. The surface of algae covered with cations exhibit a positive charge which helps in the adsorption of melanoidin. This is a multilayered adsorption process which can be explained by the Freundlich isotherm. Melanoidin is negatively charged at pH above 2.5 due to the dissociation of carboxylic and phenolic groups. Hence, the adsorption of melanoidin on algae is by hydrophobic interaction and hydrogen binding mechanism. A large number of hydrogen group bonds the C or N of algae with hydroxyl, carboxyl and phenol groups of melanoidin which additionally favors sorption. The multilayered adsorption increases the density of algal cells enabling it to sediment with the contaminants. Active uptake mechanism of algae helps in the removal of inorganic contaminants such as SO_4^{2-} (sulphate), NH^+_4 (ammonia), NO^-_2 (nitrite), NO^-_3 (nitrate), PO_4^{2-} (phosphate) and Na^+ (sodium) ions. The available of sunlight for algal growth is an indicator of the reduction of melanoidin. Since the algal treatment is after the bacterial treatment, the electrons from bacterial metabolism are taken up by algae for photosynthesis and carbon fixation which ultimately pumps out oxygen. Hence, removal of organic, inorganic contaminants by adsorption, uptake, sedimentation and supply of oxygen by algae reduces the TDS, TSS, COD, and BOD in the effluent.

Table 1-14 Assignment of bands in FTIR - adapted algal consortium un-interacted & interacted with DE

Main peak in un-interacted (cm^{-1})	Main peak in interacted (cm^{-1})	Assignment	Probable site for functional group
3286	3370	N–H and O–H stretching vibrations from polysaccharides and proteins	Cell wall – direct interaction of OH with cations
2954	2952	CH_3 asymmetric stretching from lipids, proteins, polysaccharides and nucleic acids	Proteins and carbohydrates in the cell wall
2918, 2851	2920, 2844		
2258	2267	N-H stretching	Amine hydrohalides
1648	1644	Amide I (protein C=O stretching)	Peptides – amino acids/amides
1416	1 416	carboxylate ion group (COO−)	cellular
1230	1226	P=0 stretching of phosphodiesters	Nucleic acid, polysaccharides
1153	–	Cellulose	Cell wall skeleton
1085	1068	Carbohydrate (-O-C) of polysaccharides. Nucleic acid (other phosphate containing compounds) P=0 stretching of phosphodiesters	Polysaccharides – extra cellular
701	702	CH_2 Vibrations of Polysaccharide	Polysaccharide - cell wall
585	580		

1.3.3.3 Water quality and treatment mechanism at Vuyyuru

Physico chemical properties

In raw water, the load of contaminants varied with respect to season. Initial load is very important for the treatment process. The temperature in Pre monsoon is higher than the following seasons other than the post monsoon. Temperature plays an important role in the formation of Milliard's product, melanoidin. As the temperature increases, the rate of formation of the polymer increases. Hence, the COD, BOD, TDS, TSS and other parameters hike. In the following season i.e. south west monsoon, the temperature of raw water has decreased significantly around 32°C and as a result, the quality of industrial treatment effluent which is the raw water for current treatment was better. The same observation suits to the north east monsoon. As the temperature increased in the post monsoon season which is an odd phenomenon, the increase in the concentration of different parameters is visible. The initial load is determined by the temperature in different seasons which in turn

has significant effect on the treatment process carried out in aeration tank, settlement tank and CWL.

The biological treatment is generally affected by the effluent temperature, pH, initial load of pollutants, and initial load of microbes. In aeration tank, 50% dilution of anaerobic treated distillery effluent helps in reducing the initial pollutant load and the effluent temperature. This process slows down the milliard's reaction which is then ceased as the pH increases in the aeration tank due to bacterial treatment. The bacterial growth is affected by the initial load of pollutants despite the dilution process if the anaerobic treatment by industry is not efficient. This occurs mostly during the pre monsoon and SW monsoon. But, the treatment in SW monsoon is better than pre-monsoon which is very clearly due to the decrease in temperature (38.9 to 31.8°C) which is around the optimum temperature to achieve maximum bacterial growth in aeration tank. The performance of treatment further improved in the NE monsoon season which again is due to low temperature and the inlet of better treated effluent (RW) in that season. The post monsoon season had a mild increase in the temperature and inlet load which affected the concentration of final treated water to be higher than that of NE monsoon.

In the settlement tank, algal treatment is very efficient in the SW, NE and post monsoon seasons. Whereas, in the pre-monsoon season, the initial load received in ST and its temperature were higher than other seasons which significantly affected the algal growth and the treatment adversely. Although, the algal treatment was efficient and almost similar in SW, NE and post monsoon seasons a pattern of maximum and minimum concentration of contaminants were observed in this season. The treatment was better in these seasons at minimum temperature and slightly poor as the temperature was maximum. The CWL was started in the NE monsoon. So this has been compared between two seasons i.e. NE and post monsoon. In the CWL temperature doesn't seem to play a significant role as in other treatments. This is clearly understood from the fact that even though both seasons don't have a significantly different average temperatures, the treatment in post monsoon is efficient than that in NE monsoon. This may also be attributed to the rain in the end of NE monsoon and beginning of post monsoon, which would have possibly washed away the adsorbed pollutants from CWL creating more binding sites for the following treatment cycles. This would have favored a mild improvement in the treatment in CWL in post monsoon than the previous season.

Table 1-15 Water quality status of AT in different seasons

Season	Pre Monsoon				South West Monsoon				North East Monsoon				Post Monsoon			
	Min	Max	Avg	Std. Dev	Min	Max	Avg	Std. Dev	Min	Max	Avg	Std. Dev	Min	Max	Avg	Std. Dev
pH	6.8	7.1	7.0	0.2	7	7.1	7.07	0.06	6.8	7	6.90	0.10	7.1	7.2	7.15	0.07
Eh (mV)	-4	27	7.3	17.1	2	4	3.00	1.00	1.0	9	4.67	4.04	-2.0	2.0	0.00	2.83
Ec (mS/cm)	16.4	17.1	16.9	0.4	16.6	17.1	16.87	0.25	16.7	17.3	17.00	0.30	17.4	17.7	17.55	0.21
Salinity (PPT)	8.4	8.6	8.5	0.1	8.3	8.6	8.48	0.16	8.3	8.6	8.47	0.15	8.6	8.7	8.65	0.07
Temperature (°C)	38.1	40	38.9	1.0	30.3	34.7	31.83	2.48	28.4	32.1	30.30	1.85	31.6	33.3	32.45	1.20
COD (mg/L)	17000	24000	20333.3	3511.9	10850	17200	14850.00	3481.74	10200	17350	14890.00	4063.29	16420.0	17200.0	16810.00	551.54
% Colour removal	30.1	34.2	31.8	2.2	31.4	34.1	33.07	1.46	28.0	35.4	31.70	3.70	30.4	33.1	31.75	1.91
BOD (mg/L)					4850	6900	5816.67	1029.97	3870	4420	4220.00	304.14	3125.3	5750.0	4437.67	1855.91
TDS (mg/L)					27130	38000	31750.00	5615.33	30124.0	36230	32160.67	3524.15	26523.5	28731.2	27627.35	1561.08
TSS (mg/L)					6030	7600	6710.00	805.79	3473.5	4934	3975.83	830.12	3876.7	4126.8	4001.75	176.85
Phosphate					588.1	828	681.37	128.55	515.66	603.7	570.59	47.90	678.9	725.7	702.30	33.09
Chloride					201.41	257.7	225.54	28.99	210.4	248.5	231.90	19.52	238.6	251.2	244.90	8.91
Magnesium					190.7	207.3	198.43	8.36	196.8	238.6	210.90	23.99	203.6	215.9	209.75	8.70
Sulphate					36.8	40.23	38.41	1.72	25.8	43.3	37.13	9.83	33.7	36.7	35.19	2.08
Nitrate					40.9	65.33	50.91	12.80	34.2	58.2	48.94	12.90	32.1	38.4	35.25	4.45
Calcium					51.67	70.3	59.09	9.88	55.1	63.4	60.00	4.35	50.7	52.2	51.45	1.06
DO					NA	NA	NA	NA	0.5	1.7	1	0.6245	0.8	1.3	1.05	0.35

Table 1-16 Water quality status of ST in different seasons

Season	Pre Monsoon				South West Monsoon				North East Monsoon				Post Monsoon			
	Min	Max	Avg	Std. Dev	Min	Max	Avg	Std. Dev	Min	Max	Avg	Std. Dev	Min	Max	Avg	Std. Dev
pH	7.1	7.3	7.2	0.1	7.1	7.3	7.20	0.10	7.1	7.2	7.13	0.06	7.2	7.3	7.23	0.10
Eh (mV)	-9	-4	-5.7	2.9	-16	-5	-9.67	5.69	-9.3	-4	-6.20	2.75	-9.2	-7.0	-8.10	1.56
Ec (mS/cm)	16.1	17.3	16.8	0.6	16.4	16.9	16.57	0.29	16.6	17.2	16.90	0.30	17.2	17.5	17.35	0.21
Salinity (PPT)	8.3	8.4	8.3	0.1	8.4	8.5	8.47	0.06	8.2	8.5	8.36	0.15	8.4	8.4	8.44	0.00
Temperature (°C)	35.2	39.3	37.9	2.3	28.3	32.4	30.30	2.05	28.4	33	30.51	2.34	30.3	31.6	30.93	0.89
COD (mg/L)	9100	15000	12700.0	3157.5	4900	7000	5900.00	1053.57	4300	9630	7310.00	2731.17	8100.0	8815	8457.50	505.58
% Colour removal	60.1	62.8	61.6	1.4	63.83	67	65.40	1.59	55.3	64.4	62.62	6.65	59.8	62.0	60.91	1.54
BOD (mg/L)					1000	1800	1400.00	400.00	950	2110	1449.17	596.66	1698.8	2400.0	2049.38	495.86
TDS (mg/L)					24250	32000	27750.00	3929.06	22672	27145	25432.50	2413.64	17275.7	26812.8	22044.23	6743.71
TSS (mg/L)					1520	4800	2773.33	1771.48	1228	2982	2199.00	891.98	2138.6	2684.5	2411.55	386.01
Phosphate					422.5	635.5	515.00	109.23	399.33	540.2	475.20	71.06	513.1	552.2	532.67	27.62
Chloride					167.3	192.3	178.13	12.83	164.6	192.8	177.38	14.28	173.2	183.8	178.49	7.48
Magnesium					140.33	170.48	156.40	15.17	152.3	195.2	179.29	23.50	192.8	201.5	197.14	6.16
Sulphate					15.2	18.43	17.04	1.66	16.33	20.1	17.78	2.06	18.5	19.2	18.87	0.51
Nitrate					18.71	28.3	22.87	4.92	17.2	25.3	21.32	4.05	20.6	23.4	21.99	1.96
Calcium					38.62	4.3	42.27	4.50	35.91	49.2	41.94	6.75	38.9	45.0	41.93	4.28
DO					NA	NA	NA	NA	2.3	3.4	2.76	0.56	2.7	3.5	3.1	0.56

Table 1-17 Water quality status of CWL in different seasons

Season	North East Monsoon				Post Monsoon			
	Min	Max	Avg	Std. Dev	Min	Max	Avg	Std. Dev
pH	7	7.1	7.03	0.06	7.1	7.3	7.18	0.18
Eh (mV)	-5.6	-2.0	-3.21	2.10	-4.0	-3.0	-3.50	0.71
Ec (mS/cm)	16.2	16.7	16.37	0.29	16.4	16.6	16.50	0.14
Salinity (PPT)	7.9	8	7.97	0.06	7.8	7.8	7.80	0.00
Temperature (°C)	30.3	31.7	30.78	0.78	29.4	31.2	30.28	1.30
COD (mg/L)	5200.0	6600	5666.67	808.29	4200.0	4750.0	4475.00	388.91
% Colour removal	58.0	63.1	60.79	2.56	63.1	63.4	63.26	0.20
BOD (mg/L)	955	1121.3	1010.42	95.98	952.5	1050.0	1001.25	68.94
TDS (mg/L)	22645.0	24662.8	23384.25	1111.72	17420.0	22758.5	20089.25	3774.89
TSS (mg/L)	1200.0	1793.5	1404.50	337.03	1032.5	1214.0	1123.25	128.34
Phosphate	279.2	387.7	318.78	59.94	344.4	369.7	357.03	17.91
Chloride	167.8	171.3	169.63	1.74	166.2	171.2	168.70	3.54
Magnesium	148.5	169.4	155.71	11.89	150.4	164.8	157.60	10.18
Sulphate	14.1	15.5	14.66	0.73	15.2	17.8	16.51	1.83
Nitrate	17.1	19.3	17.96	1.16	17.2	18.3	17.73	0.81
Calcium	34.2	37.4	35.48	1.72	35.1	36.3	35.68	0.88
DO	2.1	3.3	2.7	0.6	2.8	3.5	3.15	0.49

1.3.3.4 Biological properties of Vuyyuru

Soil microbial load and pathogenic poplation in reuse demonstration plots - (July 2015)
Soil sample were collected from three different points from all trial plots based on Randomised block design.

Microbial load and pathogenic population of the agricultural soil of L1
The total microbial load was log 7 CFU/g in the 19 soil samples (Fig 37-40). The pathogenic population in the different trial plot namely halophytes, tree grass and sweet corn was determined which would be the baseline data for determination of the pathogenic population in the soils treated with waste water used for irrigation purpose.

The soil samples harboured pathogenic organisms *Staphylococcus* sp. at log 5 CFU/g *Yersinia* sp. were detected at the range log 4 CFU/g and *Enterobacter* sp.; and *Shigella* sp. /*Salmonella* Type A were detected at the range log 3-4 CFU/g (Fig 37-40) .This base line data would be the reference data to monitor the changes in the pathogenic population in the agriculture soils due to irrigation with treated and untreated distillery effluent.

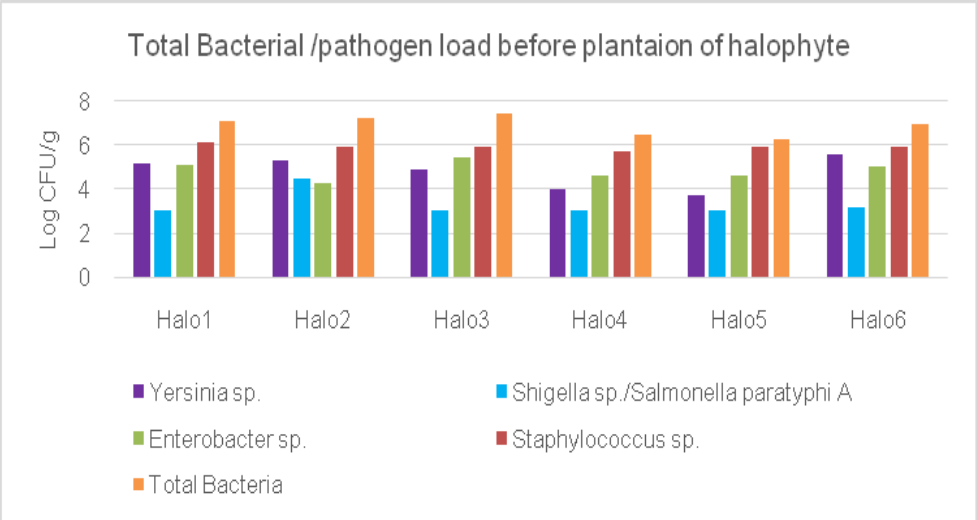


Figure 1-26 Total Bacterial / pathogen population in halophyte plantation plot

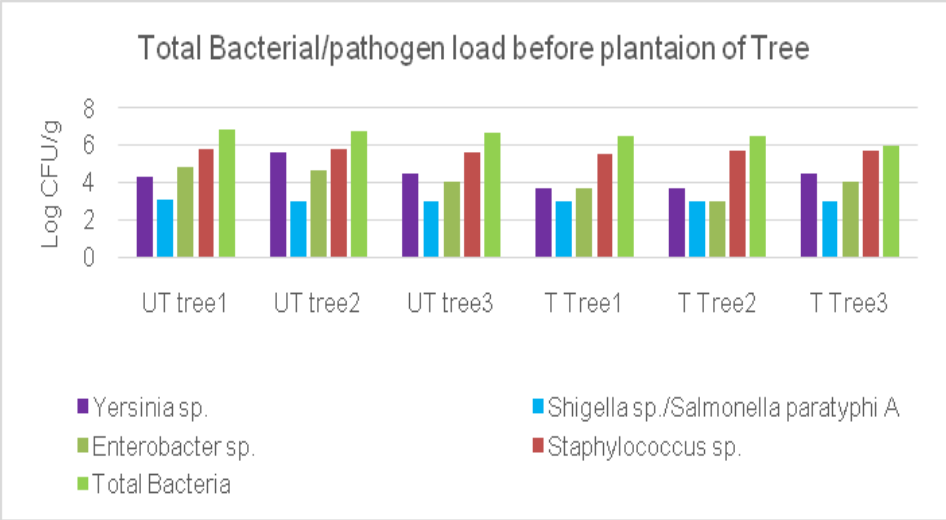


Figure 1-27 Total Bacterial and pathogen population in tree plantation plots

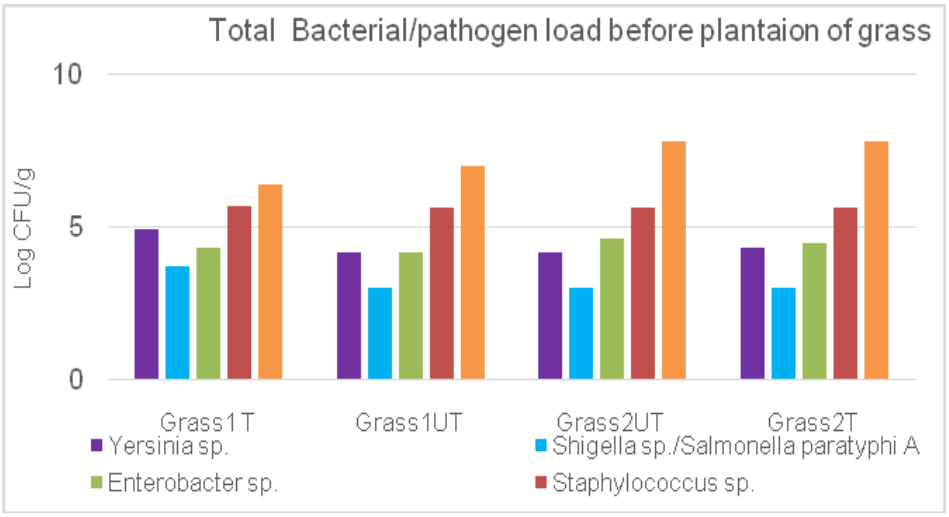


Figure 1-28 Total Bacterial /pathogen population in Grass plantation plot

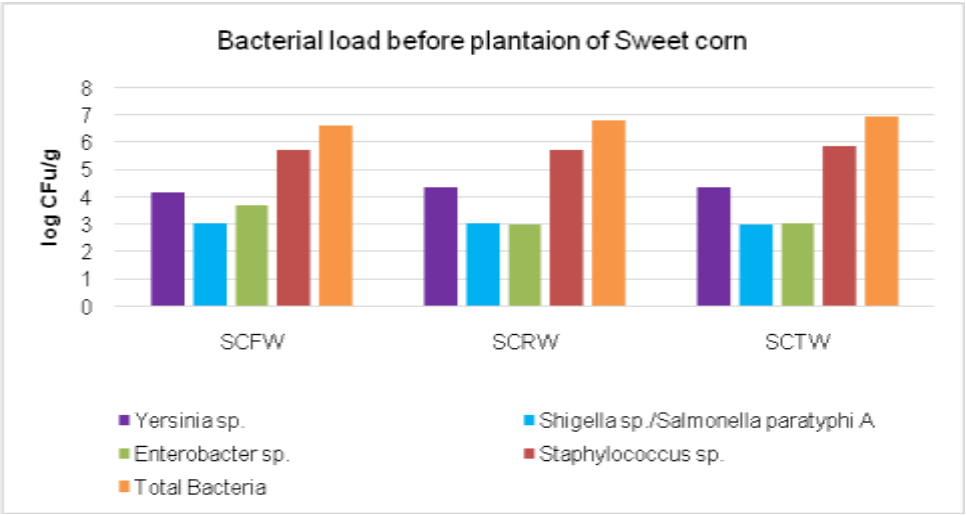


Figure 1-29 Total Bacterial / pathogen population before harvesting of Sweet corn - (SCFW – Fresh water irrigated sweet corn; SCRW – Raw water irrigated sweet corn; SCTW – Treated water irrigated sweet corn)

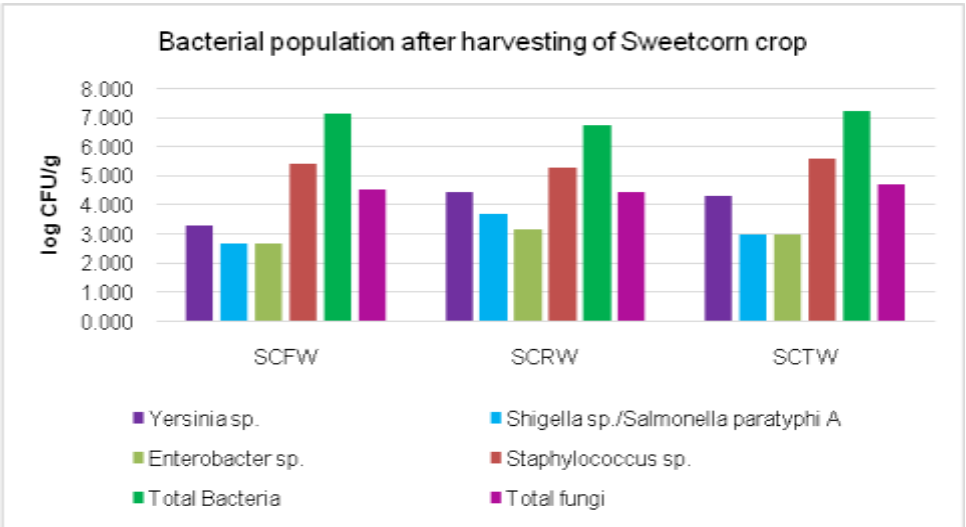


Figure 1-30 Total Bacterial / pathogen population after harvesting of Sweetcorn

The bacterial load and pathogenic population in sweet corn cultivated plots irrigated with raw Distillery wastewater, Fresh water and treated Distillery wastewater was analysed on January 2016. Total bacterial population was in the range of 6-7 log CFU/g and total fungal population was 4 log CFU/g. The soil samples harboured pathogenic organisms *Staphylococcus* sp. At log 5 CFU/g. *Shigella* sp. And *Enterobacter* sp. Were detected at ≤ log 3 CFU/g . In 0^{th} hour and post harvest soil of sweet corn the total microbial load and the pathogenic population remained the same.

1.3.3.5 Phenol Degradation

Apart from melanoidin, polyphenolic compounds released during milling process of cane also contribute to the brownish colour of the Distillery Molasses spent wash (DMSW). Hence bioremediation of phenolic compounds using phenol degrading bacterial isolates was attempted to reduce the colour intensity in DMSW.

Isolation of bacteria for the Degradation of Phenol

About 10 g of rhizosphere soil of *Cana indica*, *Typa* sp. Vyyurru soil (AnTMSW irrigated soil) and AnTMSW (5 ml) were acclimatised in increasing concentration of phenol of 600, 800 and 1000 mg/L amended in Davis minimal medium (DMM) and incubated at 150 rpm for 7 days at 30 ^{o}C. The phenol concentration in the culture filtrate was measured at every 24 h interval by the method of APHA 5530 D (2005). The consortia of Vuyyuru soil and *C. indica* showed complete degradation of 1000 mg/L of phenol at 120h and AnTMSW consortia showed complete phenol degradation in 96 h. Comparatively the typha consortia showed lesser degradation efficiency ie. upto 600mg/L and increasing concentration was inhibitory (Fig. 31).

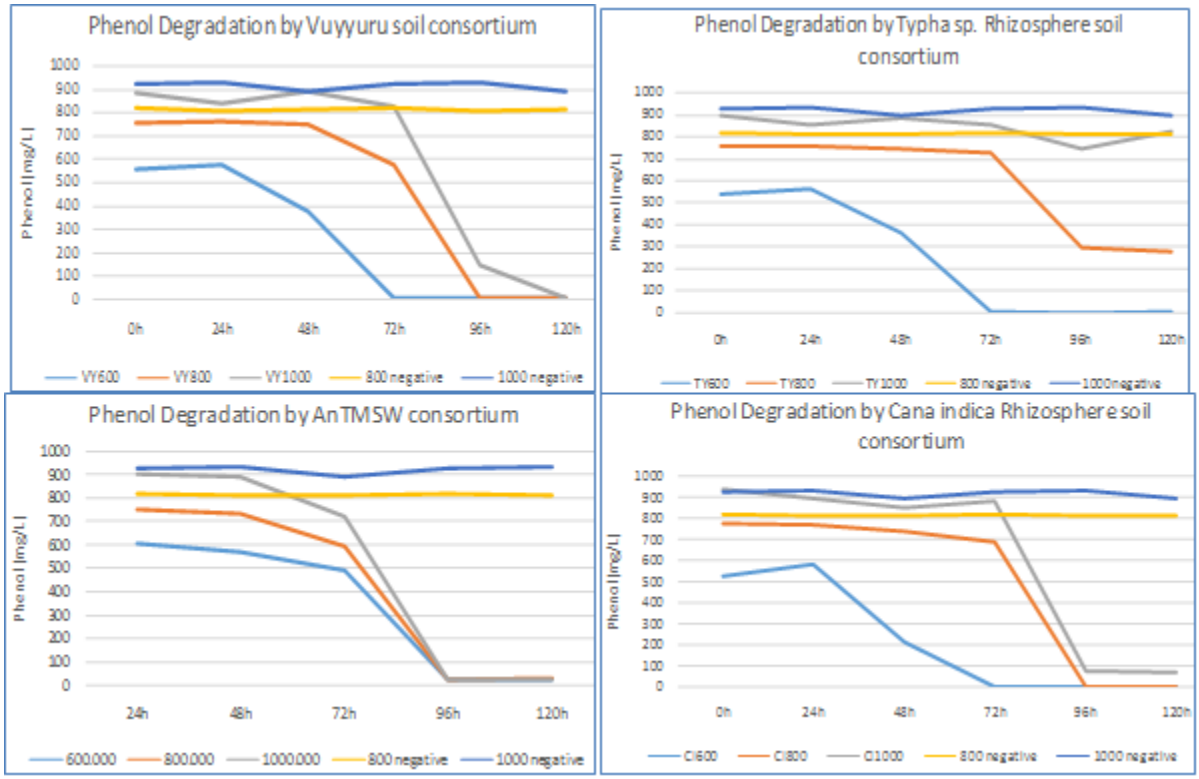


Figure 1-31Phenil degradation by bacterial consortia of different soil and AnTMSW

In order to identify the phenol degrading isolates in the consortia, serially diluted consortia was plated in DMM agar amended with 200 mg/L of phenol and incubated at 30 ^{o}C for 72h. Around 304 pure colonies were picked and screened for phenol degrading efficiency in 96 multiwell plate reader at 500 nm in *Multiskan Go* (Thermo scientific). Among the 304 cultures 39 bacterial isolates were able to degrade 1000 mg/L of phenol within 72-96 h (Fig. 32) which needs to be identified.

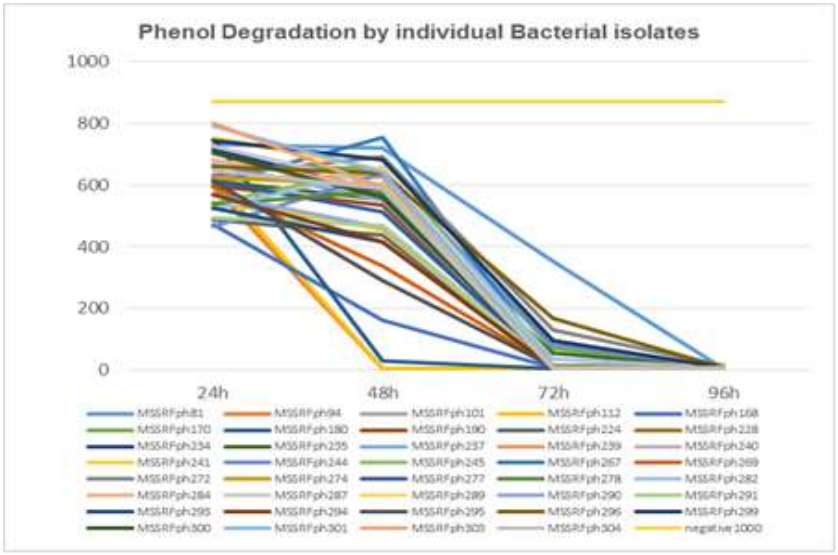


Figure 1-32 Phenol degradation by individual bacteria

PCR Box profiling of the phenol degrading isolates

Genomic DNA was isolated from all the 39 isolates following the modified method of Marmur (1969) and checked in 1% agarose gel (Fig.48).

The BOX-PCR fingerprinting of the positive isolates was performed using BOX A1R primer (CTACGGCAAGGCGACGCTGACG) (Versalovic *et al.*1995). The PCR products were separated in 1.5% agarose gels at a constant 80V voltage for 6 h, stained with ethidium bromide-(EtBr) and visualized under UV, the gel images was captured using the Bio-Rad gel documentation system (Fig. 1-33).

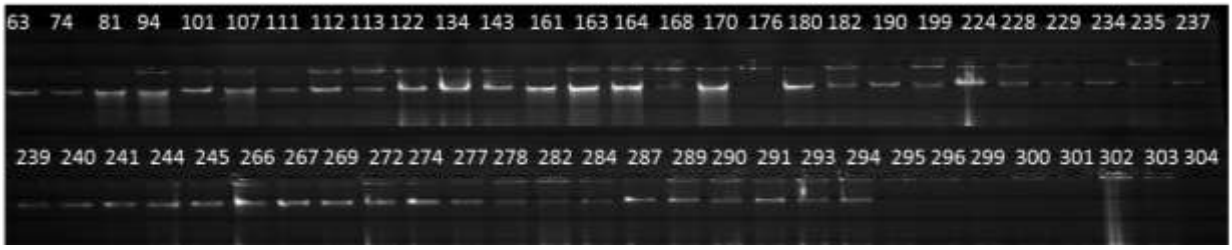


Figure 1-33 DNA from the bacterial isolates

Amplification of Phenol Hydroxylase enzyme coding Gene:

Phenol Hydroxylase is reported to be involved in hydroxylation of Phenol into catechol, an initial step in the phenol degradation, further this is broken down into two intermediate molecule; 2-Hydroxymuconic semialdehyde and *cis*, *cis* mucanic acid by catechol 2,3 dioxygenase and catechol 1,3 dioxygenase respectively which enters into the kerbs cycle (Paula *et al*., 2000).

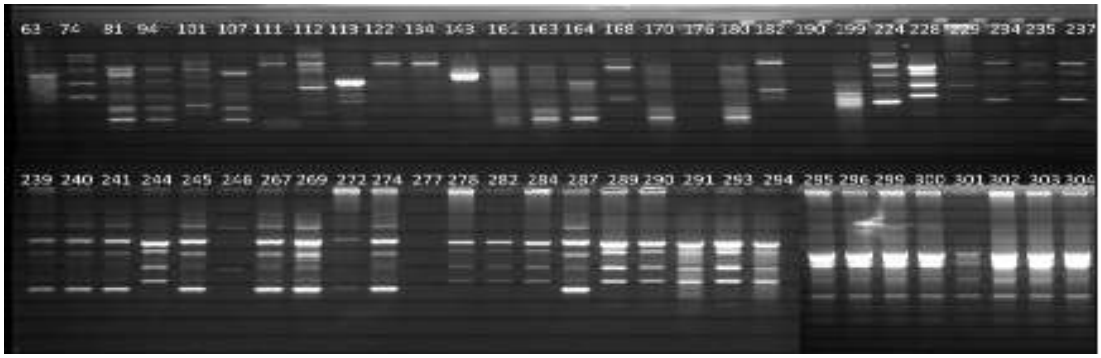


Figure 1-34 BOX PCR profile of the phenol degrading isolates

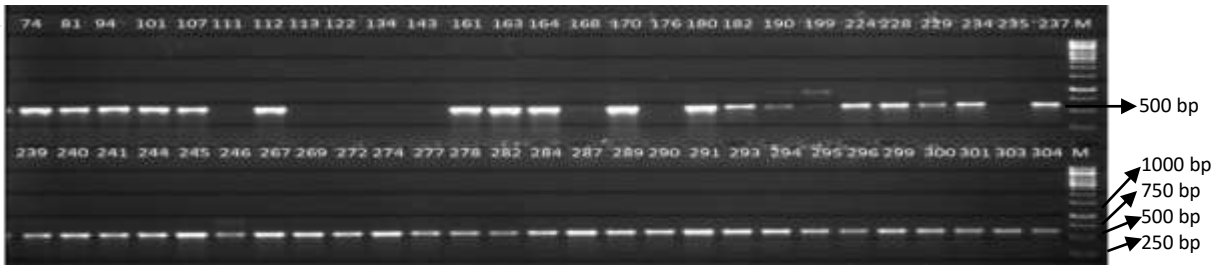
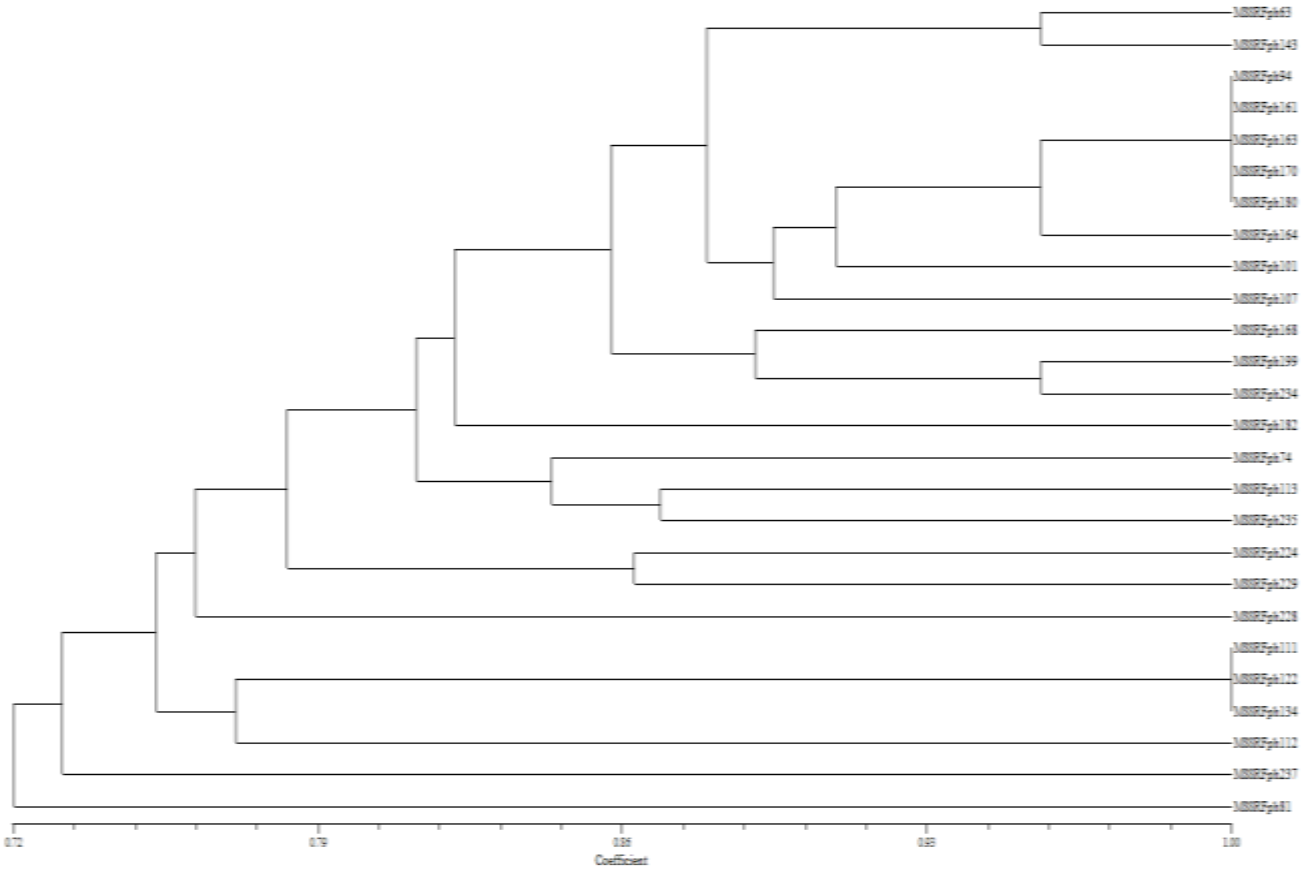


Figure 1-35 Phenol degrading bacteria harbouring phenol hydroxylase gene (M- 1kb Ladder)

All the 39 isolates produced an amplicon of 620 bp indicating positive for phenol hydroxylase gene but the phenol degrading efficiency of the positive isolates varied.



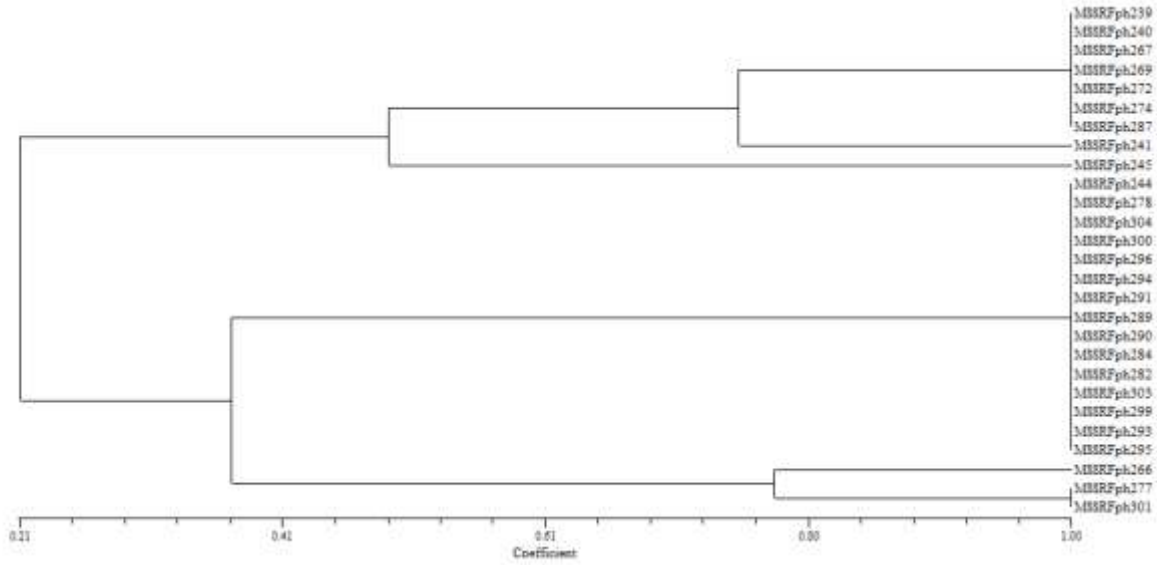


Figure 1-36 Dendrogram of phenol degrading isolates constructed using NTsys software

Based on cluster analysis 25 different group of phenol degrading bacteria isolates were represented

1.4 Carbons and membranes for the recovery of phenolics / pigments

The following progress was done during the reporting period.

Application of mixed matrix membranes (MMM) for melanoidins and polyphenols retention from distillery wastewater

Development of mixed matrix membranes by incorporation of nanocomposites in polysulfone (PSF) matrix and its application for synthetic melanoidins retention was described in previous report. This study was further elaborated and polysulfone membrane (PSF-18) with 1% nanocomposite was selected for further use. Polyvinyl alcohol (PVA) layer of varying concentrations (0.05%, 0.1%, 0.15% and 0.25%) was applied for 1 min and 2 min respectively. PVA coated mixed matrix membranes (MMM) thus obtained, were analyzed for melanoidins and polyphenols retention. Distillery wastewater (DWW) was collected from Brajnathpur distillery unit of Simbhaoli Sugars Limited, Ghaziabad district, Uttar Pradesh. DWW colored stream, generated from distillation column was used as-received. 50 mL DWW was centrifuged at 8000 rpm for 30 min. Filtration was carried out in dead end filtration unit cell procured from Millipore, USA. The cell was pressurized using nitrogen gas (99.99%, Sigma gases, New Delhi) between 6 to 20 bar.

It was observed that membrane defects were sealed with PVA coating as indicated by decrease in permeability and increase in melanoidins retention with increasing PVA concentration. 0.25 % PVA coated MMM showed approximately 78% and 64% melanoidins and polyphenols retention respectively, over a 5 h duration (Figure 1-37 and 1-38).

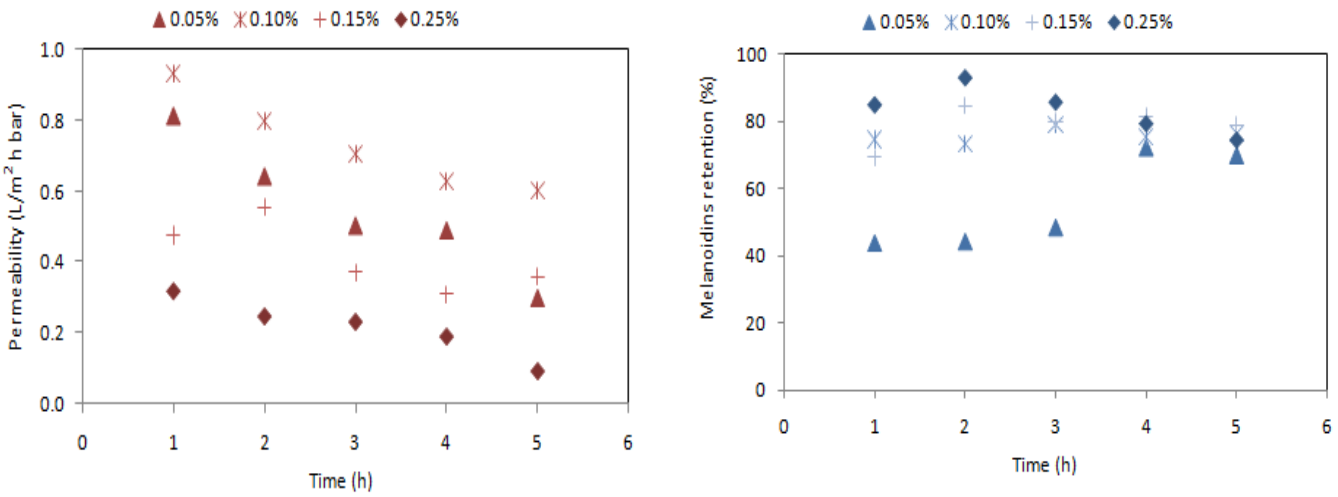


Figure 1-37 Permeability and melanoidins retention of MMM coated with different PVA concentration for DWW

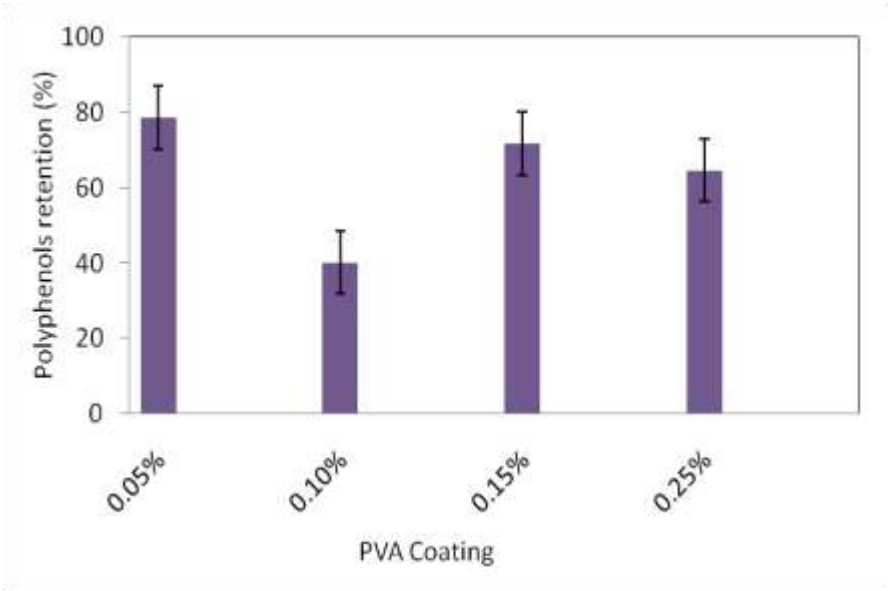


Figure 1-38 Polyphenols retention by PVA coated MMMs for DWW

Comparison of PVA coated MMM and PSF-18 membranes

A comparative study was conducted with 0.25% PVA coated MMM and 0.25% PVA coated PSF 18 membrane. The said membranes were tested in cross flow filtration unit obtained from Rayflow, France. The effective filtration area was 109 cm^2. 5 L of sieved distillery wastewater was used as feed. Masterflux pump was used for pressurizing the feed across the membranes at a pressure of 2 bars.

As observed from Figure 14, 0.25% PVA coated MMM showed higher (89%) melanoidins retention in comparison to PVA coated PSF-18 (75%). However, the flux of PVA coated MMM was considerably lower (0.326 L/m^2h) than PVA coated PSF-18 (2.63 L/m^2h). The flux and melanoidins retention thus obtained by PVA coated PSF 18 were comparable with that of commercial UF membrane making it suitable for further distillery wastewater fractionation studies.

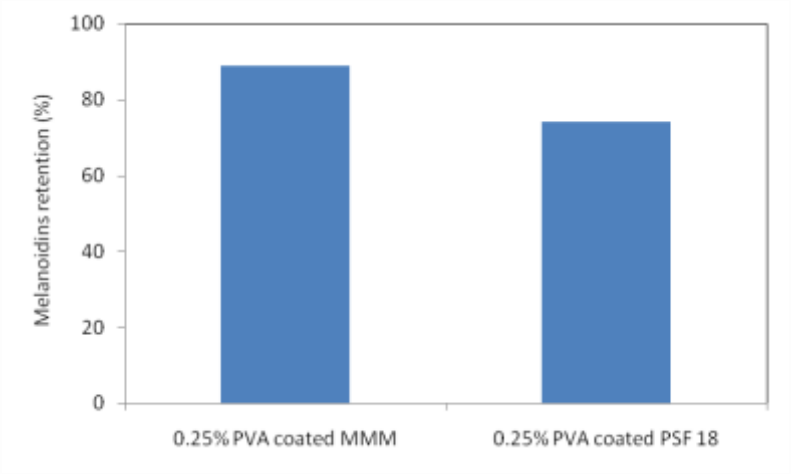
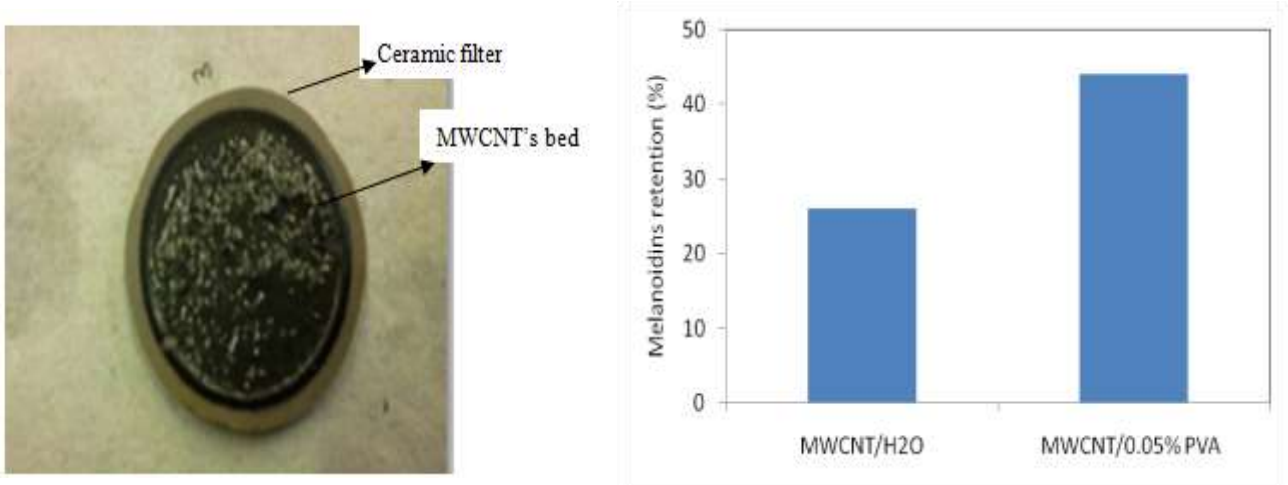


Figure 1-39 Comparison of PVA coated MMM and PSF-18 membranes for melanoidins retention in DWW

Ceramic membrane modification with multiwalled carbon nanotubes (MWCNT's)

0.005 % (w/v) MWCNT's was dispersed in Triton X-100 and sonicated for 1 h. Ceramic filter (prepared in-house from waste sugarcane bagasse ash) was placed in dead-end stirred cell filtration unit obtained from Millipore, USA. 100 mL of prepared MWCNT's solution was poured in the filtration cell assembly with continuous stirring and permeate flow was optimized to settle MWCNT's uniformly over the surface of ceramic filter (Figure 1-40a). Subsequently, the settled MWCNT's were washed with 1L of distilled water to ensure the formation of uniform packed bed of MWCNT's. In order to bind MWCNT's more tightly, similar procedure of MWCNT's bed packing on ceramic filter was followed by making 0.005% (w/v) MWCNT suspension in 0.05% PVA solution. After the coating of MWCNT's on ceramic filters, 1L of 5% centrifuged DWW was passed in dead end filtration mode at a pressure of 0.5 bar and the samples were analyzed for melanoidins retention.

As observed from Figure 1-40b, ceramic filter coated with MWCNT's in PVA solution showed higher melanoidins retention (45%) as compared to the one coated with MWCNT's in water. This can be attributed to stronger binding of CNT's with each other as well as to the ceramic filter surface due to application of PVA.



(a) (b)

Figure 1-40 a) Formation of MWCNT's bed on ceramic filter. (b) Melanoidins retention on MWCNT's coated ceramic filter.

Fractionation of distillery wastewater

Ultrafiltration (UF) of DWW was done in Sepa ST membrane cell, procured from Osmonics, USA. UF membranes, having molecular cutoff 100 and 10 kDa were obtained from Sterlitech, Mumbai. Chemicals namely 2,2'-Azobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulphate, N,N-Dimethylformamide (DMF), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, (Trolox), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), methanol, were procured from Sigma, New Delhi.

The as-received distillery wastewater (as-is DWW) sample was centrifuged at 8000 rpm for 20 minutes prior to ultrafiltration. High and low molecular weight DWW component fractions were obtained using 100 and 10 kDa UF membranes. Different fractionation schemes were followed to concentrate antioxidant compounds from distillery wastewater (Figure 1-41a and b).

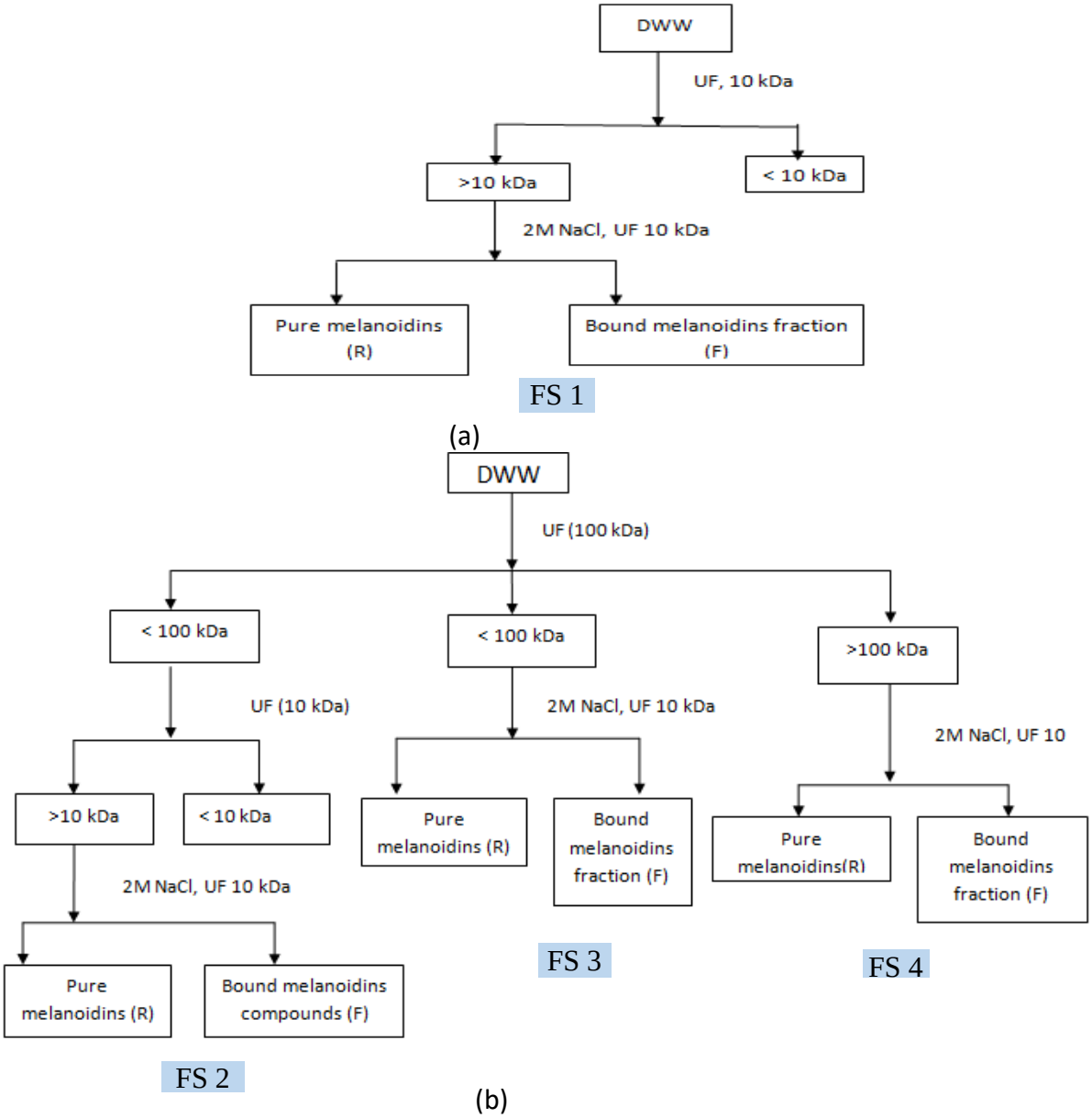


Figure 1-41 Flow chart diagram for various fractionation schemes involving (a) single step UF using 10 kDa membrane and (b) stepwise UF using 10 and 100 kDa membranes

A dead end filtration was performed using SEPA ST membrane cell with 22.4 cm height, 5.1 cm diameter and an effective membrane area of 16.9 cm^2. The filtration cell consisted of a cylindrical vessel of 300 ml capacity, equipped with Teflon coated magnet and a porous support on which membrane was placed. Nitrogen gas (99.99%, Sigma gases, New Delhi) was used to pressurize the cell and different filtration schemes as shown above were studied.

According to the first filtration scheme (FS 1), 100 ml of centrifuged DWW sample was subjected to UF using 10 kDa membrane at 4 bar. After UF, 10 ml retentate was made up to 100 ml with RO water and washed again. This washing procedure (diafiltration) was repeated at least three times to concentrate the retentate fraction. The washed and concentrated retentate was collected for further analysis. In order to obtain pure melanoidins fractions, washed retentate was incubated overnight in 2M NaCl. NaCl was used to release potential low molecular weight compounds ionically attached to melanoidins skeleton (Rufian- Henares., 2007[1]).

Thereafter, the sample was ultrafiltered again, using 10 kDa membrane. Retentate fraction thus obtained was composed of pure melanoidins while filtrate contained low molecular weight bound melanoidins compounds.

The second filtration scheme (FS 2) consisted of stepwise UF. Briefly, 100 ml centrifuged DWW was subjected to UF using 100 and 10 kDa membranes respectively. Retentate fractions were diafiltered thrice as mentioned earlier. The permeate fractions (filtrate) from 100 kDa membrane was subjected to further ultrafiltration through 10 kDa at 2 bar pressure. Pure melanoidins and bound melanoidins compounds were obtained by incubating with 2M NaCl as explained above.

In third and fourth filtration schemes (FS 3 and 4), <100 kDa and >100 kDa fractions were separately treated with 2M NaCl and pure and bound melanoidins compounds were released after UF with 10 kDa membrane.

Characterization of DWW component fractions

Antioxidant property

ABTS Assay

The antioxidant capacity of DWW fractions was estimated in terms of radical scavenging activity in aqueous solution following the procedure adapted from Delgado-Andrade et al, 2005[2]. $ABTS^+$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in dark at room temperature for 12 -16 hours before use. The $ABTS^+$ (stable for 2 days) was diluted with 5 mM phosphate buffered saline (pH 7.4) to an absorbance of 0.70± 0.02 at 734 nm. 5 mM stock solution of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was prepared in phosphate buffered saline (PBS) after dissolving it in DMF. 3ml of $ABTS^+$ was used to record the baseline absorbance ($A_{baseline}$) using spectrophotometer (Aquamate, India). After addition of 50µl of

[1] Rufian-Henares, J.A. and Morales, F.J. (2007). Functional properties of melanoidin: In vitro antioxidant, antimicrobial and antihypertensive activities. Food Research International 40, 995-1002.

[2] Delgado-Andrade, C.; Rufian-Henares, J.A.; Morales, F.J. (2005). Assessing the antioxidant activity of melanoidins from coffee brews by different antioxidant methods. Journal of Agriculture and Food Chemistry 53, 7832-7836.

sample (melanoidins fraction) to 3ml of $ABTS^+$ solution, absorbance reading (A_{sample}) was taken after 2 min. Absorbance of 50µl R.O water in 3ml $ABTS^+$ was taken as control. The calculation was as follows:

% Radical scavenging activity= 100- $[(A_{sample}/ A_{baseline})*100]$ (1)

DPPH Assay

DPPH radical scavenging activity of was evaluated according to procedure adapted from Xu and Chang, 2007[3]. 125 µl of sample was added to 3.8 ml of methanol solution of DPPH (0.1 mM). The mixture was shaken for 1 min and left to stand in the dark at room temperature for 30 min. Thereafter, the absorbance of sample was measured using spectrophotometer at 517 nm against methanol as blank. The percent of DPPH decolorization was calculated according to the following equation:

% Radical scavenging activity = $[1 – (A_{sample}/A_{control})] \times 100$ (2)

Trolox solutions were prepared at concentrations ranging from 150-1150 µM for calibration purpose. The antioxidant activity of DWW components for both ABTS and DPPH assays was expressed as an equivalent of that of trolox using the equation derived from the calibration curve for respective assays.

The results obtained for antioxidant activity for $ABTS^+$ and DPPH are shown in Figure 17. It was observed that as-is DWW showed highest antioxidant activity of 67 millimole of trolox equivalent. Pure melanoidins obtained from first fractionation scheme showed highest antioxidant activity possibly due to release of low molecular weight compounds bound to melanoidins core exhibiting low radical scavenging behavior. Similar results were obtained by Rufian-Henares and Morales, 2007 [1] during their analysis on melanoidins prepared from glucose-phenylalanine mixture. Furthermore, for high molecular weight DWW components, >100 and >10 kDa antioxidant activity was found to be low (6 and 9 mM equivalent of trolox respectively) than their respective low molecular weight fractions <100 and <10, having an antioxidant activity of 48 and 21 mM equivalent of trolox respectively as measured by ABTS assay. Similar behavior of antioxidant activity was obtained during DPPH assay. Antioxidant values obtained with DPPH differ from and are lower than those of ABTS assay, likely due to different reaction media (aqueous and methanolic for ABTS and DPPH, respectively), hence indicating subdued antioxidant behavior in methanol.

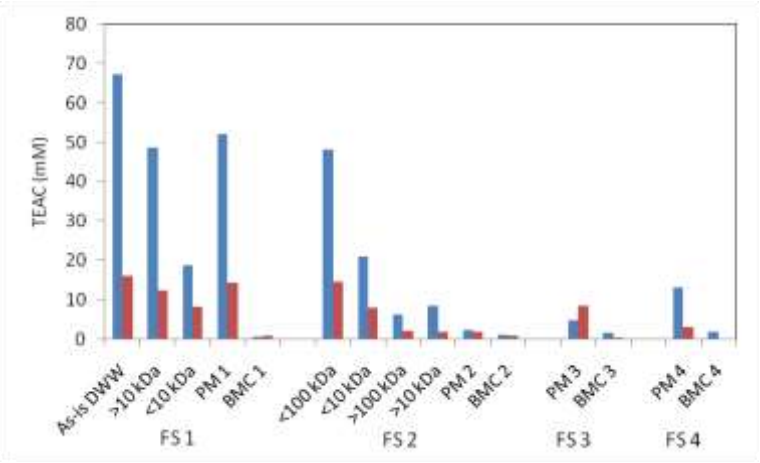


Figure 1-42 Antioxidant activity of different DWW fractions

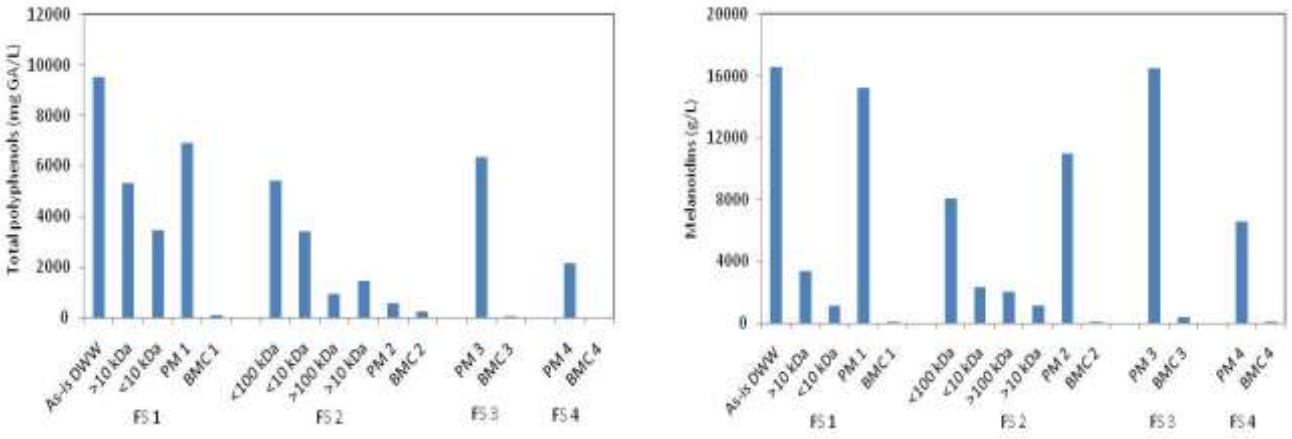
[3] Xu, B.J. and Chang, S.K.C. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of Food Science, 72, (2), 160-161.

Melanoidins and polyphenols content

Stock solution of melanoidins was prepared using glucose and glycine procured from Sigma, New Delhi, according to the procedure given by Dahiya et al., 2001[4]. Melanoidins were dialysed using 12 kDa dialysis membrane in order to remove low molecular weight components. A calibration curve was plotted between different concentrations (2 to 50 g/L) of dialysed melanoidins and their corresponding absorbance values at 475 nm. Melanoidins in DWW samples were quantified using the calibration curve.

Total polyphenols were analyzed according to Singleton's method (Singleton & Rossi., 1965[5]). For every sample appropriate dilutions were prepared with RO water. 500 µl of Folin's –Ciocalteau reagent was added to 100 µl of diluted sample. After 2 min, 1.5 ml of 7.5% sodium carbonate solution was added. Next, the sample was incubated in darkness at room temperature for 90 min. The absorbance of sample was measured at 765 nm in spectrophotometer. A calibration curve was plotted with gallic acid (10- 200 mg/L) and results were expressed as gallic acid (GA) equivalents.

As evident from Figure 1-43 (a) and (b), content of melanoidins was significantly higher than total polyphenols, in all DWW fractions. Antioxidant behaviour of fractions obtained from FS 1 and FS 2 may be attributed to the presence of melanoidins and phenolic compounds (Pastoriza et al, 2014[6]; Ludwig et al, 2012[7]; Scoma et al, 2012[8]).



(a) (b)

Figure 1-43 Antioxidant compounds in different DWW fractions (a) Polyphenols and (b) Melanoidins

[4] Dahiya, J., Singh, D., Nigam, P., 2001. Decolorization of synthetic and spentwash melanoidins using the white-rot fungus *Phanerochaete chrysosporium* JAG-40. Bioresource Technol. 78, 95–98.

[5] Singleton, V. and Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16 (3), 144-158

[6] Pastoriza, S. and Rufian-Henares, J.A. (2014). Contribution of melanoidins to antioxidant capacity of the Spanish diet, Food Chemistry, 164, 438-445

[7] Ludwig, I.A., Sanchez, L., Caemmerer, B., Kroh, L.W., Paz De Pena, M., Cid, C. (2012). Extraction of coffee antioxidants: Impact of brewing time and method, Food Research International ,48, 57-64

[8] Sacoma, A., Pintucci, C., Bertin, L., Carlozzi, P., Fava, F. (2012). Increasing the large scale feasibility of a solid phase extraction procedure for the recovery of natural antioxidants from olive mill wastewaters, Chemical Engineering Journal, 198-199, 103-109

Proteins and carbohydrates

DWW samples were further characterized for proteins and carbohydrates content. Protein content was determined by Lowry method using bovine serum albumin as the standard (Lowry et al, 1951[9]). The phenol-sulfuric acid method was used for carbohydrate determination using glucose as standard (Dubois et al, 1956[10]).

Chemical analysis of different DWW fractions revealed that they contained reasonable amounts of proteins while their carbohydrate content was significantly lower (Figure 19). It can be further deduced that antioxidant compounds like melanoidins and polyphenols, possibly exit in conjugation with proteins rather than carbohydrates (Dai and Mumper, 2010[11]).

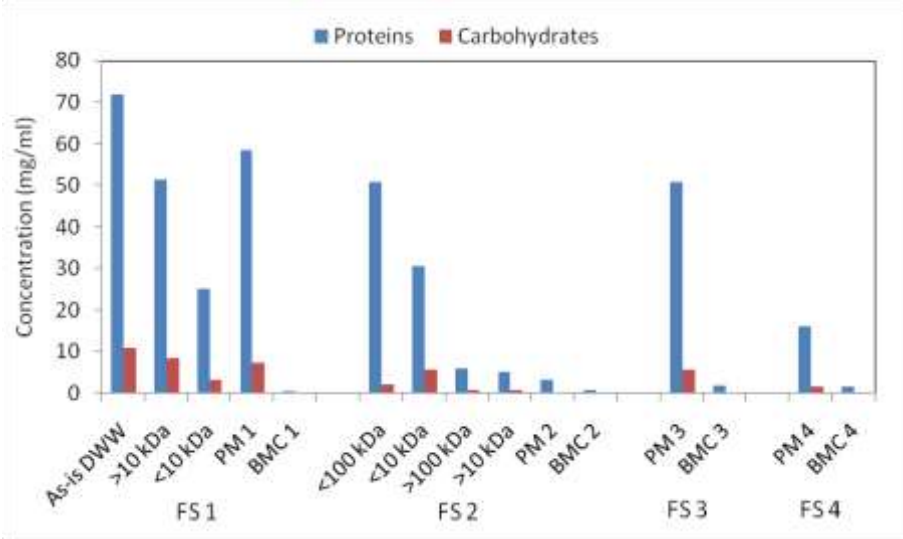


Figure 1-44 Carbohydrates and proteins content in different DWW fractions

Comparison of fractionation schemes

From different fractionation schemes it was observed that highest antioxidant activity of 67mM of trolox, was obtained when both high and low molecular weight components were present together in DWW. Though fractions obtained through FS 1 showed high antioxidant potential but the flux during this fractionation scheme was very low as the relatively tight 10 kDa membrane was used. Also, a clear picture of distribution of antioxidants with various molecular cutoffs could not be obtained through FS 1. On the contrary, FS 2 scheme generated a wider distribution of fractions according to their molecular sizes. Hence, to isolate high and low molecular weight antioxidant compounds from DWW, adsorption experiments were mainly performed on the fractions obtained from FS 2.

[9] Lowry, O.H., Rosenbrough, N.J., Farr, L., Randall. R.J., 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193, 265-275.

[10] Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28, 350-356.

[11] Dai. J and Mumper, R.J. (2010). Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules 2010, 15, 7313-7352; doi:10.3390/molecules15107313 . Review

Adsorption-desorption studies for recovery of melanoidins and polypehenols

Adsorption equilibrium experiments were carried out in order to recover antioxidant compounds from DWW. Unburnt activated carbon (UAC), commercial activated carbon (CAC) and polymeric resin XAD16 were used as adsorbents.

Unburnt activated carbon described in the earlier reports, was prepared from steam activation of unburnt carbon obtained from bagasse flyash, at 740 ºC for 4 h with 1:3 carbon to water ratio. CAC was dried in oven at 105 ºC overnight and then used for adsorption study. Prior to adsorption, XAD16 was activated using acidified ethanol (Scoma et al., 2012[8]). Adsorbent dosage was varied from 10-200 g/L. 50 mL of adsorbate was mixed with known amount of adsorbent in 100 mL conical flasks. The adsorbate-adsorbent mixture was kept in a shaker (Orbitek, Scigenics Biotech, India) at 160 rpm and equilibrated for 24 h at 25°C. One set of flasks without adsorbent addition was kept as control. After equilibrium, the suspension was vacuum filtered through 0.45 µm filter paper. Filtrate was analyzed for melanoidins and polyphenols removal as mentioned earlier. Adsorption yield (%) was calculated using the following equation:

$$\text{Adsorption yield} = \frac{\text{Mass adsorbed (g)}}{\text{Mass in feed (g)}} * 100 \qquad (3)$$

Adsorption was mainly carried out on as-is DWW, <100 and <10 kDa DWW fractions. Extensive adsorption study was done with XAD16. Effect of different temperatures (25 to 45°C) on adsorption process was studied for various time durations. A sequence of loading-regeneration cycles was performed in order to assess the stability in performance for the resin. For this, adsorption was performed for 3h as described earlier. Post adsorption, the supernatant was measured and collected for analysis. The adsorbent resin was washed with water to remove the residual DWW and the washing was analyzed. Thereafter, 50 ml of acidified ethanol was added for desorption and analyzed for melanoidins and polyphenols. These steps were repeated for 5 cycles. Single step desorption using acidified ethanol was also studied on CAC.

<u>Adsorption-desorption studies with UAC and CAC</u>

UAC and CAC were equilibrated with DWW fractions (<100 and <10 kDa respectively) at 80 g/L dosage for 24 h. As-is DWW was not directly applied on these carbons due to poor adsorption performance evident in earlier studies. Figure 20 indicates that UAC exhibited higher adsorption of polyphenols (46%) than melanoidins (24%) for both fractions. However, previous studies with UAC and 5% synthetic melanoidins showed a removal of approximately 90% melanoidins from the solution. Evidently, lower adsorption performance of UAC with DWW component fractions indicates its suitability as an adsorbent for diluted wastewater streams. On the contrary, significantly higher adsorption of polyphenols and melanoidins was achieved with CAC. Approximately, 80% melanoidins and 64% polyphenols were adsorbed from high (<100 kDa) molecular weight DWW fraction while slightly higher removal efficiencies were obtained with fractions of low (<10 kDa) molecular weight (92% melanoidins and 75% polyphenols).

Though melanoidins and polyphenols were efficiently removed through CAC, poor recovery (6%) of adsorbed compounds was achieved using acidified ethanol as desorbing agent.

Furthermore, adsorption-desorption process was rather inconvenient at high CAC dosage due to formation of adsorbate-adsorbent slurry.

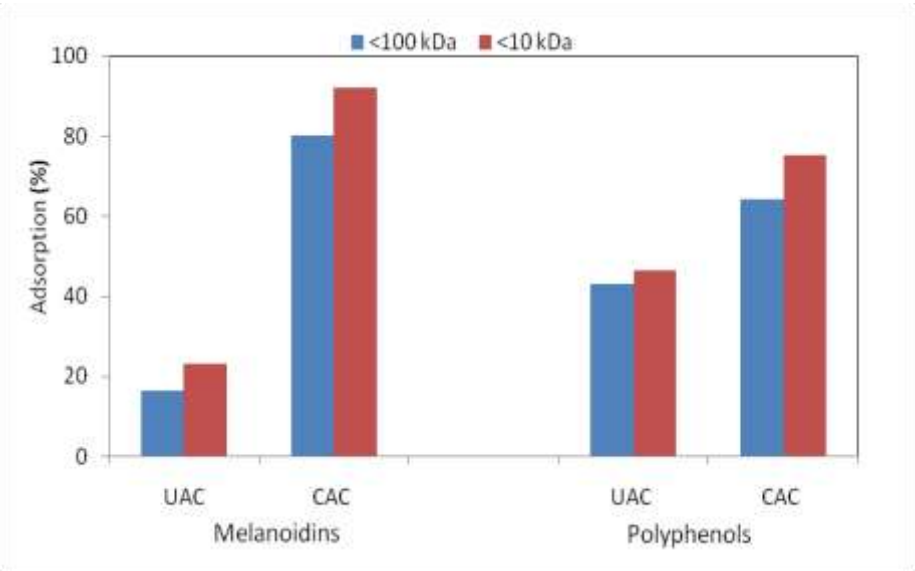


Figure 1-45 Adsorption of high and low molecular weight DWW fractions on different adsorbents

<u>Adsorption-desorption studies with XAD16</u>

In order to achieve the desired goal of recovering antioxidant compounds a polymeric, non-ionic, styrene divinylbenzene, adsorbent resin Amberlite XAD16 was used as solid adsorbing phase at a dosage of 200 g/L.

XAD16 was equilibrated with as-is DWW and <10 kDa DWW fractions for varying time and temperature conditions. It was observed that during adsorption of melanoidins and polyphenols equilibrium was attained within a span of 3 h at all temperatures for both fractions (Figure 1-46 and 47).

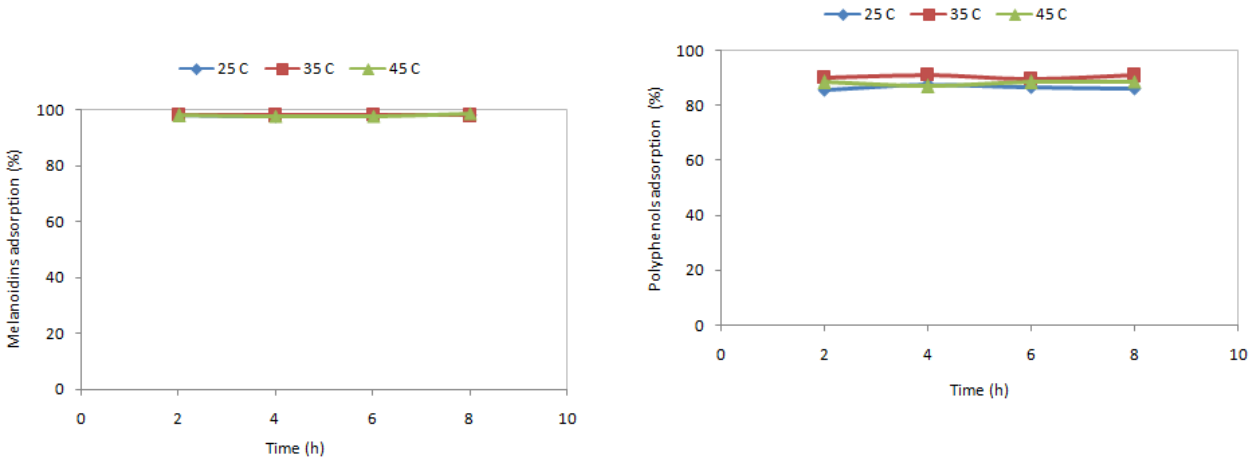


Figure 1-46 Adsorption of melanoidins and polyphenols from as-is DWW on XAD16 at various time intervals

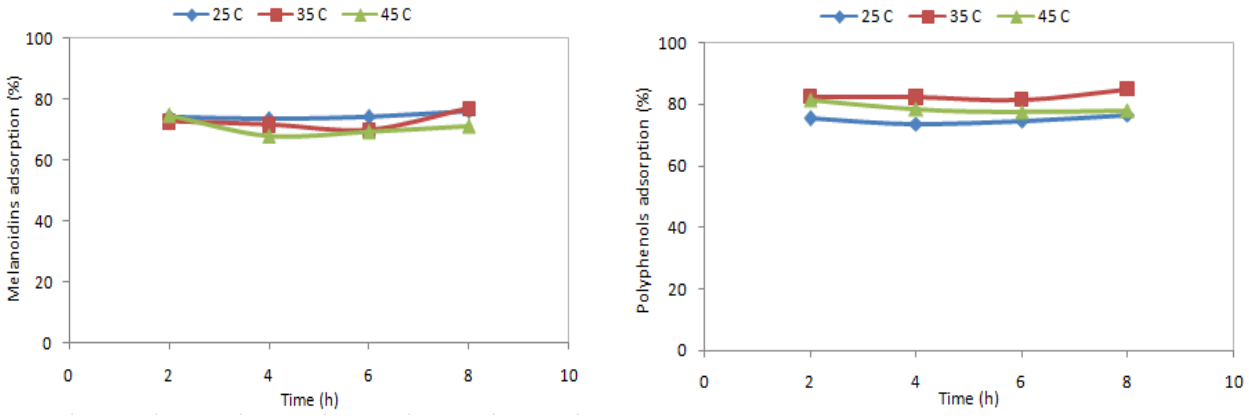


Figure 1-47 Adsorption of melanoidins and polyphenols from <10 kDa DWW fraction on XAD16 at various time intervals

A maximum of 97% melanoidins and 86% polyphenols were adsorbed from as-is DWW while about 75% of both were adsorbed from <10 kDa fraction. Furthermore, it was observed that adsorption of proteins was similar to polyphenols confirming the fact that latter compounds may be linked to proteins rather than carbohydrates (Figure 1-48).

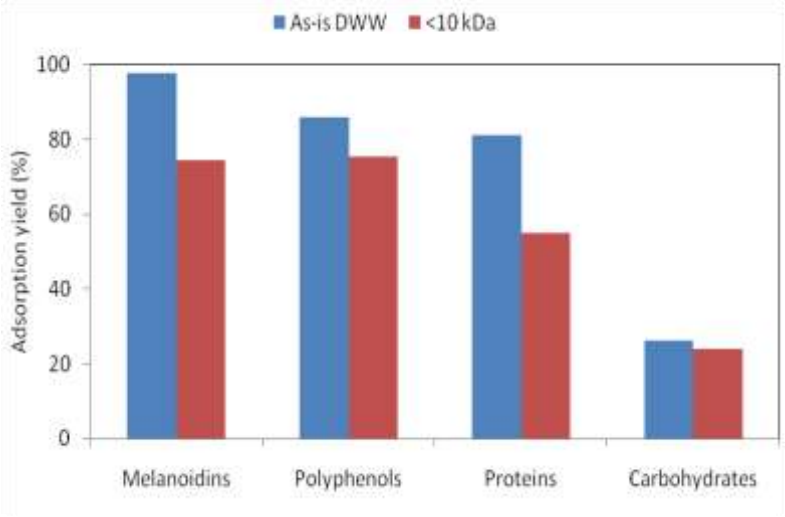
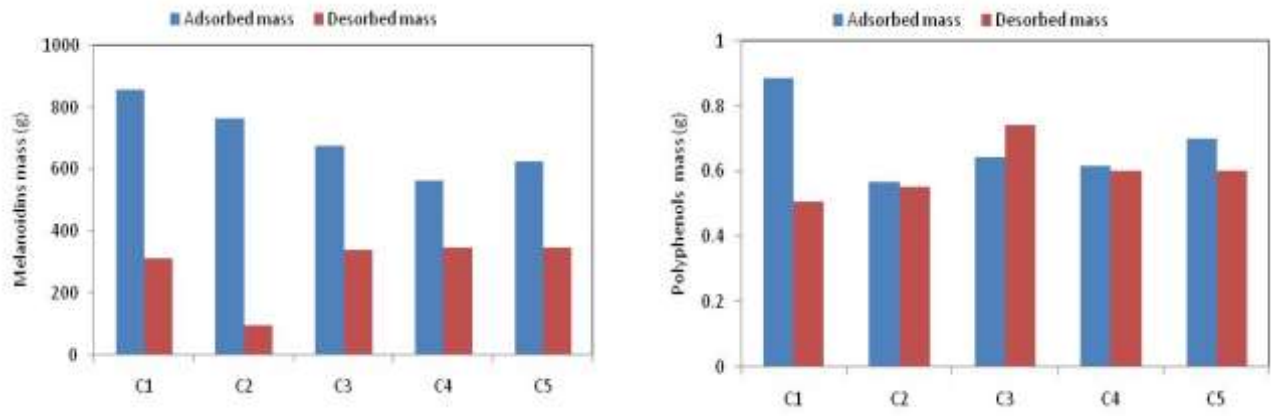


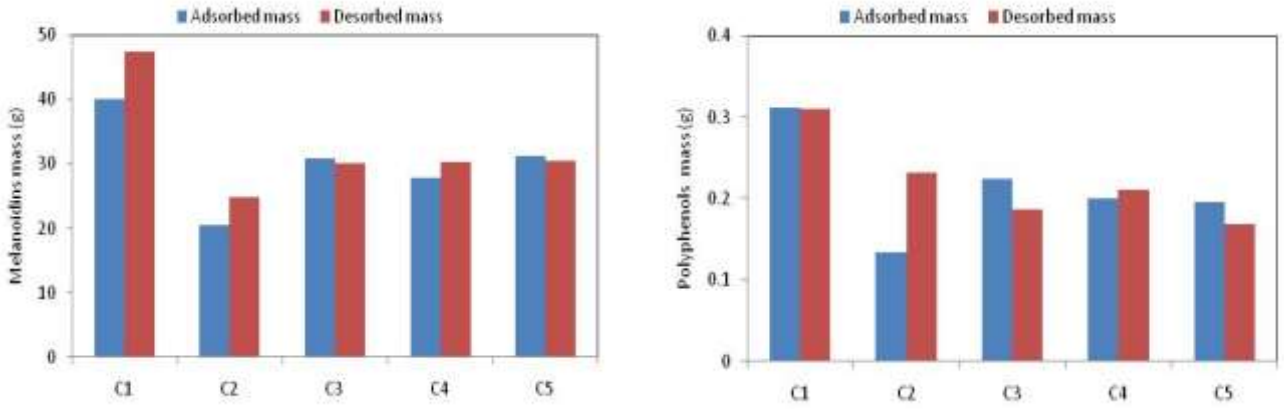
Figure 1-48 Adsorption of different compounds on XAD16

A batch study was also performed with XAD16 to apply a sequence of loading regeneration cycles with different DWW fractions in order to check the stability in behaviour for the adsorption and desorption yield over 5 cycles. Figure 24 indicates that when XAD16 was equilibrated with as-is DWW, at the end of 5th cycle nearly 80% melanoidins were adsorbed while 50% were efficiently recovered through desorption with acidified ethanol. Polyphenols were adsorbed to an extent of 70% with approximately complete desorption. In case of low molecular weight DWW fraction (<10 kDa), adsorption efficiency reduced to 50% for both melanoidins and polyphenols while the latter were recovered completely. During desorption, at some stages of the cycle it was observed that the amount of desorbed

compounds exceeded than the actual amount which was adsorbed. This may be attributed to accumulation of some compounds during preceding cycles before they were actually recovered.



(a)



(b)

Figure 1-49 Batch adsorption-desorption of melanoidins and polyphenols on XAD16 with DWW fractions for 5 cycles (a) As-is DWW and (b) <10kDa

Thus XAD16 is a promising adsorbent which can be efficiently used for the solid phase extraction of melanoidins and polyphenols from distillery wastewater while UAC seems a promising waste based adsorbent for low molecular weight polyphenols present in less concentrated streams.

1.5 Impact of treated and untreated wastewater use on soil, crop and groundwater quality

2 Work Package: Bio-treatment of Municipal Wastewater for Reuse and Bioremediation of Degraded Lands

Objectives

Selection and optimization of microbial consortium to reclaim degraded lands and bio-treatment of municipal wastewater for re-use in agriculture

2.1 Demonstration of CWs and HRTS systems

2.1.1 Column/ lysimeter experiment to evaluate the enteric pathogens removal efficiency of different substrate and vegetation from domestic wastewater

A comparative study was carried out to evaluate the efficiency of different substrate material along with macrophytes *Typha latifolia* and *Cyperus rotundus* in treating domestic wastewater intended for reuse in agriculture. Major emphasis is given on the removal of pathogens viz; total coliform, fecal coliform, *Escherchia coli, Shigella, Salmonella* and infective stages of parasites like *Ascaris lumbricoides* eggs, *Entamoeba histolytica* cyst and *Strogyloides stercoralis* larvae as their infections are more common in tropical region with warmer climate, favouring the survival of parasitic developmental stages in soil. Pathogen removal by use of readily available material like sand, marble chips and local vegetation in constructed wetland will result in a cost-effective, eco-friendly and sustainable wastewater treatment.

Study Area

Batch experiments were conducted in vertical columns under greenhouse conditions for six months, from November 2014 to April2015 at CSIR-NEERI campus, Nagpur, in Central India. Raw wastewater required for the study was collected from Nag River, which showed typical properties of domestic sewage with a high concentration of helminths eggs, protozoan cysts and other pathogens like fecal coliform, *shigella* and *salmonella*. It flows through the middle of city, Nagpur, covering the densely populated area and finally meets Kannan River in the outskirt of the city.

Experimental Set up

Eight treatments were set up in triplicate to elucidate the effects of different substrate and vegetation on bacterial pathogens and parasites removal efficacy. The experimental columns were designed using of PVC pipes of 25 cm diameter and 100 cm length with a basement and open top. Three types of substrate compositions were decided on the basis of porosity and surface area of substrate materials; i) pure sand (<2 mm) ii) pure marble chips (10-15 mm) and iii) mixture of sand and marble chips. The columns with respective filter media were further divided into subgroups with and without plants. Two types of vegetation viz., *Typha latifolia* and *Cyperus rotundus* were used in the study, summoning up to eight treatments.

Treatment Details

S= sand+ gravel

SC= sand + gravel+ *C. rotundus*

MT= marble chips + gravel + *T. latifolia*

SMT= sand + marbles chips + gravel + *T. latifolia*

ST= sand + gravel + *T. latifolia*

M= pure marble chips + gravel

MC= marble chips + gravel + *C. rotundus*

SMT= sand + marbles + gravel + *C. rotundus*

Enumeration of pathogenic bacteria and parasites

Samples were analysed for the presence of Total coliforms (TC), Fecal coliforms (FC), *E. coli* (EC), *Salmonella* and *Shigella*. The enumeration of total coliforms, faecal coliforms and *E Coli* was performed by membrane filtration on 0.45 µm pore size membranes (Millipore). *Salmonella* and *Shigella* were enumerated using standard plate count method on selective SS medium. The count was represented as Log CFU/ml.

For parasites, samples were processed using modified Bailenger method (WHO, 1989) by centrifuging at 2500 rpm. *A. lumbricoides* eggs and *S. stercoralis*larvae concentration was determined using MacMaster counting cell at 100 magnification. Isolation of *E. histilytica* cyst was carried out by zinc sulphate centrifugal technique. Microscopic observation was performed using wet mount method at 400 magnification and quantified using Neubauer chamber.

Wastewater Characterization

The initial characterization of inlet wastewater was carried out by analyzing parameters like BOD, COD, TSS, EC and pH (APHA, 2012) along with the concentration of parasites and pathogens. The inlet values of the above parameters were considered as 0-day values. The wastewater was allowed to retain in the columns for varying hydraulic retention time (HRT) i.e. from 1 to 4 days. The concentration of respective pollutants and organisms through the outlet of the column was measured daily to determine percent removal in each treatment.

Removal of enteric pathogen

The inlet concentration of different bacterial pathogens and parasites is given in Table 1. Wastewater collected from Nag River showed higher bacterial count ranging from 4.21×10^1 to 2.76×10^8. *E. histolytica, A. lumbricoides* and *S. stercoralis* were the most common parasite found in Nag River, with concentration ranging from 430 to 530 cyst/ litre, 140 to170 eggs/litre and 35 to 65 larvae/litre respectively, along with other minor parasites like *Trichuris trichura*, *Giardia* and *Trichomonas vaginalis*.

The overall performance of the different treatments for each pathogen and their removal rates with respect to time is shown in Figure 2-1.The decline in pathogens population was observed in all the treatments, and it was least at fourth day retention time, however after third day, the rate of reduction was extremely low except for *salmonella*. Even at three days retention time, all the treatments showed significantly lower concentration of fecal coliforms> 1000 which meets the quality criteria of WHO (2006) for microbiological quality guidelines for wastewater use in agriculture**.** The final concentration of the microorganisms after passing through different substrate composition at four days retention time is given in Table 2-1. Treatments planted with *T.latifolia* in sand and mix substrate were most effective in removing TC, FC, EC and *shigella* from sewage wastewater after a period of 4 days as compared to other treatments. The Higher efficiency of *T. latifolia* in pathogen removal was might be due to some antibacterial properties of this plant specially rhizome as reported by Shukla and Mishra (2013). Also, the roots of *T. latifolia* are known to colonise gram negative bacteria (Aziz et al. 2015). This might be another reason for removal of EC and other enteric pathogens as they are gram negative in origin and might have colonised in roots of the plant. However, other mechanisms like sedimentation, aggregation, oxidation, filtration, antibiosis, predation, and competitionalsoaid in a bacterial reduction in wetlands.

Table 2-1 Inlet and outlet concentration of different treatments for physico-chemical and pathological parameters

Parameters	Inlet (0 day)	Outlet (4 day)							
		S	ST	SC	M	MT	MC	SMT	SMC
pH	7.43±0.1	7.82±0.24	7.9±0.33	7.82±0.23	7.76±0.32	7.75±0.3	7.72±0.5	7.65±0.7	7.64±0.57
EC	692.6±79.85	687.4±65.6	662.8±41.9	705.6±64.55	621.6±16.23	610.2±23	659.4±35.68	703.6±62.64	717.8±68.67
TSS	655.6±39.4	11.17±2.47	8.57±1.9	7.23±3.01	16.23±3.01	13.37±0.56	13.17±2.26	12.23±3.5	12.2±3.97
COD	1350±20	120.0±7.5	110.4±5.5	145.5±5.5	95±6	90.2±5.5	97.4±3.5	110.1±3.5	100±4
BOD	155.6±4.5	10.1±0.6	8.1±0.4	10.5±1.6	14.1±1.5	17.1±2.5	15.1±2.5	13.5±2.2	12.1±2.6
Bacterial pathogens, log CFU/mL									
Total coliform	7.41±0.55	4.38±0.17	2.4±0.15	3.6±0.15	4.45±0.1	3.23±0.07	4.18±0.75	2.59±0.04	3.18±0.05
Fecal coliform	6.08±0.61	0.3±0.38	0±0	2.52±0.02	2.85±0.32	1.96±0.34	2.7±0.28	0±0	1.26±0.06
E. coli	6.47±0.45	3.41±0.63	2±0.15	3.04±0.11	3.28±0.36	2.32±0.23	3.15±1.06	2.54±0.08	2.66±0.15
Shigella	7.56±0.54	3.15±0.76	2.04±0.23	4.3±0.23	3±0.16	2.95±0.02	4.51±0.49	2.08±0.09	3.79±0.08
Salmonella	1.62±0.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Parasites, log count/ L									
E. histolytica	2.65±0.02	0.67±0.15	0.55±0.09	0.34±0.44	1.13±0.14	0.98±0.08	0.85±0.09	0.74±0.27	0.63±0.2
A. lumbricoides	2.01±0.06	0.3±0.19	-0.08±0.31	-0.3±0.22	0.99±0.11	0.83±0.12	0.7±0.2	0.48±0.14	0.3±0.25
S. stercoralis	1.6±0.09	0.11±0	-0.07±0.12	-0.06±0.14	0.97±0.09	0.84±0.11	0.81±0.1S	0.59±0.13	0.58±0.11

mean values ± standard deviation; n=12

The World Health Organisation set down intestinal nematodes as the greatest health risk involving agricultural uses of wastewater, due to the resistance of the eggs to environmental factors and also because the ingestion of fewer than ten eggs has a probability of causing infection (WHO 1986). As per WHO, infective stages of frequently occurring parasites were taken into consideration while planning the experiments. Figure 2-2 shows the performance of different treatment in parasites removal with respect to time. Like pathogens, the decline in parasites population was also observed in all the treatments however, after thirdday, the rate of removal became very slow. Maximal declination can be noticed in the treatments having sand as a substrate material along with vegetation. Stressing the importance of sand Okojokwu et al. 2014 reported that the biosand filter is more efficient in removal of helminth ova, oocysts of *Cryptosporidium* spp and *Giardia lamblia* (reduction up to 97.45%) than sewage treatment plant (52.61%). The Higher efficiency of SC and ST treatment for the removal of above parasites credited to better sieving ability of sand which aids in efficient filtration along with the profuse growth of plant roots which formed the dense mat to trap the parasites.

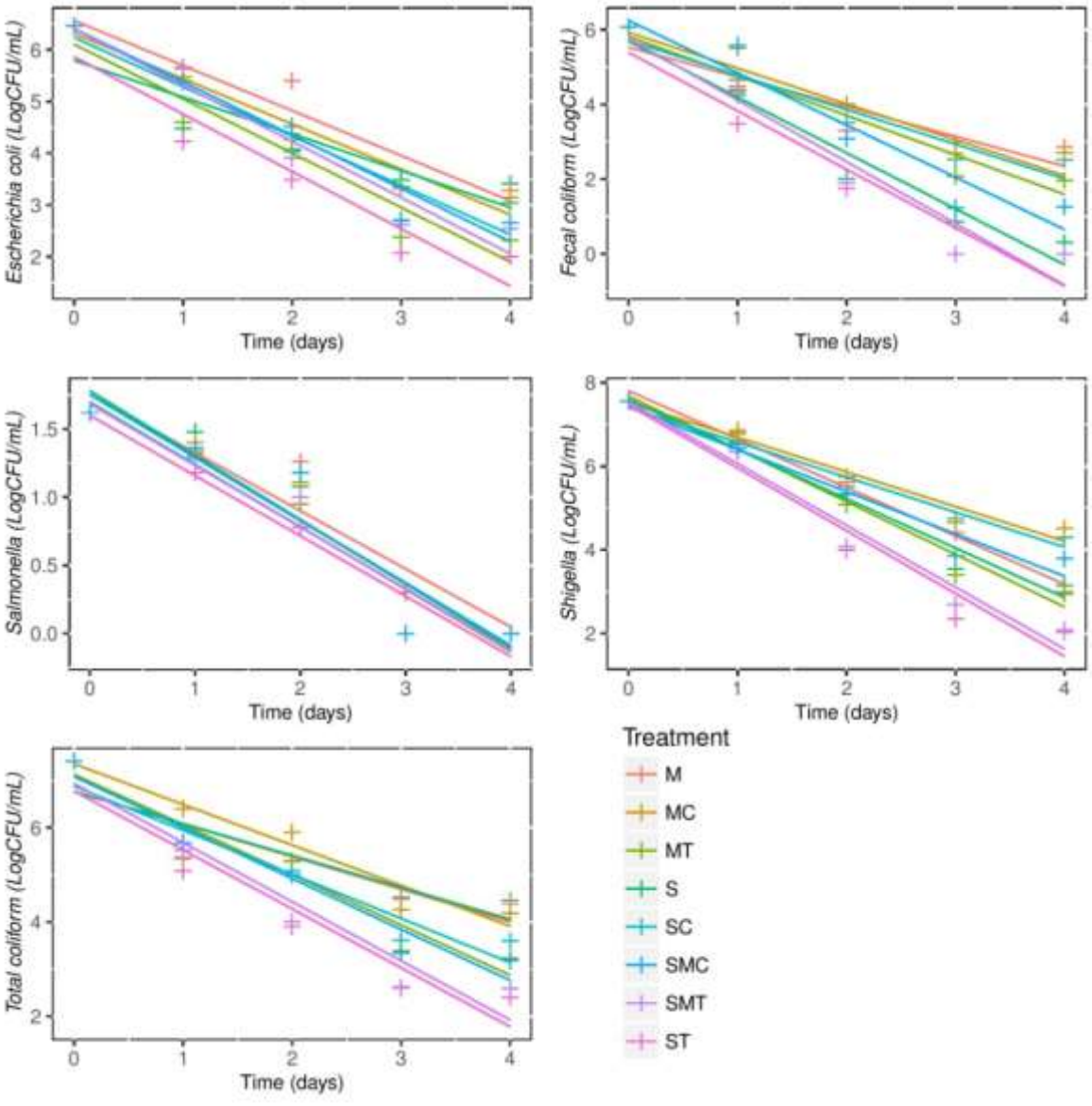


Figure 2-1 different treatments for each pathogen and their removal rates with respect to time

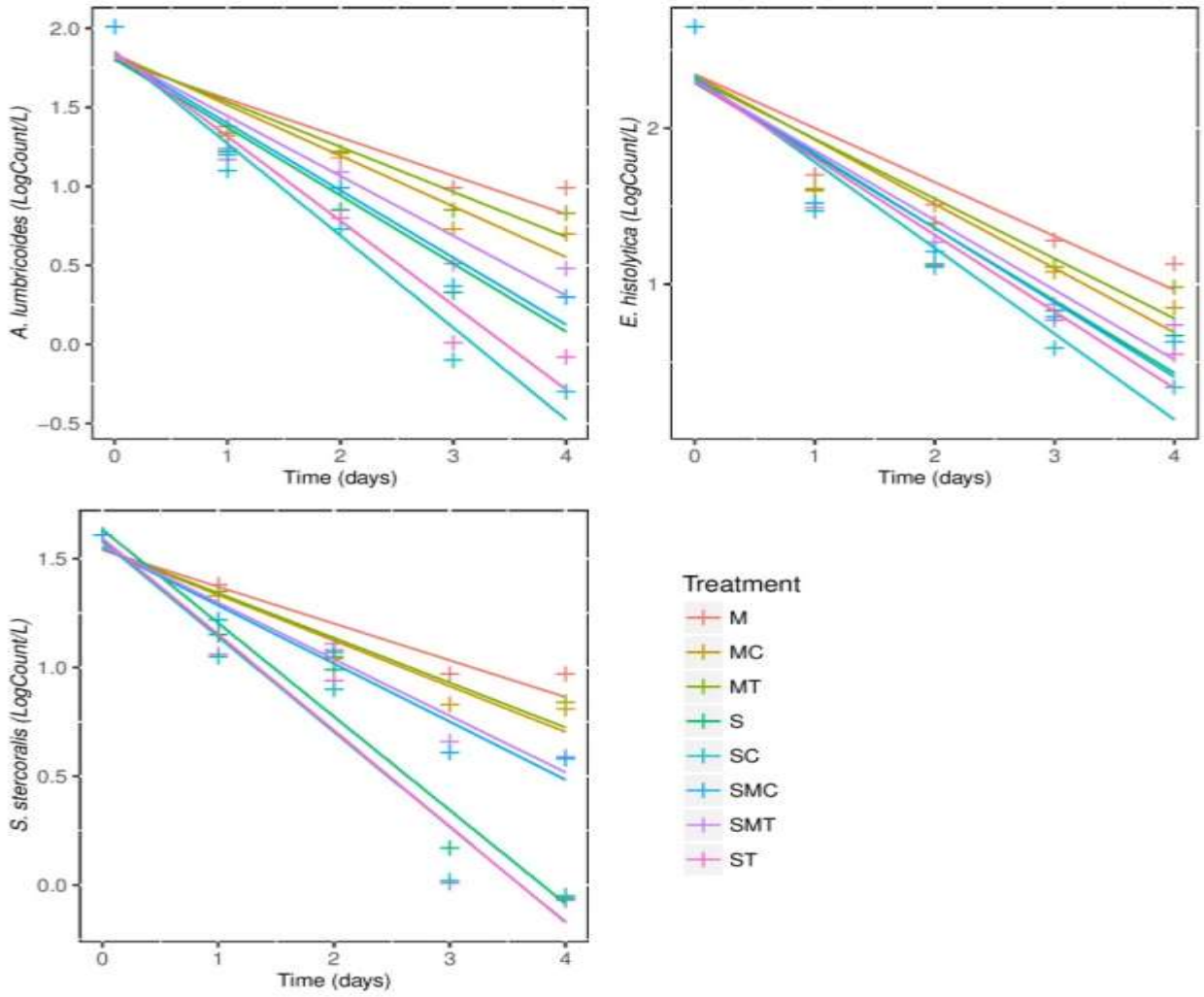


Figure 2-2 Reduction of parasites in different treatments

2.1.2 Demonstration and installation of constructed wetland at CICR-NEER (Pilot scale study)

The horizontal flow subsurface constructed wetland system was installed at the study area based on our earlier research findings that has been mentioned in the previous report (Juwarkar et al. 1995; Kadaverugu et al. 2014). The study suggests that the wetland system with locally available filter media –sand, gravel and marble chips has higher removal capacity for organic pollutants and nutrients. The wetland trough was made up of fibre reinforced plastic material with dimensions of 3 m x 1.2 m x 1 m (length x width x depth), and bed slope of 1% (Figure 2-3). The filter media for the present study was prepared by mixing sand (<2 mm), marble chips (10-15 mm) and gravel (40-60 mm), which was filled in the wetland trough up to the height of 0.6 m (porosity: 40%). A free board of 0.4 m was allowed for the safety. Wetland plant *Typha latifolia* was planted on the filter media with a density of 1 plant per 0.1 x 0.1 m^2. The wetland system was provided with 6 months of stabilization period for the plants to get acclimatized to the wastewater and filter media.

Figure 2-3 Constructed wetland installed at CSIR-NEERI

The wastewater pumped from the channel was collected in a tank for primary treatment, which was allowed to settle for 3-5 hours to remove the grit. The primary treated wastewater was then allowed to flow into the wetland trough through the porous media. The flow rate was adjusted to maintain the hydraulic retention time (HRT) of 0.8-1 day for efficient removal of pollutants. The quality of wastewater treated by pilot unit is mentioned in Table 2-2. Further the study was extended to check impacts of raw wastewater and treated wastewater irrigation on soil physico-chemical properties.

Samples of wastewater, treated wastewater and tap water were collected once in a month throughout the cropping season to characterize the physico-chemical properties. Collected samples were preserved in refrigerators maintained at 4˚C and they were examined within 24 hour of collection. The pH and Electrical Conductivity (EC) of the samples were measured using pH and conductivity meter (make Hach). Na^+ and K^+ concentrations were determined using flame photometer. Ca^{2+} and Mg^{2+} were measured by EDTA titration method. Total Suspended Solids (TSS) was determined after filtration of the water samples through glass microfiber filter paper- GF/C (47 mm diameter; Whatman, Maidstone, UK) using a vacuum system. Phosphates, sulphates and nitrates were determined using spectrophotometer. Ammonical nitrogen was estimated using titrimetric method. The Sodium Adsorption Ratio (SAR) was calculated using the formula $SAR = (Na^+)/[(Ca^{2+}+ Mg^{2+})/2]^{1/2}$ (Gatta et al. 2015). BOD_5 was analyzed by titrimetric method (incubating at 20 ^oC for 5 days) and COD was measured with open reflux method.

Physico-chemical characteristics of untreated wastewater, treated wastewater and tap water used in the study are shown in Table 2-3. It was observed that the three types of irrigation water were slightly alkaline in nature with pH ranging from 7.6 to 8.3. The influent wastewater was quite diluted as far as TSS, BOD and COD were concerned.

CW unit has reduced the concentration of TSS from 71- 84 mg/l to 4-12 mg/l in the outlet, COD from 106 -125 mg/l to 29-39 mg/l and BOD5 from 40-60 mg/l to 6-13 mg/l. One-way ANOVA suggested that the characteristics of untreated wastewater were significantly different from the treated wastewater and tap water; with respect to organics. The removal efficiency of CW was found to be quite low for some form of nutrients such as NH_4-N (47%) and PO_4 (69%). Tukey HSD test suggested that, though the concentrations of Na^+, Ca^{2+}, Mg^{2+}, K^+ and hardness were reduced in treated wastewater, the difference was not statistically significant in comparison with wastewater. Table 2-3 illustrates the heavy metals concentration in the inlet and outlet of CWs. CWs was found to be very efficient in removal of elements like B, Fe, Cd, Cu, Pb and Zn, while it showed moderate performance in case of Cr, Mn and Nickel. Key mechanisms involved in the reduction of these heavy metals in CW are complexation, precipitation and uptake by the macrophytes along with other viz., adsorption, sedimentation, erosion and diffusion.

Table 2-2 Characteristics of untreated wastewater, treated wastewater and tap water used for irrigation.

Parameter	Untreated wastewater	Treated water	Tap water
pH	7.50±0.24	7.52±0.16	8.24±0.10
EC(µS cm^{-1})	472.75±10.5	462±8.91	224.75±3.78
TSS (mg l^{-1})	78.375±5.78	8.42±3.52	NA
BOD (mg l^{-1})	49.5±5.0	8.41±3.29	NA
COD (mg l^{-1})	113.25±6.89	33.75±4.57	NA
PO_4 (mg l^{-1})	5.32±1.81	1.65±0.78	NA
NH_3-N (mg l^{-1})	1.04±0.41	0.53±0.22	NA
TKN (mg l^{-1})	8.56±0.82	3.87±0.59	NA
Na^+ (mg l^{-1})	34.95±2.78	29.18±2.34	10.76±1.48
K^+ (mg l^{-1})	4.25±0.48	3.5±0.40	1.34±0.34
Ca^{2+} (mg l^{-1})	43.92±9.64	37.99±8.02	17.8±2.24
Mg^{2+} (mg l^{-1})	19.89±3.98	17.61±3.34	10.35±1.02
Cl^- (mg l^{-1})	43.48±2.64	33.63±2.02	25.07±0.86
Hardness (mg l^{-1} $CaCO_3$)	160.75±8.70	143.95±6.24	64.55±3.84
SAR	6.23±0.28	5.75±0.28	2.86±0.44

Table 2-3 Concentration of heavy metals in raw wastewater and CWs treated wastewater

Elements	Inlet (PPM)	Outlet (PPM)	% Removal
B	170.1157±6.2880	71.18241±0.51201	58.16
Cd	0.00053±0.03523	0.000012±0.001421	97.73
Co	0.001183±0.002412	0.001089± 0.001321	7.94
Cr	0.009062±0.077273	0.007563±0.001824	16.54
Cu	0.011798±0.027543	0.006073±0.006102	48.56
Fe	0.778532±0.563926	0.032699±0.030046	95.80
Mn	0.309977±0.095	0.270037±0.005321	12.87
Ni	0.000203± 0.000352	0.000137±0.000237	32.51
Pb	0.009047+0.014156	BDL	100
Zn	0.114256±0.017125	0.032116±0.027313	71.89

2.1.3 ICRISAT Patancheru

Performance monitoring of the constructed wetland in ICRISAT campus was carried out in an attempt to understand the wastewater treatment efficiency. As different plant species and different flow types of wetlands were treating real wastewater; field scale performance comparison was carried out. The general layout of the wetland is given in Fig 2.4.

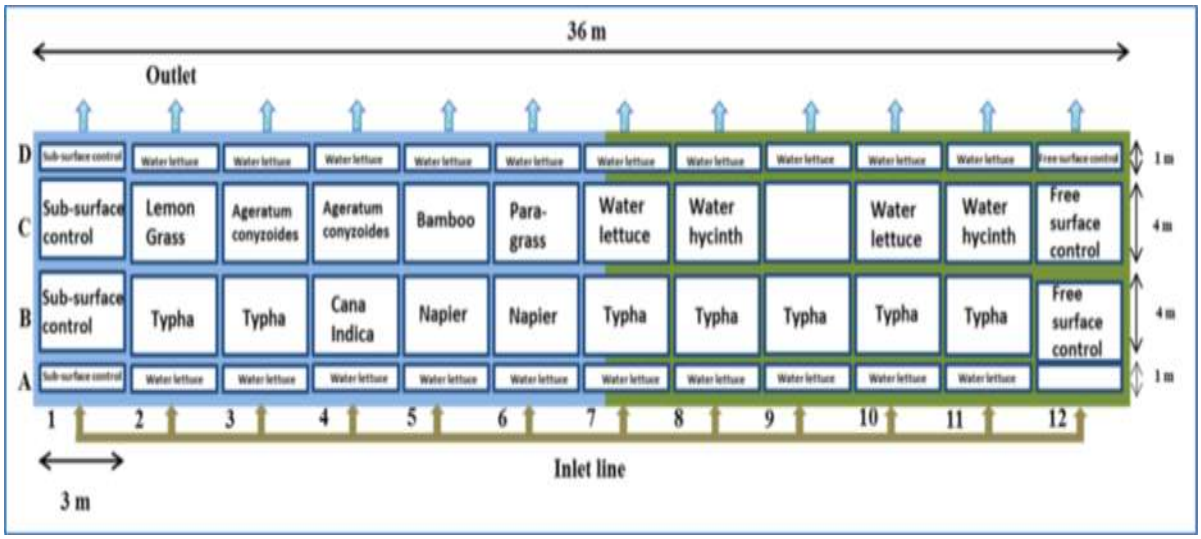


Figure 2-4 The overall layout of the constructed wetlands as on June 2016

Wastewater flow monitoring

The wastewater inflows and outflows were monitored on a daily basis with help of mechanical Itron flow meters shown in Figure 4. The inlet pipes for both CWs were fitted with a house-old tap to regulate the inlet flow. The outlets only had flow meter but no regulators. The calibrated wastewater inflow rate into 10 CWs and two controls for an eight month period was kept at 2.08 L/min (2.99 m^3/day). The hydraulic retention time (HRT) and the hydraulic loading rates (HLR) were computed using the equations 1 and 2 respectively (USEPA, 2000).

Monitoring frequency wastewater parameters and analysis methods

The wastewater samples (inlet and outlet) were collected every week each month from 10 CWs and two controls over an eight month period. The wastewater samples collected in Nalgene bottles were analyzed in the ICRISAT laboratory for NH_4-N, NO_3-N, soluble reactive phosphorus (SRP), COD and TSS using the APHA standard methods 4500-NH3 F, 4500-NO_3, 4500-P D, 2540-D and 5220-C respectively.

Coarse sand sampling and analysis methods

The coarse sand samples were collected each month at 0-5 cm depths from 10 CWs and two controls for an eight month period. The sampling was conducted using a T shape auger manufactured by AIC Agro Instruments (P) Ltd, Kolkata, India. The samples were air dried for 1-2 days and passed through 2 mm sieve and analyzed for total N and P, available P, and organic carbon. The Kjeldahl method (thiosulphate modification) was used to analyze total N (Dalal et al., 1984). The total P and available P were analyzed using the methods given in Tandon et al. (1962) and Olsen and Sommers (1982) respectively. The organic carbon was analyzed using the method of Nelson and Sommers (1982).

Wetland plant sampling and analysis methods

The above-ground biomass (stems, branches and leaves) and below-ground biomass (roots) were sampled for each wetland plant upon maturity for an eight month period. The plant samples were collected in cloth bags and kept for drying (4-5 days) in oven at 65° C. For wetland plants such as *Pistia stratiotes* and *Eichhornia crassipes*, the N and P content in the entire plant (leaves + roots) was measured. The dried plant samples were passed through a Willey grinder machine (Nebraska, USA) to make a fine powder. The dry weight of the powder for each wetland plant was recorded. The total N and P contents in the wetland plants were measured in ICRISAT laboratory using the sulphuric acid-selenium digestion method (Sahrawat et al., 2002).

Maintenance activities for all the CWs

The inlet tank (capacity-70 m^3) was cleaned every three months while the inlet and the outlet pipes were manually cleaned each week. In all the CWs, monoculture plant regime was maintained and the invasive plants were removed each week. The pipes carrying the wastewater from the inlet tank into the CWs were subject to clogging. To attenuate this problem, "U" shaped bends were installed to removed larger suspended particles by allowing them to settle and these were removed manually by opening the cap shown in figure 2-5

Maintenance of vegetation in wetlands

In many treatment cells, more than a single plant species (unplanned species that grew as weed) were found and the nutrient removal efficiency of individual plant species would have been speculative. Maintaining a single monoculture wetland species in each treatment cell was mandatory. Cell by cell cleaning and weeding of unwanted plant species was undertaken (every 15 days) to make sure that each cell contained the desired single plant species.

Figure 2-5 View of the all treatment cells of 10 ICRISAT CWs (Photo taken on May 17 2016).

Figure 2-6 Harvesting of Typha latifolia (left) and Ageratum conyzoides (right).

Wastewater Characteristics

Wastewater analysis was conducted from influent and effluent points for subsurface, surface controls and for all the 10 treatment CWs. Collected samples were immediately analyzed for pH, ORP, electrical conductivity, total dissolve salt, salinity, by using multi-parameter meter probes. In addition to these parameters, samples were also analyzed for NH4-N, NO3-N, sulphate, heavy metals and chemical oxygen demand (COD). Table 1 quantifies the average inlet concentrations for various parameters observed during this period.

Wastewater characteristics of subsurface and surface controls

The outlet pH for subsurface and surface controls averaged 8.2 and 7.56. The subsurface control reduced the TSS and COD by 48% and 41% respectively. However, the TSS and COD reduction by surface control averaged 64% and 34% respectively. In spite of the absence of vegetation cover, the reduction in the COD and TSS may be due to sedimentation, deposition, entrapment in coarse sand media and gravel layers in both the controls (Vyzamal, 2010). The sulphate reductions in subsurface and surface controls were 17% and 25% respectively.

Low removal efficiency was probably due to absence of vegetation and root-zone microbial consortia, considered as drivers of transformation and facilitation of plant uptake of sulfur moieties (Vymazal, 2007). The NH4-N reduction efficiency of subsurface and surface controls averaged 14% and 20% respectively. This NH4-N reduction could be attributed to its accumulation in the coarse sand media (adsorbed to some organic matter present in the coarse sand media) and volatilization (as occasionally the pH greater than 9.3). Subsequently, we did not observe any increase in the NO3-N concentrations in the outlet due to nitrification of NH4-N. However, some nitrification of NH4-N definitely occurred in both the controls.

Table 2-4 Average wastewater inlet and outlet characteristics of subsurface and surface controls from October 15-June 16.		
Wastewater parameters	**Subsurface control**	**Surface control**
Same inlet wastewater for subsurface and surface control (avg concentrations)		
Inlet pH	8.19	
Inlet TSS (mg/L)	75	
Inlet COD (mg/L)	131	
Inlet sulphate (mg/L)	12	
Inlet NH_4-N (mg/L	50	
Inlet NO_3-N (mg/L)	3.1	
Inlet SRP (mg/L)	3.35	
Outlet data for Subsurface control (avg concentrations)		
Outlet pH	8.2	
Outlet TSS (mg/L)	39	
Outlet COD (mg/L)	77	
Outlet sulphate (mg/L)	10	
Outlet NH_4-N (mg/L)	43	
Outlet NO_3-N (mg/L)	2.15	
Outlet SRP (mg/L)	3.00	
Outlet data Surface control (avg concentrations)		
Outlet pH	7.56	
Outlet TSS (mg/L)	27	
Outlet COD (mg/L)	87	
Outlet sulphate (mg/L)	9	
Outlet NH_4-N (mg/L)	40	
Outlet NO_3-N (mg/L)	1.99	
Outlet SRP (mg/L)	2.79	

Table 2-5 Average wastewater inlet and outlet parameters (mg/L) for each CW (total 10 CWs) from October 15-June 16

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**
pH	8.19									
Inlet COD	131									
In TSS	75									
Inlet NO_3-N	3.1									
Inlet NH_4-N	50									
Inlet SRP	3.35									
In sulphate	12									
Outlet pH	8.1	8	7.9	8.12	7.92	7.94	7.97	8.04	7.99	8.05
Outlet COD	65	79	75	70	67	63	47	69	62	64
Outlet TSS	21	25	17	11	27	22	14.5	18	27	15
Out NO_3-N	2.6	1.90	1.97	1.67	1.83	1.79	1.77	1.91	1.63	1.76
Out NH_4-N	38	43	42	41	44	43	39	44	40	35
Outlet SRP	3	3.1	3.0	2.97	3.12	2.90	2.60	3.13	3.0	2.65
Out sulphate	4.8	4.7	5.8	5.82	5.90	4.84	5.23	6.0	5.2	4.98

Note: Inlet and outlet parameters such as COD, TSS, NH_4-N, NO_3-N, sulphate and SRP are in mg/L. T1, T2, T3, T4-treatments 1, 2, 3 and 4 respectively.

2.1.4 Kothapally, Telangana

The work in Kothapally involved construction and establishment of a second constructed wetland for the treatment of wastewater from 100 households as well as performance monitoring and maintenance of the constructed wetland commissioned during the year.

Constructed Wetland 1 **(commissioned in July 2014)**:

The average inlet and outlet wastewater characteristic is given in Table 2.3. The overall TSS removal efficiency was 62 %. Resuspension of bio-particles from different plants near the outlet decreases the actual efficiency though. The removal of inorganic nitrogen and phosphate were found to be 34.93 % and 21.56 % respectively. In absence of periodic harvesting COD removal efficiency of the CW dropped steadily from initial values of around 65 % to about 30% at present. The absence of weeding

Figure 2-7 Different phases of commissioning of the constructed wetland 1 (CW-1) in Kothapally, Telengana, India.

severely affected the growth of slow growing plants like bamboo very much restricted. Average sulphate removal observed was about 24.75 %. The total biomass yield during the last one year for this CW was 3673 kg. The harvesting was done four times with an approximate interval of three months.

Table 2-6 Average Inlet and outlet wastewater characteristic for the CW -1

Parameters	Inlet	Outlet
Arsenic (mg/L)	0.01	0.01
Boron (mg/L)	0.15	0.14
Cadmium (mg/L)	BDL	BDL
Calcium (mg/L)	110.47	119.26
Chlorides (mg/L)	184.8	163.92
Chromium (mg/L)	BDL	BDL
Cobalt (mg/L)	BDL	BDL
Chemical Oxygen Demand or COD (mg/L)	294	206.5
Copper (mg/L)	BDL	BDL
Detergents (mg/L)	12.34	7.44
Electrical Conductivity or EC (ms/cm or ds/m)	2.94	2.46
Fluorides (mg/L)	1.67	1.65
Lead (mg/L)	BDL	BDL
Magnesium (mg/L)	69.19	76.68
Manganese (mg/L)	0.11	0.11
Ammoniacal-Nitrogen (mg/L)	33.73	21.66
Nickel (mg/L)	BDL	BDL
Nitrate-Nitrogen (mg/L)	6.14	4.28
pH	7.52	7.54
Phosphates (mg/L)	1.65	1.3
Potassium (mg/L)	24.2	23.19
Sodium (mg/L)	145.71	128.78
Sulfur (mg/L)	20.19	15.19
Total Alkalinity (mg/L as CaCO3)	382.4	340.5
Total Dissolved Solids or TDS (mg/L)	1799.4	1511.63
Total Hardness (mg/L as CaCO3)	684	598.75
Iron (Fe3+ and Fe2+) (mg/L)	BDL	BDL
Total Suspended Solids (mg/L)	80.2	30.5
Zinc (mg/L)	BDL	BDL

Constructed Wetland 2 **(commissioned in Aug 2015)**: The construction of this wetland took place during the summer of 2015 (Feb –April 2015. The salient features of this wetland are given in Table 2-7

Table 2-7 Salient features of CW -2

Capacity of wetland:	~20 m^3/day (100 households)
Dimension	Constructed wetland = 20 m x 4 m x 1.5 m Treated water storage tank= 20 m x 4 m x 1.5 m
Type of wetlands	Vegetated submerged bed
Filter media (from top to bottom)	Sand (50cm thick) Gravel 10 mm size (25 cm thick) Gravel 20 mm size (25 cm thick) Gravel 40 mm size (25 cm thick)
Vegetation	*Typha latifolia* and *Cana indica*
Water source for treatment	Domestic wastewater from rural households

Plantation took place during Aug 2015 and the CW was allowed to stabilize as the plants got established during the monsoon. Wastewater sampling and analysis started from July itself i.e. before the plantation. The initial phase was marked by high TSS (96 %) and moderate COD (56 %) removal.

Figure 2-8 Construction phase of CW-2

Figure 2-9 Plantation and stabilization phase of CW-2

The nitrogen removal efficiency were between 12-17 % in the months after the plantations i.e. during Aug-Oct 2015. The nitrogen removal efficiency stabilized at around 68 % from October 2015 onwards. The average inlet and outlet wastewater characteristics post stabilization phase till today i.e.e during October 2015-June 2016 is given in Table 2-8. Apart from COD and inorganic nitrogen significant removal of sulphate was observed during this period. *Cana indica* and *Typha latifolia* both exhibited high sulfate uptake capacity (as reveald by the plant tisuuse analysis), which may be the reason for such high Res observed consistently. Both *Cana indica* and *Typha latifolia* were introduced in this CW in an equal surface area (30 m^2 each) a comparative study of their biomass generation could be done.

Figure 2-10 CW-2 after stabilization (Oct, 2015)

Table 2-8 Average inlet and outlet wastewater characteristics for CW-2

SAMPLE	INLET	OUTLET
PH	7.79	8.01
EC (ms)	2.17	1.735
TDS (ppt)	1.301	1.04
TSS (mg/L)	332	15.9
Total Alkalinity (mg/L)	340	312
Total Hardness (mg/L)	630	620
COD (mg/L)	308	210.7
NH4-N (mg/L)	47.2	12.9
Nitrates (mg/L)	7.23	3.18
Phosphates (mg/L)	2.54	0.92
Chlorides (mg/L)	143.9	124.58
Potassium (mg/L)	26.11	23.98
Fluorides (mg/L)	1.181	1.341
Sulfates (mg/L)	127.4	11.15
Sodium (mg/L)	147.16	120.55

Figure 2-11 Routine harvesting of the plant biomass

Figure 2-12 The CW-2 as on 20th June 2016

2.1.5 UAS, Dharwad

Model engineered constructed wetland (ECWL) was established in UAS, Dharwad as demonstration unit on pilot basis and to monitor the water quality characteristics due to wetland treatment (plate. 1). ECWL of size 10m X 8 m X 1.2 m with treatment capacity of 50 m^3 per day was constructed during 2014-15. Macrophytes i.e. *Typha latifolia* and *Bracharia mutica* have been established in the wetland (plate.2). For the filter material, 40 mm and down size pebbles of thickness 60 cm and sand 20 cm thick were placed. Water proofing at sides and bottom of the ECWL was included to prevent any possible seepage. The water quality parameters as influenced by the different species of the macrophytes in water quality improvement are presented in the table 1. The wastewater (black and grey water) generated from the University of Agricultural Sciences (UAS) campus, Dharwad is the water source for ECWL treatment. UAS campus, Dharwad generates on an average of 1.5 lakh liters per day of domestic wastewater.

The treated wastewater is utilized for the comparative studies on crop response. Effect of the wastewater, treated wastewater, fresh water and conjunctive utilization of different

sources of water are being evaluated during 2015-16 (plate**.** 5, 6 and 7) and 2016-17 for the vegetable crop performance (Chilli, Brinjal, Cluster bean, Ridgeguard, Bitterguard, Okra, Tomato etc). Another new pilot ECWL was established and commissioned during 2015-16. ECWL is of 12.5 m x 11 m x 1.2 m with capacity of treating 75 m^3 per day (plate.3 and 4). Wetland was filled with 40 mm and down size pebbles of depth 60 cm and 20 cm depth of sand. Water proofing at both the sides and bottom were taken prevent any possible seepage. The performance of *Canna indica* on water treatment through ECWL is under progress.

2.1.5.1 Effect of Engineered constructed wetland on water characteristics

Effect of the ECWL on the water quality improvement was recorded for a period of one year i.e., April 2015- March 2016. The quality of the water due to ECWL treatment was monitored on the monthly basis (Fig 2-13, 2-14, 2-15 and 2-16) and the mean of the parameters are presented in table 2-9. The effect of the ECWL on observed parameters was profound. ECWL treatment resulted in 33.7, 43.8 and 28.5 per cent reduction of total solids, total suspended solids and total dissolved solids, respectively. With respect to the nutrient load in the wastewater and the treated water with ECWL; per cent reduction was to the extent of 39.7, 46.5, 42.0, 44.6, 29.4 and 30.2 in total nitrogen, nitrate nitrogen, ammoniacal nitrogen, phosphate, chlorine and sodium, respectively. Appreciable improvement in the COD, BOD, RSC and SAR was noticed due to ECWL treatment.

Table 2-9 Influence of the engineered constructed wetland on the water characteristics (mean data of values from April 2015- March 2016)

Parameter	Untreated domestic sewage water	ECWL treated water	Per cent reduction over untreated sewage water
TS (mg l^{-1})	1209	801	33.7
TSS (mg l^{-1})	413	232	43.8
TDS (mg l^{-1})	796	569	28.5
BOD (mg l^{-1})	185	125	32.4
COD (mg l^{-1})	333	219	34.2
Total- N (mg l^{-1})	20.4	12.3	39.7
NO_3^- N (mg l^{-1})	4.3	2.3	46.5
NH_4^+- N (mg l^{-1})	11.9	6.9	42.0
P (mg l^{-1})	10.1	5.6	44.6
Cl (mg l^{-1})	5.1	3.6	29.4
Na (meq l^{-1})	9.6	6.7	30.2
RSC (meq l^{-1})	-0.34	-0.30	11.8
SAR	4.83	4.0	17.0

Plate 1. Established typha and Paragrass in ECWL at Dharwad

Plate 2. Periodic harvesting and monitoring the effect of macrophtyes

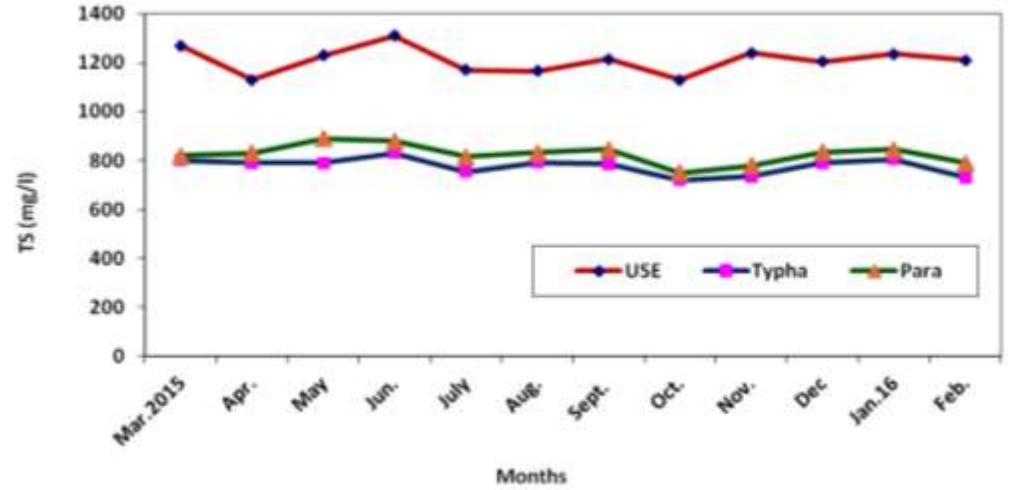


Figure 2-13 Variation in total solids under untreated and ECWL treated sewage wastewater

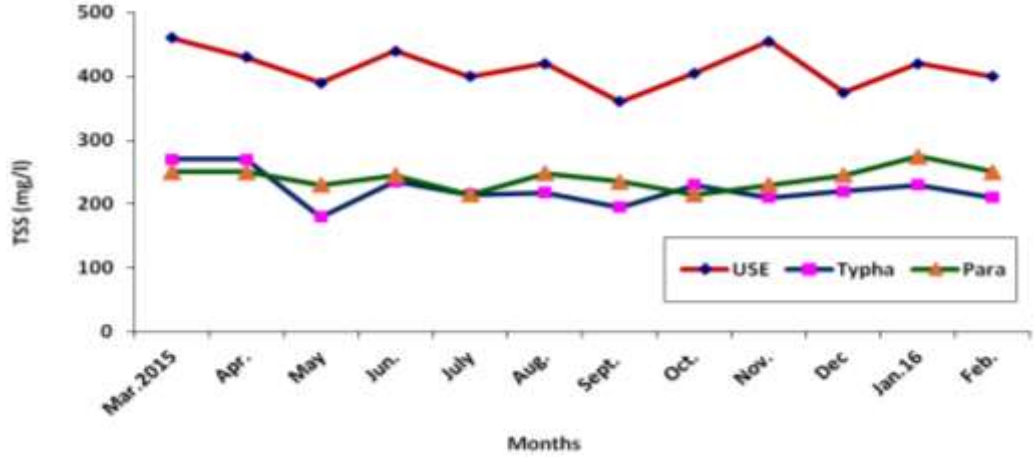


Figure 2-14 Variation in total suspended solids under untreated and ECWL treated sewage wastewater

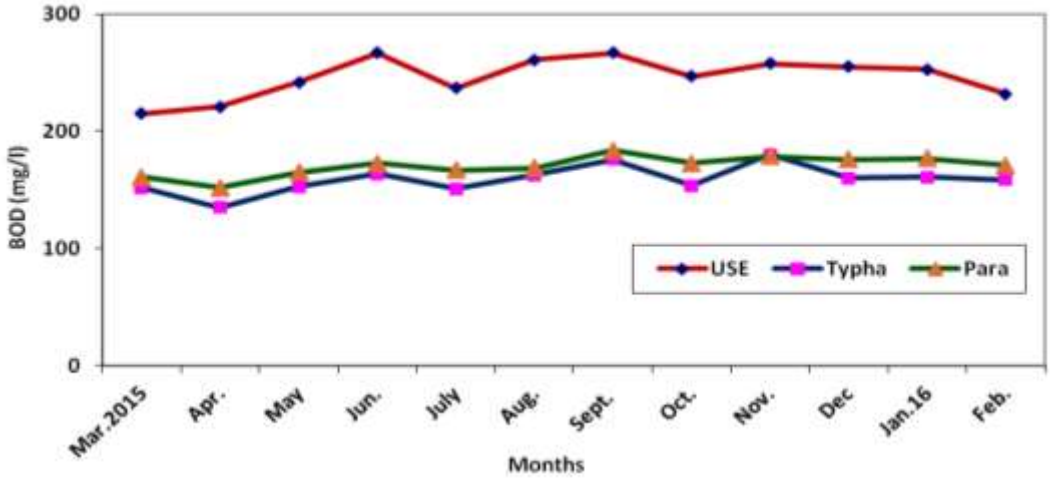


Figure 2-15 Variation in BOD under untreated and ECWL treated sewage wastewater

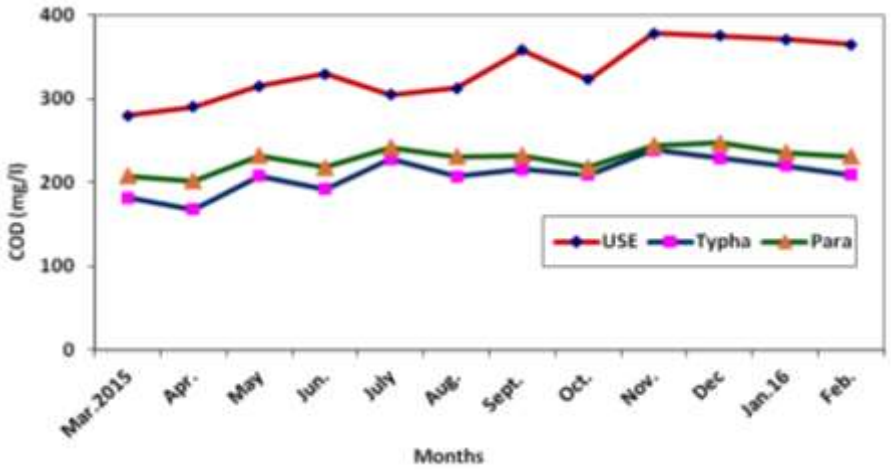


Figure 2-16 Variation in COD under untreated and ECWL treated sewage wastewater

Plate 3. Newly constructed wetland commissioned during 2015-16 at UAS, Dharwad

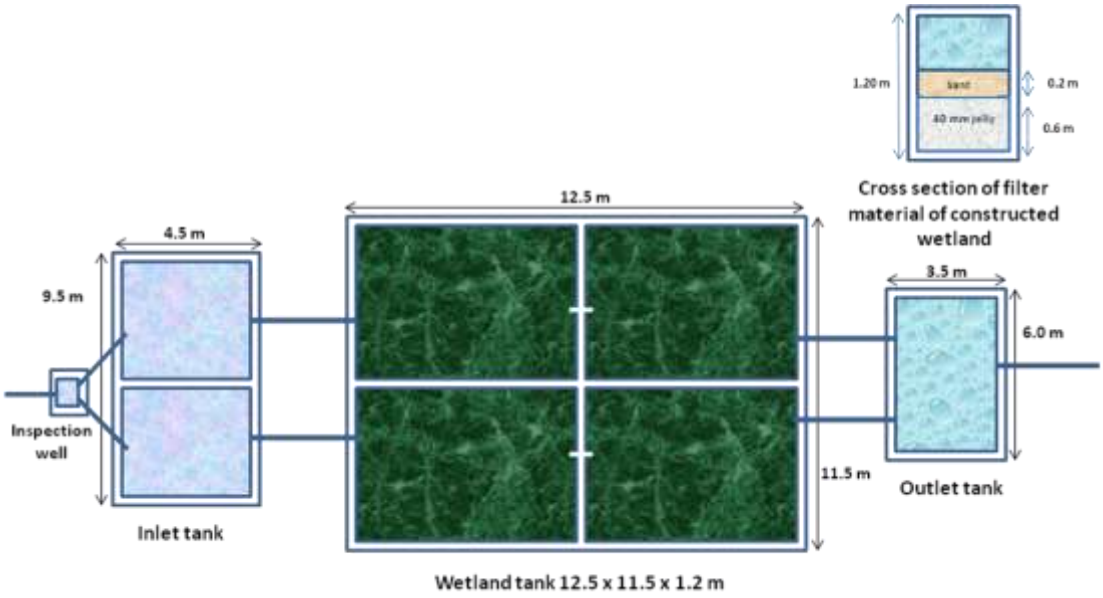


Plate 4. Specification of newly commissioned engineered constructed wetland at UAS, Dharwad

2.1.5.2 Evaluation of performance of Macrophytes (Typha latifolia and Bracharia mutica) and ECWL on water quality

Macrophytes are established in the wetland facilitated accumulation and periodic removal of the negate factors by the plants resulting in improvement of the wastewater. Macrophytes of typha and paragrass which are capable of re-growth after removal of the flush on periodic basis were used in the ECWL pilot study. Two compartments each of the mentioned species was used in the present evaluation. Typha was subjected to pruning once in average of 3 months and whereas paragrass was subjected to pruning average on monthly duration. Pruning of the re-growth of the macrophytes was regulated to maintain the growth of the macrophytes.

Variation in the water quality improvement with respect to the different species of macrophyte was observed and presented in table 2-10. Macrophyte Typha (*Typha latifolia*) induced greater reduction of TS (35.5 %), TSS (42.9 %), TDS (31.6 %), BOD (34.9 %), COD (37.3 %), and Na (35.3 %). Para grass (*Bracharia mutica*) was efficient in reducing total nitrogen (39.3 %), nitrate nitrogen (45.4 %) ammoniacal nitrogen (40.8 %) and phosphates (45.9 %). Both *Typha latifolia* and *Bracharia mutica* induced moderation in the quality of the sewage water in respect of SAR, RSC and chloride content (5.94, - 0.40 and 3.2; 6.61, -0.36 and 3.8, respectively) as against the raw sewage water (7.86, -0.36 and 5.1). Results indicated that use of combination of macrophytes is ideal for wetland planting for overall improvement in the quality of the domestic sewage water for its utilization.

Table 2-10 Effect of the macrophytes on the quality of the wastewater (mean data from April 2015 to March 2016).

Parameter	Untreated domestic sewage water	Typha treated water	Per cent reduction over untreated wastewater	Paragrass treated water	Per cent reduction over untreated wastewater
TS (mg l^{-1})	1209	780	35.5	828.13	31.5
TSS (mg l^{-1})	413	236	42.9	253.54	38.6
TDS (mg l^{-1})	796	543	31.6	574.58	27.8
BOD (mg l^{-1})	185	120	34.9	129.88	29.8
COD (mg l^{-1})	333	208	37.3	227.67	31.6
Total-N (mg l^{-1})	20.4	12.1	40.0	11.94	41.5
NO_3^- N (mg l^{-1})	4.3	2.8	33.6	2.28	47.0
NH_4^+-N (mg l^{-1})	11.9	6.7	43.6	7.05	40.8
P (mg l^{-1})	10.1	5.5	46.0	5.80	42.6
Cl (mg l^{-1})	5.1	3.3	38.0	3.8	24.8
Na(meq l^{-1})	9.6	5.4	32.6	11.27	28.1
RSC(meq l^{-1})	-0.34	-0.40	-10.0	-0.34	7.4
SAR	7.86	5.94	24.4	6.61	15.8

Microbial studies of different sources of irrigation used in the investigations were evaluated and are presented in table 2-11. ECWL treated water resulted in lesser bacteria (16.6 %), fungi (17.4%) and *E. coli* (47.7%) as compared to untreated sewage wastewater and the study is under progress with time.

Table 2-11 Microbial analysis of the source of water

Water quality parameter	Fresh water	Untreated sewage water	ECWL Treated wastewater	Percent reduction over untreated sewage wastewater
Bacteria (cfu's/ml x 10^6)	2.50	42.33	35.30	16.6
Fungi (cfu's/ml x 10^4)	0.85	3.33	2.75	17.4
E. coli (cfu's/ml x 10^4)	0.0	6.50	3.4	47.7

The impact of the different sources of irrigation and fertilizer application on soil nutrient dynamics, crop performance and quality aspects is under progress and will be continued during 2016-17. Long term effects of the different sources of irrigation on soil properties are being evaluated.

2.2 Bio-remedial measures tested to improve degraded lands due to use of wastewater

2.2.1 Remediation of land previously loaded with biorefinery wastewater through biological means (UAS, Dharwad)

A field experiment was conducted during 2015-16 to study the effect of different drainage systems and soil fertility management on bio-remediation of lands previously loaded with bio-refinery wastewater in Maize - Wheat cropping system. The study was conducted at Ugar khurd, Ugar, Belagavi Dist, Karnataka. The experimental details are given in Table 2-12.

Table 2-12 Details of the experiment

Treatment details	
Main plot (Drainage methods)	**Sub plot (Soil fertility)**
D_1 – Surface drainage system D_2 – Sub-surface drainage system D_3 – No drainage (Control)	S_1 – Green manuring in-situ (Dhaincha – wheat) S_2 – Use of press mud (Maize - wheat) S_3 – Microbial culture (Maize-wheat) S_4 – S_1 + microbial culture (Dhaincha-wheat) S_5 – S_2+ microbial culture (Maize – wheat) S_6- Control
Experimental design	Split plot design
Replication	Three
Plot size	9 m x 6 m
Season	*Kharif,2015* and *Rabi/summer,* 2015-16
Variety	*Kharif, 2015* : Maize: 900 M Gold *Rabi/summer*: Wheat:MACS-6222

For sub-surface drainage system, corrugated and perforated pipes of 10 cm diameter with synthetic envelop were installed at a depth of 1.00 m below ground level at a drain spacing of 30 m. In case of surface drainage system, open drains were excavated at a depth of 0.5 m with a spacing of 30 m. The experimental results are presented below;

Effect of different drainage systems and soil fertility management on maize

Maize crop cv 900 M Gold was sown during *kharif*, 2015 [25-07-2015] following the recommended package of practices. The amount of rainfall received during the cropping period was 228 mm. In all, a total of 4 irrigations each of depth 6 cm were applied to the crop. The total water applied including the effective rainfall was 39.36 cm. The crop was harvested on 24-11-2015.

Effect of different drainage systems on maize yield (Table 2-13) was significant with higher yields recorded in D2 i.e., sub-surface drainage (4775 kg/ha) and lower yields were recorded under D3 i.e., no drainage (3952 kg/ha). However, the yield levels in case of control were on par with that of surface drainage treatment (4475kg/ha). This indicated that sub-surface drainage system was effective in bio-remediation of degraded lands compared to surface drainage system. Among the various soil fertility management treatment; use of press mud + microbial culture (S5) resulted in higher maize yield (4992 kg/ha). Significant difference in crop yield was recorded among the interaction combination of different drainage systems and soil fertility management aspects. Higher maize yield (5659 kg/ha) was observed in sub-surface drainage system combined with use of press mud + microbial culture (D2S5).

Higher water productivity of 121.31 kg/ha-cm was achieved in case of sub-surface drainage system as against lower water productivity of 100.40 kg/ha-cm under control (Table 2-13). Among the different soil fertility management aspects, higher water productivity of 126.82 kg/ha-cm was recorded in case of press mud + microbial culture (S5) followed by use of press mud (S_2) and lower water productivity of 94.71 kg/ha- cm was recorded under control (S6).

Among the different drainage methods, higher gross returns (Rs 62071/ha), net returns (Rs 20337/ha) and B: C ratio (1.64) were recorded (Table 2-14) with sub-surface drainage (D2). Among the different soil fertility management aspects, higher gross returns (Rs 64902/ha), net return (Rs 20245/ha) and B: C ratio (1.62) were recorded with use of press mud + microbial culture (S5). Sub-surface drainage combined with use of press mud + microbial culture (D2S5) resulted in higher gross return (Rs 73568/ha), net return (Rs 28911/ha) and B: C ratio (1.81).

Table 2-13 Effect of different drainage systems and microbial culture inoculation on seed yield and water productivity of maize in maize-wheat cropping system

Treatments	Maize seed yield (kg/ha)				Water productivity (kg/ha-cm)			
	D1	D2	D3	Mean	D1	D2	D3	Mean
S2	5109	4807	3530	4482	129.80	122.12	89.68	113.87
S3	4122	5157	3921	4400	104.72	131.02	99.61	111.78
S5	5068	5659	4251	4992	128.76	143.77	108.00	126.82
S6	3601	3476	4106	3728	91.48	88.31	104.31	94.71
Mean	4475	4775	3952	-	113.69	121.31	100.40	-
	SEm±	CD (p=0.05)						
Main (D)	104	359						
Sub (S)	134	383						
DXS	268	766						

Table 2-14 Effect of drainage systems and microbial culture inoculation on gross returns, net returns and B:C ratio of maize in maize-wheat cropping system

Treatments	Gross returns (Rs/ha)				Net returns (Rs/ha)				B:C ratio			
	D1	D2	D3	Mean	D1	D2	D3	Mean	D1	D2	D3	Mean
S2	66421	62487	45885	58265	15065	17830	1228	11374	1.43	1.58	1.16	1.39
S3	53592	67046	50970	57203	8935	22389	6313	12546	1.36	1.67	1.29	1.44
S5	65879	73568	55259	64902	21221	28911	10601	20245	1.66	1.81	1.38	1.62
S6	46815	45184	53377	48459	13849	12218	20412	15493	1.67	1.51	1.77	1.65
Mean	58177	62071	51373		14768	20337	9638		1.53	1.64	1.40	
	SEm±	CD (p=0.05)			SEm±	CD (p=0.05)			SEm±	CD (p=0.05)		
Main (D)	1348	4666			1527	5284			0.04	0.13		
Sub (S)	1742	4978			1806	5162			0.05	0.15		
DXS	3483	9955			3612	10324			0.11	0.30		

Effect of different drainage systems and soil fertility management on wheat

Wheat crop cv. MACS – 6222 was sown during *Rabi/summer*, 2015-16 [12-12-2015] following the recommended package of practices. The amount of rainfall received during the cropping period was 9.50 mm. In all, a total of 8 irrigations each of depth 6 cm was applied to the crop. The total water applied including the effective rainfall was 48.95 cm. The crop was harvested on 25-03-2016. Effect of the different drainage systems on wheat yield (Table 2-15) was significant with higher yields recorded in D2 i.e., sub-surface drainage (1843 kg/ha) and lower yields under D3 i.e., no drainage (1045 kg/ha). However, the yield levels in case of control was on par with that of surface drainage treatment (1219 kg/ha). This indicated that, sub-surface drainage system was effective in bio-remediation as compared to surface drainage system. Among the different soil fertility management treatments, green manuring *in situ* of Dhaincha + microbial culture application (S4) resulted in significantly higher seed yield (1780 kg/ha). However, lower seed yield was recorded in control (656 kg/ha). Positive interaction effect of the drainage systems and soil fertility managment practices was observed. Significantly higher seed yield (2786 kg/ha) was recorded with sub-surface drainage + *in situ* green manuring of Dhainca (*kharif*) with microbial culture application (D2S4) and lower seed yield 646 kg/ha was recorded under control plot.

Higher water productivity of 37.65 kg/ha-cm was achieved in case of sub-surface drainage system, where as lower water productivity of 21.34 kg/ha-cm was recorded under control. Among the different soil fertility management aspects, highest water productivity of 36.36 kg/ha-cm was recorded in case of green manuring *in situ* of dhaincha + microbial culture application and lowest water productivity was recorded in-case of control i.e., 13.40 kg/ha-cm (S6). Among the different drainage methods, higher gross returns (Rs. 44239/ha), net returns (Rs. 27360/ha) and B: C ratio (2.59) were recorded with D2 i.e., sub-surface drainage (Table 2-16). Among the different soil fertility management aspects, higher gross return (Rs 42715/ha), net return (Rs 25521/ha) and B: C ratio (2.48) were recorded with green manuring *in-situ* of Dhaincha + microbial culture application (S4) followed by use of press mud (S2) and lowest in case of control (S6). Subsurface drainage combined with green manuring *in situ* of Dhaincha + microbial culture application (D2S4) recorded highest gross return (Rs 66864/ha), net return (Rs 49670/ha) and B: C ratio (3.89).

Based on the study, it was observed that combination of sub-surface drainage system with incorporation of *in-situ* green manuring + application of pressmud along with microbial culture recorded higher crop yield, water productivity, net income and B:C ratio in maize and wheat cropping system.

Table 2-15 Effect of different drainage systems and microbial culture inoculation on yield and water productivity of wheat in maize-wheat cropping system

Treatments	Wheat yield (kg/ha)				Water productivity (kg/ha cm)			
	D1	D2	D3	Mean	D1	D2	D3	Mean
S1	1423	1440	1137	1333	29.07	29.41	23.22	27.23
S2	1270	2163	1183	1539	25.94	44.18	24.16	31.44
S3	1223	1973	1081	1426	24.98	40.30	22.08	29.13
S4	1313	2786	1240	1780	26.82	56.91	25.33	36.36
S5	1410	2047	983	1480	28.80	41.81	20.08	30.23
S6	671	650	646	656	13.70	13.27	13.19	13.40
Mean	1219	1843	1045		24.90	37.65	21.34	
	SEm+	CD (p=0.05)						
D	70	241						
S	73	208						
DXS	146	416						

Table 2-16 Effect of drainage systems and microbial culture inoculation on gross returns, net returns and B:C ratio of wheat in maize-wheat cropping system

Treatments	Gross returns (Rs/ha)				Net returns (Rs/ha)				B:C ratio			
	D1	D2	D3	Mean	D1	D2	D3	Mean	D1	D2	D3	Mean
S1	34160	34560	27280	32000	16966	17366	10086	14806	1.99	2.01	1.59	1.86
S2	30480	51920	28400	36933	13286	34726	11206	19739	1.77	3.02	1.65	2.15
S3	29360	47360	25944	34221	12166	30166	8750	17027	1.71	2.75	1.51	1.99
S4	31520	66864	29760	42715	14326	49670	12566	25521	1.83	3.89	1.73	2.48
S5	33840	49120	23600	35520	16646	31926	6406	18326	1.97	2.86	1.37	2.07
S6	16104	15608	15512	15741	804	308	212	441	1.05	1.02	1.01	1.03
Mean	29244	44239	25083	-	12366	27360	8204	-	1.72	2.59	1.48	-
	SEm+	CD (p=0.05)			SEm+	CD (p=0.05)			SEm+	CD (p=0.05)		
D	1672	5786			1672	5786			0.10	0.34		
S	1748	4997			1748	4997			0.10	0.29		
DXS	3497	9995			3497	9995			0.20	0.58		

2.2.2 Remediation of land previously loaded with biorefinery wastewater through biological means (TERI)

The following progress was done in the reporting period:

Previously, out of 57 isolates obtained from soil of sugarcane fields of Ugar Sugar, located in the southern part of India, have extremely adverse land where salinity level is high with high toxic levels of sodium, calcium, iron which affect the availability of several important nutrients to plants resulting in low yield. Only 12 isolates out of 57 isolates have shown salt tolerance upto 10% and 4 isolates have shown tolerance upto 15%. Among 12 salt tolerant bacteria, 8 isolates have showed phosphate solubilizing activity. All the isolates showed siderophore production and 10 isolates showed acid production out of 12 salt tolerant bacteria. All 12 salt tolerant bacteria showed IAA production. Antagonistic and synergistic activities of these salt tolerant bacteria were also carried out last year with 16S rDNA sequencing for molecular identification.

12 salt tolerant bacterial isolates are selected for green house experiment on sweet sorghum as model plant in soil with effluent from 15 years. Evaluation of nutrient uptake and soil properties in different treatment under greenhouse experiments were carried out this year for the selection of best consortia. This consortium farther tested with TERI -mycorrhizal consortium on sweet sorghum for evaluation of nutrient uptake and soil properties as well as determination of compatibility. The consortium was mass multiplied and was applied to the site field with subsurface drainage and surface drainage system for bioremediation of the land.

Qualitative and quantitative estimation of biofilm formation of salt tolerant bacteria on polystyrene surface

All bacteria were cultured overnight in Brain Infusion Broth (BHI-0.25 glucose at 30°C). The culture was diluted 1:20 in fresh BHI plus (0.25%) glucose at 30°C. This suspension (200 µL) was utilized to inoculate sterile 96-well-polystyrene microtiter plates. The plates were incubated at 30°C aerobically for 24h. The cultures were eliminated and the microtiter wells were washed twice with phosphate-buffered saline (7 mM Na_2HPO_4, 3 mM NaH2PO4 and 130 mM NaCl at pH 7.4) to remove non-adherent cells and were dried in an inverted position. Then, bacteria that not adherate to microtiter plates were stained with 1% Crystal violet for 15 min. The wells were

washed once more and the Crystal violet was dissolved in 200 µL of ethanol (95%). An automated PR3 100 TSC (Bio-Rad) was used to measure the absorbance at 550 nm (OD550). Each essay was performed in triplicate.

Formation of biofilm or micro colonies by PGPR in the plant surrounding causes their efficient colonization with their host which promotes the plant microbe interaction. Four isolates were non-biofilm forming on polystyrene surfaces with an OD550≤0.1. While, A25 produced a very large amount of biofilm (OD550 = 2.102) was strongly adhesive to polystyrene with a value of 1.238 at 550 nm. SRA25 have shown medium adhesive to the abiotic surface (Figure 1)

Control	SRA19	SRA25	SRA26	SRA10	SRC5	SRC9
0.19	1.184	1.647	0.178	0.195	0.294	0.258
0.195	0.943	1.523	0.221	0.216	0.22	0.239
0.181	1.128	1.19	0.195	0.244	0.22	0.246
0.196	0.845	1.48	0.234	0.251	0.272	0.255
0.192	0.924	2.27	0.188	0.21	0.27	0.245
0.201	1.145	1.441	0.203	0.237	0.245	0.248
0.198	0.911	1.435	0.195	0.288	0.272	0.246
0.2	0.877	1.522	0.174	0.223	0.266	0.223

Figure 2-17 Biofilm formation by bacterial strain tested on 96 well titre plates

Selection of bacteria for best consortia from Microcosm experiments (experimental ecosystem) under green house condition

Microcosm (experimental ecosystem) experimental setup was carried out under green house condition for determining the effectiveness of the 12 isolated bacteria and their Synergistic isolates as bio-inoculants. 5ml microtips filled with 10g soil samples [1. Above 15 years Company plot 242 (Normal) 2. Above 15 years Company plot 242 (autoclaved), 3. Normal Soil (autoclaved)] Seeds of sweet shorgam (*Sorghum bicolor* L. Moench) were sown and irrigated daily. Evaluation of nutrient uptake and soil properties in different treatment under greenhouse experiments are carried out (Figure 2-18).

Figure 2-18 Effect on plants after application of bacteria under the microcosm (experimental ecosystem) setup for determination for selecting best consortia

The results that bacterial isolates inoculation led to reduction of in the Na, Fe, Ca and Cu content of soil indicating a possible role in improving the translocation of micronutrients. For sodium (Na) concentration decreases the most in the soil inoculated with *B. licheniformis SRA25*. While cupper (Cu) and iron (Fe) concentration is lowest in the soil inoculated with *B. pumilus* SRA10, *B. marisflavi* SRA26, *B. amyloliquefaciens* SRC 5 (Table 2-17). Among micronutrients total nitrogen phosphorus and potassium content in sweet sorghum leaves grown on non- autoclaved soil (above 15 years company plot 242), autoclaved soil (above 15 years company plot 242) and autoclaved soil (Normal) found significantly higher after inoculation of *B. licheniformis SRA25, B. pumilus* SRA10, *B. marisflavi* SRA26, *B. amyloliquefaciens* SRC 5 respectively (Table 2-18).

These four species of *Bacillus* were selected for mass multiplication and further tested with TERI -mycorrhizal consortium.

Table 2-17 Total Sodium (Na), Cupper (Cu), Calcium (Ca), Iron (Fe) in rhizosphere soil (Mean ± SE)

	Total Na (ppm)	total Cu (ppm)	Total Ca (ppm)	Total Fe (ppm)
NON- AUTOCLAVED SOIL (Above 15 years Company plot 242**)**				
B. pumilus SRA9	260.0±0.01	128.3±1.7	8024.5±5.2	8650.9±7.5
B. pumilus SRA10	260.0±0.01	123.5±2.2	9572.6±9.8	7504.7±0.8
Brevibacterium iodinum SRA19	260.0±5.77	121.1±1.7	7636.1±8.1	7948.9±2.7
B. licheniformis SRA25	253.3±6.67	115.8±4.9	7512.1±7.4	7754.6±4.6
B. marisflavi SRA26	270.0±5.77	84.0±2.1	9210.6±4.3	5300.2±4.5
B. licheniformis SRA 31	320.0±5.08	117.0±1.7	9088.5±8.7	6044.3±1.5
B. cereus SRA 33	383.3±3.80	119.3±0.1	10510.9±4.2	8863.0±2.1
Brevibacterium linens SAC 3	396.7±6.67	118.3±2.3	10119.7±9.8	8572.5±0.2
B. amyloliquefaciens SRC 5	406.7±6.67	103.1±1.7	8628.8±2.5	1373.2±1.2
Enterobacter cloacae SRC 9	423.3±8.82	119.0±6.9	8756.4±2.4	8302.9±1.6
Achromobacter xylosoxidans SRC15	400.0±1.00	109.2±7.0	9295.6±9.6	7612.3±3.5
B. safensis SRC23	430.0±1.02	122.0±1.1	8222.3±0.8	8534.6±3.7
Consortia	416.7±4.08	105.6±6.6	8455.0±0.8	6117.3±3.2
Control	423.3±5.77	128.4±2.8	13605.3±1.1	9098.3±4.2
AUTOCLAVED SOIL (Above 15 years Company plot 242)				
B. pumilus SRA9	456.7±3.1	121.9±0.4	8862.4±0.9	8103.4±4.8
B. pumilus SRA10	423.3±3.3	120.0±1.9	8592.0±0.8	7794.6±6.3
Brevibacterium iodinum SRA19	346.7±6.7	89.5±1.5	6766.1±9.0	6427.8±4.4
B. licheniformis SRA25	326.7±3.3	159.1±2.0	11832.1±3.4	10448.5±3.9
B. marisflavi SRA26	380.0±6.7	148.2±2.0	9298.5±9.8	9159.8±3.0
B. licheniformis SRA 31	460.0±4.8	149.2±1.8	12097.1±3.4	7340.3±7.4
B. cereus SRA 33	383.3±6.7	159.0±0.8	9723.8±5.6	7119.9±4.2
Brevibacterium linens SAC 3	386.7±2.5	150.0±3.9	9002.5±3.2	8229.4±6.9
B. amyloliquefaciens SRC 5	383.3±8.8	70.3±7.3	5376.1±9.7	3951.9±3.9
Enterobacter cloacae SRC 9	446.7±2.3	92.9±1.5	6392.8±7.1	4685.9±3.3
Achromobacter xylosoxidans SRC15	473.3±5.0	88.9±1.5	7404.5±2.6	6067.6±4.1
B. safensis SRC23	436.7±3.4	52.5±3.7	4425.7±1.2	3756.2±5.4
Consortia	403.3±4.4	122.7±5.3	9163.3±0.3	6981.5±2.3
Control	480.0±3.3	104.16±5.6	8568.7±3.1	6144.1±2.1
AUTOCLAVED SOIL (Normal)				
B. pumilus SRA9	166.7±3.4	5.7±1.5	3017.6±9.3	1626.8±6.9
B. pumilus SRA10	190.0±3.3	6.8±1.5	2521.4±8.1	1607.2±5.9
Brevibacterium iodinum SRA19	236.7±2.0	3.1±1.5	3398.5±2.9	1606.8±3.7
B. licheniformis SRA25	146.7±3.3	7.0±0.7	3588.2±2.1	1607.5±5.6
B. marisflavi SRA26	153.3±0.01	4.4±0.9	2238.1±4.7	1618.4±5.9
B. licheniformis SRA 31	243.3±2.9	0.6±0.2	3135.8±7.8	1670.3±3.7
B. cereus SRA 33	246.7±3.3	0.7±0.2	2441.0±1.8	1673.1±8.9
Brevibacterium linens SAC 3	249.9±3.3	17.5±1.2	1979.8±3.8	1414.3±5.2
B. amyloliquefaciens SRC 5	153.3±3.3	1.2±0.1	2569.4±93.3	1488.7±5.0
Enterobacter cloacae SRC 9	253.3±0.01	0.1±1.5	2658.4±8.9	1529.4±1.7
Achromobacter xylosoxidans SRC15	250.0±3.3	2.6±0.6	2115.9±3.1	1544.2±5.0
B. safensis SRC23	253.3±3.3	0.6±0.5	2234.4±3.2	1336.1±4.1
Consortia	155.3±6.7	0.5±0.3	1761.3±4.5	1604.9±10.0
Control	246.7±2.7	3.4±0.4	1637.6±5.7	1609.5±3.2

Table 2-18 Macronutrient status (Mean±SE) of sweet sorghum shoots

	Total N%	Total P (ppm)	Total K (ppm)
NON- AUTOCLAVED SOIL (Above 15 years Company plot 242**)**			
B. pumilus SRA9	0.021±0.001	34.1±2.5	1418±46.3
B. pumilus SRA10	0.034±0.001	32.9±0.7	2155±67.1
Brevibacterium iodinum SRA19	0.024±0.002	33.0±0.6	1452±78.6
B. licheniformis SRA25	0.028±0.004	34.1±1.1	1470±27.5
B. marisflavi SRA26	0.035±0.001	31.2±3.2	1565±10.0
B. licheniformis SRA 31	0.021±0.002	29.1±2.2	1427±53.3
B. cereus SRA 33	0.028±0.002	30.4±2.7	1465±67.6
Brevibacterium linens SAC 3	0.024±0.001	31.8±1.1	1477±16.4
B. amyloliquefaciens SRC 5	0.027±0.001	39.3±1.1	1315±17.6
Enterobacter cloacae SRC 9	0.028±0.000	37.7±0.5	1342±46.4
Achromobacter xylosoxidans SRC15	0.027±0.002	35.3±2.6	1265±14.1
B. safensis SRC23	0.031±0.000	31.3±1.3	1528±78.0
Consortia	0.031±0.003	32.5±1.0	1415±84.3
Control	0.024±0.001	29.7±1.9	1377±56.3
AUTOCLAVED SOIL (Above 15 years Company plot 242)			
B. pumilus SRA9	0.030±0.000	25.6±0.1	1325±68.4
B. pumilus SRA10	0.034±0.002	25.1±1.8	1212±79.1
Brevibacterium iodinum SRA19	0.031±0.003	30.8±1.6	1153±41.8
B. licheniformis SRA25	0.030±0.001	33.2±0.6	1587±77.1
B. marisflavi SRA26	0.034±0.002	32.1±0.6	2588±10.4
B. licheniformis SRA 31	0.031±0.002	32.9±3.4	1172±7.3
B. cereus SRA 33	0.029±0.002	25.8±1.6	1158±31.8
Brevibacterium linens SAC 3	0.029±0.002	27.5±1.3	1128±30.9
B. amyloliquefaciens SRC 5	0.033±0.002	30.2±2.7	1212±49.4
Enterobacter cloacae SRC 9	0.028±0.003	27.2±1.3	1218±14.2
Achromobacter xylosoxidans SRC15	0.034±0.002	24.0±0.3	1355±32.5
B. safensis SRC23	0.031±0.001	23.9±0.3	1265±77.1
Consortia	0.031±0.002	26.6±2.3	1692±16.9
Control	0.025±0.004	28.9±2.0	1203±45.4
AUTOCLAVED SOIL (Normal)			
B. pumilus SRA9	0.008±0.001	1.5±0.1	1760±3.00
B. pumilus SRA10	0.006±0.002	1.8±0.1	651±2.59
Brevibacterium iodinum SRA19	0.006±0.001	0.8±0.1	400±4.38
B. licheniformis SRA25	0.007±0.003	1.7±0.1	490±5.94
B. marisflavi SRA26	0.003±0.000	1.5±0.1	700±5.04
B. licheniformis SRA 31	0.003±0.001	0.2±0.1	330±5.36
B. cereus SRA 33	0.004±0.001	0.3±0.1	340±6.14
Brevibacterium linens SAC 3	0.004±0.001	1.0±0.0	630±4.14
B. amyloliquefaciens SRC 5	0.004±0.001	1.8±0.1	451±2.17
Enterobacter cloacae SRC 9	0.004±0.001	1.1±0.1	441±7.53
Achromobacter xylosoxidans SRC15	0.005±0.001	1.0±0.0	441±8.24
B. safensis SRC23	0.004±0.001	1.3±0.1	541±8.09
Consortia	0.004±0.001	1.2±0.1	571±4.92
Control	0.004±0.001	1.1±0.2	390±3.50

Developing of consortium with selected bacteria by optimizing fermentation process and formulation with TERI- AMF consortium and carrier.

Operation of 16L fermenter

After sterilization and addition of medium components, pH of the batch medium was set to 6.8. The bioreactor was set at 30^0C and 200 rpm. A polarization time of about 6h was given for DO probe to give a stabilized output. The cultivation was started in batch phase by inoculating the bioreactor medium with inoculum. The bacterial strains were cultivated in fermenter with 1.5L initial working volume. The temperature was controlled automatically at 30 °C by electrical heating/ chilled water. The aeration rate was fixed at a minimum value of 0.1 vvm (air flow rate= 150ml/ minute) and agitation at a minimum value of 200 rpm . The pH set point was 6.8±0.05. The set point for DO was 30% saturation value. Whenever, DO come down and approaches the set point, agitation was increased in a step of 50 rpm. This action was repeated until the rpm comes to its maximum limit of 500. Thereafter, the air was enriched with pulses of pure oxygen automatically. Towards the end of batch phase (~20-24 h), first pH and then DO began to rise steadily. During this phase, acidic metabolites produced, thus far, were consumed causing pH to go up steadily. When the pH comes to 7.5, the cell biomass reaches a maximum of about 15-20 OD. The fermenter is cooled to about 28^0C to slow down metabolic activity of the culture and broth was harvested for further processing in downstream operations. Bacterial cultural broth was harvested by centrifuging at 10,000 rpm for 10mins. The pellet was resuspended into 10% sucrose and was lyophilized. 0.01gm of lyophilized material was transferred into series of eppendorffs at 10-fold dilutions each and streaked on 0.9% saline to get CFU (colony forming unit) of bacterial culture after lyophilization. Pre-autoclaved terragreen was mixed with lyophilized bacterial inocula and TERI AMF Consortium according to FCO guidelines ($5x10^{11}$ CFU per hectare).

***In vivo* test on sweet sorghum with selected bacterial consortium from microcosoms experiments with TERI AMF consortium**

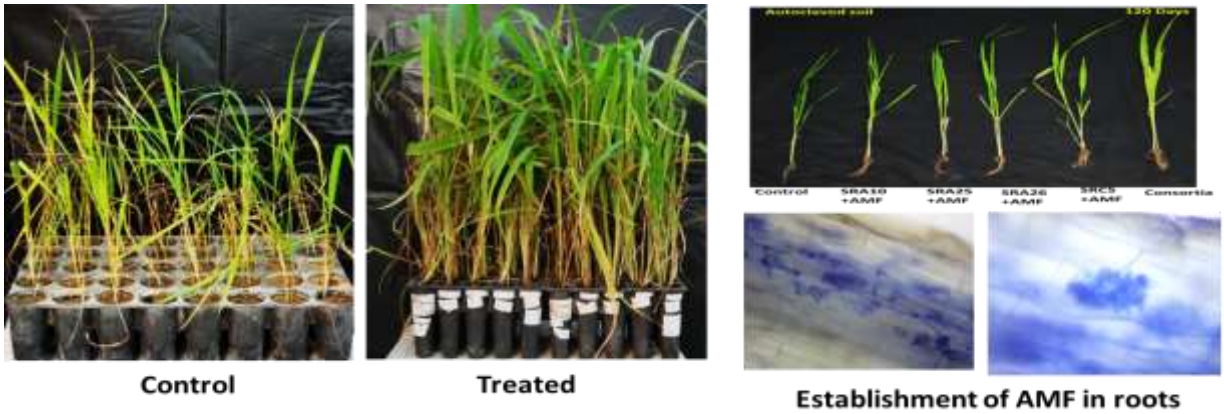
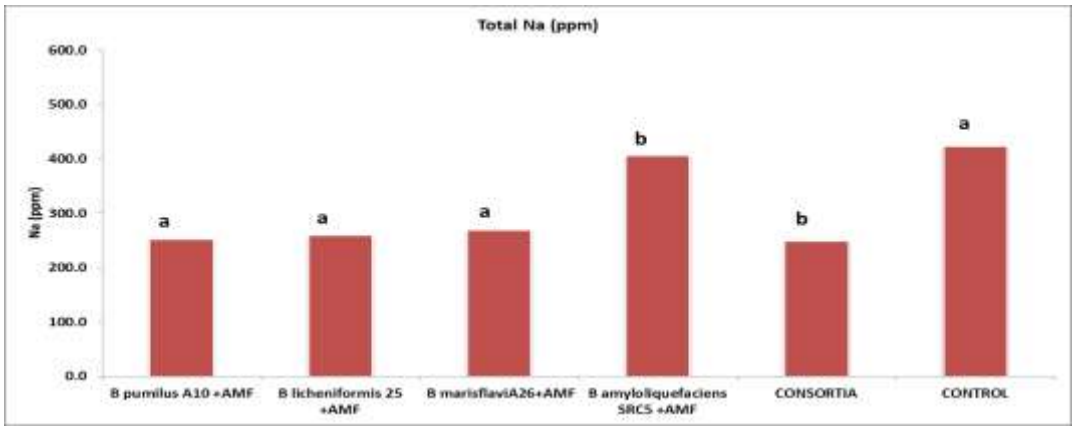
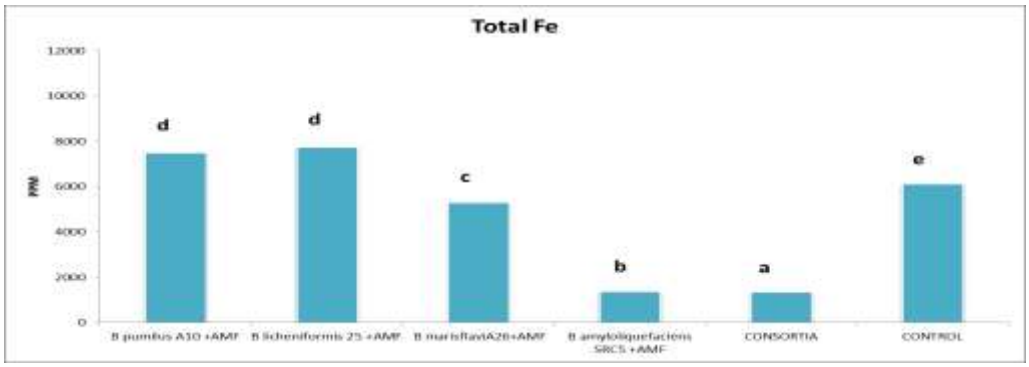


Figure 2-19 In vivo test on sweet sorghum with selected bacterial consortium with TERI AMF consortium.

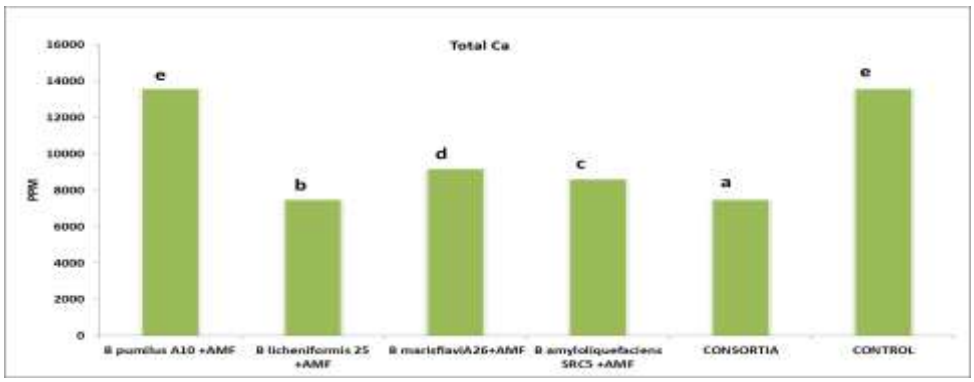
Four bacterial consortium Bacillus species *B. pumilus* (SRA 10), *B. licheniformis* (SRA 25), *B. marisflavi* (SRA 26), *B. amylosliquefaciens* (SRC 5) screened from microcosoms experiments with TERI consortium of AMF species.



a, b, c …values indicate significant different at P< 0.05 according to Duncan multiple range test

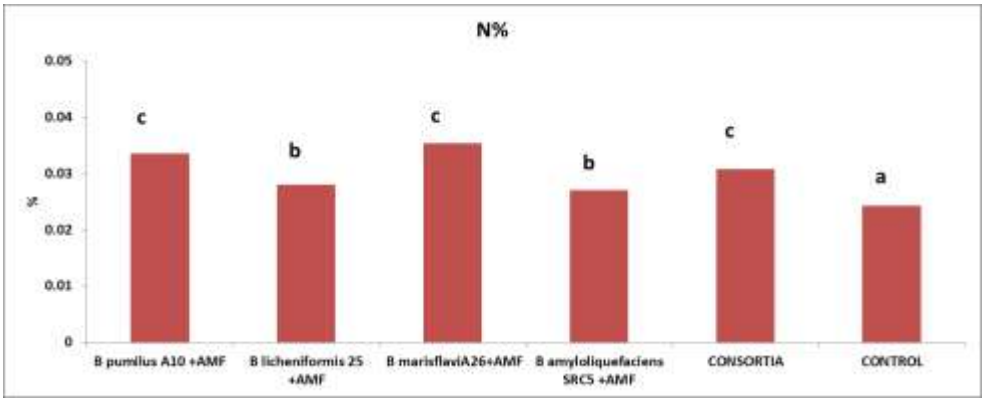


a, b, c …values indicate significant different at P< 0.05 according to Duncan multiple range test

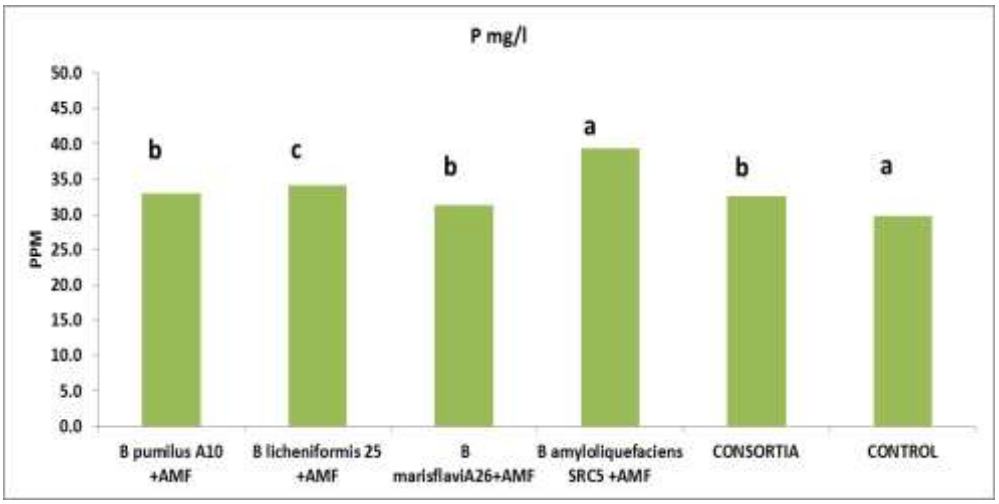


a, b, c …values indicate significant different at P< 0.05 according to Duncan multiple range test

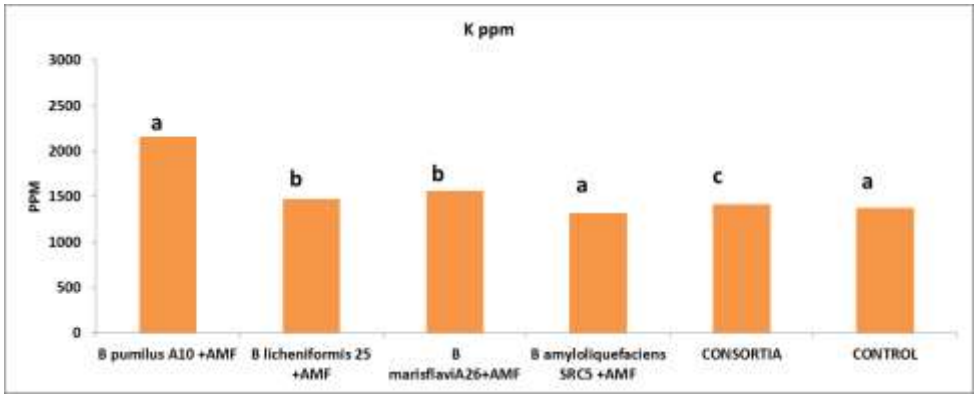
Figure 2-20 Significant decrease (p<0.05) in total content of Na , Ca and Fe in compare to control was found in treated non- autoclaved soil (above 15 years Company plot 242) after 120 days of growth period



a, b, c …values indicate significant different at P< 0.05 according to Duncan multiple range test



a, b, c …values indicate significant different at P< 0.05 according to Duncan multiple range test



a, b, c …values indicate significant different at P< 0.05 according to Duncan multiple range test

Figure 2-21 Significant increase (p<0.05) in total Nitrogen, Phosphorus and Potassium in the shoot, was found after the treatment of non- autoclaved soil (above 15 years Company plot 242) after 120 days of growth period

AMF consortia was used for further *In vivo* test on sweet sorghum for evaluation of nutrient uptake and soil properties as well as determination of their compatibility. Seeds of sweet

sorghum were sown in root trainers under soil surface. Pots were inoculated with all given consortium (1mg/pot). After 4 months of planting, plants were harvested and measured for different growth parameters fresh and dry weight for shoot and root as well as used for analysis of total nitrogen (%), phosphorus (ppm) and potassium (ppm). Soil from pots of harvested plants were dried and grounded and the digested samples were used for analysis of micronutrients (Fe, Cu, Ca and Na) concentration with an atomic absorption spectrophotometer at the most sensitive wavelengths for Fe (248.3 nm), Cu (324.8 nm) Ca (422.7 nm) and Na (589.0 nm). 60-70 % of AMF colonization was found in the root of sweet sorghum (Figure 2-19). Significant decrease ($p<0.05$) in total content of sodium (Na), calcium (Ca) and iron (Fe) in compare to control was found in treated soil (Company plot 242) after 120 days of growth period (Figure 2-20; Table 8) as well as significant increase ($p<0.05$) in percentage of total Nitrogen (N), Phosphorus (P) and Potassium (K) in ppm level in the shoot (Figure 2-21), was observed after the treatment.

Table 2-19 Total Sodium (Na), Cupper (Cu), Calcium (Ca), Iron (Fe) in rhizosphere soil (Mean ± SE) in the *in vivo* test on sweet sorghum

	Total Na (ppm)	**Total Ca (ppm)**	**Total Fe (ppm)**
AUTOCLAVED SOIL (Above 15 years Company plot 242)			
B pumilus SRA10 +AMF	423.3+3.3	8592.0+0.8	10300.0+3.9
B licheniformis SRA25 +AMF	460.0+4.8	8568.7+3.4	7794.6+6.3
B marisflavi SRA26+AMF	383.3+8.8	9298.5+9.8	9159.8+3.0
B amyloliquefaciens SRC5 +AMF	403.3+4.4	7820.0+9.7	7951.9+4.9
Consortia	380.0+3.3	5376.1+0.3	6144.1+2.3
Control	480.0+2.5	11832.1+3.1	10448.5+2.1
AUTOCLAVED SOIL (Normal)			
B pumilus SRA10 +AMF	150.3+0.1	2521.4+8.1	1607.2+5.9
B licheniformis SRA25 +AMF	190.0+3.3	1761.3+2.1	1607.5+5.6
B marisflavi SRA26+AMF	146.7+3.3	2238.1+4.7	1618.4+5.9
B amyloliquefaciens SRC5 +AMF	146.7+3.3	2569.4+3.3	1604.9+10.0
Consortia	180.0+6.7	1637.6+4.5	1488.7+5.0
Control	253.3+2.7	3588.2+5.7	1609.5+3.2

The microbial consortium developed in this project promotes exchange of sodium (Na) ions from the soil particle followed by leaching, which results the improvement in soil aggregation property. The use of microbial consortium improves the soil quality by reducing sodium and other salt. The microorganisms are able to grow in deeper soil layer and reclaim the soil into sub-surface layers. Along with this, production of plant growth promoting substances by the microbes presents in the consortium increase the plant macro-nutrient (Nitrogen, Phosphorus, Potassium) status, biomass as well as length of shoot and root (Table 2-20, 2-21 & 2-22) . The consortium reduces sodium absorption ratio, displays exchange of the sodium ions from the exchange complex of the soil and the subsequent leaching from soil particles, improving soil aggregation, thereby improving water holding capacity and texture of soil. This method provides the use of consortium of halotolerant and acid producing microbial composition of bacteria and mycorrhiza that lowers down the soil salts and improved method for reclamation of saline soil by towards the normality and also enhances the plant health status.

Table 2-20 Macronutrient status (Mean±SE) of sweet sorghum shoots

	Total N%	Total P (ppm)	Total K (ppm)
AUTOCLAVED SOIL (Above 15 years Company plot 242)			
B pumilus SRA10 +AMF	0.031±0.002	25.1+1.8	1212+7.1
B licheniformis SRA25 +AMF	0.030±0.001	26.6+2.3	1203+4.4
B marisflavi SRA26+AMF	0.034±0.002	32.1+0.6	1692+1.4
B amyloliquefaciens SRC5 +AMF	0.033±0.002	30.2+2.7	1212+4.4
Consortia	0.034±0.002	33.2+0.6	2588+1.9
Control	0.025±0.004	28.9+2.0	1587+7.1
AUTOCLAVED SOIL (Normal)			
B pumilus SRA10 +AMF	0.004+0.001	1.2+0.1	571+4.9
B licheniformis SRA25 +AMF	0.006+0.002	1.7+0.1	490+5.9
B marisflavi SRA26+AMF	0.004+0.001	1.5+0.1	651+2.5
B amyloliquefaciens SRC5 +AMF	0.004+0.001	1.8+0.1	451+2.1
Consortia	0.007+0.003	1.8+0.1	700+5.0
Control	0.003+0.001	1.1+0.2	390+3.5

Table 2-21 Effect of consortium applied *in vivo* on shoot and root biomass of sweet sorghum (fresh and dry weight) grown under controlled conditions in polyhouse at TERI GRAM, Gurgaon, India

Treatments	Fresh weight (g)	Dry weight (g)
Control	0.29±0.21a	0.08±0.01a
B pumilus SRA10 +AMF	0.56±0.05ab	0.14±0.04ab
B licheniformis SRA25 +AMF	0.91±0.04b	0.38±0.05c
B marisflavi SRA26+AMF	0.66±0.21ab	0.34±0.05c
B amyloliquefaciens SRC5 +AMF	1.53±0.04c	0.26±0.04bc
Consortia	1.61±0.05c	0.80±0.05d

a, b, c …values indicate significant different at P< 0.05 according to Duncan multiple range test

Table 2-22 Effect of consortium applied *in vivo* on shoot and root length of sweet sorghum grown under controlled conditions in polyhouse at TERI GRAM, Gurgaon, India

Treatments	Root length (cm)	Shoot length (cm)
Control	8.3±0.5a	9.3±1.1a
B pumilus SRA10 +AMF	13.4±2.8a	13.8±1.8ab
B licheniformis SRA25 +AMF	14.3±1.7a	14.7±1.2ab
B marisflavi SRA26+AMF	14.1±3.6a	15.5±4.6ab
B amyloliquefaciens SRC5 +AMF	14.3±3.1a	13.2±4.4ab
Consortia	14.5±1.2a	19.4±1.5ab

a, b, c …values indicate significant different at P< 0.05 according to Duncan multiple range test

Bioremediation of the land using TERI consortia in sugarcane

A consortium of 4 bacterial isolates and TERI's mycorrhiza consortium was applied in the field trial with assistance of UAS Dharwad University and Ugar Sugar, Karnataka as per recommendation in the "2nd Annual Review and Planning Meeting" for the India sub component from 28-30 September at MSSRF, Chennai., under two kind of drainage system: one is Subsurface drainage and another is Surface drainage system. The field trial was initiated on 25, January, 2016 (Figure 2-22 and 2-23).

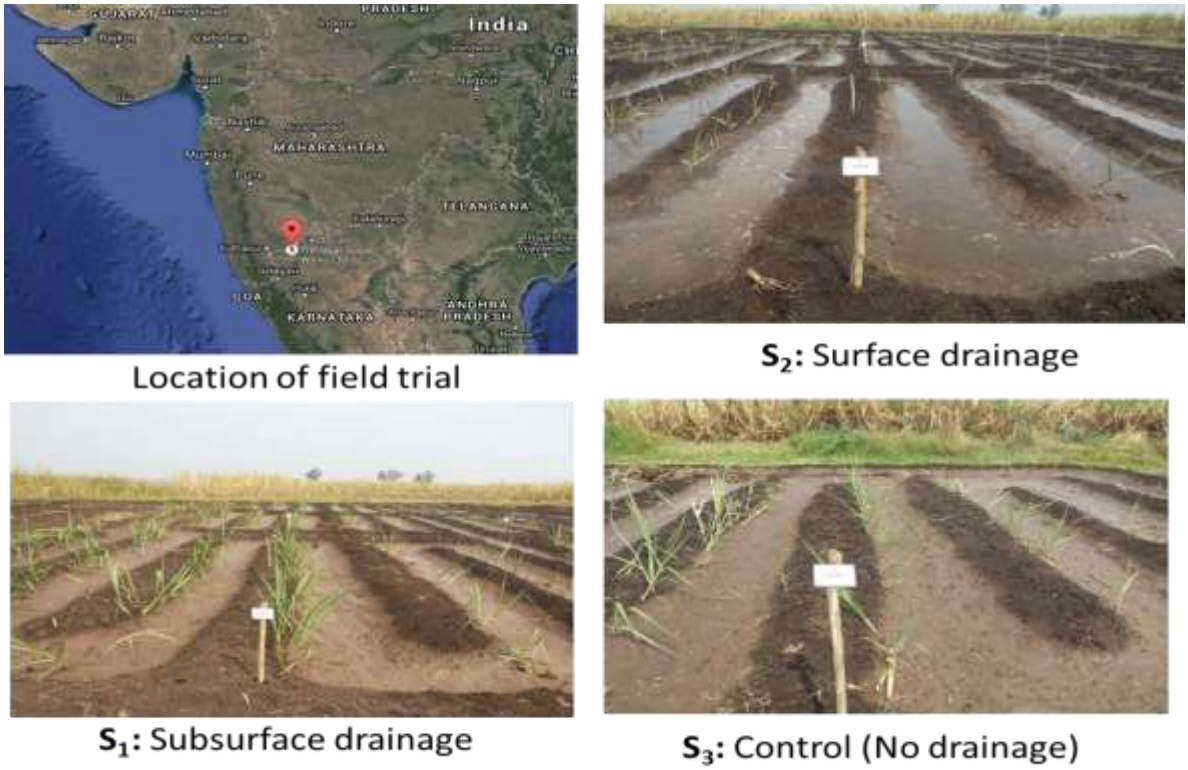


Figure 2-22 The location of the field trial and the treatments

Figure 2-23 Snap shots of field trials at Ugar Sugar, Karnataka

The following are the accountabilities which were carried out by each institute

UAS Dharwad University

- Drainage system Engineering (Subsurface, surface and no drainage)
- Installation of Drainage system
- Assistance in plot designing

Ugar Sugar, Karnataka

- Provided land
- Provided Salt tolerant sugarcane variety
- Assistance in field trial
- Arrangement of irrigation
- Record of data

TERI, New Delhi

- Development of microbial consortia (Bacteria and AMF)
- Designing of treatment
- Application of consortia
- Data analysis

Treatment details

Main plot: (Drainage)

S_1: Subsurface drainage

S_2: Surface drainage

S_3: Control (No drainage)

Subplot: (Fertilizer and consortium)

F_1: RDF (100%)

F_2: RDF (75%)

F_3: RDF (50%)

F_4: Microbial consortia + RDF (100%)

F_5: Microbial consortia + RDF (75%)

F_6: Microbial consortia + RDF (50%)

Replication: 3

Plot size: 3 m X 6 m

Crop: Sugarcane

Spacing: 4 feet inter row spacing

RDF for sugarcane: 25 tonnes of FYM or 2.5 tonnes of vermicompost with 250:75:190 kg NPK ha^{-1} (Package of practices, UAS, Dharwad)

20-25 kg of Fe SO_4 ha^{-1} and 20-25 kg of Zn SO_4 ha^{-1} applied in case of deficiency based on the soil test report.

10 % of recommended Nitrogen + 100 % P +100 % K basal dose

20 % Nitrogen- 6^{th} week after planting

30 % Nitrogen- 10^{th} week after planting

40 % Nitrogen- 14^{th} week after planting

Calculation of 50 %, 75 % and 100% of the RDF as mentioned in the treatment to done and indicated for accuracy of imposition of the treatments.

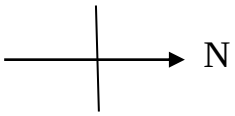
For the purpose of the implementation and getting the individual effect of the drainage, consortia and fertilizer and corresponding interaction effects of the inputs, two treatments No drainage with 75 % RDF and No drainage with 50 % RDF have been included. The treatments can be ignored while analyzing the combined interaction of drainage+ consortia+ fertilizers.

The surface drainage systems, which start functioning as soon as there is an excess of rainfall or irrigation applied, operate entirely by gravity. The surface drainage systems consist of check gates placed in the embankments surrounding flat basins, such as those used for fields in flat lands. These fields are usually submerged and only need to be drained on certain occasions (e.g. at harvest time). Most of the known criteria for these systems concern the efficiency of the techniques of land levelling and earthmoving.

The subsurface drainage systems consist of horizontal or slightly sloping channels made in the soil; which are open ditches, trenches, filled with buried perforated plastic (PE or PVC) pipe lines wrapped with an envelope or filter material to improve the permeability around the pipes and to prevent entry of soil particles, which is especially important in fine sandy and saline soils. When the drain discharge takes place entirely by gravity. They can save much irrigation water. When the discharge takes place by pumping, the drainage can be checked simply by not operating the pumps or by reducing the pumping time.

The design and the layout of the experiment with the details of the treatment as furnished are given below.

Layout:



R1	R 2	R 3
$S_1 F_4$	$S_1 F_5$	$S_1 F_1$
$S_1 F_2$	$S_1 F_6$	$S_1 F_4$
$S_1 F_1$	$S_1 F_1$	$S_1 F_6$
$S_1 F_3$	$S_1 F_2$	$S_1 F_5$
$S_1 F_6$	$S_1 F_4$	$S_1 F_3$
$S_1 F_5$	$S_1 F_3$	$S_1 F_2$
$S_2 F_1$	$S_2 F_6$	$S_2 F_4$
$S_2 F_5$	$S_2 F_3$	$S_2 F_1$
$S_2 F_3$	$S_2 F_1$	$S_2 F_6$
$S_2 F_6$	$S_2 F_2$	$S_2 F_5$
$S_2 F_4$	$S_2 F_5$	$S_2 F_3$
$S_2 F_2$	$S_2 F_4$	$S_2 F_2$
$S_3 F_6$	$S_3 F_5$	$S_3 F_3$
$S_3 F_3$	$S_3 F_4$	$S_3 F_2$
$S_3 F_4$	$S_3 F_1$	$S_3 F_5$
$S_3 F_1$	$S_3 F_3$	$S_3 F_6$
$S_3 F_2$	$S_3 F_6$	$S_3 F_4$
$S_3 F_5$	$S_3 F_2$	$S_3 F_1$

3.0 m

Service Road towards Rail

Treatment combinations details

S_1F_1: Subsurface drainage + RDF (100%)
S_1F_2: Subsurface drainage **+** RDF (75%)
S_1F_3: Subsurface drainage + RDF (50%)
S_1F_4: Subsurface drainage +Microbial consortia + RDF (100%)
S_1F_5: Subsurface drainage + Microbial consortia + RDF (75%)
S_1F_6: Subsurface drainage **+** Microbial consortia + RDF (50%)
S_2F_1: Surface drainage + RDF (100%)
S_2F_2: Surface drainage **+** RDF (75%)
S_2F_3: Surface drainage + RDF (50%)
S_2F_4: Surface drainage +Microbial consortia + RDF (100%)
S_2F_5: Surface drainage + Microbial consortia + RDF (75%)
S_2F_6: Surface drainage **+** Microbial consortia + RDF (50%)
S_3F_1: No drainage + RDF (100%)- Absolute control
S_3F_2: No drainage **+** RDF (75%)
S_3F_3: No drainage + RDF (50%)
S_3F_4: No drainage +Microbial consortia + RDF (100%)
S_3F_5: No drainage + Microbial consortia + RDF (75%)
S_3F_6: No drainage **+** Microbial consortia + RDF (50%)

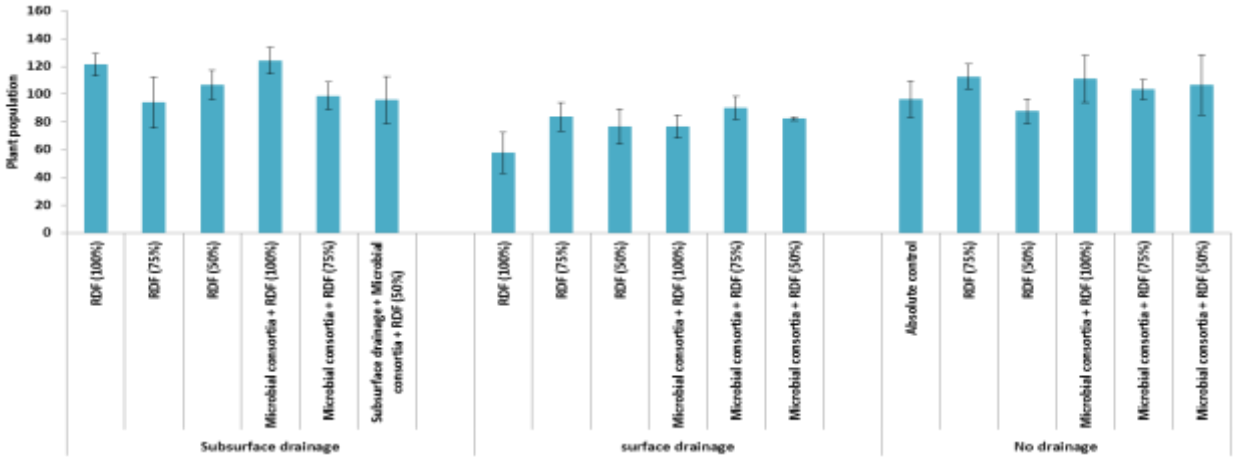


Figure 2-24 Effect on Plant Population after Treatment (Data recorded on 6 March, 2016)

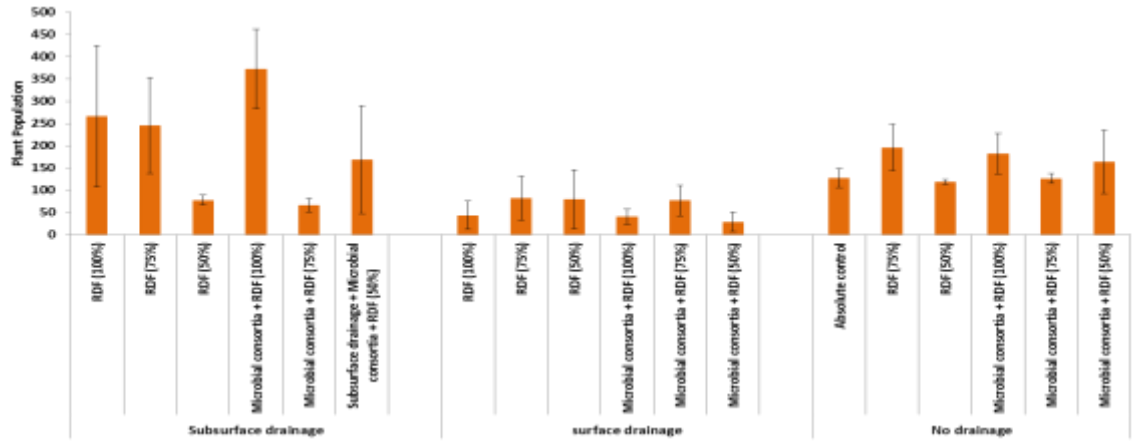
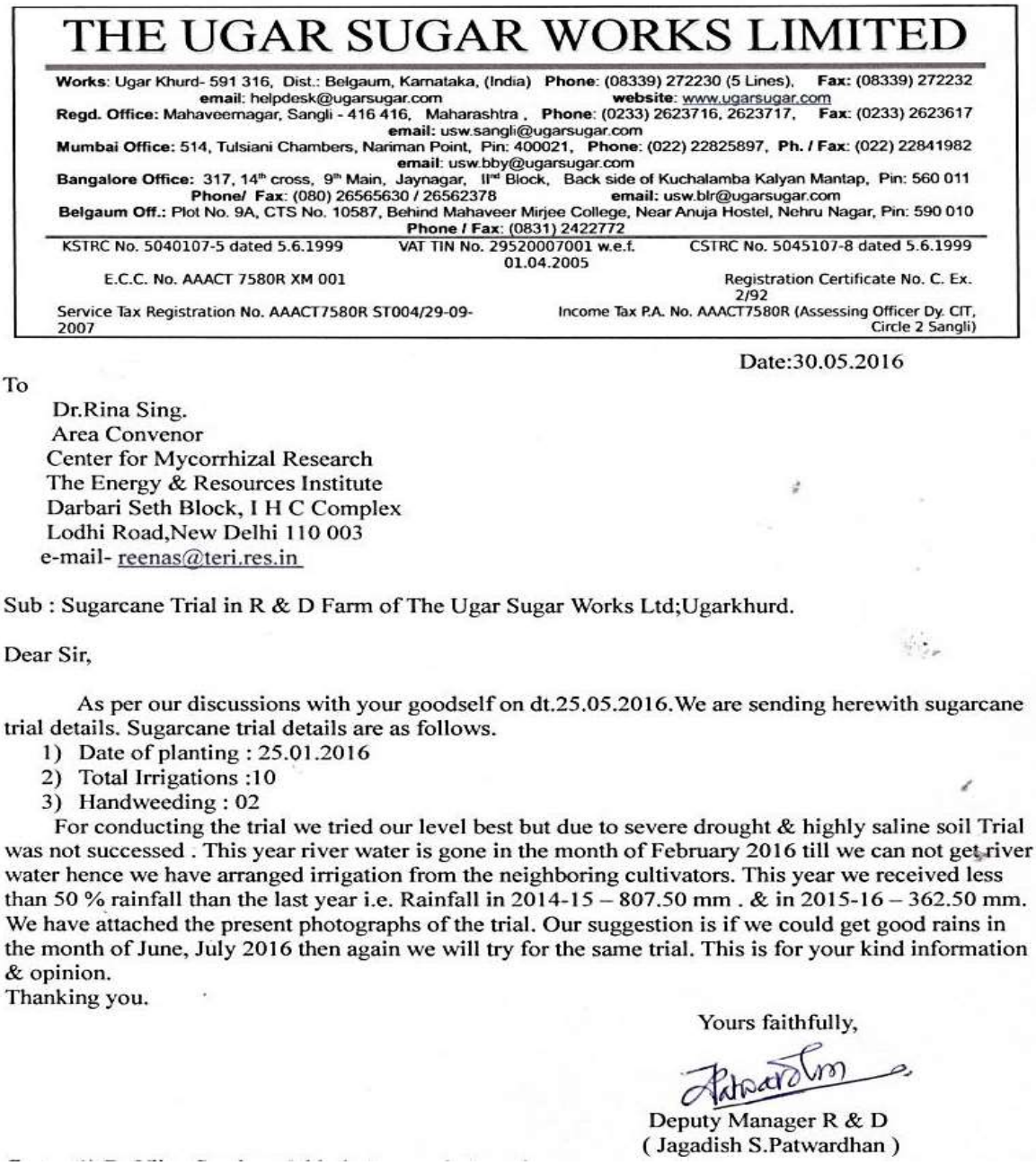


Figure 2-25 Effect on Plant Population after Treatment (Data recorded on 25 May, 2016

Figure 2-26 Current status of field trials at Ugar Sugar, Karnataka



THE UGAR SUGAR WORKS LIMITED

Works: Ugar Khurd- 591 316, Dist.: Belgaum, Karnataka, (India) **Phone**: (08339) 272230 (5 Lines), **Fax**: (08339) 272232
email: helpdesk@ugarsugar.com **website**: www.ugarsugar.com
Regd. Office: Mahaveernagar, Sangli - 416 416, Maharashtra , **Phone**: (0233) 2623716, 2623717, **Fax**: (0233) 2623617
email: usw.sangli@ugarsugar.com
Mumbai Office: 514, Tulsiani Chambers, Nariman Point, Pin: 400021, **Phone**: (022) 22825897, **Ph. / Fax**: (022) 22841982
email: usw.bby@ugarsugar.com
Bangalore Office: 317, 14th cross, 9th Main, Jaynagar, IInd Block, Back side of Kuchalamba Kalyan Mantap, Pin: 560 011
Phone/ Fax: (080) 26565630 / 26562378 **email:** usw.blr@ugarsugar.com
Belgaum Off.: Plot No. 9A, CTS No. 10587, Behind Mahaveer Mirjee College, Near Anuja Hostel, Nehru Nagar, Pin: 590 010
Phone / Fax: (0831) 2422772

KSTRC No. 5040107-5 dated 5.6.1999 | VAT TIN No. 29520007001 w.e.f. 01.04.2005 | CSTRC No. 5045107-8 dated 5.6.1999

E.C.C. No. AAACT 7580R XM 001 | Registration Certificate No. C. Ex. 2/92

Service Tax Registration No. AAACT7580R ST004/29-09-2007 | Income Tax P.A. No. AAACT7580R (Assessing Officer Dy. CIT, Circle 2 Sangli)

Date:30.05.2016

To

Dr.Rina Sing.
Area Convenor
Center for Mycorrhizal Research
The Energy & Resources Institute
Darbari Seth Block, I H C Complex
Lodhi Road,New Delhi 110 003
e-mail- reenas@teri.res.in

Sub : Sugarcane Trial in R & D Farm of The Ugar Sugar Works Ltd;Ugarkhurd.

Dear Sir,

As per our discussions with your goodself on dt.25.05.2016.We are sending herewith sugarcane trial details. Sugarcane trial details are as follows.

1) Date of planting : 25.01.2016
2) Total Irrigations :10
3) Handweeding : 02

For conducting the trial we tried our level best but due to severe drought & highly saline soil Trial was not successed . This year river water is gone in the month of February 2016 till we can not get river water hence we have arranged irrigation from the neighboring cultivators. This year we received less than 50 % rainfall than the last year i.e. Rainfall in 2014-15 – 807.50 mm . & in 2015-16 – 362.50 mm. We have attached the present photographs of the trial. Our suggestion is if we could get good rains in the month of June, July 2016 then again we will try for the same trial. This is for your kind information & opinion.

Thanking you.

Yours faithfully,

Deputy Manager R & D
(Jagadish S.Patwardhan)

Figure 2-27 Intimation letter from R&D of Ugar Sugar, Karnataka about the field trial under drought condition with their recommendation

After initiation of the field trial on 25th February, 2016 the effect on plant population after treatment was recorded on 6th March, which was presented in the Figure 2-24. Further data was recorded on 25th may, 2016 and found horrific effect on the plant population which reduced less than 80% (Figure 2-25). This was occurred due to severe drought condition **(**Figure 2-26). This year the entire area was received less than 50% rainfall than the last year rainfall. The last year (2014-15) rainfall was 807.50mm whereas this year the rainfall was around 362.50 mm. The field trial will be repeated in the month of June –July, 2016 depending on the monsoon rain as per the suggestion of UAS Dharwad University and Ugar Sugar, Karnataka (Figure 2-27).

2.3 Impact assessment of treated wastewater use in agriculture

Subtask 3.1 Assess effect of untreated and treated wastewater on soil properties (Pilot scale)

Study Area; CSIR-NEERI

Design of Constructed wetland; As described above

Field Design

The field trial was carried out for three seasons with tomato (*Solanum lycopersicum* L. ; variety: Lakshmi), red gram crop (*Cajanas cajan*; variety: Ankur -Prabha) and Brinjal (*Solanum melongena*; variety; Pusa Ankur). Tomato was cultivated in the first season (February, 2014 to April, 2014), red gram crop in second season (July, 2014 to January, 2015) and Brinjal crop in third season (June, 2015 to August,2015). The field experiment was laid out in randomized block design with three main treatments of wastewater, treated wastewater and tap water irrigation. Each treatment had three replications. A field design consist of an area 11 x 11 m^2 which was prepared near the pilot CW unit and it was divided into to 9 uniform plots of size 3.5 x 3.5 m^2 forming a 3 x 3 matrix. Standard agronomic practices as per the local area were performed. Recommended dose of farm yard manure was given to each crop at the time of land preparation to boost the growth of plants initially. **Table 5** shows physico-chemical properties of experimental soil at three different depths. Currently, cultivation of Maize crop is under progress.

Treatment details;

WW- Wastewater irrigated plots

TW- Treated wastewater irrigated plots

PW- Tap water irrigated plots

Figure 2-28 Tomato crop at experimental site

Figure 2-9 Red gram crop at experimental site

Figure 2-9 Brinjal crop at experimental site

Irrigation with wastewater caused increased in the soil EC for the first layer which could be due to upward movement of water and soluble salts by evaporation and capillary rise that resulted in the accumulation of salts at the soil surface. There was no significant effect on soil pH due to wastewater application but significant increase in EC was observed in WW plots followed by TW and PW. Organic carbon content of WW plots was found to be higher than TW and PW plots and it showed decreasing trend with deeper depth. ECW was found to be very efficient in the removal of organic loads from the treated wastewater and thus it also reduced the organic carbon content of TW plots.

Both TW and WW plots showed higher concentration of total nitrogen as compared to PW plots in both the seasons but depth wise, the concentration was reduced in second and third soil layer of these plots. Maximum accumulation of P in the third soil layer of WW and TW plots in second and third season resulted from the downward movement of P in the soil. Movement of P for large distances can occur when the soil reaches its maximum adsorption capacity as also noted by Heidarpour et al. (2007). The differences found between K concentrations of the plots treated with different types of irrigation water related to K concentration of the applied water (Saffari and Saffari2013). As expected it was higher in WW and TW plots. In the second and third season, K concentration of PW plots was slightly increased than at the first season. This result might be due to application of farm yard manure (FYM) at the time of field preparation.

A slight decrease in Ca concentration was observed for the first layer of WW and TW plots in the first season. This could be due to leaching, plant uptake and reaction of Ca with carbonate and sulfate, which were present in the applied water as noted by (Heidarpour et al. 2007). Ca is known to moderate the influence of Na on soil physical properties. Therefore, Ca removal from the soil by any other means can lead to the damage of soil due to accumulation of Na (Jnab et al. 2001). However, during the second season more accumulation of Ca in the soil was noted probably due to the continuous application of irrigation water (untreated and treated wastewater).WW plots showed higher concentration of Na in the first soil layer followed by TW and PW at the end of study. As the Na concentration increased in the first layer the soil tends to become more dispersed, which results in the breakdown of soil aggregates and deterioration of physical conductivity. However, the influence of Na on soil particle depends on the total electrolyte concentration in the soil (Feigin et al. 2012). As the exchangeable Na values were found within the normal range of 0.60 -1.50 % in all the season, there was no adverse effect on the soil. Moreover, high level of Na causes soil degradation at concentrations greater than 15% of the cation exchange capacity (Pescod 1992).

Physical properties of the soil like bulk density, water holding capacity and porosity remained unaffected by any of the treatment. This could be due to fact that the soil was more resistant to physical changes unlike the chemical properties, and it takes longer duration of wastewater application to produce any drastic effect. Porosity and WHC of the soil decreased with the time which could be due the dispersion and sedimentation of clay particles (Abedi-Koupai et al. 2006).

Heavy metal concentration in soil

Heavy metal concentration of untreated, treated wastewater and tap water irrigated plots is given in Table 2-25. WW plots showed slightly higher concentration of all heavy metals than those irrigated with treated wastewater. Higher cobalt concentration was observed in top most soil layer and decreased with depth. No significant difference was found between plots irrigated with untreated and treated wastewater in case of cobalt irrespective of depth and season. It ranged from 25 to 31 mg/kg in soil. A remarkable increased in Chromium concentration was observed as the season proceeds. In the first season the concentration was higher in upper layer but with each season it got accumulated in deeper layer. Its concentration found to be higher in wastewater irrigated plots in comparison to others. Copper concentration in soil remains more or less constant in all the seasons. In the first season its concentration showed no significant difference with different depths but as the

season proceed, increased concentration could be observed in the upper soil layer of wastewater irrigated plots. Soil at experimental site was quite rich in iron content (concentration varies from 25,000 to 34,000 mg/Kg) which is evident by the values in the table 2-22. Iron concentration increased with each passing season in WW and TW plots and it was found significantly higher in WW plots followed by TW and PW plots. Its concentration deceased with deeper depth in all the treatments and seasons. Like iron the present soil was also rich in manganese content. Higher concentration of manganese was observed in WW plots followed by TW and PW. There was no specific pattern in depth wise distribution of this element until the last season where the increased concentration could be seen in the top most soil layer. Nickel concentration in soil exceeds the maximum permissible limits given by USEPA which is 50 mg/kg. At the initial stage of experiment, it varied between 40 to 45 mg/kg but with continuous application of wastewater the concentration reached up to 61 to 66 mg/kg in WW plots and 55 to 59 g/kg in TW plots at last season. Like manganese, nickel concentration in soil did not follow any specific pattern depth wise. There was no significant difference observed in Lead and Zink concentration of WW plots and TW plots. Only in the last season it showed slightly higher concentration in upper layers of WW plots. From the obtained data and pattern on heavy metals, it can be noticed that accumulation of heavy metals occur over a period of time and significant difference between WW and TW plots can only be detected at third season. As the constructed wetland removes reasonable amount of heavy metals from wastewater, TW plots showed comparatively lower concentration of these metals than WW plots.

Effects of wastewater, treated wastewater and tap water on crop growth yield

Wastewater irrigated crops (tomato, red gram and Brinjal) showed slightly higher yield than treated wastewater irrigated crop but there was no significant difference in between them. It proved efficiency of treated wastewater to provide nutrients through irrigation with negligible deterioration of soil health. Some of the growth parameter and yield of both the crop is given in the Table 2-23 below.

Table 2-23 Growth parameters and yield of the Tomato, Red gram and Brinjal crop

Season	Treatment	Germination (%)	Height at the time of harvesting (cm)	Branches /plant	Yield (Quintal/ hectare)
Season 1 (Tomato)	WW	80	24.45	11.02	137.48
	TW	80	23.13	10.31	112.77
	PW	85	20.15	9.56	79.56
Season 2 (Red gram)	WW	75	165.21	8.12	21.28
	TW	78	153.89	7.88	18.5
	PW	80	134.75	6.11	9.12
Season 3 (Brinjal)	WW	65	65.42	6.86	320
	TW	72	62.81	7.18	311
	PW	70	60.02	5.23	260

Table 2-24 Physico- chemical Characteristics of soil at experimental field

Season	Treatment	Depth (cm)	pH	EC (mS/cm)	Organic Carbon (%)	Total N (%)	Total P (%)	Total K (%)	Na ($Cmol^+$/kg soil	K ($Cmol^+$/kg soil	Ca ($Cmol^+$/kg soil	Mg ($Cmol^+$/kg soil	CEC (meq/100gm)	ESP (%)	BD (gm/cc)	WHC (%)	POR (%)
At the beginning	T1	0-15	8.23	0.17	0.58	0.06	0.07	0.28	0.42	0.68	28.89	6.70	42.69	1.13	1.25	58.16	49.14
		15-30	8.25	0.18	0.43	0.05	0.07	0.30	0.37	0.63	28.62	7.05	42.66	1.00	1.26	55.37	47.63
		30-45	8.21	0.15	0.36	0.04	0.07	0.29	0.25	0.76	29.09	5.95	42.05	0.69	1.28	51.92	46.11
	T2	0-15	8.24	0.12	0.91	0.09	0.07	0.25	0.36	0.47	29.26	7.94	44.02	0.93	1.24	58.55	48.10
		15-30	8.15	0.12	0.61	0.07	0.07	0.28	0.29	0.75	28.26	5.98	42.28	0.81	1.26	54.07	47.53
		30-45	8.14	0.13	0.58	0.05	0.07	0.30	0.26	0.87	27.27	4.94	40.35	0.79	1.27	53.88	47.29
	T3	0-15	8.38	0.14	0.49	0.07	0.07	0.27	0.38	0.51	30.03	8.32	45.23	0.96	1.25	57.97	48.91
		15-30	8.32	0.13	0.45	0.06	0.07	0.25	0.34	0.68	27.61	7.10	42.73	0.95	1.27	54.05	46.20
		30-45	8.20	0.14	0.44	0.05	0.07	0.26	0.29	0.60	26.84	5.73	41.46	0.85	1.29	52.33	44.75
Season 1 (Tomato)	T1	0-15	8.46	0.20	0.88	0.09	0.09	0.37	0.51	0.86	26.79	7.13	42.28	1.43	1.23	57.99	46.94
		15-30	8.58	0.20	0.87	0.08	0.07	0.32	0.46	0.92	28.53	7.21	43.13	1.25	1.27	55.09	43.14
		30-45	8.56	0.20	0.86	0.07	0.09	0.28	0.47	0.88	28.91	7.54	43.80	1.23	1.29	52.21	41.36
	T2	0-15	8.51	0.16	0.87	0.09	0.08	0.34	0.36	0.87	25.85	5.34	39.41	1.12	1.25	58.62	46.81
		15-30	8.36	0.17	0.86	0.08	0.08	0.32	0.34	0.89	27.31	6.00	41.54	1.00	1.28	54.36	42.70
		30-45	8.25	0.17	0.72	0.07	0.09	0.32	0.32	0.90	27.07	6.25	41.54	0.93	1.29	54.19	41.41
	T3	0-15	8.34	0.15	0.78	0.05	0.07	0.30	0.30	0.77	25.06	6.69	41.83	0.93	1.25	56.10	45.52
		15-30	8.31	0.15	0.71	0.06	0.07	0.25	0.31	0.74	26.51	6.46	41.02	0.91	1.28	53.23	41.84
		30-45	8.31	0.15	0.59	0.05	0.08	0.26	0.30	0.75	26.40	6.24	41.70	0.90	1.30	51.58	40.61
Season2 (Red gram)	T1	0-15	8.21	0.23	0.95	0.09	0.10	0.44	0.59	1.49	31.22	10.43	47.74	1.36	1.27	53.73	46.22
		15-30	8.20	0.21	0.86	0.08	0.08	0.47	0.51	1.39	28.31	9.10	45.32	1.31	1.34	48.04	40.10

Season	Treatment	Depth (cm)	pH	EC (mS/cm)	Organic Carbon (%)	Total N (%)	Total P (%)	Total K (%)	Na ($Cmol^+$/kg soil	K ($Cmol^+$/kg soil	Ca ($Cmol^+$/kg soil	Mg ($Cmol^+$/kg soil	CEC (meq/100gm)	ESP (%)	BD (gm/cc)	WHC (%)	POR (%)
		30-45	8.21	0.19	0.75	0.07	0.09	0.43	0.49	1.25	30.30	8.73	46.77	1.21	1.42	42.61	40.31
	T2	0-15	8.19	0.19	0.78	0.07	0.08	0.43	0.47	1.39	30.57	10.23	47.67	1.11	1.34	56.12	52.96
		15-30	8.08	0.18	0.75	0.07	0.08	0.41	0.47	1.19	29.34	9.54	45.53	1.15	1.41	51.61	48.15
		30-45	8.20	0.18	0.70	0.06	0.09	0.41	0.46	1.26	26.18	7.47	42.37	1.30	1.34	51.19	46.70
	T3	0-15	8.09	0.16	0.73	0.06	0.07	0.32	0.33	0.85	27.57	6.91	42.65	0.92	1.35	57.28	52.23
		15-30	8.12	0.16	0.61	0.07	0.07	0.26	0.33	0.86	26.99	6.69	41.87	0.95	1.38	54.95	49.23
		30-45	8.07	0.17	0.60	0.05	0.07	0.22	0.33	0.84	26.08	5.84	40.09	1.00	1.39	51.95	45.57
Season2 (Brinjal)	T1	0-15	7.83	0.23	1.12	0.12	0.12	0.59	0.59	1.17	31.29	11.58	46.63	1.27	1.29	52.13	47.42
		15-30	8.12	0.21	0.93	0.10	0.12	0.48	0.59	1.10	33.58	10.72	47.98	1.23	1.36	46.04	41.40
		30-45	7.98	0.24	0.90	0.09	0.11	0.426	0.65	1.10	35.38	8.78	47.91	1.35	1.45	41.51	40.01
	T2	0-15	8.13	0.20	1.06	0.11	0.11	0.52	0.58	1.08	33.42	11.36	48.44	1.19	1.34	52.22	51.76
		15-30	7.93	0.21	0.95	0.11	0.12	0.44	0.54	1.11	29.44	10.48	43.56	1.24	1.42	49.71	47.95
		30-45	7.91	0.19	0.75	0.09	0.11	0.42	0.49	0.90	26.25	7.24	40.87	1.20	1.40	50.01	45.43
	T3	0-15	8.12	0.19	0.83	0.09	0.11	0.44	0.42	0.97	30.15	8.18	41.72	1.01	1.36	57.08	51.34
		15-30	7.81	0.20	0.73	0.07	0.11	0.32	0.47	0.88	27.32	8.35	40.01	1.17	1.38	53.95	49.66
		30-45	7.93	0.21	0.54	0.05	0.11	0.39	0.48	0.69	28.51	8.84	41.53	1.16	1.40	50.85	46.87

Table 2-25 Mean concentration of total heavy metals at experimental field

Season	Treatment	Depth (cm)	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
At the beginning	T1	0-15	30.04	48.36	60.02	28443.70	1023.18	44.33	7.82	54.60
		15-30	27.57	50.90	61.00	28557.03	1033.65	42.33	7.84	51.62
		30-45	25.67	58.00	64.99	26330.37	1047.38	44.88	10.29	72.64
	T2	0-15	31.98	36.46	68.53	28103.70	1122.25	38.39	6.72	75.37
		15-30	27.59	57.66	67.52	28530.37	1083.78	45.88	9.74	78.71
		30-45	24.19	54.12	68.08	24777.03	1123.45	38.95	13.07	79.90
	T3	0-15	30.23	41.87	42.72	26950.37	1048.18	40.47	9.43	55.04
		15-30	31.85	38.67	63.79	28243.70	1106.12	40.66	9.23	72.41
		30-45	29.01	40.32	64.54	25737.03	1086.85	46.99	10.62	52.31
Season 1 (Tomato)	T1	0-15	29.47	63.83	66.42	35014.45	1183.57	53.98	12.22	70.03
		15-30	28.09	65.34	61.74	33414.63	1199.34	51.56	9.01	64.98
		30-45	28.38	61.22	69.53	32824.10	1231.21	54.11	10.62	69.45
	T2	0-15	27.47	58.11	62.46	30282.27	1073.93	48.89	8.81	75.60
		15-30	26.05	55.60	59.93	28275.54	1195.14	45.55	10.11	62.18
		30-45	26.26	53.01	57.62	29071.87	1186.70	44.53	11.98	58.33
	T3	0-15	24.12	56.78	54.86	28024.55	968.01	45.63	8.28	61.63
		15-30	22.59	47.39	53.78	26367.01	925.73	40.95	9.28	68.19
		30-45	24.78	49.19	59.86	28070.24	950.85	42.49	7.81	66.65
Season2 (Red gram)	T1	0-15	30.63	80.08	73.31	33327.46	1129.62	52.32	9.98	78.83
		15-30	28.46	93.73	69.02	32125.02	1104.47	58.22	11.19	76.94
		30-45	27.02	93.28	61.39	29906.80	1036.28	61.79	14.20	72.68
	T2	0-15	25.70	82.45	65.22	28536.77	953.06	42.32	9.10	76.74
		15-30	26.08	83.23	65.03	28002.54	1053.95	40.01	9.83	70.81
		30-45	25.37	88.27	63.01	27826.51	1090.93	56.39	12.27	76.61
	T3	0-15	24.87	71.69	62.45	27063.00	945.61	43.24	9.98	73.81
		15-30	24.48	58.81	61.99	22284.33	1019.00	44.36	10.76	67.68
		30-45	25.01	72.43	69.67	27113.95	955.96	43.77	11.36	70.57
Season 3 (Brinjal)	T1	0-15	33.24	108.43	69.31	34380.18	1273.78	64.23	12.23	79.32
		15-30	32.27	122.17	68.25	32376.86	1226.63	61.22	12.17	75.82
		30-45	33.00	116.03	67.67	32330.62	1214.14	66.08	10.95	76.58
	T2	0-15	29.78	83.04	58.86	27805.29	1076.98	59.89	10.92	71.01
		15-30	28.99	64.78	57.52	26639.07	1041.00	55.41	10.53	68.48
		30-45	29.75	90.27	57.41	26523.96	1032.10	59.28	10.51	69.65
	T3	0-15	30.14	74.68	59.21	25428.32	1043.16	51.68	12.02	74.55
		15-30	29.46	56.22	57.84	24751.98	1022.73	51.60	9.16	69.99
		30-45	29.50	97.24	58.16	24730.84	1017.38	51.49	9.11	70.88

Unit: mg/ kg soil

3 Work Package: Agricultural Water Management

Objectives

- Baseline characterization of five benchmark sites with respect to climate, soil, crops and irrigation
- Improving water use efficiency through efficient irrigation systems, strategies and improved agronomic practices
- Assess the impact of waste and low quality water on crop produce, soil and groundwater quality
- Validate simulation models for assessing water use efficiency in the targeted production systems
- Build capacity of community and stakeholders for improving saline wastewater use efficiency through integrated agro-aqua farming system

3.1 Efficient irrigation system evaluated

3.1.1 Location of study area

The experiment was conducted on two different locations Jain Plastic Park and Jain Valley, Jain Irrigation Systems Ltd., Jalgaon (Maharashtra). The Jain Plastic Park lies between 75°32'55" E to 75°33'20" E longitude and 21°00'05" N to 21°00'20" N latitude. It is about 227 m above mean sea level. Jain Valley lies between 21° 05' N latitude, 75° 40'E longitude and at an altitude of 209 m above mean sea level. The climate of the area is semi - arid with 690 mm mean annual rainfall. The laboratory test was carried at the Jain Plastic Park and field evaluation was done in Jain Valley.

Three water sources were selected for the experiment are Treated Fruit Waste Water (TFWW), Treated Onion Waste Water (TOWW), and Bore Well Fresh Water (BWFW). The sample of irrigation water was collected in 1000 ml of polythene bottle from each source. The parameters like TDS, EC, pH, BOD, COD, TH, Nitrate-N, Total-P, K, Na, Ca, Mg, Chloride and Sulphate-S were analysed in the laboratory. The water analysis was done at 01 DAS, 30 DAS, 60 DAS, 90 DAS and120 DAS. The results of monthly analysis of TFWW, TOWW and BWFW are presented in Table 3-1, Table 3-2, and Table 3-3.

Table 3-1 Monthly analysis of treated fruit waste water (TFWW)

Parameters	01 DAS	30 DAS	60 DAS	90 DAS	120 DAS	Average
TDS (ppm)	820.5	780	770.6	789.1	893.1	810.67
EC (dS/m)	1.142	1.165	1.362	1.224	1.388	1.22
Ph	7.20	7.50	7.10	7.30	7.25	7.27
BOD (ppm)	9.2	8.43	8.87	7.45	7.90	8.83
COD (ppm)	65.5	57.8	110.78	78.77	72.43	78.03
TH (ppm)	210	225	278	221	265	237.67
Nitrate - N (ppm)	5.55	0.36	0.78	2.33	0.66	2.23
Total - P (ppm)	0.092	1.04	0.81	0.78	0.90	0.65
K (ppm)	14.70	62	56	72	64	44.23
Na (ppm)	60	221	246	249	233	175.67
Ca (ppm)	96	80	88	92	86	88.00
Mg (ppm)	78	40	43	44	54	53.67
Chloride (ppm)	132	165	128	120	134	141.67
Sulphate - S (ppm)	43	18	82	87	97	47.67

Table 3-2 Monthly analysis of treated onion waste water (TOWW)

Parameters	01 DAS	30 DAS	60 DAS	90 DAS	120 DAS	Average
TDS (ppm)	928	1050	1112	960	987	1030.00
EC (dS/m)	1.681	1.484	1.62	1.53	1.45	1.60
pH	7.73	7.42	7.74	7.67	7.69	7.63
BOD (ppm)	10.2	18.43	16.87	13.45	19.90	15.17
COD (ppm)	105.5	97.8	112.78	158.77	98.43	105.36
TH (ppm)	324	285	378	281	295	329.00
Nitrate - N (ppm)	0.37	1.04	0.43	0.56	0.46	0.61
Total - P (ppm)	18	0.52	0.26	0.36	1.23	6.26
K (ppm)	27.6	49.8	57.87	66.8	73.20	45.09
Na (ppm)	71.8	182.6	41.98	56.7	84.30	98.79
Ca (ppm)	153	121	96.99	90.12	93.67	123.66
Mg (ppm)	123.5	58.74	36.31	43.24	56.89	65.45
Chloride (ppm)	53.45	128.6	130.65	122.0	116.67	104.23
Sulphate - S (ppm)	15.6	49.25	56.75	65.45	63.67	40.53

Table 3-3 Monthly analysis of bore well fresh water (BWFW)

Parameters	01 DAS	30 DAS	60 DAS	90 DAS	120 DAS	Average
TDS (ppm)	788.68	760.50	780.80	735.55	875.88	788.30
EC (dS/m)	1.03	1.08	1.12	1.18	1.09	1.10
pH	6.54	6.77	6.99	6.79	6.58	6.734
BOD (ppm)	0.80	0.40	0.60	0.40	0.60	0.56
COD (ppm)	1.04	0.80	0.97	0.86	0.98	0.93
TH (ppm)	130	118	185	153	166	135.50
Nitrate - N (ppm)	0.35	0.43	0.52	0.86	0.21	0.48
Total - P (ppm)	0.92	1.30	1.60	1.93	1.67	1.48
K (ppm)	31.50	48.50	63.98	58.67	52.04	50.94
Na (ppm)	19.50	21.50	24.54	23.45	27.83	23.36
Ca (ppm)	48	165.50	71.2	89.67	103.86	95.65
Mg (ppm)	48.5	23.5	36.64	32.43	30.67	34.35
Chloride (ppm)	0	0	0	0	0	0
Sulphate - S (ppm)	0	0	0	0	0	0

3.1.2 Laboratory study

3.1.2.1 Emitter exponent prior to the clogging test

Emitter exponent was determined by measuring the discharge of 25 emitters at different pressure levels for Model A2.0, Model A4.0 , Model B2.0, Model B4.0, Model D1.1, Model D1.7 types of emitters were 0.5, 1.0, 1.2, 1.5 and 2.0 kg cm^{-2} and for Model C1.6 and Model C2.0 types of emitters were 1.0, 1.2, 1.5, 1.8 and 2.0 kg cm^{-2}. To obtain emitter exponent, four emitters were selected for measurement of discharge

having position 7, 12, 13 and 21 from the first catch can or emitter and its values are presented in Table 3-4. The highest emitter exponent was found in Model A2.4 and Model A4.0 emitter *i.e.* 0.52 and lowest emitter exponent was found in Model D1.1 *i.e.* 0.01. Higher the emitter exponent greater a variation in the emitter discharge and vice versa.

Table 3-4 Emitter exponent obtained by catch can method

Emitter Type	Technical Details	Emitter Exponent
Model A2.4	Non pressure compensating (NPC)	0.52
Model A4.0	Non pressure compensating (NPC)	0.52
Model B2.0	Non pressure compensating (NPC)	0.48
Model B4.0	Non pressure compensating (NPC)	0.49
Model C1.6	Pressure compensating, compensating non leakage, anti-syphon (PC CNL AS)	0.02
Model C2.0	Pressure compensating, compensating non leakage, anti-syphon (PC CNL AS)	0.02
Model D1.1	Pressure compensating and anti-syphon (PCAS)	0.01
Model D1.7	Pressure compensating and anti-syphon (PCAS)	0.02

Table 3-5 Effect of TFWW on the clogging resistance of emitters

Emitter type	Number of emitter clogged within 15 days of test (test sample containing 10 emitters)															Number of emitter clogged at end of test	Percent of emitter clogged during test
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Model A2.4	0	0	0	0	0	1	0	0	1	1	1	2	2	2	2	2	20
Model A4.0	0	0	0	1	1	0	0	0	0	1	2	2	2	2	3	3	30
Model B2.0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	10
Model B4.0	0	0	0	0	0	0	1	0	0	1	1	2	2	2	2	2	20
Model C1.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Model C2.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Model D1.1	0	0	1	0	2	2	3	4	5	6	7	7	8	8	8	8	80
Model D1.7	0	0	1	1	1	2	2	3	5	6	7	8	8	9	9	9	90
Avg. pH								7.27									
Avg. EC (dS/m)								1.22									
Avg. TDS (ppm)								810.67									

Table 3-6 Effect of TOWW on the clogging resistance of emitters

Emitter type	Number of emitter clogged within 15 days of test (test sample containing 10 emitters)															Number of emitter clogged at end of test	Percent of emitter clogged during test
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Model A2.4	0	0	0	1	1	2	2	2	1	1	1	2	2	3	3	3	30
Model A4.0	0	0	0	1	1	1	1	1	1	2	2	2	2	2	3	3	30
Model B2.0	0	0	0	0	0	0	0	0	1	0	0	1	1	2	2	2	20
Model B4.0	0	0	0	0	1	1	1	1	1	1	1	2	2	2	2	2	20
Model C1.6	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Model C2.0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Model D1.1	0	0	1	0	2	2	3	4	6	6	8	8	9	9	8	8	80
Model D1.7	0	0	1	1	1	2	2	3	5	6	7	9	8	10	10	10	100
Avg. pH								7.63									
Avg. EC (dS/m)								1.60									
Avg. TDS (ppm)								1030.00									

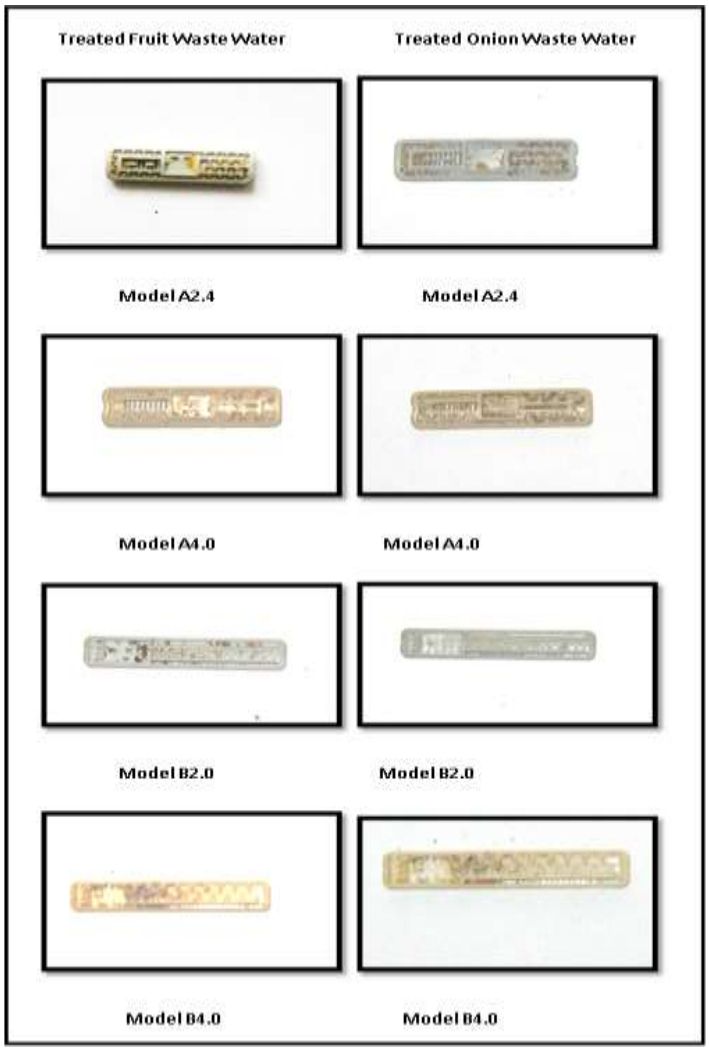


Figure 3-1 Clogging resistance of NPC emitters against TFWW and TOWW

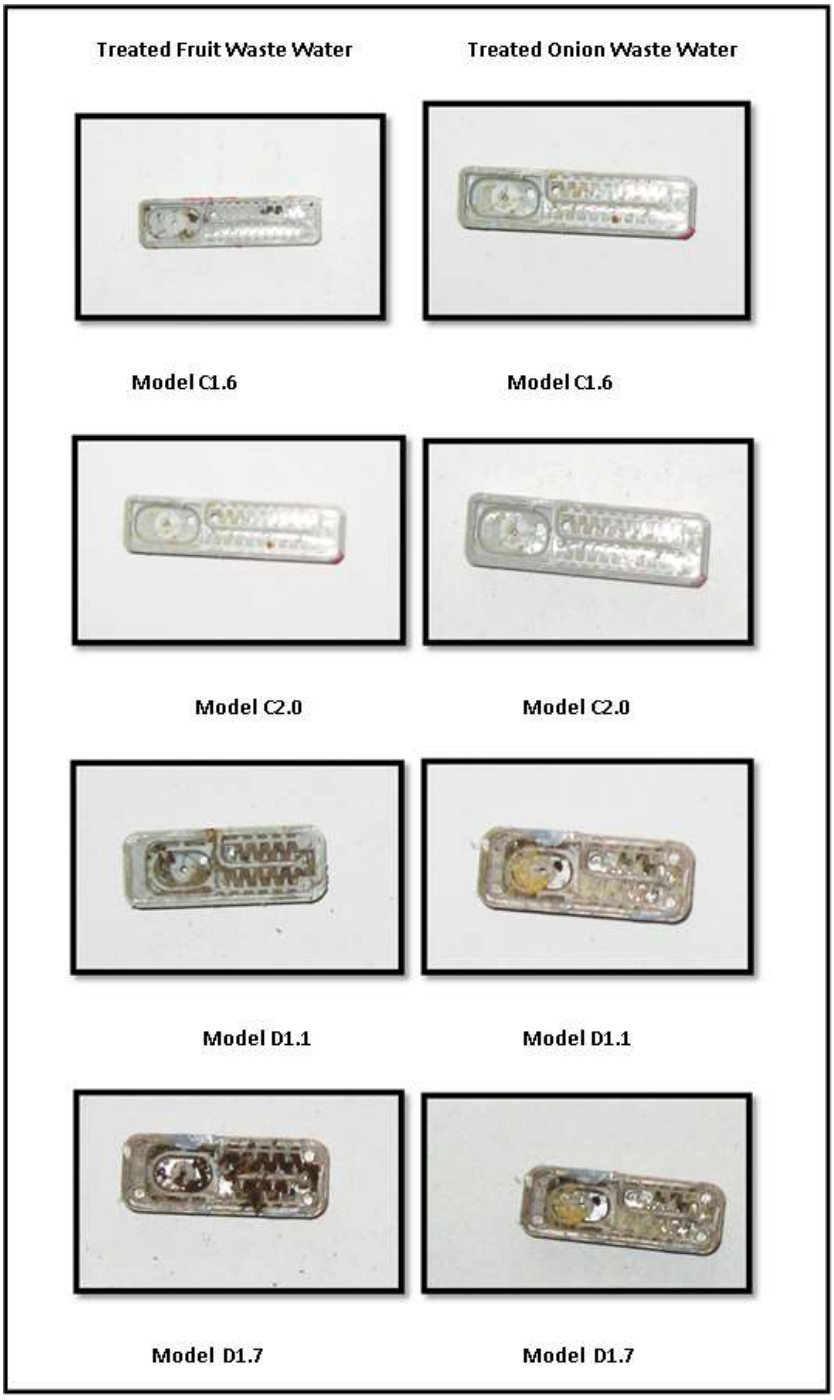


Figure 3-2 Clogging resistance of PC, CNL, AS emitters against TFWW and TOWW.

3.1.2.2 Selection of suitable emitter geometry by using clogging test method

Eight different types of emitter were tested to find out the suitable emitter geometry under TFWW and TOWW. Emitter clogging percentage of all the selected emitters after 15 days of test is presented in Table 3-5 and Table 3-6. To find the clogging points in emitters, emitters were peeled out through lateral as presented in the Figure 3.1 and 3.2.

It was found that flow path of Model B2.0, Model B4.0, Model C1.6, Model C2.0 type of emitter were having high clogging resistance for TFWW and TOWW than Model D1.1 and

Model D1.7 type of emitters which was severely clogged for same water. Model A2.4 and Model A4.0 type emitters were also clogging moderately against TFWW and TOWW except Model A2.4 type emitter has shown better resistance to TFWW

3.1.3 Field experiments

The crop was sown on 30th January 2014. The mean maximum and minimum temperature was about 37^{o}C and 20^{o}C, respectively, with mean maximum and minimum relative humidity was 68 per cent and 32 per cent were recorded during the period of experimentation. The crop was harvested on 30th May 2014. There was no precipitation during the entire crop growth period. So, the crop was irrigated frequently as and when required on the basis of daily evapotranspiration. Selection of suitable emitter geometry for field experiment was based on clogging test of emitter in the laboratory. On the other hand, analysis of soil and water helped to understand the necessary changes occurred into it during the experiment. Effect of different irrigation treatments and sub treatment on irrigation scheduling, uniformity coefficient, distribution uniformity, water use efficiency and maintenance scheduling are explained below;

3.1.3.1 Irrigation scheduling

It was observed that, water requirement of maize crop was 12.60 mm / day in the month of May and 2.66 mm / day in month of February. This may be the attributed to growth stage of crop, the higher ambient temperature and higher evaporation losses during this month. Total water requirement of maize crop during its growing season is 855.46 mm.

3.1.3.2 Determination of uniformity coefficient

The data pertaining to uniformity coefficient of drip irrigation system at different stages (01, 30, 60, 90 and 120 DAS) of crop growth as influenced by different irrigation treatments and different emitter types as well as their interactions are presented in the
Table 3-7There was significant effect of different irrigation treatments on the uniformity coefficient throughout the experiment, except at the end of experiment i.e. 120 DAS, there was not much difference in the uniformity under each treatment due to maintenance of drip system. The highest uniformity coefficient about was 96.07 per cent (01 DAS) was observed under TFWW. On the contrary, lowest uniformity coefficient of 90.07 per cent (90 DAS) was observed under TOWW.

There was no any significant effect among the different emitter types, hence the highest uniformity coefficient of 96.04 per cent was observed under Model C2.0 type emitter (01 DAS). On the contrary, lowest uniformity coefficient of 88.60 per cent (90 DAS) was observed under Model B2.0 type emitter. Only after 90 DAS, the statistically significant uniformity coefficient was observed in Model C2.0 type of emitter (95.09 per cent) than Model B2.0type emitter (88.60 per cent).

Interaction effect was significant among different irrigation sources and emitter types on uniformity coefficient except TFWW was having non-significant effect on 01 DAS, 30 DAS and 60 DAS. The Model C.20 type emitter was having higher uniformity coefficient than Model B2.0 type emitter.

3.1.3.3 Determination of distribution uniformity

The data pertaining to distribution uniformity of drip irrigation system at different stages (01, 30, 60, 90 and 120 DAS) of crop growth as influenced by different irrigation treatments and different emitter types as well as their interactions are presented in Table 3.8. Significant distribution uniformity was observed in each treatment during the experiment except at the end of experiment i.e. 120 DAS. The highest distribution uniformity 92.99 per cent (01 DAS) was observed under BWFW. On the contrary, lowest distribution uniformity of 78.80 per cent (90 DAS) was observed under TOWW.

Among the different emitter types, the highest distribution uniformity of 92.57 per cent was observed under Model C2.0 type emitter (01 DAS). On the contrary, lowest distribution uniformity of 78.61 per cent (90 DAS) was observed under Model B2.0 type emitter.

There was no significant effect among the interaction between different irrigation sources and emitter types on distribution uniformity except TOWW. The Model B2.0 type emitter under TOWW had a significant result to clogging at 90 DAS and 120 DAS but the Model C2.0 type emitter was having good clogging resistance throughout the experiment. The Model C.20 type emitter was having higher distribution uniformity than Model B2.0 type emitter.

Table 3-7 Effect of different water treatments on uniformity coefficient of emitters.

M: Different irrigation sources	**Uniformity coefficient (%)**				
	01 DAS	**30 DAS**	**60 DAS**	**90 DAS**	**120 DAS**
M1	96.07	95.95	95.74	91.72	94.07
M2	95.14	94.80	94.74	90.07	94.31
M3	95.28	95.14	95.06	93.74	94.39
S.Em ±	0.14	0.12	0.17	0.28	0.40
C. D. (5%)	0.42	0.36	0.51	0.86	1.24
CV	0.46	0.39	0.55	0.97	1.35
S : Different emitter types					
S1	96.04	95.89	95.85	95.09	95.61
S2	94.95	94.71	94.51	88.60	92.90
S.Em ±	0.46	0.54	0.56	1.13	0.54
C. D. (5%)	1.40	1.65	1.72	3.48	1.66
CV	0.67	0.80	0.83	1.74	0.81
Interaction					
MxS					
S.Em ±	0.29	0.34	0.35	0.71	0.34
C. D. (5%)	0.89	1.04	1.09	2.20	1.05
CV	0.67	0.80	0.83	1.74	0.81

Where, M1 : Treated fruit waste water (TFWW)
M2 : Treated onion waste water (TOWW)
M3 : Bore well fresh water (BWFW)
S1 : Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter.
S2 : Non pressure compensating (NPC) emitter.

Table 3-8 Effect of different water treatments on distribution uniformity of emitters.

M: Different irrigation sources	Distribution uniformity (%)				
	01 DAS	30 DAS	60 DAS	90 DAS	120 DAS
M1	92.24	92.11	91.54	84.53	89.29
M2	91.61	90.82	90.28	78.80	89.80
M3	92.99	92.77	92.03	88.01	89.02
S.Em ±	0.18	0.38	0.53	1.38	0.97
C. D. (5%)	0.56	1.19	1.62	4.25	2.99
CV	0.63	1.33	1.83	5.20	3.43
S : Different emitter types					
S1	92.57	92.50	92.56	88.95	91.54
S2	91.99	91.30	89.98	78.61	87.20
S.Em ±	1.10	1.38	1.78	3.35	2.20
C. D. (5%)	3.39	4.25	5.48	10.32	6.78
CV	1.69	2.12	2.76	5.66	3.48
Interaction					
MxS					
S.Em ±	0.70	0.87	1.13	2.12	1.39
C. D. (5%)	2.14	2.69	3.47	6.53	4.29
CV	1.69	2.12	2.76	5.66	3.48

Where,M1 Treated fruit waste water (TFWW)
M2 : Treated onion waste water (TOWW)
M3 : Bore well fresh water (BWFW)
S1 : Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter.
S2 : Non pressure compensating (NPC) emitter.

3.1.3.4 Effect on soil

Soil analysis prior to sowing of crop and after the treatments (60 DAS and 120 DAS) is presented in the Table 3-9. The soil analysis was carried after every two months to check the effect of different water treatments on soil parameters. This data was necessary to know the changes arose in the macro and micro nutrient level under each water treatment on soil parameters.

3.1.3.5 Effect on crop

At germination stage, there was no significant effect of different irrigation treatments and emitter types on plant population (Table 3.10). In different irrigation treatments plants population varied between 59100 (TOWW) to 59300 (TFWW) and in different emitter type plant population varied between 59160 Model B2.0 type emitter to 59266.67 Model C2.0 type emitter. The interaction effects due to different irrigation treatment and emitter types on plant population during germination were found to be non- significant.

At harvesting stage, there was no significant effect of different irrigation treatments and emitter types on plant population. In different irrigation treatments plants population varied between 57540 (TOWW) to 57900 (BWFW) and in different emitter type plant population varied between 57546.67 Model B2.0 type emitter to 57853.33 Model C2.0 type emitter. The interaction effects due to different irrigation treatment and emitter types on plant population during harvesting were found to be non- significant except TFWW. The

Model C2.0 type emitter was having significantly plant population (58280) than Model B2.0 type emitter (57040).

Table 3-9 Effect of different water treatments on soil properties after 60 and 120 DAS of maize.

Parameters	Before Treatmen	TFWW		TOWW		BWFW	
		60 DAS	120 DAS	60 DAS	120 DAS	60	120 DAS
N (kg/ha)	188.16	325.21	481.88	313.41	444.61	261.6	265.58
P (kg/ha)	16.43	102.06	260.49	131.10	254.02	89.19	108.91
K (kg/ha)	92.91	505.0	865.71	649.70	731.84	397.0	585.88
Ca (%)	0.14	0.17	0.64	0.16	0.71	0.17	0.59
Mg (%)	0.08	0.12	0.18	0.14	0.16	0.12	0.14
Fe (ppm)	4.96	10.17	12.01	9.98	11.20	8.85	10.65
Mn (ppm)	2.36	6.78	12.20	7.46	10.52	7.77	9.84
Zn (ppm)	0.52	1.26	1.83	0.854	1.48	1.13	2.854
Cu (ppm)	2.58	3.62	4.73	4.082	5.58	3.734	4.014
S (ppm)	8.06	10.65	13.03	9.05	9.85	8.63	8.95
Bulk density	1.30	1.32	1.31	1.30	1.33	1.31	1.30
Field capacity (%)	33.24	32.66	32.68	32.52	33.05	32.67	32.62
Permanent wilting	21.76	22.18	21.10	21.97	22.01	21.32	21.04
Texture	clay loam	clay loam	clay loam	clay loam	clay loam	clay loam	clay loam

Table 3-10 Effect of different water treatments on plant population per hectare.

M: Different irrigation sources	Plant population per hectare	
	Germination	Harvesting
M1	59300.00	57660.00
M2	59100.00	57540.00
M3	59240.00	57900.00
S.Em ±	149.56	244.82
C. D. (5%)	460.83	754.34
CV	0.80	1.34
S : Different emitter types		
S1	59266.67	57853.33
S2	59160.00	57546.67
S.Em ±	464.40	376.39
C. D. (5%)	1430.96	1159.76
CV	1.11	0.92
Interaction		
MxS		
S.Em ±	293.72	238.05
C. D. (5%)	905.02	733.50
CV	1.11	0.92

Where,M1 Treated fruit waste water (TFWW)
M2 : Treated onion waste water (TOWW)
M3 : Bore well fresh water (BWFW)
S1 : Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter.
S2 : Non pressure compensating (NPC) emitter.

Grain yield

The data pertaining to grain yield influenced by different irrigation treatments and different emitter types as well as their interactions are presented in the

Table 3-11. Significantly highest grain yield was observed under BWFW was about 9.92 tha^{-1}. On the contrary, lowest grain yield 7.15 tha^{-1} was observed under TOWW but among treated waste water TFWW (8.34 tha^{-1}) has significant effect on grain yield. Among the different emitter types, significantly highest grain yield of 9.04 tha^{-1} was observed under Model C2.0 type emitter. On the contrary, lowest grain yield was 7.15 tha^{-1} under Model B2.0 type emitter.

Table 3-11 Effect of different water treatments on maize grain yield.

M: Different irrigation sources	Grain yield (t/ha)
M1	8.34
M2	7.15
M3	9.92
S.Em ±	0.10
C. D. (5%)	0.30
CV	3.63
S : Different emitter types	
S1	9.04
S2	7.89
S.Em ±	0.17
C. D. (5%)	0.54
CV	2.90
Interaction	
MxS	
S.Em ±	0.11
C. D. (5%)	0.34
CV	2.90

Where, M1	Treated fruit waste water (TFWW)
M2 :	Treated onion waste water (TOWW)
M3 :	Bore well fresh water (BWFW)
S1 :	Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter.
S2 :	Non pressure compensating (NPC) emitter.

Interaction effects due to different irrigation treatments and emitter types on grain yield were found to be significant. The more difference in the grain yield was observed in the BWFW followed by TFWW and TOWW. The maximum grains yield was observed in the Model C2.0 type emitter (10.56 tha^{-1}) in BWFW and minimum in Model B2.0 type emitter (6.62 tha^{-1}) in TOWW. The influence of different irrigation treatments and different emitter types as well as their interactions on quality parameters of maize such as protein, carbohydrates, fats, ash, crude fiber and energy are presented in the followingTable 3.12.

Cost economics of maize crop under drip irrigation by using different irrigation treatments and sub treatments are presented in Table 3.13.

Table 3-12 Effect of different water treatments on the quality parameter of maize

Sr. No.	Parameters	TFWW	TOWW	BWFW
1	Protein (%)	7.82	7.62	4.92
2	Carbohydrates (%)	82.48	82.24	87.70
3	Fat (%)	3.01	3.21	1.28
4	Ash (%)	1.67	1.74	2.12
5	Crude Fiber (%)	1.36	2.15	1.46
6	Energy (kcal)	388.31	388.34	382.03

Table 3-13 Economics of maize under treated waste water by using drip irrigation system.

Treatments	Yield (kg ha^{-1})	Cost of cultivation (Rs)	Gross returns (Rs)	Net returns (Rs)	Returns per rupee of investment
M1S1	8901	133950	284832	186582	1.39
M1S2	7770	133950	248640	150390	1.12
M2S1	7675	133950	245600	147350	1.10
M2S2	6618	133950	211776	113526	0.85
M3S1	10559	133950	337888	239638	1.79
M3S2	9278	133950	296896	198646	1.48

Where, M1 Treated fruit waste water (TFWW)
M2 : Treated onion waste water (TOWW)
M3 : Bore well fresh water (BWFW)
S1 : Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter.
S2 : Non pressure compensating (NPC) emitter.

3.1.3.6 Field water use efficiency (WUE)

The data pertaining to WUE of crop growth as influenced by different irrigation treatments and different emitter types as well as their interactions are presented in the Table 3-14. Significantly highest WUE 11.60 $kgha^{-1}mm^{-1}$ was observed under BWFW. On the contrary, lowest WUE 8.36 kg $ha^{-1}mm^{-1}$was observed under TOWW. Among the different emitter types, significantly highest water use efficiency 10.58 kg $ha^{-1}mm^{-1}$was observed under Model C2.0 type emitter. On the contrary, lowest WUE of 9.23 $kgha^{-1}mm^{-1}$ was observed under Model B2.0 type emitter. Interaction effect was significant among different irrigation sources and emitter types on WUE. The Model C2.0 type emitter was having higher WUE than Model B2.0 type emitter in each treatment. Significantly highest WUE was observed in BWFW 12.35 kg $ha^{-1}mm^{-1}$under Model C2.0 type emitter and lowest in TOWW 7.74 kg $ha^{-1}mm^{-1}$in Model B2.0 type emitter.

Table 3-14 Effect of different water treatment on water use efficiency of maize.

M: Different irrigation sources	WUE (kg/ha/mm)
M1	9.75
M2	8.36
M3	11.60
S.Em ±	0.11
C. D. (5%)	0.35
CV	3.63
S : Different emitter types	
S1	10.58
S2	9.23
S.Em ±	0.20
C. D. (5%)	0.63
CV	2.90
Interaction	
MxS	
S.Em ±	0.13
C. D. (5%)	0.40
CV	2.90

Where, M1 Treated fruit waste water (TFWW)
M2 : Treated onion waste water (TOWW)
M3 : Bore well fresh water (BWFW)
S1 : Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter.
S2 : Non pressure compensating (NPC) emitter.

3.2 Impact assessment of wastewater on crops, soil and groundwater documented

3.2.1 Assessing Suitability of Brewery Wastewater as Irrigation in Field Crops Using In-vitro bioassay and pot culture

3.2.1.1 Pot culture

A pot culture experiments were conducted in glasshouse at International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India (17.53° N, 78.27° E) to study the effects of wastewater on soil and crop growth. The soil taken for pot culture was medium black clayey (Vertisols). The initial characteristics of soil are mention in Table 1. Pearl millet (ICMV221), pigeon pea (Asha), maize (kauvery 235), okra (MH10), tomato (lakshmi) and sorghum (CSV15) were grown during the experiments.The experiment consisted of four treatments with three replications in a Completely Randomized design (CRD). Four treatments of source of irrigation water were good quality groundwater (T_1), untreated domestic wastewater (T_2), treated effluent from brewery (T_3) and partially treated effluent from brewery (T_4). Seventy two pots containing 10 kg of soil was taken for the experiment. Out of seventy two pots, twelve pots were assigned for each crop. Five seeds were dibbled in soil at the depth of 3-5 cm in each pot. Fertilizer scheduling was followed as per the soil

test based recommendation specific to each crop. Full dose of P, K, Zn, B, S and 50% of N dose was applied basally before sowing and remaining 50 % N dose was top dressed.

Two different sources of water are used for conducting the experiment. One water source was taken from SAB Miller factory at Sangareddy particularly Effluent Treatment Plant (ETP) and UASB (Up flow Anaerobic Sludge Bed) reactor and the other source of water was drawn from Bharat Heavy Electrical Limited (BHEL).Irrigation plays a vital role in global food security. About 40% of global food production consumes 70% of fresh water therefore when wastewater is used for irrigating the land by altering the existing irrigation infrastructure and scheduling will totally reduce the amount of fresh water and this was the strategy expectation. Irrigation water quality can be measured by total salt content, sodium, pH (alkalinity-Carbonate and bicarbonate), and specific ions such as chloride, sulfate, boron and nitrate. In first season of pot culture experiment CROPWAT 8.0 was used for scheduling the irrigation and in the second season of pot culture experiment irrigation scheduling was done by alternate wet and dry method.

Crop parameters such as biomass and height were measured. Biomass was done at postharvest stage of the crop and height was measured from peak vegetative stage (30DAS) to preflowering stage (45DAS -60DAS) respective to the specific crop duration.In each treatment and replication, three plants were selected and their respective shoot and root mass was recorded. In each treatment and replication, three plants were selected and the weight of seeds per plant was recorded. The grains were dried in oven at 60^0 C for 24 hours and 100 grain weight for each treatment (in three replications) was recorded. The average was calculated and expressed in grams. The yield of pods per pot^{-1} of each treatment and replication was recorded and expressed as gram per pot.

Soil samples are drawn at the vegetative (30DAS), flowering (45-60DAS) and post harvest stage respective to the crop duration. The soil samples were collected from 15-20 cm depth before the conduct of the experiment and after the harvest of the crop. Samples were air dried, sieved through 2 mm mesh and used for the soil nutrient estimation. pH and EC was measured with soil water extract (Jackson, 1973) and particle size analysis performed by hydrometer method (Day, 1965). The organic carbon was estimated by the method proposed by Walkley and Black (1934). Available soil phosphorus was estimated by the procedure outlined by Bray *et al* (1954), available potassium by neutral ammonium acetate by flame photometric method (Stanford and English, 1949) and available sulphur by 0.01M $CaCl_2$ extract (Randall, 1988). Available micronutrients (Zn, Fe, Mn, Cu was estimated by DTPA extract outlined by (Lindsay and Norvell, 1978).To determine the plant nutrient uptake parameters such as dry weight and nutrient content are required. Dry weight was measured after the postharvest of the crop and nutrient content was estimated by selenium sesquioxide method (Sahrawat, 2002).

Effect of wastewater on soil

The soil samples were collected from surface (0-15 cm) of the pots irrigated with good quality ground water (T_1), untreated domestic wastewater (T_2), treated effluent from brewery (T_3) and partially treated effluent from brewery (T_4). The chemical characteristics of the wastewater on soil in different crops are presented in

Table 3-15.The results indicated that the pH of the soil varied in the range of 7.21 to 7.84 in first pot culture experiment and 7.4 to 7.8 in second pot culture experiment with an electrical conductivity ranging from 287 to 559.5 µS in first pot culture experiment and 6730 to 17600 µS in second pot culture experiment respectively. The pH and EC of the wastewater irrigated soil were slightly higher than control due to the presence of sodium ions in wastewater which is used as a supply of irrigation source. As per the data the pH values tends to vary from neutral to slightly alkaline in condition and therefore falls within the permissible limits ranging from 6.0 to 9.0 as mentioned by Patel *et al* (2004).Similarly the electrical conductivity of wastewater irrigated soils are higher but found to be within the toxic limit as per the USSL (1954). The organic carbon content of surface soils irrigated with wastewater in first pot culture experiment varies from 0.51 per cent to 0.63 per cent and 0.43 to 0.49 per cent in second pot culture experiment. Based on low (<0.5 per cent), medium (0.5-0.75 per cent) and high (> 0.75 per cent) status, all values in first pot experiment fell under medium level of organic carbon and second pot experiment values fell under low level of organic carbon. This decrease in the level of organic carbon content may be due to utilization of carbon by the crop as a source of nitrogen.

Table 3-15 Effect of wastewater irrigation on chemical properties of sandy clay loam soil in different crops (*sorghum bicolor & Solanum lycopersicum*)

Crop	Treatment	pH		EC(µS)		OC(%)	
		P-I	P-II	P-I	P-II	P-I	P-II
Sorghum	Control-T_1	7.21	7.4	292.1	6900	0.57	0.46
	BHEL untreated-T_2	7.24	7.6	517.3	9050	0.51	0.45
	ETP-T_3	7.30	7.8	387.4	11200	0.51	0.45
	UASB-T_4	7.31	7.6	394.6	11000	0.54	0.47
Tomato	Control-T_1	7.21	7.6	336.8	6730	0.59	0.46
	BHEL untreated-T_2	7.44	7.7	287.0	8070	0.54	0.49
	ETP-T_3	7.84	7.7	559.5	12500	0.63	0.43
	UASB-T_4	7.44	7.6	514.9	17600	0.54	0.46

The available phosphorous content in soil varies from 2.77 to 57.7 ppm. In first pot culture experiment in sorghum and tomato crop maximum phosphorous value was reported in untreated domestic wastewater (T_2). But in second pot culture experiment in tomato crop highest value was observed in partially treated effluent from brewery (T_4) and in sorghum highest value was obtained in untreated domestic wastewater (T_2).All the values fell under the very high. In first pot experiment in sorghum crop $T_1$and T_4 falls under the category of very low level (0-3 ppm),T_2 and T_3 fell under low level (4-7 ppm).In tomato all the treatments falls under low level (4-7 ppm).In such cases build up recommendation will benefit the crop production. In second pot experiment in sorghum and tomato crop all the values falls under the category of very high range (16+ ppm).Therefore maintenance recommendation should be taken up to maintain the soil quality and health. Application of domestic wastewater along with the recommended NPK dose increases the phosphorous content in soil (Ladwani *et al*, 2012).

The exchangeable potassium content in soil varies from 162 to 298 ppm. In first pot culture experiment in sorghum and tomato crop maximum potassium value was reported in treated effluent from brewery (T_3). But in second pot culture experiment in tomato and tomato crop

highest value was observed in partially treated effluent from brewery (T_4).In first and second pot experiment in tomato and sorghum crop all the values got from all the treatments falls under very high range of concentrations (161+ ppm).In general there is no need to add fertilizer to the soil. The available calcium content in soil varies from 6914 to8168 ppm. In first and second pot culture experiment in sorghum crop highest value was obtained in untreated domestic wastewater (T_2) and in tomato crop maximum value was observed in ground water application (T_1).All the values obtained falls under the category of very high range of concentration (4500+ ppm).The available sodium content in soil varies from 219 to 2429 ppm. In first pot culture experiment in sorghum and tomato crop highest value was obtained in treated effluent from brewery (T_3). In second pot culture experiment in sorghum highest value was obtained in partially treated effluent from brewery (T4) but in tomato highest value was observed in treated effluent from brewery (T_3). Similar type of results was observed in Beta vulgaris (Anita singh and MadhoolikaAgrawal, 2012).

Table 3-16 Effect of wastewater irrigation on macro and micronutrient status in vertisol in different crops (*sorghum bicolor & Solanum lycopersicum*)

Crop	Treatment	Avail-P (ppm)		Exch-K (ppm)		Avail-Zn(ppm)		Avail-B (ppm)		Avail-S(ppm)		Na(ppm)	
		P-I	P-II	P-I	P-II	P-I	P-II	P-I	P-II	P-I	P-II	P-I	P-II
Sorghum	Control-T_1	2.77	28.3	201	162	1.52	234.0	0.7	3.51	20.7	43	219	395
	BHEL untreated-T_2	5.65	22.4	205	145	3.24	207.1	1.3	4.58	31.0	22	334	541
	ETP-T_3	3.73	22.3	209	247	1.62	253.7	0.8	3.71	26.2	42	621	2281
	UASB-T_4	2.89	29.6	200	262	0.90	114.1	0.7	3.06	23.7	43	463	2440
Tomato	Control-T_1	4.48	36.9	174	186	1.66	147.3	0.6	4.44	25.6	66	256	479
	BHEL untreated-T_2	5.87	57.7	187	228	1.86	125.8	1.0	4.43	24.3	60	358	710
	ETP-T_3	4.84	36.5	229	256	1.80	230.5	1.0	4.32	26.8	37	1519	2429
	UASB-T_4	4.70	42.5	207	298	1.26	211.7	0.9	6.80	24.6	67	1160	2254

Table 3-17 Effect of wastewater irrigation in nutrient uptake of different crops (*sorghum bicolor & Solanum lycopersicum*)

Crop	**Treatment**	**N (mg)**		**P(mg)**		**K(mg)**		**Zn(mg)**		**B(mg)**		**S(mg)**	
		P-I	**P-II**	**P-I**	**P-II**	**P-I**	**P-II**	**P-I**	**P-II**	**P-I**	**P-II**	**P-I**	**P-II**
Sorghum	Control-T_1	2.29	1.33	0.12	0.08	2.49	1.23	54.69	73.54	17.69	18.38	1638.49	899.06
	BHEL untreated-T_2	2.41	1.90	0.12	0.25	2.34	1.34	56.75	73.19	20.49	21.61	1505.69	1135.99
	ETP-T_3	1.43	1.11	0.06	0.08	2.23	1.21	26.55	74.83	11.4	2.25	1143.77	720.46
	UASB-T_4	2.24	1.30	0.11	0.11	2.16	0.82	51.65	55.22	14.24	5.72	1528.29	790.20
Tomato	Control-T_1	1.66	4.21	0.12	0.21	3.25	3.32	74.41	183.16	64.03	89.08	4839.57	6148.09
	BHEL untreated-T_2	2.03	6.74	0.14	0.39	3.67	4.77	69.31	339.48	58.82	102.54	4822.49	9016.14
	ETP-T_3	1.69	6.19	0.09	0.28	2.61	3.34	95.95	204.47	61.63	72.05	3952.81	6865.92
	UASB-T_4	2.76	5.51	0.13	0.25	3.02	4.01	92.72	200.18	57.65	65.38	5484.38	4646.71

The available sulphur content in soil varies from 20.7 to 67 ppm. In first pot culture experiment in sorghum crop highest value was obtained in untreated domestic wastewater (T_2) and in tomato maximum value recorded in treated effluent from brewery (T_3).In second pot experiment maximum value was observed in ground water application (T_1) and partially treated effluent from brewery (T_4).In both the pot experiment all values falls under very high concentration (15+ ppm).Therefore external source of addition of fertilizer is not needed and in turn reduces the fertilizer cost and its application.

Effect of wastewater on crops

Plant samples were collected and dry weight and nutrient content was measured and further used for the calculation of nutrient uptake. The nitrogen uptake in crop ranges from 1.11 to 6.74 mg. In first pot experiment in sorghum maximum N uptake obtained in untreated domestic wastewater (T_2) and minimum uptake observed in Treated effluent domestic wastewater (T_3).In tomato maximum uptake observed in partially treated effluent from brewery (T_4) and minimum uptake in ground water application. But in second pot experiment for both sorghum and tomato crop maximum uptake was observed in untreated domestic wastewater (T_2) and in sorghum and tomato minimum uptake recorded in treated effluent domestic wastewater (T_3) and ground water respectively.

The phosphorous uptake in crop ranges from 0.06 to 0.39 mg. In first and second pot experiment for both tomato and sorghum crops maximum P uptake was observed in untreated domestic wastewater (T_2) and minimum uptake was recorded in treated effluent domestic wastewater (T_3). The potassium uptake in crop ranges from 0.82 to 4.77 mg. In first pot experiment in sorghum maximum K uptake observed in ground water (T_1) and minimum in partially treated effluent from brewery (T_4) but in case of tomato crop maximum K uptake was recorded in untreated domestic wastewater (T_2).In sorghum and tomato minimum K uptake was obtained in partially treated effluent from brewery (T_4) and ground water (T_1) respectively.

Wastewater irrigation effect on aboveground biomass:

Generally there is an increase in plant shoot biomass was observed when wastewater is used as an irrigation source consequently for two years (2013-2014). In first pot culture experiment in tomato and sorghum maximum shoot biomass was obtained in ground water (T_1) followed by treated effluent from brewery (T_3). But in the case of second pot culture experiment in tomato and sorghum highest biomass recorded in untreated domestic wastewater (T_2) followed by treated effluent from brewery (T_3).Comparison between the biomass of 2013 and 2014 there was a significant increase in biomass was observed in 2014.This may be due to the enrichment of soil with nutrients when wastewater is used as an irrigation source and in turn enables the plant growth (Yasser *et al*, 2013).

Influence of wastewater on crop yield

From the data it was inferred that in first pot culture experiment tomato and sorghum obtained maximum yield in partially treated effluent from brewery (T_4) followed by ground water treatment (T_1). But in second crop season in tomato and sorghum crop highest yield was obtained in ground water (T_1) followed by untreated domestic wastewater (T_2). The use of wastewater significantly increased the yield of the crop. This may be due to continuous supply of wastewater over a year results in build up of nutrients in the top layers of the soil. Among the two crops (sorghum bicolor & solanum lycopersicum) used for the study tomato was observed to have obtained higher yield than sorghum (Ladwani *et al*, 2012). Decrease in yield in sorghum may be due to the phytotoxicity effect of some heavy metal accumulation.

3.2.2 Response of brinjal to different sources of irrigation water

The experiments were carried out at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka during *kharif* 2015 to study the effect of different source of water on yield, water productivity and its economic feasibility of brinjal.

Treatment details:

T_1: Domestic wastewater (DWW)
T_2: Engineered constructed wetland treated wastewater (TWW)
T_3: Fresh/good water (FW, bore well water)
T_4: Freshwater alternated with domestic wastewater (FW-DWW)
T_5: Freshwater alternated with ECWL treated wastewater (FW-TWW)
T_6: ECWL treated wastewater alternated with domestic wastewater (TWW-DWW)

The experimental site is situated at a latitude of 15º 26' N and longitude of 75º 07' E with an altitude of 678 m above mean sea level. The soil of the experimental site was red sandy clay loam. The rainfall received during the cropping period was 410 mm while, rainfall received during *kharif, 2015* was 620 mm.

Experiment details:

Treatments	6
Replication	4
Design	Randomized complete block design (RCBD)
Crop spacing	75 cm x 60 cm
Plot size	4.20 m x 6 m
Fertilizer dose	125:100:50 kg N, P_2O_5, K_2O /ha
Date of planting	08/07/2015
Variety:	Manjeeri
Crop season	*Kharif* , 2015
Soil type:	Red sandy clay loam
Average annual rainfall	780 mm
Rainfall during 2015:	620 mm
Rainfall during cropping season	410 mm

The results showed that significantly higher plant height and number of fruits per plant of brinjal were recorded in plots irrigated with domestic wastewater (114 cm and 53.09, respectively) compared to freshwater (109 cm and 34.79, respectively) but it was on par with conjunctive use of domestic wastewater altered with treated wastewater irrigation (113 cm and 47.98, respectively) (Table 3-18). Significantly higher fruit weight per plant were observed under domestic wastewater (1251 g/plant) followed by treated wastewater + domestic wastewater irrigation (1197 g/plant) compared to all other treatments. Lower fruit weight per plant was recorded in crop irrigated with fresh water and treated water alone (874 and 960 g/plant, respectively).

Brinjal yields were significantly higher with domestic wastewater irrigation (17.43 t/ha) which was closely followed by treated wastewater alternated with domestic wastewater treatment (17.37 t/ha) and freshwater alternated with domestic wastewater treatment (16.96 t/ha). The lowest yield of brinjal was registered with freshwater irrigation (14.52 t/ha) while, direct irrigation with treated wastewater recorded a yield of 15.32 t/ha.

Rainfall received during the cropping period was 410 mm with an effective rainfall of 246.12 mm. In all a total of 11 irrigations were applied to the crop which includes two common irrigation. The total water applied including effective rainfall was 602 mm for all the treatments. Water productivity was significantly higher with domestic wastewater irrigation (289.5 kg/ha-cm) which remained on par with conjunctive use of treated wastewater and domestic wastewater irrigation (288.5 kg/ha-cm). Lower values were recorded for freshwater (253.8 kg/ha-cm) and treated wastewater (254.5 kg/ha-cm) irrigation (Table 3-19).

Table 3-18 Growth and yield parameter of brinjal as influenced by different source of water

Treatment	Plant height (cm)	Number of branches/ plant	Number of fruits/ plant	Fruit wt./plant (g)	Yield (t/ha)
T_1: Domestic wastewater (DWW)	114	7.25	53.09	1251	17.43
T_2: Treated wastewater (TWW)	111	8.75	44.79	960	15.32
T_3 : Freshwater (FW)	109	8.42	34.79	874	14.52
T_4: Freshwater + Domestic wastewater	113	9.33	40.81	1070	16.96
T_5 : Freshwater + Treated wastewater	113	8.92	48.54	1013	15.94
T_6: Treated wastewater + Domestic wastewater	113	8.83	47.98	1197	17.37
SEm±	5	0.56	1.71	52.55	0.59
CD (P=0.05)	12	1.70	5.17	158.4	1.78

Table 3-19 Water applied, yield and water productivity of brinjal as influenced by different source of water

Treatment	No. of common irrigation	Depth of common irrigation (mm)	No. of treat-mental irrigation	Mean depth of irrigation (mm)	Effective rainfall (mm)	Total water applied (mm)*	Yield (kg/ha)	Water productivity (Kg/ha-cm)
T_1: Domestic wastewater (DWW)	2	30	9	32.88	246.12	602	17430	289.5
T_2: Treated wastewater (TWW)	2	30	9	32.88	246.12	602	15320	254.5
T_3 : Freshwater (FW)	2	30	9	32.88	246.12	602	14520	253.8
T_4: Freshwater + Domestic wastewater	2	30	9	32.88	246.12	602	16960	281.7
T_5 : Freshwater + Treated wastewater	2	30	9	32.88	246.12	602	15940	264.8
T_6: Treated wastewater + Domestic wastewater	2	30	9	32.88	246.12	602	17370	288.5

Common irrigation – Bore well water through sprinklers * including effective Rainfall (mm). Significantly higher net returns and B:C ratio were recorded in domestic wastewater (Rs. 2,18,355/ha and 2.68) as compared to freshwater (Rs. 1,75,348/ha and 2.35) and treated wastewater (Rs. 1,76,148/ha and 2.36). However, treated wastewater alternated with domestic wastewater irrigation resulted in par net returns and B:C ratio (Rs. 2,17,035/ha and 2.67) as like domestic wastewater (Table 3-20). The highest and lowest net profit of Rs 3627and 2913 were

obtained per cm use of water in case of domestic wastewater and fresh water irrigated brinjal. However the combination of FW with DWW and TWW with DWW also recorded higher net profit of Rs 3471 and 3605 per cm use of water, respectively.

Table 3-20 Net returns, B:C ratio and net profit per cm of water applied used of brinjal as influenced by different source of water

Treatment	Yield (t/ha)	Gross returns (Rs/ha) #	Net returns (Rs/ha)	B:C ratio	Net profit/cm of water applied (Rs/cm)
T_1: Domestic wastewater (DWW)	17.43	348646	218355	2.68	3627
T_2: Treated wastewater (TWW)	15.32	306400	176148	2.36	2926
T_3: Freshwater (FW)	14.52	305639	175348	2.35	2913
T_4: Freshwater + Domestic wastewater	16.96	339240	208949	2.60	3471
T_5 : Freshwater + Treated wastewater	15.94	318785	188494	2.45	3131
T_6: Treated wastewater + Domestic	17.37	347326	217035	2.67	3605
SEm±	0.59	11843	11843	0.09	-
CD (P=0.05)	1.78	30500	30450	0.27	-

#Market price for the produce @ Rs. 20 /kg

Effect of different sources of water on soil chemical properties at different depths were studied in brinjal crop during *kharif* 2015 at harvest stage. The results are presented in table 4 and 5. Soil reaction (pH) and conductivity (EC) showed no difference among treatments at 0-20 cm and 20-40 cm soil depth. Available nitrogen differed significantly in soil due to source of the irrigation water at both the depths of soil observed. Significantly higher available nitrogen content (222.66 kg/ha) was recorded in the treatment irrigated with the domestic sewage water (T1) at 0-20 cm depth. However it was on par with treated wastewater alternated with domestic wastewater (T6), fresh water alternated with treated wastewater (T5) and treated wastewater (T2). Lower available nitrogen content (169.34 kg/ha) was recorded in the soil irrigated with fresh water as the source of irrigation (T3). Similar trend with respect to the available nitrogen content in the soil was observed at 20-40 cm depth with higher and on par values recorded in T6, T1, T5, T2 and T4 and lower value recorded with fresh water irrigation (T3). Conjunctive use of the domestic wastewater with treated wastewater or with freshwater contributed higher amount of the nitrogen to soil through the water source resulting in higher content of the available nitrogen in soil which was comparable to the application of the domestic wastewater alone. With respect to the potassium content in the soil at different depths, sources of irrigation resulted in non-significant difference. Calcium concentration showed no difference among the water source treatments at 0-20cm and 20-40 cm soil depth (table 5). Similar trend was recorded with respect to the magnesium concentration at both soil depths of 0-20cm and 20-40 cm.

Table 3-21 Effect of sources of water on soil pH, EC, available nitrogen and potassium

Source of irrigation water	pH		EC (dS/m)		Nitrogen (kg/ha)		Potassium (kg/ha)	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
T1: Domestic wastewater (DWW)	7.61	7.57	0.405	0.403	222.66	208.95	452.40	275.58
T2: Treated wastewater (TWW)	7.64	7.62	0.437	0.410	197.57	194.43	421.95	287.25
T3: Fresh water (FW)	7.67	7.63	0.398	0.392	169.34	163.07	460.78	280.68
T4: Fresh water altered with Domestic wastewater	7.70	7.65	0.420	0.381	181.89	194.43	433.65	274.15
T5: Fresh water altered with Treated wastewater	7.64	7.64	0.431	0.396	206.98	200.71	424.70	257.68
T6: Treated wastewater altered with Domestic wastewater	7.63	7.63	0.411	0.409	213.25	212.09	433.98	267.33
SEm±	0.03	0.03	0.022	0.020	9.37	10.18	26.84	12.65
CD(p=0.05)	NS	NS	NS	NS	28.25	30.68	NS	NS

Table 3-22 Effect of sources of water on soil calcium and magnesium concentration at different depths

Source of irrigation water	Ca (meq/100g)		Mg (meq/100g)	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm
T1: Domestic wastewater (DWW)	21.75	20.50	14.50	13.25
T2: Treated wastewater (TWW)	19.50	18.50	12.25	12.75
T3: Fresh water (FW)	18.25	16.25	13.00	12.50
T4: Fresh water altered with Domestic wastewater	19.25	18.00	14.00	14.00
T5: Fresh water altered with Treated wastewater	19.50	19.25	13.50	13.25
T6: Treated wastewater altered with Domestic wastewater	22.75	19.50	15.50	13.50
SEm±	1.24	1.15	0.81	0.83
CD(p=0.05)	NS	NS	NS	NS

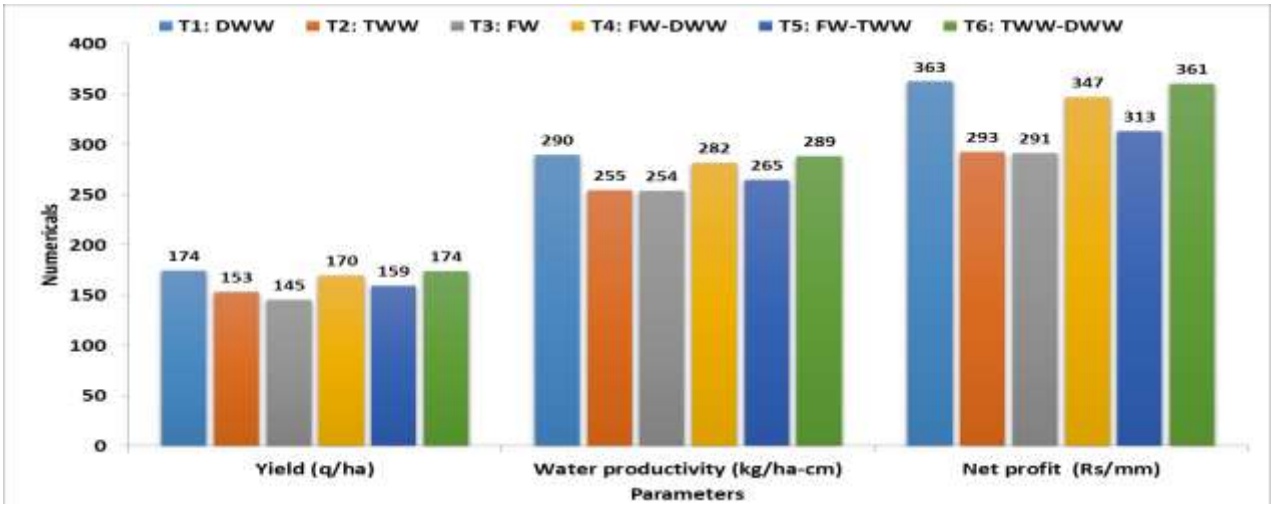


Figure 3-3 Yield, water productivity and net profit per cm of water used as influenced by sources of irrigation in Brinjal

3.2.3 Response of chilli to different sources of irrigation water

This experiment was carried out at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad Karnataka during *kharif* 2015 to study the effect of different source of water on the performance of chilli

Treatment details:

T_1: Domestic wastewater (DWW)
T_2: Engineered constructed wetland treated wastewater (TWW)
T_3: Fresh/good water (FW, bore well water)
T_4: Freshwater alternated with domestic wastewater (FW-DWW)
T_5: Freshwater alternated with ECWL treated wastewater (FW-TWW)
T_6: ECWL treated wastewater alternated with domestic wastewater (TWW-DWW)

Treatment:	6
Replication:	4
Design:	Randomized Complete Block design (RCBD)
Spacing:	75 X 45 cm
Plot size:	4.20 X 6 m
Fertilizer dose:	100:50:50 kg N,P_2O_5, K_2O /ha
Date of planting:	08/07/2015
Variety:	Byadagi Dabbi
Crop season:	*Kharif* ,2015
Soil type:	Red sandy clay loam (Alfisol)
Average annual rainfall	780 mm
Rainfall during 2015:	620 mm
Rainfall during cropping season	410 mm

Influence of the different sources of irrigation water on growth and yield attributes of chilli was found to be significant (Table 3-23). Domestic wastewater irrigation recorded significantly higher plant height and number of branches (78.76 cm and 13.41) as compared to freshwater irrigation (67.17 cm and 10.75, respectively). Maximum number of fruits and fruit weight per plant were recorded in plots irrigated with DWW (99.89 and 749 g/plant) compared to FW (76.45 and 583 g/plant) and TWW alone (77.67 and 611 g/plant) and was on par with TWW alternated with

DWW (90.13 and 739 g/plant). Higher chilli yield were recorded with DWW (17.58 t/ha) compared to FW (13.31 t/ha) and TWW alone (14.63 t/ha). However it was on par with TWW alternated with DWW (16.28 t/ha), FW alternated with DWW (16.18 t/ha) and fresh water alternated with TWW (15.61 t/ha).

Rainfall received during the cropping period was 410 mm with an effective rainfall of 246.12 mm. In all a total of 11 irrigations were applied to the crop which includes two common irrigation. The total water applied including effective rainfall was 654 mm for all the treatments. Higher water productivity of chilli were obtained in case of DWW (268.8 kg/ha-cm) followed by 248.9, 247.4 and 238.7 kg/ha-cm (table 3-24 and figure 3-4) in TWW alternated with DWW, FW alternated with DWW and fresh water alternated with TWW, respectively as compared to FW (203.5 kg/ha-cm) and TWW (223.7 kg/ha- cm).

Higher net returns of Rs 220553 per ha was obtained in case of domestic wastewater irrigation and lower net return of Rs 134147 per ha was observed under fresh water irrigation. Similar trend of results was recorded in case of B:C ratio.
The highest and lowest net profit of Rs 3372 and 2066 were obtained per cm use of water in case of domestic wastewater and fresh water irrigated chilli. However the combination of FW with DWW and TWW with DWW also recorded higher net profit of Rs 2943 and 2973 per cm use of water, respectively.

Table 3-23 Growth and yield parameters of green chilli as influenced by different sources of water

Treatment	Plant height (cm)	No. of branches/ plant	Number of fruits /plant	Fruit wt./plant (g)	Yield (t/ha)
T_1: Domestic wastewater (DWW)	78.76	13.41	99.89	749	17.58
T_2: Treated wastewater (TWW)	72.92	11.75	77.67	611	14.63
T_3 : Freshwater (FW)	67.17	10.75	76.45	583	13.31
T_4: Freshwater + Domestic wastewater	70.46	12.92	81.08	541	16.18
T_5 : Freshwater + Treated wastewater	67.58	10.17	83.40	626	15.61
T_6: Treated wastewater + Domestic wastewater	74.75	12.75	90.13	739	16.28
SEm±	2.53	0.81	4.85	46	0.94
CD (P=0.05)	7.64	2.43	14.63	138	2.83

Table 3-24 Water applied, yield and water productivity of chilli as influenced by different source of water

Treatment	No. of common irrigation	Depth of common irrigation (mm)	No. of treatment irrigation	Mean depth of irrigation (mm)	Effective rainfall (mm)	Total water applied (mm) *	Yield (t/ha)	Water productivity (Kg/ha-cm)
T_1: Domestic wastewater (DWW)	2	30	9	38.65	246.12	654	17.58	268.8
T_2: Treated wastewater (TWW)	2	30	9	38.65	246.12	654	14.63	223.7
T_3 : Freshwater (FW)	2	30	9	38.65	246.12	654	13.31	203.5
T_4: Freshwater + Domestic wastewater	2	30	9	38.65	246.12	654	16.18	247.4
T_5 : Freshwater + Treated wastewater	2	30	9	38.65	246.12	654	15.61	238.7
T_6: Treated wastewater + Domestic wastewater	2	30	9	38.65	246.12	654	16.28	248.9

Table 3-25 Net returns, B:C ratio and net profit per cm water of chilli as influenced by different source of water

Treatment	Yield (t/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	B:C ratio	Total water applied (mm) *	Net profit /cm of water applied (Rs)
T_1: Domestic wastewater (DWW)	17.58	351692	220553	2.68	654	3372
T_2: Treated wastewater (TWW)	14.63	292635	161546	2.23	654	2470
T_3 : Freshwater (FW)	13.31	266236	135147	2.03	654	2066
T_4: Freshwater + Domestic wastewater	16.18	323576	192487	2.47	654	2943
T_5 : Freshwater + Treated wastewater	15.61	312212	181123	2.38	654	2769
T_6: Treated wastewater + Domestic wastewater	16.28	325552	194463	2.48	654	2973
SEm±	0.94	18766	18766	0.14	-	-
CD (P=0.05)	2.83	56566	56566	0.43	-	-

Influence of different sources of water on soil chemical properties at different depths were studied in chilli crop during *kharif* 2015 at harvest stage. The results are presented in table 3-26 and 3-27. Insignificant difference with respect to soil reaction (pH) and conductivity (EC) was recorded with different sources of irrigation at 0-20cm and 20-40 cm depth. Significant difference was recorded with respect to available nitrogen content observed at both depths due to different source of the irrigation water. Higher available nitrogen content (247.72 kg/ha) was recorded with domestic sewage water irrigation (T1) at 0-20 cm depth, which was on par with TWW alternated with DWW (T6), FW with TWW (T5) and FW alternated with DWW (T4). Lower available nitrogen content (191.30 kg/ha) was recorded in the soil irrigated with fresh water as the source of irrigation (T3). Similar trend with respect to the available nitrogen content in the soil was observed at 20-40 cm depth with higher available nitrogen content in T1 (216.37 kg/ha) and was on par with T6, T5 and T2 whereas lower available nitrogen recorded with fresh water irrigation(T3). Effect of conjunctive use of the domestic wastewater with treated wastewater or with freshwater resulted in higher available nitrogen in soil through the water source resulting in higher content of the available nitrogen in soil which was comparable to the application of the domestic wastewater alone. Insignificant difference was recorded with respect to the potassium content in the soil at different depths due to different sources of irrigation.

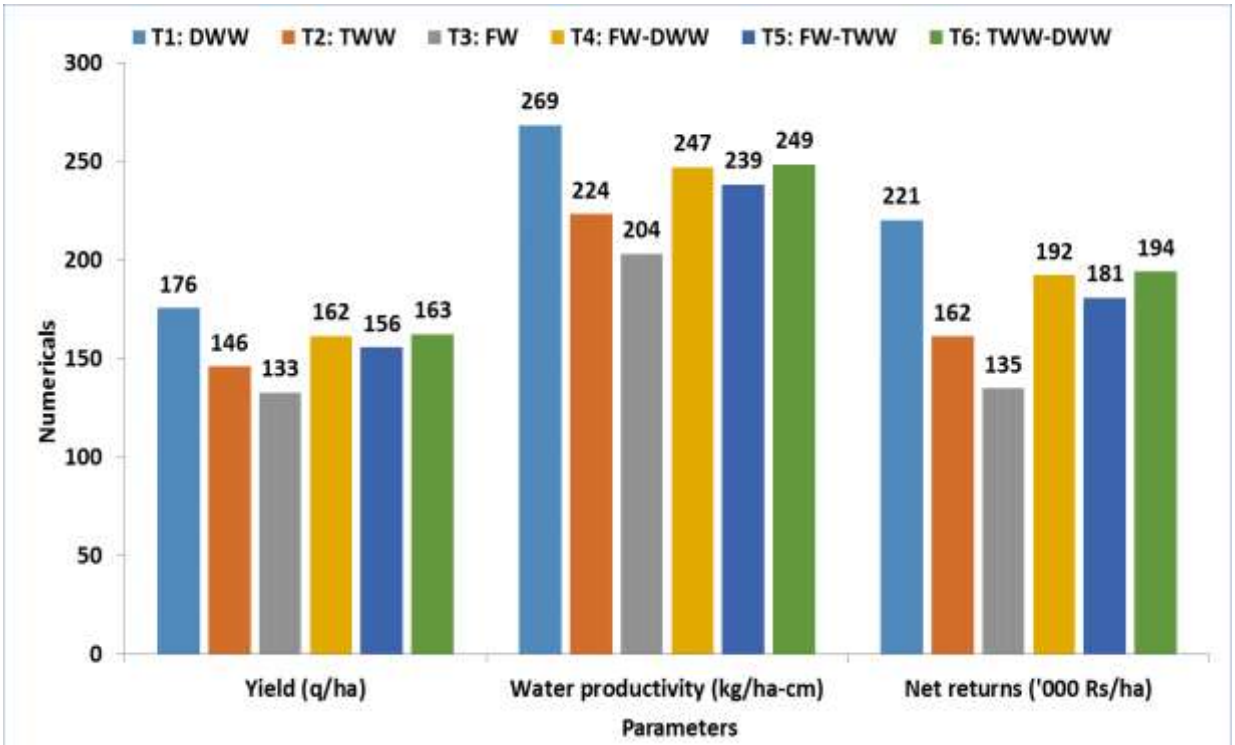


Figure 3-4 Yield, Water productivity and net returns as influenced by sources of irrigation in Chilli

Influence of different sources of water on concentration of calcium and magnesium at different depths in chilli crop at harvest stage are presented in table 10. Variation in calcium and magnesium concentration in the soil at 0-20 cm and 20-40 cm depth due to the source of the irrigation water was insignificant.

Table 3-26 Effect of source of water on soil pH, EC, available nitrogen and potassium

Treatment	pH		EC (dS/m)		Nitrogen (kg/ha)		Potassium (kg/ha)	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
T_1: Domestic wastewater (DWW)	7.65	7.66	0.388	0.408	247.72	216.37	304.65	198.38
T_2: Treated wastewater (TWW)	7.64	7.69	0.384	0.398	216.39	194.44	288.15	172.90
T_3 : Freshwater (FW)	7.74	7.61	0.357	0.347	191.30	156.81	284.15	181.63
T_4: Freshwater + Domestic wastewater	7.70	7.68	0.383	0.367	225.79	175.63	296.83	170.95
T_5 : Freshwater + Treated wastewater	7.76	7.77	0.350	0.372	222.66	181.90	307.93	178.70
T_6: Treated wastewater + Domestic wastewater	7.62	7.59	0.350	0.357	228.93	197.57	301.95	197.93
SEm±	0.05	0.06	0.013	0.019	9.39	11.33	12.16	10.36
CD (P=0.05)	NS	NS	NS	NS	28.29	34.16	NS	NS

Table 3-27 Effect of sources of water on soil calcium and magnesium concentration at different depths

Treatment	Ca (meq/100g)		Mg (meq/100g)	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm
T_1: Domestic wastewater (DWW)	17.50	17.00	14.75	13.75
T_2: Treated wastewater (TWW)	18.00	14.25	16.25	12.75
T_3 : Freshwater (FW)	16.50	16.50	14.25	14.25
T_4: Freshwater + Domestic wastewater	15.50	17.50	13.00	14.75
T_5 : Freshwater + Treated wastewater	19.25	15.75	15.75	12.75
T_6: Treated wastewater + Domestic wastewater	16.00	15.25	14.75	13.50
SEm±	0.92	1.29	1.35	0.85
CD (P=0.05)	NS	NS	NS	NS

Application of wet land treated wastewater or untreated sewage water in conjunction with good quality water was found to be significantly on par in terms of crop yield, water productivity, net returns B: C ratio and net profit per cm of water used with untreated wastewater irrigation alone.

3.2.4 Effect of different sources of water on soil health, yield and quality of ridge gourd.

The experiment was carried-out at the Main Agricultural Research Station, Dharwad, Karnataka during *rabi 2015-16* to study the effect of different sources of water on the performance of ridge gourd.

Treatment details:

T_1: Domestic wastewater (DWW)

T_2: Engineered constructed wetland treated wastewater (TWW)

T_3: Fresh/good water (FW, bore well water)
T_4: Freshwater alternated with domestic wastewater (FW-DWW)
T_5: Freshwater alternated with ECWL treated wastewater (FW-TWW)
T_6: ECWL treated wastewater alternated with domestic wastewater (TWW-DWW)

Treatment:	6
Replication:	4
Design:	Randomized Complete Block design (RCBD)
Spacing:	120 X 60 cm
Plot size:	4.20 m X 6 m
Fertilizer dose:	100:50:50 kg N, P2O5, K_2O /ha
Date of planting:	11/01/2016
Variety:	JL

Ridge gourd was sown on 11/01/2016 during *rabi/summer* 2015- 16 following the recommended package of practices. Two common irrigations with a depth of 30 mm with good quality bore well water was applied for establishment through sprinklers. Irrigation treatments were imposed after 15 days of sowing. Fifteen irrigations were given with an average depth of 31.2 mm for each irrigation and total water applied during the entire cropping period was 551.4 mm which includes effective rainfall of 23.4 mm.

Influence of sources of water on crop performance was found to be non significant with respect to plant height and number of vines per plant. However number of fruits per plant, fruit weight/ plant and yield were significantly influenced by different source of irrigation (table 3-28). Higher number of fruits/plant (13.21), and fruit weight/plant (962 g) were obtained with domestic wastewater irrigation as compared to fresh water and treated waste water but it was on par with other conjunctive sources of irrigation applied. Yield response to the sources of irrigation was found to be significant. Higher fruit yield (7038 kg/ ha) was recorded with domestic wastewater as compared to fresh water alone (5975 kg/ha) and treated wastewater (6304 kg/ha). However, yield was on par with other irrigation sources (Table 3-29).

Higher net return (Rs 1,56,429/ha) and B: C ratio (3.87) were observed with application of domestic wastewater as compared to freshwater (Rs.1,24,547/ha and 3.30, respectively) and treated wastewater (Rs.1,34,417/ha and 3.50, respectively) (Table 3-29). The highest and lowest net profit of Rs 2837 and 2259 were obtained per cm use of water in case of domestic wastewater and fresh water irrigated ridge gourd. However the combination of FW with DWW and TWW with DWW also recorded higher net profit of Rs 2603 and 2744 per cm use of water, respectively. The total water applied including effective rainfall was 551.4 mm for all the treatments. Higher water productivity was achieved with domestic wastewater (127.64 kg/ha-cm) followed by application of domestic wastewater alternated with treated wastewater (124.53 kg/ha-cm) and fresh water alternated with domestic wastewater (119.85 kg/ha-cm). Lower water productivity was achieved in case of freshwater alone (108.37 kg/ha-cm) and treated wastewater (114.34 kg/ha-cm) (Table 3-30 and figure 3-5). Post harvest soil analysis of effect of the sources of irrigation on ridge gourd is under progress.

Table 3-28 Response of ridge gourd to sources of water on growth and yield parameters and its economics

Treatment	Plant height (cm)	Vines /plant	Number of Fruits /plant	Fruit weight/ plant (g)	Yield (kg/ha)	Gross return (Rs/ha)	Net return (Rs/ha)	B:C ratio	Net profit /cm of water applied (Rs)
T_1: Domestic wastewater (DWW)	174.24	12.33	13.21	962	7038	211140	156429	3.87	2837
T_2: Treated wastewater (TWW)	158.47	11.90	10.98	808	6304	134417	134417	3.50	2438
T_3 : Freshwater (FW)	144.07	10.17	9.78	697	5975	179250	124547	3.30	2259
T_4 : Freshwater + Domestic wastewater	149.42	10.00	10.43	818	6609	198270	143545	3.64	2603
T_5: Freshwater + Treated wastewater	153.00	10.25	11.12	764	6222	186660	131950	3.44	2393
T_6: Treated wastewater + Domestic wastewater	169.50	12.66	11.76	833	6867	206010	151280	3.78	2744
SEm±	**10.52**	**1.36**	**0.62**	**57.8**	**282**	**8445**	**8445**	**0.15**	-
CD (P=0.05)	**NS**	**NS**	**1.88**	**174.3**	**849**	**25456**	**25456**	**0.45**	-

*Effective rainfall during cropping period is 23.4 mm
Market rate of ridge gourd Rs. 30/kg

Table 3-29 Water applied, yield and water productivity of ridge gourd as influenced by different source of water

Treatment	No. of common irrigation	Depth of common irrigation (mm)	No. of treat-mental irrigation	Mean depth of irrigation (mm)	Effective rainfall (mm)	Total water applied (Including effective RF cm)*	Yield (kg/ha)	Water productivity (kg/ha-cm)
T_1: Domestic wastewater (DWW)	2	30	15	31.2	23.4	551.4	7038	127.64
T_2: Treated wastewater (TWW)	2	30	15	31.2	23.4	551.4	6304	114.34
T_3 : Freshwater (FW)	2	30	15	31.2	23.4	551.4	5975	108.37
T_4 : Freshwater + Domestic wastewater	2	30	15	31.2	23.4	551.4	6609	119.85
T_5: Freshwater + Treated wastewater	2	30	15	31.2	23.4	551.4	6222	112.84
T_6: Treated wastewater + Domestic wastewater	2	30	15	31.2	23.4	551.4	6867	124.53

*Effective rainfall during cropping period is 23.4 mm

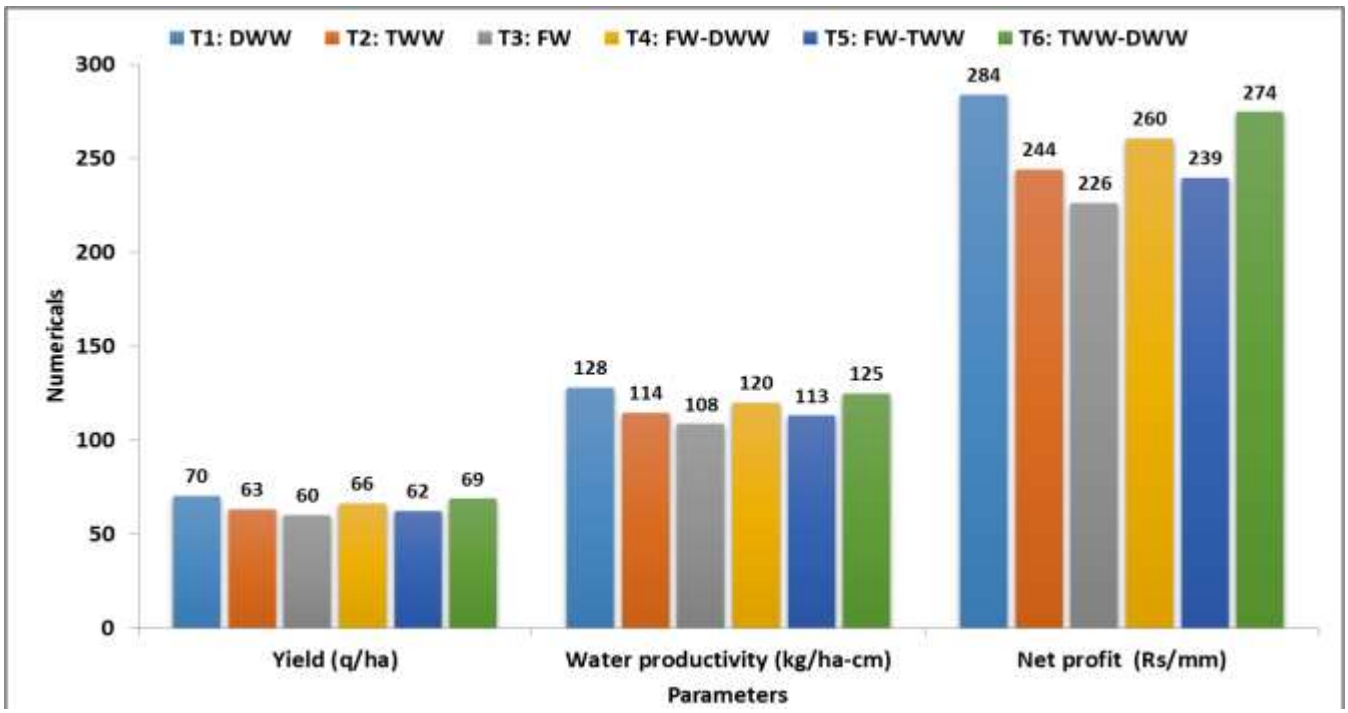


Figure 3-5 Yield, water productivity and net profit per cm of water used as influenced by sources of irrigation in ridge gourd

3.2.5 Effect of different sources of water on soil health, yield and quality of clusterbean

Field experiment was conducted to evaluate the performance of clusterbean to different source of irrigation water during *rabi/ summer* 2015-16 with following objectives:

1. To know the response of different sources of irrigation on growth and yield of cluster bean
2. To know the effect of sources of irrigation on soil properties.
3. To study the effect of sources of irrigation on water productivity and their economic feasibility

Treatment details:

T_1: Domestic wastewater (DWW)
T_2: Engineered constructed wetland treated wastewater (TWW)
T_3: Freshwater (FW)
T_4: Freshwater alternated with domestic wastewater
T_5: Freshwater alternated with ECWL treated wastewater
T_6: ECWL treated wastewater alternated with domestic wastewater

Treatment:	6
Replication:	4
Design:	Randomized Complete Block design (RCBD)
Spacing:	45 x 20 cm
Plot size:	4.20 m x 6 m
Fertilizer dose:	25:75:60 kg N, P2O5, K_2O /ha
Date of planting:	12/01/2016
Variety:	PNB

Clusterbean was sown on 12/01/2016 during *rabi/summer* 2015-16 following the recommended package of practices. Two common irrigations with a depth of 30 mm with good quality bore well water was applied for establishment through sprinklers. Irrigation treatments were imposed after 15 days of sowing. Thirteen irrigations were given with an average depth of 35.2 mm for each

irrigation and total water applied during the entire cropping period was 541.4 mm which includes effective rainfall of 23.4 mm. Influence of sources of water on plant height was found to be significant with domestic wastewater irrigation with higher plant height (75.15cm) as compared to freshwater (63.48) (Table 3-30). Yield attributes includes number of fruits/plant (70.79), fruit weight / plant (141.57 g) were found to be higher in domestic wastewater followed by treated wastewater alternated with domestic wastewater (67.07 and 134.15 g, respectively). Lesser number of fruits and fruit weight per plant were recorded in case of fresh water alone (58.37 and 119.23 g). Higher yield (8874 kg/ ha) was recorded with application of domestic wastewater as compared to fresh water (5145 kg/ha). However it was on par with treated wastewater alternated with domestic wastewater (7703 kg/ha) (Table 3-31).

Significantly higher net return (Rs 1,34,440/ha) and B:C (4.12) were observed with application of domestic wastewater as compared to all other treatments except treated waste water altered with domestic waste water (Rs.1,11,023/ha and 3.58, respectively). The total water applied including effective rainfall for all the treatments was 541 mm. Higher water productivity was observed with domestic wastewater (164.03 kg/ha-cm) followed by conjunctive use of wastewater treated wastewater with domestic wastewater (Table 3-32 and figure 3-6). The highest and lowest net profit of Rs 2485 and 1106 were obtained per cm use of water in case of domestic wastewater and fresh water irrigated clusterbean. However the combination of TWW with DWW also recorded higher net profit of Rs 2052 per cm water used.

Post harvest soil analysis of influence of the sources of irrigation on cluster bean is under progress. Application of wetland treated wastewater or untreated sewage water in conjunction with good quality water was found to be significantly on par in terms of crop yield, water productivity, net returns and B: C ratio with untreated wastewater irrigation alone.

Table 3-30 Response of cluster bean to sources of water on growth and yield parameters yield and economics

Treatment	**Plant height (cm)**	**Branches/ plant**	**Fruits /plant**	**Fruit weight /plant (g)**	**Yield (kg/ha)**	**Gross return (Rs/ha)**	**Net return (Rs/ha)**	**B:C ratio**
T_1: Domestic wastewater (DWW)	75.15	7.48	70.79	141.57	8874	177480	134440	4.12
T_2: Treated wastewater (TWW)	68.52	6.92	65.87	131.73	6229	124580	81540	2.89
T_3 : Freshwater (FW)	63.48	6.75	58.37	119.23	5145	102900	59849	2.39
T_4 : Freshwater + Domestic wastewater	69.11	7.00	64.34	130.83	6682	133640	90592	3.10
T_5: Freshwater + Treated wastewater	64.60	6.50	65.41	128.68	6202	124040	80992	2.88
T_6: Treated wastewater + Domestic wastewater	71.92	7.67	67.07	134.15	7703	154060	111023	3.58
SEm±	2.85	0.47	2.17	4.34	436	**6754**	6754	0.16
CD (P=0.05)	8.58	NS	6.54	13.09	1243	**23419**	23419	0.47

Market rate of cluster bean Rs. 20/kg

Table 3-31 Water applied, yield and water productivity of cluster bean as influenced by different source of water

Treatment	No. of common irrigation	Depth of common irrigation (mm)	No. of treat-mental irrigation	Mean depth of irrigation (mm)	Effective rainfall (mm)	Total water applied (mm)*	Yield (kg/ ha)	Water productivity (Kg/ha-cm)	Net profit/cm of water (Rs)
T_1: Domestic wastewater (DWW)	2	30	13	35.2	23.4	541.4	8874	164.03	2485
T_2: Treated wastewater (TWW)	2	30	13	35.2	23.4	541.4	6229	115.14	1507
T_3: Freshwater (FW)	2	30	13	35.2	23.4	541.4	5145	95.10	1106
T_4: Freshwater + Domestic wastewater	2	30	13	35.2	23.4	541.4	6682	123.51	1674
T_5: Freshwater + Treated wastewater	2	30	13	35.2	23.4	541.4	6202	114.64	1497
T_6: Treated wastewater + Domestic wastewater	2	30	13	35.2	23.4	541.4	7703	142.38	2052

*Effective rainfall during cropping period is 23.4 mm

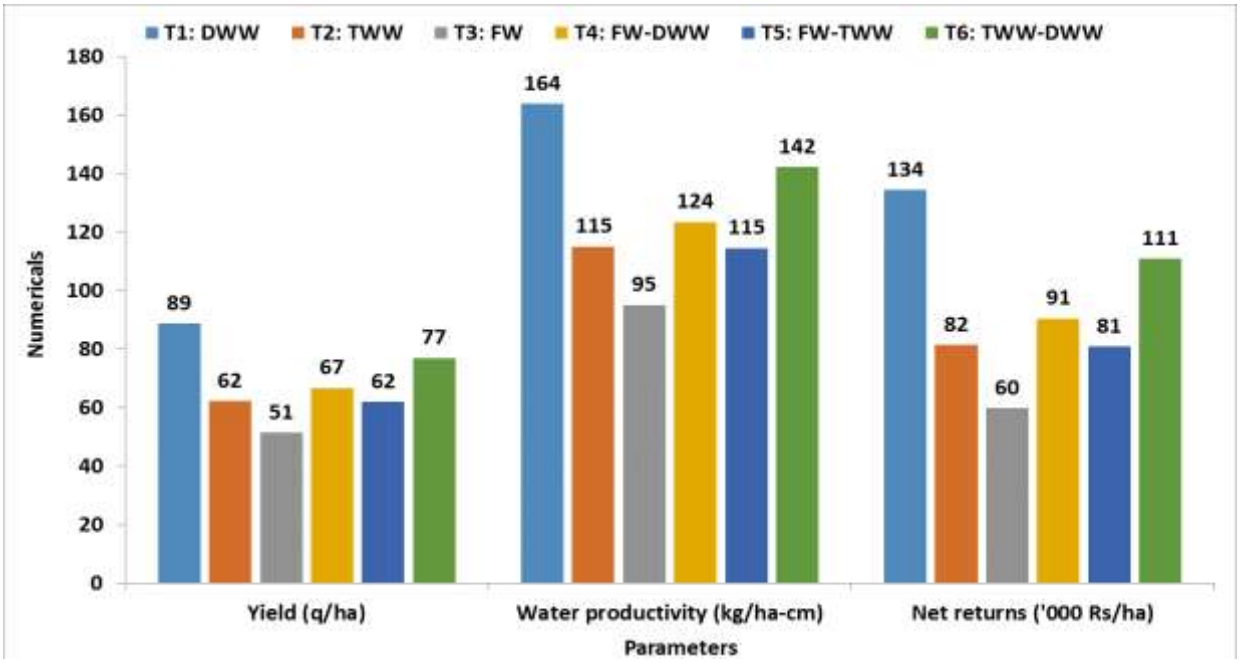


Figure 3-6 Yield, water productivity and net returns as influenced by sources of irrigation in clusterbean

WP3.5: Effect of different sources of water and fertilizer soil health, yield and quality of Bitter gourd

Field experiment to evaluate the effect of the graded levels of fertilizer with different sources of irrigation water on bitter gourd was conducted in Main Agricultural Station, University of Agricultural sciences, Dharwad, Karnataka with following objectives:

1. To know the response of different sources of irrigation and fertilizer levels on growth and yield of bitter gourd
2. To study the effect of sources of irrigation on soil properties.
3. To study the effect of sources of irrigation on water productivity and their economic feasibility

Treatment details:
Main plot:

I_1: Domestic wastewater (DWW)
I_2: Engineered constructed wetland treated wastewater (TWW)
I_3: Freshwater (FW)
I_4: Engineered constructed wetland treated wastewater alternated with domestic wastewater

Sub plot:

F_1: Control (no RDF)
F_2: 50 % RDF
F_3: 75 % RDF
F_4: 100 % RDF

Treatment combination:	16 (4- main plot treatments x 4- sub plot treatments)
Replication:	3
Design:	Split plot
Spacing:	120 X 60 cm
Plot size:	3.0 X 6 m
Fertilizer dose:	62.5:50:0 kg N,P_2O_5, K_2O /ha
Date of planting:	11/01/2016
Variety:	Monika
Season:	*Rabi/Summer* 2015-16

Effect of source of irrigation water (domestic wastewater, treated wastewater, fresh wastewater and conjunctive use of wastewater and treated wastewater) along with graded levels of fertilizer (0, 50, 75 and 100 % RDF) was studied in bitter gourd. Effect of source of irrigation was found to be non significant with respect to plant height. Influence of fertilizer levels on plant height was significant. Application of 100 per cent RDF recorded significantly higher plant height (286.3 cm) as compared to no fertilizer (194.1 cm). Interaction effect with respect to plant height was significant. Combination of domestic wastewater and application of 100 per cent RDF recorded significantly higher plant height (320.8 cm) as compared to other sources of water with no fertilizer, i.e., I2F1, I3F1 and I4F1 (198.9, 179.7 and 176.7 cm, respectively) but it was on par with I1F4 (294.4 cm). Influence of sources of irrigation, on vines/plant was significant. Higher number of vines was observed in domestic wastewater (7.5) which was significantly superior over freshwater (6.2) but it was on par with treated wastewater and domestic wastewater alternated with treated wastewater (6.7 and 7.2). Fertilizer levels significantly influenced the vines/plant to graded levels of fertilizers. Application of 100 per cent RDF recorded significantly higher vines (8.4) as compare to no fertilizer (4.9). Interaction effect was significant with respect to number of vines/plant,

combination of domestic wastewater and application of 100 per cent RDF recorded significantly higher number of vines per plant (9.9) as compared to other sources of water with no fertilizer, ie., I2F1, I3F1 and I4F1 (5.2 ,4.3, 5.5 and 4.8, respectively) but it was on par with I2F4.

Effect of sources of water on number of fruits per plant was not significant. However fertilizer levels showed significant effect with maximum number of fruits in treatment applied with 100 per cent RDF (22.7) as compared to no fertilizer (21.0). Combination of domestic wastewater and application of 100 per cent RDF recorded significantly higher number of fruits per plant (23.2) as compared to other sources of water with no fertilizer, *i.e.,* I1F1, I3F1 and I4F1 (21, 21 and 19.6, respectively) but it was on par with I4F4 (23.0).

Influence of sources of irrigation was significant, higher fruit weight per plant was observed in domestic wastewater (1041.5 g) which is significantly superior over freshwater (920.6) but it was on par with domestic wastewater alternated with treated wastewater (980.8 g). Significant difference was recorded with respect to fertilizer levels on fruit weight per plant. Significantly more number of fruit weight per plant was observed in treatment applied 100 per cent RDF (1287.6 g) as compared to no fertilizer (666.4 g). Interaction effect with respect to fruit weight per plant was significant. Domestic wastewater and application of 100 per cent RDF recorded significantly higher fruit weight per plant (1374.7 g) as compare to all other sources of water with no fertilizer, i.e., I1F1 I2F1, I3F1 and I4F1 (784.0, 601.3, 576.3 and 704.0 g, respectively) but it was on par with rest of the treatment combination. Similar trend was observed with yield/ha. Significantly higher yield was noticed in domestic wastewater (13840 kg/ha) as compared to freshwater, treated wastewater and combination of domestic wastewater alternated with treated wastewater (12484, 12490 and 12593 kg/ha, respectively).

However, higher fruit yield per ha was observed in treatment which received 100 per cent RDF (17525 kg/ha) as compared to treatment which received no fertilizer, F2 and F3 (8612, 11373 and 13896 kg/ha, respectively). Interaction effect source of irrigation water and fertilizer levels differed significantly with respect to yield/ha. Domestic wastewater and application of 100 per cent RDF recorded significantly higher fruit yield (18664 kg/ha) as compared to all other sources of water with no fertilizer, ie. I1F1 I2F1, I3F1 and I4F1 (9378, 8076, 7767 and 9228 kg/ha, respectively) but it was on par with I4F4 (18220 kg/ha).

Higher net returns was noticed in domestic wastewater (Rs 1,84,751/ha) as compared to freshwater, treated wastewater and combination of domestic wastewater alternated with treated wastewater (Rs. 1,57,635; 1,57,745 and 1,59,801/ha, respectively). Fertilizer levels resulted in significant difference with respect to net returns. Significantly higher net returns was observed in treatment which was applied with 100 per cent RDF (Rs 2,56,999/ha) as compared to no fertilizer (Rs 82,063/ha) followed by 75 per cent RDF (Rs 1,85,253/ha). Interaction effect of source of irrigation water and fertilizer levels recorded significant difference with respect to net returns. Domestic wastewater and application of 100 per cent RDF recorded significantly higher net returns (Rs. 2,79,771/ha) as compared to all other sources of water with no fertilizer, ie. I1F1 I2F1, I3F1 and I4F1 (Rs. 97,369; 71,339; 65,136 and 94,384/ha, respectively) but it was on par with I4F4 (Rs. 2,70,902/ha). Effect of sources of water on B:C ratio was significant. Higher B:C ratio was observed with application of domestic wastewater (3.00). However fertilizer levels resulted in significant difference. Significantly higher B:C ratio was observed in treatment applied with 100 per cent RDF (3.75) as compared to no fertilizer, 50 per cent and 75 per cent (1.91, 2.48 and 3.00, respectively). Interaction effect of sources of irrigation water and fertilizer levels was found to be significant. Domestic wastewater and application of 100 per cent RDF

recorded B:C ratio (3.99) as compared to all sources of water with no fertilizer, i. e., I1F1 I2F1, I3F1 and I4F1 (2.08, 1.79, 1.72 and 2.05, respectively).

Table 3-32 Effect of sources of water and fertilizer levels on growth, yield parameters and water productivity of bitter gourd

Treatment details	Plant height (cm)	Vines per plant	No. of fruits per plant	Fruits weight per plant (g)	Yield (kg/ha)	Gross returns (Rs/ha)	Net returns (Rs./ha)	B:C ratio	Water productivity (kg/ha-cm)
I1	250.6	7.5	22.09	1041.5	13840	276801	184751	3.00	234.4
I2	248.2	6.7	22.06	920.6	12490	249795	157745	2.70	211.5
I3	230.9	6.2	21.75	923.5	12484	249685	157635	2.70	211.5
I4	247.6	7.2	21.84	980.8	12593	251851	159801	2.73	213.3
SEm±	7.1	0.2	0.2	17.5	208	4160	4160	0.04	3.5
CD (p=0.05)	24.7	0.7	0.6	60.7	720	14394	14394	0.15	**12.2**
F1	194.1	4.9	21.0	666.4	8612	172247	82063	1.91	145.9
F2	228.4	6.8	22.1	834.9	11373	227459	135616	2.48	192.6
F3	268.5	6.4	21.9	1077.4	13896	277925	185253	3.00	235.4
F4	286.3	8.4	22.7	1287.6	17525	350500	256999	3.75	296.8
SEm±	7.4	0.4	0.3	39.6	490	9803	9803	0.11	8.3
CD (p=0.05)	21.6	1.1	0.8	115.7	1431	28613	28613	0.31	**24.2**
I1F1	221.4	5.2	21.0	784.0	9378	187553	97369	2.08	158.8
I1F2	226.4	7.2	21.8	893.0	12207	244138	152295	2.66	206.8
I1F3	260.1	7.7	22.4	1114.3	15112	302240	209568	3.26	256.0
I1F4	294.4	9.9	23.2	1374.7	18664	373272	279771	3.99	316.1
I2F1	198.9	4.3	22.6	601.3	8076	161523	71339	1.79	136.8
I2F2	254.2	6.7	22.1	787.3	10746	214920	123077	2.34	182.0
I2F3	278.3	6.0	21.2	1015.3	13776	275514	182842	2.97	233.3
I2F4	261.5	9.8	22.4	1278.3	17361	347225	253724	3.71	294.1
I3F1	179.5	5.5	21.0	576.3	7767	155347	65163	1.72	131.6
I3F2	217.9	6.6	22.6	829.7	11171	223413	131570	2.43	189.2
I3F3	257.5	5.7	21.1	1121.3	15144	302881	210209	3.27	256.5
I3F4	268.6	7.1	22.2	1166.7	15855	317100	223599	3.39	268.5
I4F1	176.7	4.8	19.6	704.0	9228	184568	94384	2.05	156.3
I4F2	214.9	6.6	21.9	829.7	11368	227366	135523	2.48	192.6
I4F3	278.1	6.5	22.9	1058.7	11553	231065	138393	2.49	195.7
I4F4	320.8	9.8	23.0	1330.7	18220	364403	270902	3.90	308.6
SEm±	14.8	0.7	0.5	79.3	980	19606	19606	0.21	16.6
CD (p=0.05)	43.1	2.1	1.5	231.3	2861	57227	57227	0.62	48.5

Market price Rs.20/kg

Effective rainfall received during the cropping period was 23.4 mm. In all a total of 19 irrigations were applied to the crop which includes two common irrigation. The total water applied including effective rainfall was 590.4 mm for all the treatments. Source of irrigation water induced difference with respect to water productivity (table 3-32 and 3-33; figure 3-5). Among the sources of water, significantly higher water productivity was noticed in domestic wastewater (234.4 kg/ha-cm) as compared to freshwater, treated wastewater and combination of domestic wastewater alternated with treated wastewater (211.5, 211.5 and 213.3 kg/ha-cm, respectively). Other sources of irrigation water recorded on lower and par water productivity. Water productivity of bitter gourd was significantly influenced by fertilizer level treatments. Application of 100 per cent RDF resulted in significantly higher water productivity (296.8 kg/ha-cm) whereas lower water productivity was recorded with no fertilizer treatment (145.9 kg/ha-cm).

Table 3-33 Effects of sources of water and fertilizer levels on growth, yield parameters and water productivity of bitter gourd

Treatment details	No. of common irrigation	Depth of common irrigation (mm)	No. of treat-mental irrigation	Mean depth of irrigation (mm)	Effective rainfall (mm)	Total water applied (mm)*	Yield (kg/ ha)	Water productivity (kg/ha-cm)	Net profit per cm of water applied (Rs.)
I1F1	2	30	17	30.06	23.04	590.4	9378	158.8	1649
I1F2	2	30	17	30.06	23.04	590.4	12207	206.8	2580
I1F3	2	30	17	30.06	23.04	590.4	15112	256.0	3550
I1F4	2	30	17	30.06	23.04	590.4	18664	316.1	4739
I2F1	2	30	17	30.06	23.04	590.4	8076	136.8	1208
I2F2	2	30	17	30.06	23.04	590.4	10746	182.0	2085
I2F3	2	30	17	30.06	23.04	590.4	13776	233.3	3097
I2F4	2	30	17	30.06	23.04	590.4	17361	294.1	4297
I3F1	2	30	17	30.06	23.04	590.4	7767	131.6	1104
I3F2	2	30	17	30.06	23.04	590.4	11171	189.2	2228
I3F3	2	30	17	30.06	23.04	590.4	15144	256.5	3560
I3F4	2	30	17	30.06	23.04	590.4	15855	268.5	3787
I4F1	2	30	17	30.06	23.04	590.4	9228	156.3	1599
I4F2	2	30	17	30.06	23.04	590.4	11368	192.6	2295
I4F3	2	30	17	30.06	23.04	590.4	11553	195.7	2344
I4F4	2	30	17	30.06	23.04	590.4	18220	308.6	4588

Interaction effect source of irrigation water and fertilizer levels was found to be significant. Domestic wastewater with application of 100 per cent RDF recorded higher water productivity (316.1 kg/ha-cm) as compared to all sources of water with no fertilizer, ie. I1F1 I2F1, I3F1 and I4F1 (158.8, 136.8, 131.6 and 156.3 kg/ha cm, respectively) but it was on par with I4F4, I2F4 and I3F4 (308.6, 294.1 and 268.5 kg/ha-cm, respectively). The highest and lowest net profit of Rs 4739 and 1104 were obtained per cm use of water in case of combined application of domestic wastewater with 100 % RDF and fresh water irrigation with no fertilizer, respectively. However the combination of TWW with DWW combined with 100 per cent RDF recorded higher net profit of Rs 4588 per cm use of water, respectively.

In view of the above results of the study it was observed that, combined application of Domestic waste water with 100% RDF and treated waste water with 100% RDF recorded higher crop yield, water productivity, net income and B:C ratio in bittergourd. Post harvest soil analysis of influence of the sources of irrigation on cluster bean is under progress.

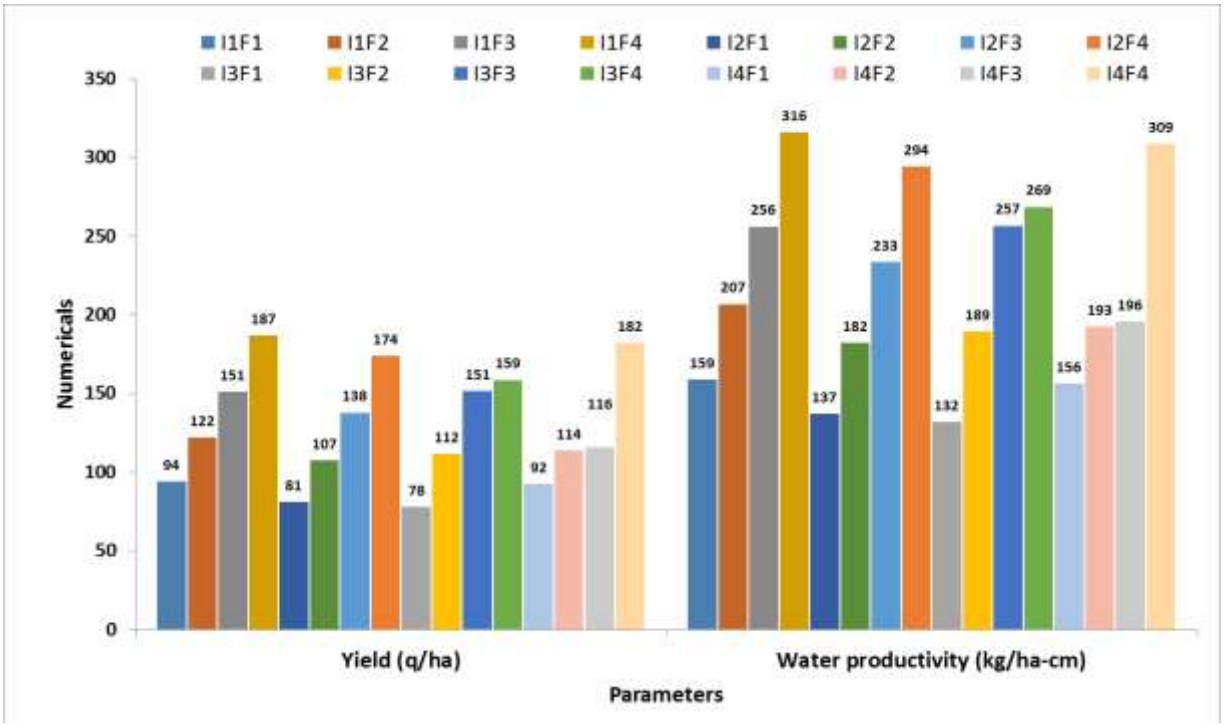


Figure 3-7 Yield and water productivity of bittergourd as influenced by irrigative sources and fertilizer level

WP 3.6: Effect of sources and methods of irrigation on growth, yield, quality and water productivity of okra

Field experiment was laid out with four sources of irrigation and four methods of irrigation. The experiment was initiated during summer 2013-14 and 2014-15 at "H" block, main agriculture research station, UAS, Dharwad with the following objectives:

1. To study the effect of sources and methods of irrigation on yield of okra.
2. To study the effect of sources and methods of irrigation on water productivity in okra with economics.

Treatment Details

Main Plot: Sources of irrigation (I)

I_1- ECWL treated wastewater
I_2- Fresh water [Bore well water]
I_3- Sewage water alternated with fresh water
I_4-Farmers practice (untreated sewage water)

Subplot: Methods of Irrigation (M) at 30 per cent depletion.

M_1-Ridge and furrow (Farmers practice)
M_2- Alternatively alternate furrow irrigation
M_3-Ridge and furrow at 50 % depletion of soil moisture
M_4-Basin irrigation,

Experimental Design:	Split plot
Replication:	3
Treatments:	4 x 4= 16 treatments
Gross plot size:	6 m X 6 m
Variety:	Arka anamika
Experimental site:	UAS, Dharwad

Experiment to evaluate the effect of the deficit irrigation using different irrigation methods and different source of irrigation water on response of okra was conducted for two years 2013-14 and 2014-15. Irrigation was scheduled for okra based on 30 and 50 per cent depletion of soil moisture. Total number of irrigations given was sixteen for 30 per cent depletion and thirteen irrigations for 50 per cent depletion. Initially three irrigations were given with fresh water for all the treatments for better seed germination and proper establishment. The treatment imposition with different sources of water was taken up 15 DAS. The amount of irrigation water applied to each treatment ranged between 42 cm to 52 cm.

Experimental results during 2014 indicated significant difference with respect to the sources of irrigation water (table 3-34). Among the source of irrigation farmers practice (untreated wastewater) recorded significantly higher yield (5.59 t/ha) over other treatments, but it was on par with treated waste water (4.92 t/ha). Similarly in 2015, significantly higher green pod yield was recorded with farmers practice (untreated waste water) treatment (5.06 t/ha) over fresh water. But it was on par with treated wastewater and sewage water alternated with fresh water (4.60 and 4.73 t/ha, respectively). Among the different methods of irrigation, ridges and furrow irrigation recorded significantly higher pod yield (5.36 t/ha) as compared to rest of the treatments during 2014. Similarly trend was recorded with respect to the yield obtained during 2015. Interaction effect with respect to pod yield during 2014 was insignificant, but difference was significant during 2015. Significantly higher pod yield was obtained in combination untreated waste water with basin irrigation (5.91t/ha) compared to all other treatment combinations, lower pod yield was observed in fresh water with ridge and furrow applied at 50 per cent depletion of soil moisture (3.43 t/ha).

Influence of sources of irrigation and methods of irrigation with respect to net returns of the okra was significant (table 3-35). Among the sources of irrigation, crop irrigated with untreated wastewater recorded significantly higher net returns (Rs.64,441/ha) over other treatments, but it was on par with treated wastewater (Rs.52,175/ha) during 2014. While in 2015, net returns per ha was significantly higher (Rs. 67,837/ha) in untreated wastewater over treated water (Rs.51,419/ha). Among the methods of irrigation, during 2014 ridges and furrow irrigation method recorded significantly higher net returns (Rs. 60,852/ha) as compared alternatively alternate furrow irrigation (Rs.34,885/ha). During 2015, significantly higher net returns was observed in basin irrigation (Rs. 74,282/ha) as compared to other treatment combinations, but it was on par with crop irrigated with ridges and furrow irrigation (Rs. 70,893/ha). Interaction effect with respect to B:C ratio during 2014 non significant, but it was significant during 2015. Significantly higher net returns was obtained in combination untreated wastewater with basin irrigation (Rs. 1,01,319/ha) compared to all other treatment combinations. Lower net returns of Rupees 39,976 per ha was observed in fresh water with ridge and furrow at 50 per cent depletion of soil moisture.

Table 3-34 Green pod yield (t /ha) of okra as influenced by sources and methods of irrigation (2014 and 2015)

Treatment	Green pod yield (t /ha)									
	2014					2015				
	Method of irrigation (M)					Method of irrigation (M)				
Source of irrigation (I)	M_1	M_2	M_3	M_4	Mean	M_1	M_2	M_3	M_4	Mean
I_1 Treated wastewater	5.58	3.89	4.82	5.38	4.92	4.60	3.87	3.61	4.45	4.60
I_2 Sewage water alternated with fresh water	5.10	3.69	4.15	4.29	4.31	4.73	3.56	3.54	3.82	4.73
I_3 Fresh water	4.92	3.46	3.92	4.43	4.18	4.34	3.75	3.43	5.10	4.34
I_4 Farmer's practice (untreated sewage water)	5.85	5.47	5.38	5.68	5.59	5.06	3.61	3.70	5.91	5.06
Mean	5.36	4.13	4.57	4.94		4.60	3.87	3.61	4.45	
For comparing means of	S.Em±		CD at 5 %			S.Em±		CD at 5 %		
Source of irrigation (I)	0.27		0.92			0.13		0.45		
Method of irrigation (M)	0.11		0.33			0.12		0.35		
I at same level of M	0.22		NS			0.24		0.69		

Table 3-35 Net returns (Rs. /ha) of okra as influenced by sources and methods of irrigation (2014 and 2015)

Treatment	Net returns (Rs/ha)									
	2014					2015				
	Method of irrigation (M)					Method of irrigation (M)				
Source of irrigation (I)	M_1	M_2	M_3	M_4	Mean	M_1	M_2	M_3	M_4	Mean
I_1 Treated wastewater	65185	31452	50809	61252	52175	68619	50304	43805	64819	56887
I_2 Sewage water alternated with fresh water	55585	27385	37342	39385	39925	71915	42617	42078	49067	51419
I_3 Fresh water	52052	22852	32809	42319	37508	62952	48042	39976	81925	58224
I_4 Farmer's practice (untreated sewage water)	70585	57852	62009	67319	64441	80084	43805	46142	101319	67837
Mean	60852	34885	45742	52569		70893	46192	43000	74282	
For comparing means of	S.Em±			CD at 5 %		S.Em±			CD at 5 %	
Source of irrigation (I)	5668			19614		3285			11367	
Method of irrigation (M)	2153			6283		2970			8669	
I at same level of M	4305			NS		5940			17338	

Data pertaining to the B:C ratio as influenced by the source and methods of irrigation of okra during 2013-14 and 2014-15 are presented table 3-36. Significantly higher B:C ratio was observed in crop irrigated with farmers practice (untreated waste water) (2.40) over treated waste water and fresh water (1.81 and 1.87) but it was on par with sewage water alternated with fresh water (2.13). While in 2015, B:C ratio was significantly higher in farmers practice (untreated waste water) (2.46) over, sewage water alternated with fresh water (2.11) but it was on par with fresh water and treated water (2.23 and 2.28). Among the methods of irrigation, during 2014 ridges and furrow irrigation was recorded significantly higher B:C ratio (2.32) as compared to all other treatments. Similarly, during 2015, significantly higher B:C ratio was observed in basin irrigation (2.61) as compared to other treatment combinations, but it was on par with crop irrigated with ridges and furrow irrigation (2.53). Interaction effect was non significant with respect to B:C ratio during 2014, but it was significant during 2015. Significantly higher B:C ratio was obtained in combination untreated waste water with basin irrigation (3.19) compared to all other treatment combinations, least B:C ratio was observed in fresh water with ridge and furrow at 50% depletion of soil moisture (1.88).

Table 3-36 B:C ratio of okra as influenced by sources and methods of irrigation (2014 and 2015)

Treatment	**B:C ratio**									
	2014					**2015**				
	Irrigation Methods (M)									
Irrigation Source (I)	**M_1**	**M_2**	**M_3**	**M_4**	**Mean**	**M_1**	**M_2**	**M_3**	**M_4**	**Mean**
I_1 Treated wastewater	2.41	1.68	2.11	2.32	2.13	2.48	2.09	1.95	2.40	2.23
I_2 Sewage water alternated with fresh water	2.21	1.59	1.82	1.85	1.87	2.55	1.92	1.91	2.06	2.11
I_3 Fresh water	2.12	1.49	1.72	1.91	1.81	2.38	2.05	1.88	2.79	2.28
I_4 Farmer's practice (untreated sewage water)	**2.52**	2.25	2.37	2.45	**2.40**	2.73	1.95	2.00	3.19	**2.46**
Mean	**2.32**	1.75	2.01	2.13	2.05	2.53	2.00	1.93	**2.61**	
For comparing means of	**S.Em±**		**CD at 5 %**			**S.Em±**		**CD at 5 %**		
Source of irrigation (I)	0.12		0.43			**0.07**		**0.25**		
Method of irrigation (M)	0.05		0.14			**0.06**		**0.19**		
I at same level of M	0.09		NS			**0.13**		**0.38**		

Data pertaining to the water applied, yield, water productivity and net return as influenced by the source and methods of irrigation of okra (mean of two years) are presented table 20 and fig 6. Based on two year of study (2014 and 2015), it was found that, basin method of irrigation and furrow irrigation with untreated waste water recorded higher okra green pod yield (5800 and 5460 kg/ha, respectively), water productivity (110.58 and 112.46 kg/ha-cm, respectively) and net income (Rs 84300 and 75360/ha, respectively). Highest (Rs1608) and lowest (Rs731) net profit per cm of water used was recorded with untreated sewage water irrigation applied through basin method of irrigation (I4M4) and fresh water application through alternatively alternate furrow irrigation (I3M3).

Highest (Rs 1608) and lowest (Rs 731) net profit per cm of the water used was recorded with application of untreated sewage applied through basin method of irrigation and application of fresh water through alternatively alternate method of irrigation, respectively.

Table 3-37 Total water applied, yield and water productivity of okra under different methods and source of water [mean data of 2014 and 2015]

Treatment	Total water applied (cm)	Okra green pod yield [kg/ha]	Water productivity (kg/ha-cm)	Net return (Rs/ha)	Net profit per cm of water used (Rs/cm)
$T_1 (I_1M_1)$	52.02	5090	97.86	66900	1286
$T_2 (I_1M_2)$	42.47	3880	91.36	40860	962
$T_3 (I_1M_3)$	48.96	4220	86.09	47280	966
$T_4 (I_1M_4)$	52.52	4920	93.58	63060	1201
$T_5 (I_2M_1)$	50.96	4920	96.45	63780	1252
$T_6 (I_2M_2)$	46.52	3630	77.93	34980	752
$T_7 (I_2M_3)$	51.09	3850	75.27	39720	777
$T_8 (I_2M_4)$	51.40	4060	78.89	44220	860
$T_9 (I_3M_1)$	52.86	4630	87.59	57480	1087
$T_{10} (I_3M_2)$	44.60	3610	80.83	35460	795
$T_{11} (I_3M_3)$	49.85	3680	73.72	36420	731
$T_{12} (I_3M_4)$	49.34	4770	96.58	62100	1259
$T_{13} (I_4M_1)$	48.51	5460	112.46	75360	1553
$T_{14} (I_4M_2)$	44.08	4540	103.00	50820	1153
$T_{15} (I_4M_3)$	47.88	4540	94.82	54060	1129
$T_{16} (I_4M_4)$	52.41	5800	110.58	84300	1608

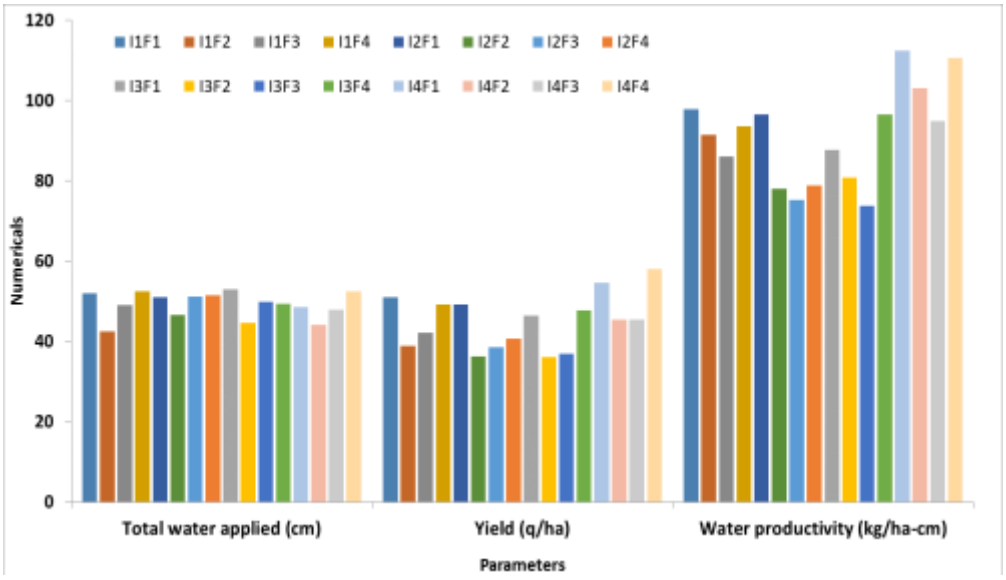


Figure 3-8 Total water applied, yield and water productivity of okra as influenced by irrigated sources and methods

Studies on the effect of ECWL treated wastewater on vegetable production in comparison to untreated wastewater and freshwater for Brinjal (*kharif* , 2016) – Cluster bean (*rabi/summer*, 2016-17) and Chilli (*kharif* ,2016) - Ridge gourd (*rabi/summer* -2016-17) cropping sequence is under progress. Further nutrient dynamics under different source of irrigation and graded levels of fertilizers in Bittergourd (*rabi/summer*, 2015-16) - French bean (*kharif*,2016); Chilli (*rabi/summer*,2015-16) – Okra (*kharif*,2016) and Brinjal (*rabi/summer*, 2015-16) – Sweet corn (*kharif*, 2016) cropping sequence is under progress.

3.2.6 Sweet corn cultivation using bio-treated wastewater

A common variety i.e. F1 Hybrid Sweet Gold 95 used by local farmers was selected for studying the reuse potential of bio-treated distillery effluent. The experiment was designed to study in comparison with irrigating anaerobic treated distillery effluent keeping fresh water as control. The experiment was carried out in randomized block design (RBD) with 7 replicates in each of the three treatments where T1 is bio-treated distillery effluent, T2 anaerobic treated distillery effluent and T3 fresh water as control.

Figure 3-10 ***Sweet corn cultivation at Vuyyuru reusing bio-treated distillery effluent***

The experimental field within the industrial premise was prepared using tractor mounted disc plough and leveling was done before laying down the replicates as per the plan. Area of cultivation of sweet corn was 262.5 m^2, 1512 m^2, and 1512 m^2 for cycle 1 from Sep 14 to Dec 14, cycle 2 from Feb 15 to May 15 and cycle 3 from Sep 15 to Dec 15 respectively. Seeds were dibbled at a depth of 2 to 3 cm in the soil followed by slight irrigation using fresh water across the treatments to ensure proper and uniform germination. About 10g of each Urea, Potash and Super phosphate were given to each plant of all the three treatments on the 20 days after sowing (DAS). The same amount of Potash and Urea was applied on the 50th DAS. Chlopyrifos 50% was sprayed on 40th DAS and subsequently on 55th day to control shoot borer. In addition, 5 g of Forge 3 was applied on 40th DAS for each crop. Hand weeding was done as and when required. Irrigation was done as alternate day wetting by localized irrigation method wetting the root zone only. The application rate was adjusted based on the crop growth where 2 L/day was

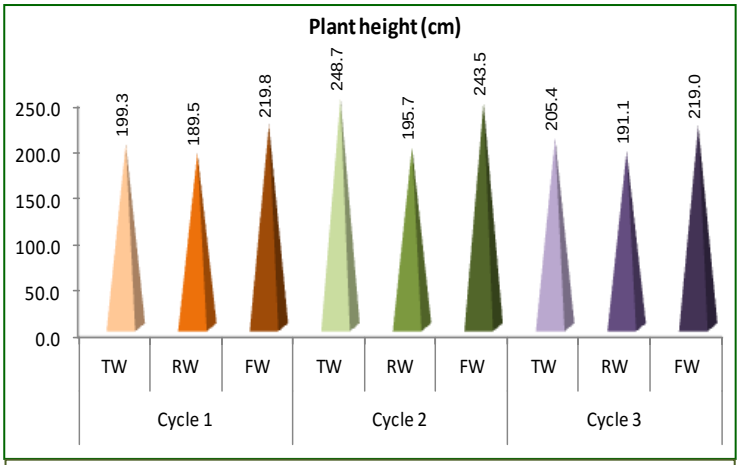


Figure 3-9 Plant height comparisons of 3 treatments & 3 cycles

applied till 50th DAS after which it was increased to 4 L/day. Harvesting was done by observing maturity signs like full size green cobs with tight husk, dry brown silks, smooth and plumpy kernels which exude milky liquid when punctured. The cobs were harvested and the biometric observations were recorded.

The sweet corn cultivation of three cycles was monitored periodically for growth characteristics and yield attributes. Three plants per replicate were tagged randomly in each treatment and their biometrics was recorded at 30 DAS, 60 DAS and during harvest. Average plant height in T1, T2 and T3 of cycle 1 and cycle 3 was similar while cycle 2 had better plant height of 248.7 cm in T2 even greater than T3 which was 243.5 cm. Other parameter like number of leaves was 11 on an average for three cycles and three treatments, number of cobs was 1, index leaf length was 69 cm and width was 6.9 cm. These parameters were near similar in cycle 1 and 3 of all the treatments. It is observed in all the biometric measurements the performance of raw water irrigated plants are lesser compared to bio-treated and fresh water. In T1 and T3 the parameters were measured almost similar. Tough the biometric parameters of plants in T1, T2 and T3 of cycle 2 performed well the yield attributes varied. The tagged plants were uprooted after harvest and were sun dried initially and subsequently dried in hot air oven at 60° C. The weight of dried plant was measured and the weights ranged as T3>T2>T1.

Similar to the biometrics studied the yield attributes were also studied and fig 43 shows the comparison details of yield attributes of T1 and T2 of three cycles. The number of cobs per plant did not show any significant variation in different treatments and in the different cycles. However, significant differences were observed in cob length, cob width, cob weight, No. of kernels, 100 kernels weight and total kernels weight in varied sources of irrigation. The cob length in T1 was 17.8% higher than in T2 of cycle 1 and similarly greater in cycle 2 and cycle 3. The cob girth of T1 was 9.4% greater than that in T2 of cycle 2 and a similar trend was observed in other cycles. The cob weight was always greater in T1 than T2 and was the maximum (292.3 g) in cycle 3 and the minimum (97.5 g) was observed in T2 of cycle 2. The sweet corn kernels were removed from the cob and counted to compare with T1 and T2 were the highest was 562.3 in T1 of cycle 2 and the lowest was 111.5 in T2 of cycle 2. However on an average with all the three cycles, the number of kernels in T1 is 36.8% greater than T2. The 100 kernel weight showed no much significant difference between the cycles however a very negligible difference was observed

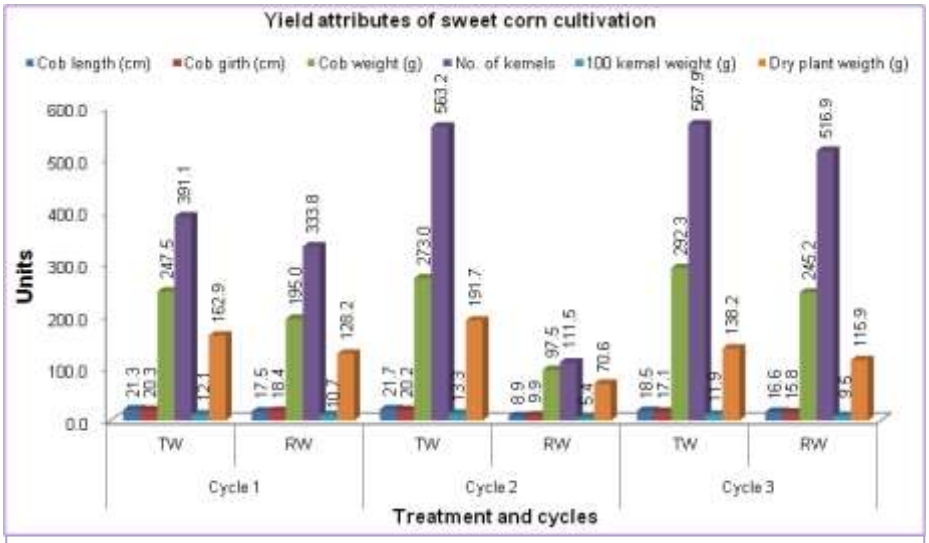


Figure 3-11 Compared yield attributed of 3 cycles of sweet corn cultivation

between the treatments. The dry weight of whole plant was also measured and observed that the weight ranged from 138.2 g to 19.7 g as the lowest and highest in T1 while in T2 it was 70.6 g to 128.2 g of lowest and highest weights of three cycles.

Overall yield of sweet corn compared with three cycles, cycle 3 had maximum yield of 18.7 t/ha of sweet corn while cycle 2 and cycle 1 had 13.6 t/ha and 5.9 t/ha combining all the three treatments yield. However when comparing within treatments of all the cycles the yield of sweet corn irrigated with bio-treated distillery effluent i.e T1 had better yield of 16.5 t/ha while T2 had 7.2 t/ha and T3 had 14.4 t/ha of yield. On a percentage basis 12.7% greater yield is obtained in bio-treated distillery effluent compared with fresh water, while 56.4% greater yield is obtained in irrigating bio-treated distillery effluent compared with anaerobic treated distillery effluent.

The crop analysis is mainly carried out to study the accumulation of macro and micronutrients as an effect of irrigating sweet corn with bio-treated distillery effluent T1, anaerobic treated distillery effluent T2 and fresh water T3 as control. The bio-accumulation of the nutrients becomes toxic in plants, in turn for animals and human when they are available in excess than that the plant needs. The cobs were harvested and the corn kernels from cobs of each treatment were separated and the samples were dried, ground and passed through 2 mm mesh. Similarly the stalk and leaves of the sweet corn crop was oven dried at 50° C for 24 hours. Dried samples were ground and sieved. 10 g of each prepared samples were sent to ICRISAT Center in Patancheru for analysis. The samples were analyzed for N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu and B on dry weight basis. The samples were analyzed using autoanalyzer, atomic absorption spectrometer and ICP-AES with respect to the parameters.

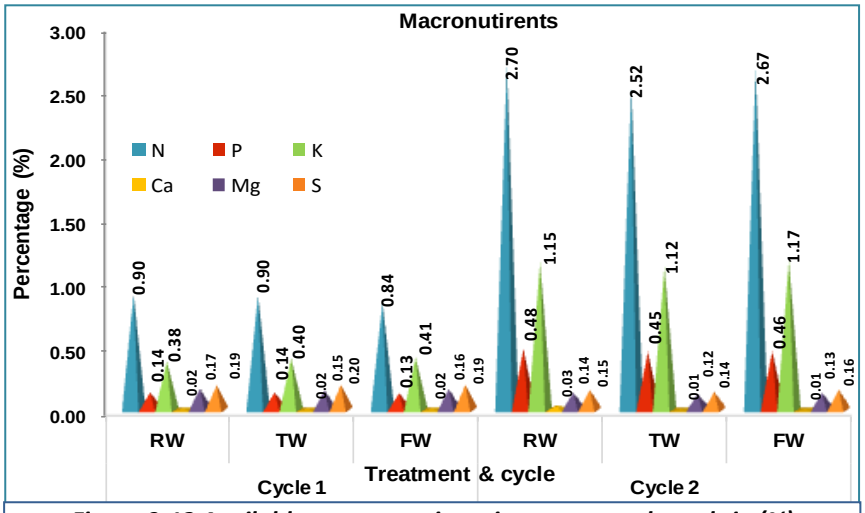


Figure 3-12 Available macro nutrients in sweet corn kernels in (%)

The application of waste water for irrigation leads to changes in soil and in the uptake by plants. The concentration of macronutrients taken up by sweet corn in different treatments and two different cycles is shown in fig. 3-10. It is observed that the concentration of the macronutrients N, P, K, Ca, Mg and S do not show significant difference with respect to difference in treatments with anaerobic treated distillery effluent and fresh water in both the cycle. The macro nutrients concentration are ordered as N >K >P >S >Mg> Ca available in both the cycles and in different treatments. The macro nutrients N, P, K and Ca are below the sufficiency level ranging as 2.50% – 3.50%, 0.25% – 0.40%, 1.6% –2.5%, and 0.2% – 0.8%

respectively in the first cycle while it was improved in the second cycle. Mg and S in both the cycles were within the sufficiency level ranged as 0.12% – 0.50 % and 0.12% - 0.40% respectively. In cycle 2 the analysis of sweet corn stalk along with leaves were also carried out for the purpose of those to be used as fodder for cattle, and the results revealed that the macro nutrients all ranged below the sufficiency level and not found to be toxic for the consumption of cattle.

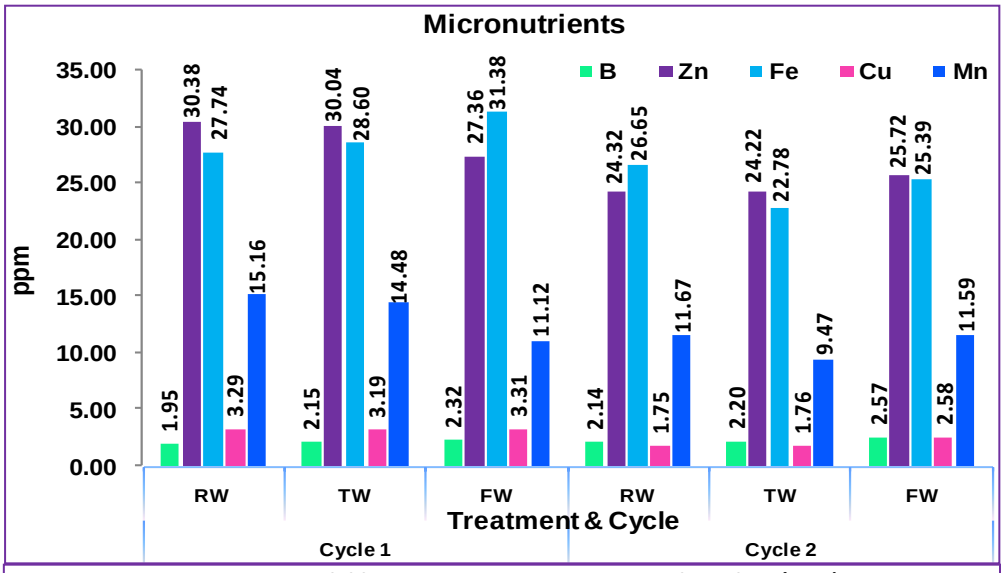


Figure 3-13 Available micro nutrients in sweet corn kernels in (ppm)

Micronutrients in plants play an essential role in balancing the plant nutrition. Deficiency in micronutrients mainly limits the growth of plant even if other macro nutrients are adequate. Fig. 3-11 shows the graphical representation of varying concentration of micronutrients with respect to different irrigation treatments given for sweet corn in cycle 1 & 2. The micro nutrients B, Fe, Cu, and Mn were below the sufficiency level ranging as 3 – 20 ppm, 30 – 250 ppm, 4 – 20 ppm and 15 – 150 ppm in both the cycles respectively while Zn was found to be within the sufficiency level 16 – 50 ppm in both the cycles. The statistical analysis revealed that the micro nutrients present in sweet corn kernels showed no significant difference between treatments. However in cycle 2 the micronutrients available in plant stalk was also analyzed and the results were below the sufficiency level which indicates no toxicity in the stalk due to available micro nutrients and does not hinder in fodder for cattle consumption.

The overall results on crop analysis of two cycles on irrigating sweet corn with the bio-treated, anaerobic treated distillery effluent and fresh water showed no significant difference in the uptake of macro and micronutrients. However the crop analysis of sweet corn kernels showed that the uptake of elements was near to sufficiency range which is evident that it is not harmful or toxic to plant itself, animals or human consumption. To know the safe use of stalk as fodder for cattle, the stalk and leaf analysis also revealed nutrients near the sufficiency range and nothing to be toxic. The crop analysis results comparing with cycles 1 and 2 reveals that adequate addition of macro and micro nutrients plays a major role in contributing to the plant yield. The analysis for other toxic elements was not carried out since they were not present in the irrigated water.

Sugarcane cultivation reusing treated sugar effluent

The second cycle fish culture was initiated on 4 Nov 2015. Based on the observations from first fish crop harvest, the better performing *Rohu* sp. alone was culture with a stocking density of 1.7/m^2. The initial weight of fingerlings ranged from 80 to 120g. Fishes are fed on daily basis with rice bran at 5% feeding level to the body weight. They are monitored on regular intervals for growth performance and better health. The length and weight of fishes have doubled in 3 months from the time if stocking. Regular monitoring of water quality and maintenance of water levels are taken care for obtaining best growth and conversion to give good yield form fish culture.

5.3 Sugarcane cultivation

5.3.1 Details of irrigation and cultural operations

Irrigation for sugarcane is done by furrow irrigation method and the frequency of irrigation is set as 15 and 10 days for clay soil and sandy soil respectively. The quantity of irrigation and its frequency was arrived based on the crop water requirement, soil moisture content and water holding capacity of clay and sandy soil and they also vary with the growth stages of sugarcane. Fertilizers, pesticides and weedicides were given, initially on 18 DAS bio-fertilizer consisting of nitrogen and phosphorous bacteria, mycorrhiza and haritha growth promoter was applied at the rate of 3 kg per replicate. Fertilizer was applied twice in the sugarcane growing cycle, about 25 kg of urea, 50 kg of single super phosphate and 10 kg of potash was applied 67 DAS and 15 kg of urea, 20 kg of single super phosphate and 10 kg of potash was applied on 140 DAS. Weedicide like gramoxon 200 g and 200 ml of fermoxon was mixed with 120 L of fresh water and sprayed and a manual weeding was done after 60 days of sowing. Attack by early shoot borer was observed at the end of second month after sowing and it was controlled by applying coragen 6 ml mixing in 120 L of fresh water. Bund mixing and a manual weeding were done three months after sowing. Tying knots of the sugarcane leaves was done end of six months after sowing.

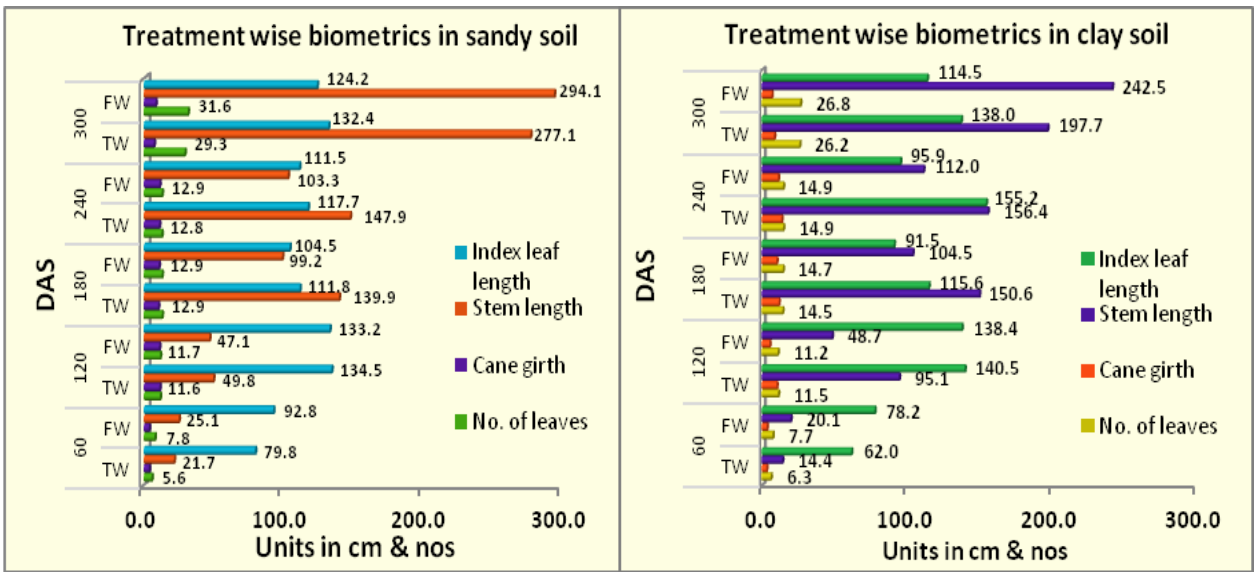


Figure 3-14 Biometrics of sugarcane in clay and sandy soil

5.3.2 Biometrics of sugarcane

Initially the germination percentage was calculated as 65%, 65.8%, 76.8% and 73.7% for clay soil irrigated with treated water, clay soil irrigated with fresh water, sandy soil irrigated with treated water and sandy soil irrigated with fresh water respectively. The biometric parameters like (i) index leaf length, (ii) stem length, (iii) cane girth and (iv) number of leaves were monitored at regular intervals of plant growth cycle. Data of biometrics taken on 60 DAS, 120 DAS, 180 DAS, 240 DAS and 300 DAS are presented in fig. 3-12 attributing to the difference with treatment in clay soil and sandy soil with difference in treatment. The overall observation reveals that the stem length seems to be better in fresh water sandy soil as compared to clay soil. While considering cane girth and index leaf length, sugarcane grown in clay soil irrigated with treated water was greater. The number of leaves in sugarcanes grown in sandy soil had greater numbers compared to clay soil in both the treatments. Between treatments comparison shows that there is high variation in stem length followed by index leaf length while the variation in number of leafs is not that significant both in clay as well as in sandy soil. Variation in cane girth is negligible in both the soil types. Considering the differences with respect to DAS it is observed that the cane girth has shown a decreasing trend closer towards the maturity stage (270 – 360 DAS) while the other parameters have an increasing trend in all the growth stages.

5.3.3 Sugarcane harvest

Figure 3-15 ***Sugarcane cultivation, biometric measurement and harvest***

Sugarcane crop was cultivated and grown over a period of 353 days. Before the harvest, leaf sheaths were collected and analysed to find the moisture content in leaf. The moisture content was greater than 80% in all the plots. Moisture content of the leaf sheaths are related to the growth and indicative of high reducing sugar content. The yield attributes of sugarcane for different soil type and different treatments is presented in fig. 3-14. The figure indicates that the yield of millable of cane in clay soil irrigated

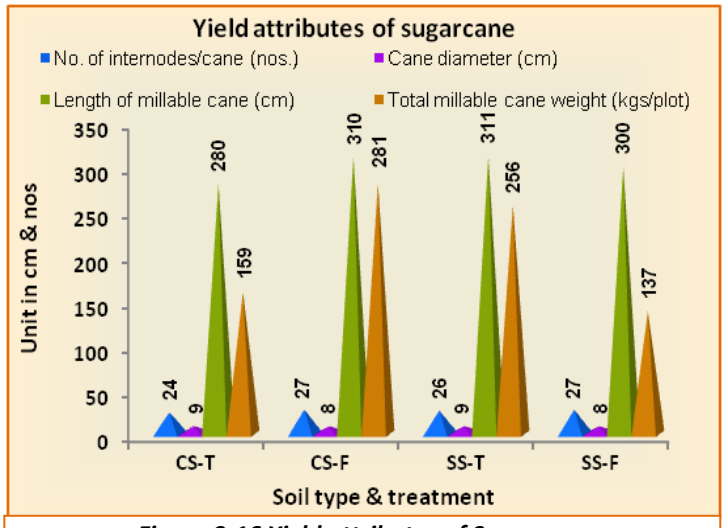


Figure 3-16 Yield attributes of Sugarcane

with fresh water > sandy soil irrigated with treated water > sandy soil irrigated with fresh water > clay soil irrigated with treated water. While length of millable cane, also follows the same trend. The number of internodes and the cane girth in different soil type and different treatment does not vary. The productivity and juice quality of sugarcane varies with respect to climatic conditions affecting the quality of sugar. In India the average productivity in tropical and sub tropical region are 077 t/ha and 63 t/ha respectively. The overall yield of sugarcane in different soil type and treatment is 87.7 t/ha in clay soil irrigated with fresh water, followed by 80.1 t/ha in sandy soil irrigated with treated water, 49.7 t/ha in clay soil irrigated with treated water and the least 42.8 t/ha in sandy soil irrigated with fresh water.

Table 3-38 Quality characteristics of sugarcane juice analysis

Sample	Cane weight (Kg)	Juice weight (Kg)	Polarity	Brix %	Sucrose %	Commercial cane sugar %	Purity %
Sandy soil - FW	2.6	1.18	70.18	17.99	17.05	12.51	94.86
Sandy soil - TW	3.0	1.38	77.74	19.66	18.77	13.82	95.55
Clay soil - FW	2.6	1.13	74.22	19.20	17.97	13.11	93.60
Clay soil - TW	2.9	1.35	70.64	18.46	17.15	12.47	92.87

Quality characteristics of sugarcane is as important as that of the productivity, hence juice analysis of sugarcanes grown in each treatment was analyzed separately for various parameters at Vuyyuru Sugarcane Research Institute, that helpe0d determine the quality of sugarcane and the results are shown in table 13. Brix % is the total solids content present in the juice including sugar and non-sugar and ranges from 15% to 23%, while purity % is the amount of sucrose present in the total solids content in the juice. Genreally minimum of 16% sucrose and 85% of purity is accepted. Commercial cane sugar % is the total recoverable sugar percent in the cane. From the table 13 it is understood that sandy soil irrigated with treated water gives a higher percentage of commercial cane sugar percentage.

Juice analysis of sugarcane

5.4 Modeling the impact of treated SE on soil and crop using SALTMED

The present study aimed at analyzing the impact of rawwastewater and bio-treated water onsoil physico-chemical properties in the process of sugarcane cultivation.Models are very useful tools in agriculture water management. It helps in predictionof yield, measurement of soil salinity, irrigation schedulingand estimation of crop water requirements. SALTMED model has been developed as a generic model which can be used for a variety of irrigation systems, soil types, soil stratifications, crops, water application strategies and different water qualities.

In water 4 crops project, primary treated wastewater from the Lakshmipuram sugar factory, was treated using hybrid model constructed wetland. The water from the constructed wetland passes through the fish tank where tertiary treatment takes place. Tertiary treated water from the fish tank is used for sugarcane cultivation in four different treatment plots. SALTMED model was used to simulate the salinity, nitrogen and moisture profiles for

sugarcane cultivation in four treatment plots namely fresh clay, treated clay, fresh sandy and treated sandy. The type of irrigation chosen was furrow irrigation.

The daily meteorological data (evaporation by pan evaporimeter, daily rainfall, wind speed, sunshine hours, maximum and minimum temperature) were obtained from the Meteorological Station located at the Sugar Industry in Lakshmipuram. The irrigation data like daily application rate (l/hr), irrigation start time, irrigation end time, fertilization start and end time (if present), salinity (dS/m), nitrogen (mg/l) and urea (mg/l) content, the daily nitrogen input data in g-N/m^2 in the form of NO_3fertilizers or NH_4fertilizers, combination of both fertilizers and urea fertilizer content were measured during irrigation practices in the field in Lakshmipuram.

The latitude, longitude value and elevation above mean sea level of Lakshmipuram area were also obtained. Profiles of the soil layer depths were also obtained. The crop coefficient (K_c), basal crop coefficient (K_{cb}), fraction cover Fc , osmotic pressure at which crop growth is reduced by 50% (π50), minimum root depth (m), maximum root depth (m), unstressed crop yield (t ha^{-1}) for sugarcane crop were obtained from the Sugarcane Research Institute, Vuyyur.

SALTMED model was run for a period from 10.12.2014 to 5.12.015 to assess its performance in simulating the salinity, soil moisture and soil nitrogen. The salinity, nitrogen and moisture profiles were obtained from the SALTMED model as output. Calibration of SALTMED model was carried out from 10.12.2014 to 5.11.2015. The graphical output indicates the salinity, moisture and nitrogen profiles. Electrical conductivity was measured in three layers viz 0-30 cm, 30-60 cm and 60-90 cm in all the four treatments like fresh clay, treated clay, fresh sandy and treated sandy.

5.4.1 Clay soil irrigated with fresh water

5.4.1.1 Soil moisture plot

From the soil moisture plot in fig. 3-18 it could be inferred that the soil moisture was minimum (0.040 – 0.064) upto 1.4 m. The moisture trend was such that the soil moisture increases gradually with increase in depth from 1.4 m to 2 m. The reason for the increase in soil moisture beyond 1.4 m may be due to absence of sub surface drainage and also due to water holding capacity of clay soil.

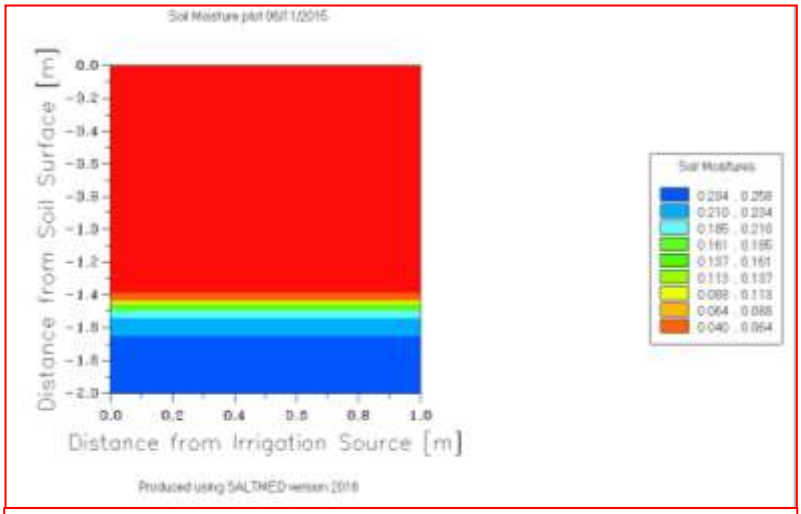


Figure 3-17 Clay soil-Fresh water: Soil moisture

5.4.1.2 Soil salinity plot

The fig. 3-19 above indicates that at the top most layer of the soil the salinity was in the range of 8.8dS/m to 3.6dS/m and later on with the increase in depth, the salinity decreases and remains constant from the depth of 0.3 to 1.4 m with salinity range of 2.6 dS/m to3.6dS/m. The salinity level at the depth of 1.4 m to 2.0 mis 0.5dS/m to 1.5dS/m. When compared with the measured EC values, the high level of salinity at the top layer shows that model overestimated the salinity.

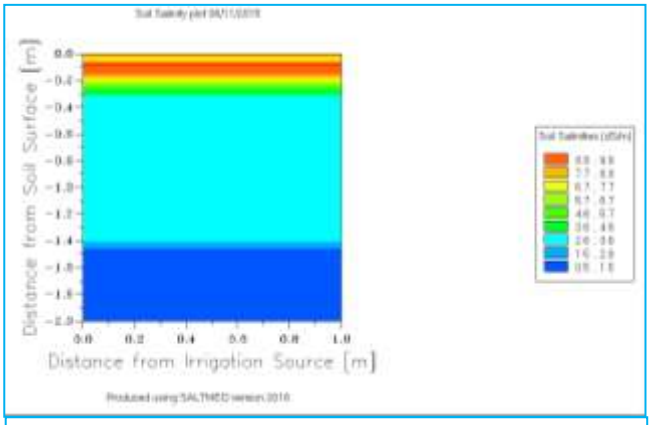


Figure 3-18 Clay soil-Freshwater: Soil salinity

5.4.1.3 Soil nitrogen plot

Figure 3-19 shows the graph representing soil nitrogen variation at various depths. The nitrogen content varies from 321 mg/l to 41 mg/l in the top 30cm depth then it decreases and attains constantrange of 0 mg/l – 41 mg/l upto 2 m.

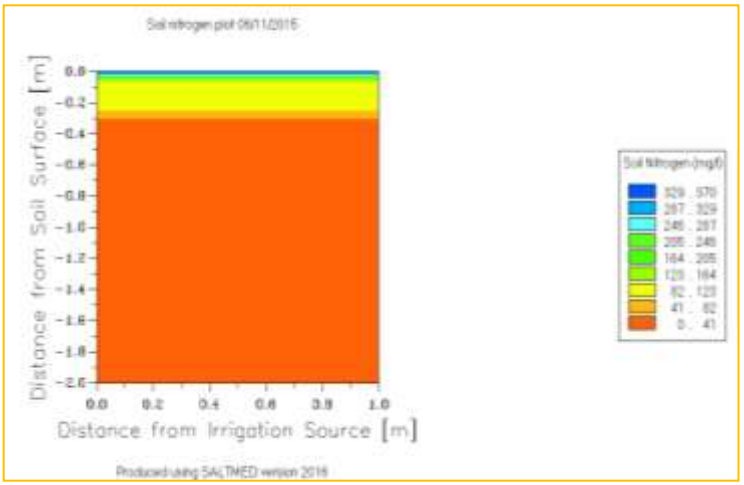


Figure 3-19 Clay soil-Freshwater: Soil nitrogen

5.4.2 Clay soil irrigated with treated water

5.4.2.1 Soil moisture plot

It is observed from fig.3-20 the soil moisture gradually increases with increase in depth and is maximum at the depth of 1.7m. This might be due to clayey soil, which naturally has the tendency of water logging. The moisture level can be corrected by adjusting the irrigation rate or frequency in order to get uniform moisture profile and also proper surface drainage could be provided.

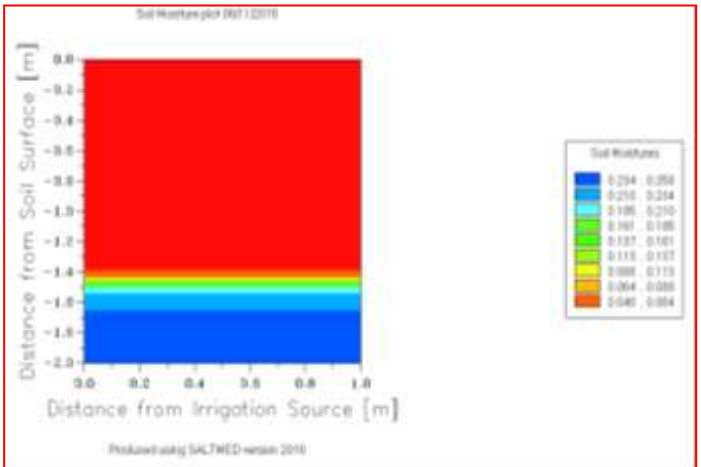


Figure 3-20 Clay soil-Treated water: Soil moisture

5.4.2.2 Soil salinity plot

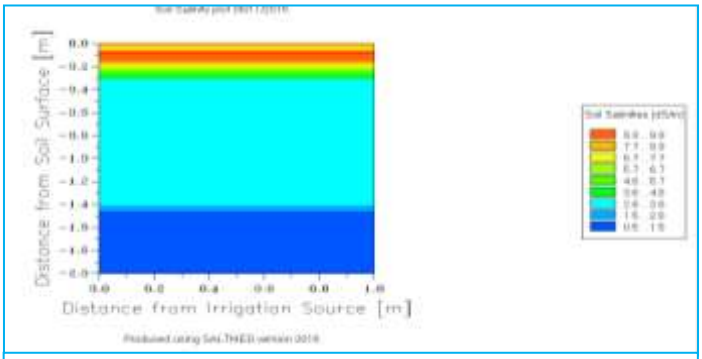


Figure 3-21 Clay soil-Treated water: Soil salinity

From fig. 3-21 it is clear that simulated soil salinity is maximum at the depth of 0.2 m (3.5dS/m to 4.5dS/m), which matches with the observed salinity of 3.55 dS/m to 1,22dS/m. This salinity level exceeds the FAO prescribed standard of 2 dS/m. Hence clay soil with treated wastewater irrigation needs proper drainage and leaching of the soil.

5.4.2.3 Soil nitrogen plot

Figure 3-40 shows the variation in soil nitrogen at various depths. The nitrogen content varies from 82 mg/l to 123 mg/l in the top 30 cm depth then it decreases and attains constant range of 0 mg/l – 41 mg/l upto 2 m.

5.4.3 Sandy soil irrigated with fresh water

5.4.3.1 Soil moisture plot

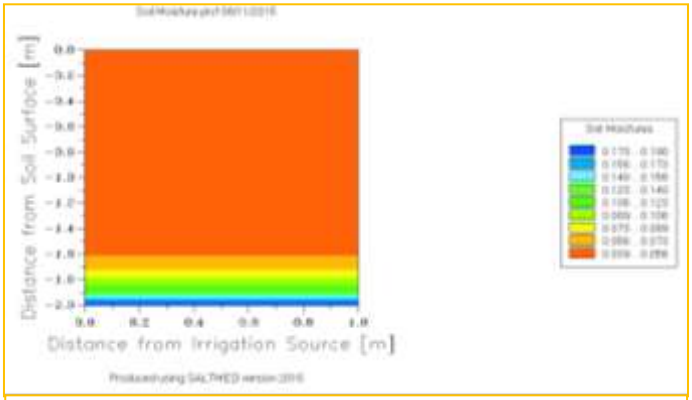


Figure 3-22 Sandy soil: Soil moisture

Figure 3-22 shows that the soil moisture was minimum (0.039-0.056) from the surface level to the depth of 1.6 m and is maximum at the depth of 2.0 m.This may be due to higher water table in that region. Sub-surface drainage could be adopted to overcome this problem.

5.4.3.2 Soil salinity plot

From the figure 3-23, it can be inferred that soil salinity was varying gradually in the range from 4.52dS/m to 5.09dS/m upto 0.30 m then the salinity increases to a range of 5.09 dS/m to 5.66 dS/m from 0.3 m to 0.6 m in depth. Beyond 0.6 m the salinity gradually decreases. The salinity ranged between 2.22 dS/m and 2.79 dS/m in the layer of 0.6 m to 0.9 m. The primary reason would be the accumulation of salt at the surface region.

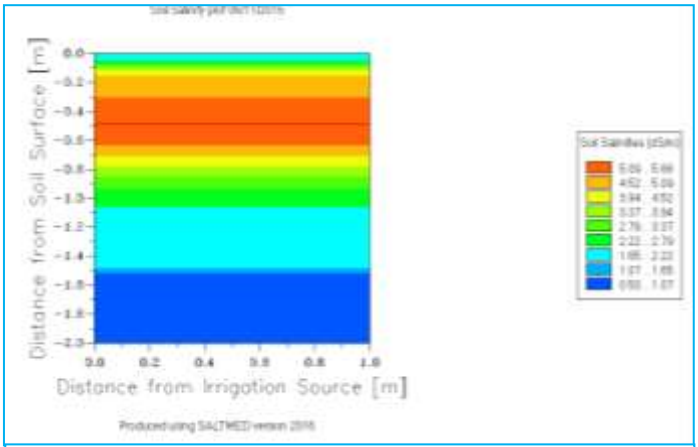


Figure 3-23 Sandy soil: Soil salinity

5.4.3.3 Soil nitrogen plot

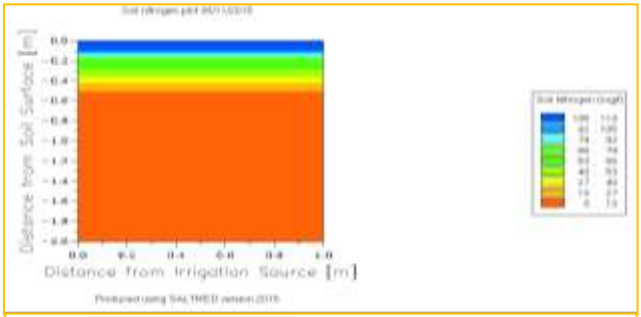


Figure 3-24 Sandy soil: Soil nitrogen

From fig 3-24 it is observed that the top 15 cm layer of soil has nitrogen content in the range of 106 mg/l to 118 mg/l. Then it gradually reduces to a range of 53mg/l to 40 mg/l. Beyond 50 cm depth the nitrogen content range between 0 mg/l to 13 mg/l.

5.4.4 Sandy Soil Irrigated With Treated Water

5.4.4.1 Soil moisture plot

From the figure 3-25 it could be seen that the soil moisture was minimum (0.039 - 0.056) till the depth of 1.6m. The soil moisture increases with increase in depth. The reason for the low moisture content at that depth may be due to high percolation rate of sandy soil and it may also be due to absence of sub surface drainage.

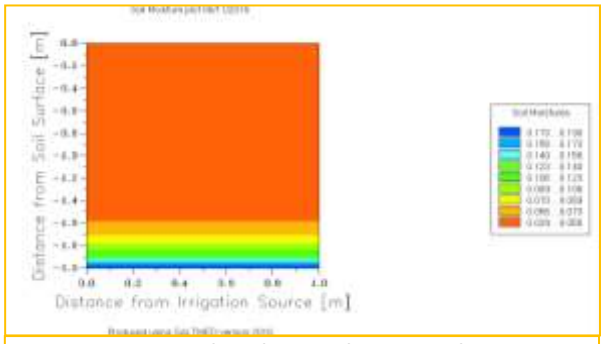


Figure 3-25 Sandy soil-Treated water: Soil moisture

5.4.4.2 Soil salinity plot

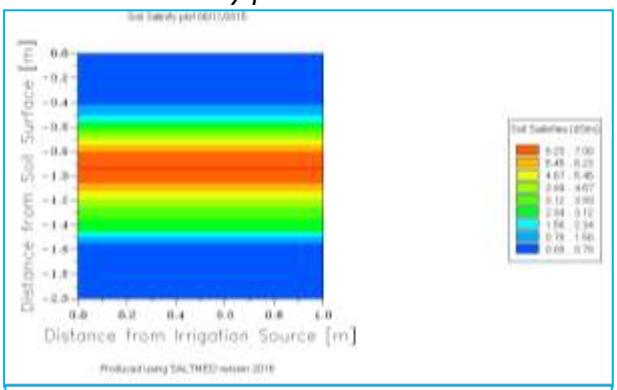


Figure3-26 Sandy soil-Treated water: Soil salinity

From figure 3-26 it could be seen that soil salinity was very minimum (0.00dS/m to 0.78dS/m) at the depth of 0 m to 0.4 m. Then it gradually increases and reached a maximum salinityof6.29dS/m to 7.00dS/m.At a depth of 1.2 m to 1.5 m the salinity decreases and finally at a depth of 1.5 m to 2.0 m the salinity becomes very minimum.Higher salinity level at the middle layer may be due accumulation of salt. Proper management practiceslike leaching could help in good plant growth.

5.4.4.3 Soil nitrogen plot

Figure 3-27 illustrates that the top 10 cm of the soil has higher nitrogen content of 85 mg/l to 95 mg/l. Then the nitrogen content reduces gradually and attains a minimum range of 1 mg/l to 11 mg/l. Thus to increase the nitrogen content in the soil and to ensure even distribution among all the layers, sun hemp is cultivated and mixed thoroughly.

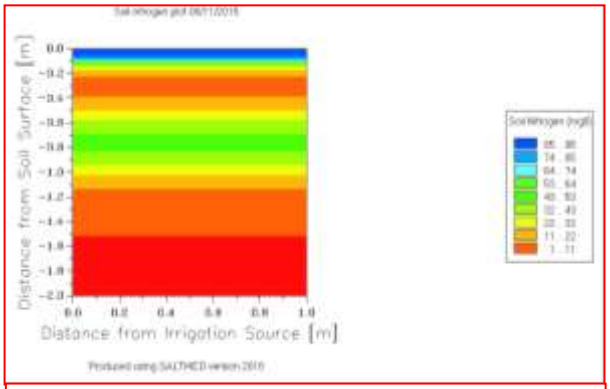


Figure 3-27 Sandy soil-Treated water: Soil nitrogen

Table 3-39 Comparison between treatments

Various depth	Comparison	0-30	30-60	60-90
Fresh clay	Simulated	3.60	2.50	2.50
	Observed	1.02	0.47	0.53
Treated clay	Simulated	3.60	2.50	2.50
	Observed	3.55	2.39	1.22
Fresh sand	Simulated	5.06	5.09	2.22
	Observed	0.07	0.10	0.15
Treated sand	Simulated	0.10	2.10	6.23
	Observed	0.14	0.17	0.21

Comparison between simulated and observed EC is illustrated in table 3-39. Goodness of fit between observed and simulated EC values was measured by calculating root mean square error (RMSE), coefficient of residual mass (CRM) and regression coefficient (R^2)

Figure 3-28 shows that there is a good correlation between the observed and the simulated electrical conductivity in fresh clay and treated clay treatment. In the fresh sandy and treated sandy treatment the correlation between simulated and observed is poor.

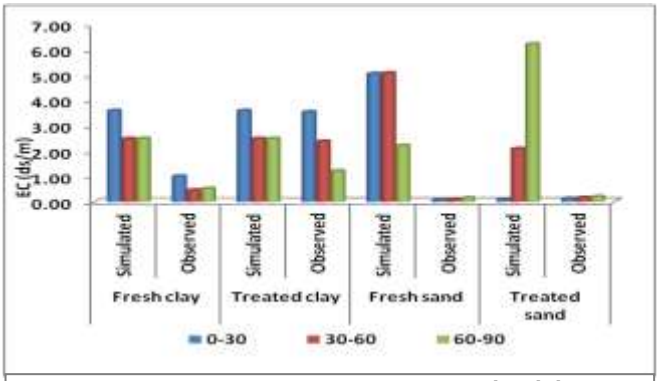


Figure 3-28 Comparison of electrical **conductivity**

Table 3-40 Comparison of simulated and measured sugarcane yield

Soil treatment	Yield (t/ha)		% deviation in yield
	Simulated	measured	
Fresh clay	55	88	37.5
Treated clay	55	50	10
Fresh sandy	36	43	16
Treated sandy	56	80	30

Table 3-40 shows the simulated and measured sugarcane yield in all the four treatments. The percentage deviation seems to be less in treated clay and fresh sandy when compared to the other two treatments.

5.4.5 Conclusion

In the above study, the moisture profiles in all the four treatments indicate that the top most layer has very less soil moisture when compared to other layers. This proves that water used for irrigation is in optimum level and hence measured yield also indicate that the values are in par with the standard yield of that sugarcane variety. Thus it could be

concluded that there is good water use efficiency in sugarcane cultivation in all the treatments. Salinity profiles confirm that the salt content in the soil in three treatments namely clay soil irrigated with fresh water, clay soil irrigated with treated water and sandy soil irrigated with fresh water exceeded the FAO standard of 2 dS/m. This high salinity can be attributed to nature of the saline soil prevalent in that location. The nitrogen profiles indicate that nitrogen content in the topmost layer in all the treatments are sufficient for the growth and yield of the sugarcane still the layers below are deficit of nitrogen. Hence sunhemp is grown and ploughed insitu for further cultivation of crops.

3.3 Increased land and saline wastewater productivity in 20 ha

3.3.1 Moringa oleifera cultivation using bio treated distillery effluent

Moringa oleifera, tree as a whole is a source of nutrition, medicine, cosmetics, biofuel and water purification. PKM 2 a type of moringa which is largely grown in Tamil Nadu was selected to study the reuse potential of irrigating bio-treated distillery effluent (T1) compared with anaerobic treated distillery effluent (T2) and fresh water (T3).

Moringa cultivation resusing bio-treated distillery effluent

Initially a nursery was raised by the end of September 2015, soil mixture with 50 kg Vermi compost + 100 kg Pressmud + 300 kg soil + 2 kg Trichoderma + 2 kg Pseudomonas was prepared and four hours soaked seeds were sown at one to two cm depth in nursery bags. The bags were placed under net to avoid pest acts until they germinate. After 20 days the geminated saplings were transferred to land with different treatments plots T1, T2 and T3 of area 55 sq m, 55 sq m and 70 sq m respectively.

Localized irrigation of four litres at two days interval is done regularly to the plants irrespective of difference in the source of irrigated water. The first flowering was observed 30 days after transplanting and neem oil was sprayed 10 days after flowering to avoid pest attack on flowers and during fruit bearing stage. The saplings were observed to grow well without any mortality with respect to different treatments, however the plants grown irrigated with anaerobic treated distillery effluent were observed to break just above the root and fall dead as shown in the picture. The reason for such breaking of stems is yet to be identified. The growth of Moringa is observed to be similar in plots T1 and T3 except that the places which fall under shade have shortened in growth. The first yield of Moringa is yet to be harvested and thus the yield attributes and other comparative observations will be recorded in the coming days.

3.3.2 Cultivation of halophytes reusing bio-treated distillery effluent

3.3.2.1 Cultivation of halophytes reusing bio-treated distillery effluent

Halophytes are saline loving plants and demonstration of reusing bio-treated distillery effluent is one of the key objectives of the project. Through the sequential bio-treatment process with bacterial and algal consortium the salinity was reduced to 8.4 PPT however the irrigation standard is ranging from 0 to 3 mS/cm. An attempt to grow halophytes likes *Sesuvium portulacastrum* and *Suaeda maritima* using the bio-treated distillery effluent was made in the first cycle and it was demonstrated successfully with luxurious growth and survival of the plants. Further to the first cycle a demonstration with halophytic plant species of *Sesuvium portulacastrum, Suaeda maritime* and *Suaeda nudiflora,* halophytic grass species of *Aerulopus lagopoides* and *Paspalum vaginatum* were planted. These halophytic plants are saline loving and this can be highly efficient crop for soil reclamation. The second trial of halophyte cultivation was initiated from mid August 2015 with treatments using bio-treated distillery effluent (T1) and anaerobic treated distillery effluent (T2) were established with 7 replicates each for each species. Plot area of each replicate was 5m x 5m and the total area of each treatment for *Sesuvium portulacastrum*, *Suaeda maritima* and *Suaeda nudiflora* is 175 m^2 respectively while the halophytic grass *Aerulopus lagopoides* and *Paspalum vaginatum* were planted in a plot of area 16m x 18.9m with 2 replicates each and total area for each treatment is 604 m^2

Biometrics and other observations

Table 3-41 Biometrics of halophytes

Species	Biometric	TW	RW
Suaeda maritima	Plant height (cm)	94.3	84.9
Suaeda nudiflora	Plant height (cm)	98.1	99.8
Sesuvium portulacastrum	Plant height (cm)	107.4	95.9
Aerulopus lagopoides	Biomass (g/sq ft)	0.19	0.15
Paspalum vaginatum	Biomass (g/sq ft)	0.44	0.29

These plants were irrigated with bio-treated distillery effluent T1 and anaerobic treated distillery effluent T2 was irrigated respectively 4L on every alternate day. Localized irrigation method is adopted where water is applied around each plant to wet only the root zone. Periodical monitoring and recording of growth and survival is done. Mortality and stunted growth was observed in T2 while plants in T1 grew luxuriantly. Plant biometrics is recorded at regular intervals and the details are given in table 1 which was measured at 150 DAS. The average circumference of *Suaeda maritima* is 2.5 m in T1 and 2.1 m in T2, while in *Suaeda nudiflora* the circumference is 2.2 in both T1 and T2. For the halophytic grass species no plant height measurements are done while the biomass of grass grown in 1 sq ft is measured and given in table 3-41. Both plant and grass species had grown luxuriantly in bio-treated distillery effluent than that in anaerobic treated distillery effluent.

3.4 Package of agro-aqua farming system available for replication

First cycle fish culture in integrated aqua-agro farming system

An extensive farming system with Indian Major Carp viz., Rohu (*Labeo rohita*) and Catla (*Catla catla)* with a low stocking density of 240 Catla and 160 Rohu with initial weight ranging from 150 to 200g is cultured. Apart from the naturall available feed within the treated water, supplementary feed is given on daily basis with rice bran and groundnut oil cake with proximate protein content ranging from 16 to 18%. 50% of water is replenished every 14 days from the sedimentation tank and water from fish tank is taken for irrigation.

The water quality analysis for water from fish tank is done on regular intervals and is maintained within the aquaculture standards. A water circulating unit is operated in the morning and evening to enhance dissolved oxygen availability for fishes. First cycle fish culture is mainly to understand and observe the survival, growth and health of fishes growing in treated sugar effluent.The fishes were grown for 274 days with a survival rate of 92% and 76% for *Catla* sp. and *Rohu* sp. respectively. The losses are mainly accounted to mammal and bird predation, human theft during crushing season at the industry. The overall net production for both species is 76.88 kg. The maximum grown *Rohu* sp. weighed up to 1.3 kg which a very good marketable size. The food conversion ratio (FCR) of *Rohu* sp. is 6.64 which is performs better than *Catla* sp. with 25.74 FCR. Similarly the percentage weight gain of *Rohu* sp. is greater than *Catla* sp shown in table 3-42. From the harvest details it is observed that the growth and performance of *Rohu* sp. is better than *Catla* sp.

Table 3-42 First cycle fish harvest details

Parameters	Catla sp.	Rohu sp.	Total
Stocked biomass (kg)	36.00	24.00	60.00
Harvested biomass (kg)	57.44	79.44	136.88
Net production (kg)	21.44	55.44	76.88
Weight gain (%)	59.55	231.00	128.13
Food conversion ratio	25.74	6.64	11.96
Specific growth rate (%)	0.17	0.44	0.30

Second crop stocking

Second cycle fish culture in integrated aqua-agro farming system

The second cycle fish culture was initiated on 4 Nov 2015. Based on the observations from first fish crop harvest, the better performing *Rohu* sp. alone was cultured with a stocking density of 1.7/m^2. The initial weight of fingerlings ranged from 80 to 120g. Fishes are fed on daily basis with rice bran at 5% feeding level to the body weight. They are monitored on regular intervals for growth performance and better health. The length and weight of fishes have doubled in 3 months from the time if stocking. Regular monitoring of water quality and maintenance of water levels are taken care for obtaining best growth and conversion to give good yield form fish culture.

4 Work Package: Development of Water Efficient Crop Varieties

4.1 Objectives

- Cross-species comparison for biomass production and water use efficiency in maize, sorghum, pearl millet and tomato
- Better understanding of mRNA and mRNA transcriptome of sorghum and pearl millet
- Mapping and characterization of quantitative trait loci (QTL) for drought tolerance related traits in maize, sorghum, pearl millet and chickpea
- Improving drought adaptation using marker-assisted breeding and trait-based selection approaches in maize, sorghum, pearl millet and chickpea
- Capacity building on NARS in research on drought adaptation of crops and integrated breeding for drought adaptation

4.2 Task 4.1a: Analyze comparative abilities of maize, sorghum and millet association panel genotypes for biomass production and water use efficiency (Lead institute: ICRISAT, Lead scientist: Vincent Vadez)

Activity 1: Characterize the *vapor pressure deficit* (VPD) response in parental lines of a backcross nested association mapping (*BCNAM)* BCNAM scheme to identify population segregating for the trait and assessment of transpiration efficiency (TE) in these lines

Rationale – We have shown that large variations existed for transpiration efficiency in sorghum. These differences are: (i) related to differences in the capacity to restrict transpiration under high VPD – i.e. there is then a mean to pre-screen for high TE by screening for the transpiration response to high VPD; (ii) higher TE led to higher yield under water stress conditions; (iii) TE discriminated sorghum races, with Guinea landraces having lower TE than Durra. This preliminary work then opens the scope for improving TE and therefore yield in Guinea-type cultivars for West Central Africa, which is currently the object of the development of BCNAM population using high TE germplasm as donor parents. Before this material becomes available, the parental lines of existing BCNAM populations developed for the WCA regions are planned to be screened for potentially important traits, in particular the capacity to restrict transpiration under high VPD.

Methodology: For the measurement of TE in some of the BCNAM parents, we have used the lysimetric system described in many papers by the group (LysiField) (See Vadez et al, 2014, J Exp. Bot doi:10.1093/jxb/eru040). In short, sorghum plants were grown in 2.0-m length and 25-cm diameter PVC tubes filled with Alfisol, with one plant per tube, with tube arrangement giving a spacing of 10 plant m^{-2}. At three weeks after sowing, plant water use monitoring started by regularly weighing the cylinders. Prior to that, cylinders were brought to field capacity and soil surface covered with a plastic sheet and a 2-cm layer of bead to limit soil evaporation. Five replicated tubes were used for each genotype-by-treatment combination. Plant water use monitoring took place until maturity, when harvest was done and agronomic parameters were collected. Two water treatments were used, a fully irrigated treatment and a terminal water stress treatment where irrigation was stopped at 5 weeks after sowing.

For the transpiration response to increasing VPD conditions, this consisted in growing plants in 8” pots under fully irrigated conditions. At about 4 weeks after sowing, plants were covered with a plastic sheet and beads to prevent soil evaporation, watered abundantly, let

to drain overnight, and transferred to a growth chamber for one day acclimation. The following day, transpiration was assessed gravimetrically (consecutive pot weighings) every hour. At each weighing, the vapor pressure deficit (VPD) in the chamber was increased by 0.5-0.8 kPa. The ladder of VPD then started from very low values, below 1 kPa up until above 6 kPa. A full set of 40 lines imported from Mali were tested. These included the parents of the BCNAM populations developed in Mali in the background of Lata3, Grinkan and Keninkeni.

Finally, LeasyScan assessments of a range of sorghum genotypes has continued in 2015, including assessment of the reference collection (384 entries), repeated assessment of a set of staygreen QTL introgression lines in S35 and R16 backgrounds, and parents of recombinant inbred line populatons.

Results and discussion:

The lysimeter data were collected in the postrainy season 2015-16 and resulting transpiration efficiency measurements are shown below. Clearly the experiment confirmed the TE differences between low and high TE lines. These were selected from earlier and larger germplasm screening. Here it should be noted that a one unit TE difference is quite substantial. Two things were noticeable and exciting: (i) the Maldandi germplasm are cultivars bred and adapted to the sorghum post-rainy season in India. They had high TE which was likely a consequence of a progressive breeding for it; (ii) the BCNAM parents, all mostly cultivars or germplasm with adapted to the Soudano-Sahelian region of West Central Africa had low TE on average, at the level of the low TE genotypes, opening a great scope for increasing TE and then yield in these genetic materials.

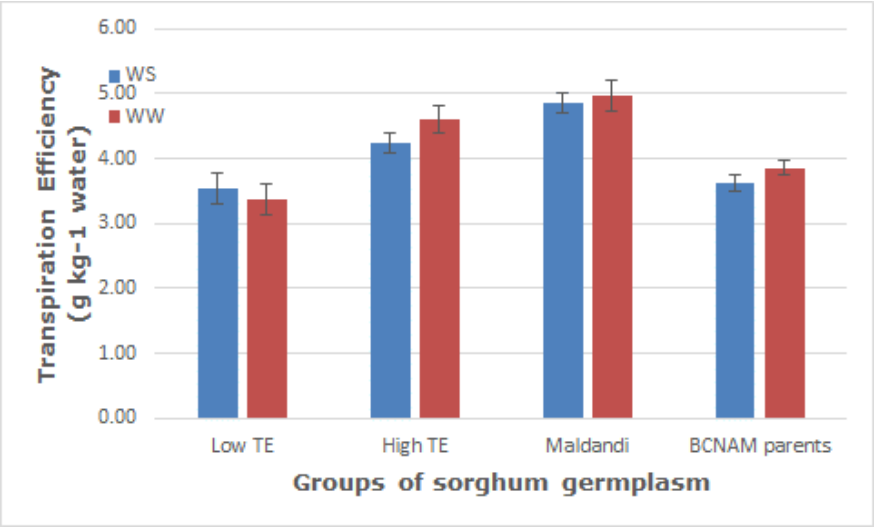


Figure 4-1 Transpiration efficiency in groups of sorghum germplasm (low TE, n=6; high TE, n=14; Maldandi, n=5; BCNAM parents, n=11). Data are mean plus SE of 5 replicated lysimeter per water treatment (WW, well-watered; WS, water stress).

The analysis of the LeasyScan data in an on-going process that involves the analysis of time-series, therefore datasets generated over several seasons. It is on-going. For the transpiration response to VPD, below is a representation of the variation that was found earlier, showing differences among genotypes. For instance, Lata3 and CSM63E showed large contrast and slope differences.

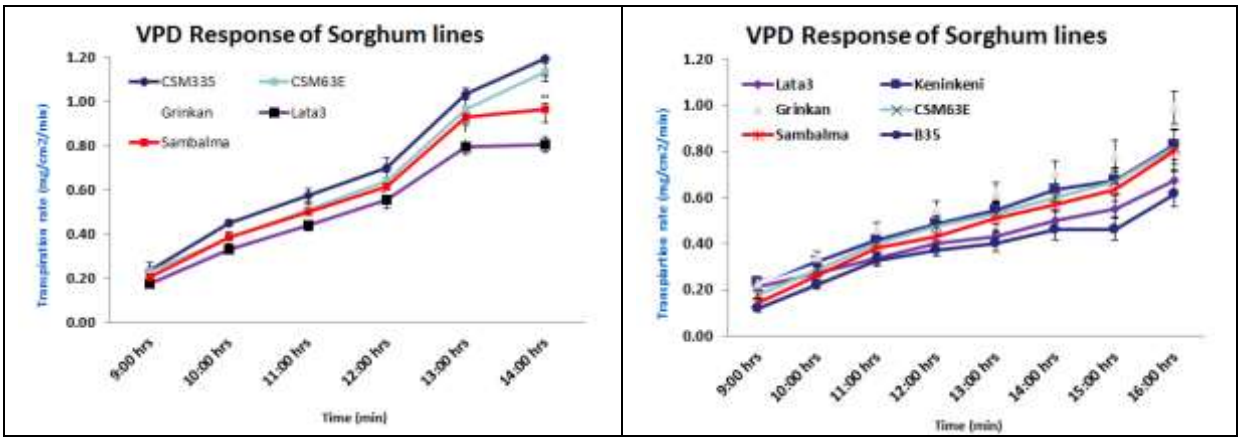


Figure 4-2 Transpiration response to increasing VPD (the graph indicates the timing of measurement – VPD was gradually increased in the chamber in India and gradually increased in a glasshouse conditions in Mali) in parental lines of existing BCNAM population developed in Mali. The left graph is the evaluation in India while the right graph is the evaluation in Bamako-Mali.

Table 4-1 Slopes of the transpiration response to VPD in parents of BCNAM populations. The left pane is from measurements carried out in India, while the right pane represents measurements in Mali.

Genotypes	Slope ± SE	X-intercept	R^2
CSM63E	0.5122 ± 0.04182	0.6107	0.974
Grinkan	0.4348 ± 0.04003	0.5212	0.9672
Lata3	0.3725 ± 0.02927	0.4772	0.9759
Sambalma	0.4423 ± 0.03747	0.5063	0.9721

Genotypes	Slope ± SE	X-intercept	R^2
CSM63E	0.1627 ± 0.01107	0.6618	0.973
Grinkan	0.1932 ± 0.01097	0.6815	0.981
Lata3	0.1202 ± 0.005560	0.1986	0.9873
Sambalma	0.1645 ± 0.01277	0.8646	0.9651

Summary and conclusions: We have shown that parents of a set of existing BCNAM populations of sorghum contrast for a trait that is essential for the adaptation to drought in semi-arid regions. In parallel to this, we have shown through crop simulation that this trait would be highly beneficial for sorghum production environments (not reported here). The work done here then opens the possibility to harness QTLs for this important trait. About 1100 population entries from 12 BCNAM population are currently in post-quarantine in India and await to be tested for that trait in the LeasyScan platform.

Compile data sets and analyze Year 2 & 3 data – In the previous two years we have been comparing a set of maize, sorghum and pearl millet genotypes. Several experiments have been carried out on these materials. A research paper is being finalized for submission to peer-reviewed journal.

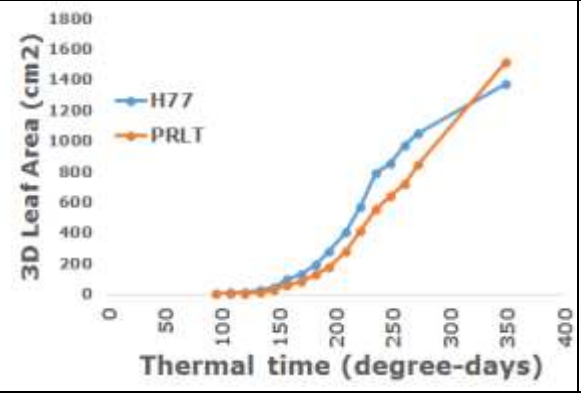
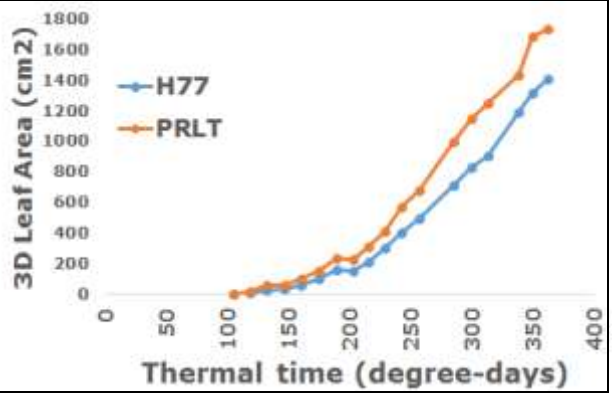
Activity 2: Compare leaf development response of maize, sorghum, and pearl millet under different VPD regimes (LeasyScan) – on-going rolling experiment across VPD experiments

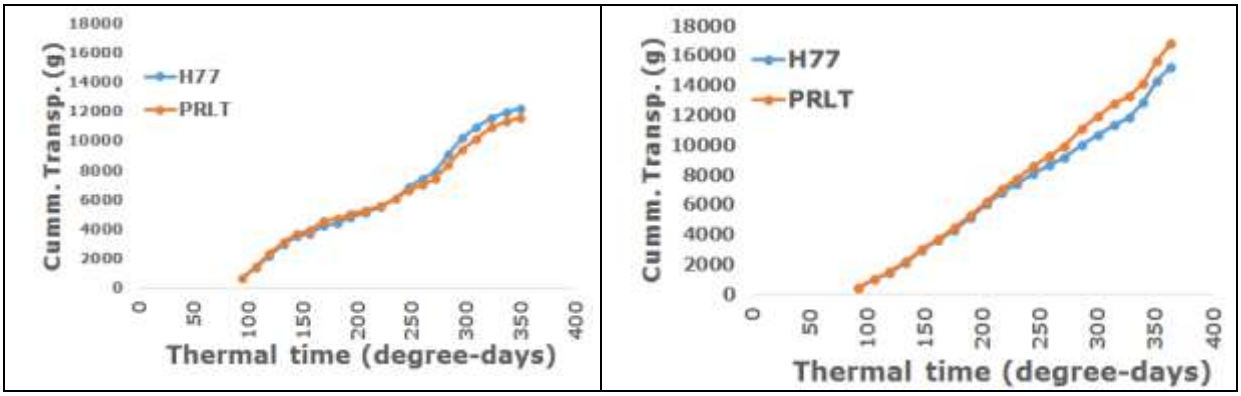
Rationale – Over the years and in this project we have been comparing species for the capacity to restrict transpiration under high vapor pressure deficit (VPD), being one of the key traits explaining TE differences in germplasm. It is also known from work in maize (F. Tardieu's group) that high VPD reduces the leaf expansion rate in certain maize genotypes. This work was carried out on leaf 6 of maize and would need to be validated in an entire canopy. Here what we did was to attempt validating this to whole canopy measurement, and using natural conditions (instead of growth chambers). This is therefore a methodological attempt to measurement phenotypic variation for a trait (the capacity to maintain leaf expansion rate under high VPD) that has large consequence on the eventual crop productivity.

Methodology: For that purpose we use the 3D-scanning system of the LeasyScan facility. Eight genotypes of sorghum, maize and Pearl millet have been grown in large pots, 6 replications per genotype. The scanning usually has taken place for about 4-5 weeks after sowing. The same experiment has been repeated over times (so far 6 replicated experiments have been carried out). The essential purpose of each of these experiments is to grow the crops under differing VPD conditions. Therefore, each experiment represents a single data point of leaf expansion rate for each genotype in the experiment. Once enough data points are collected, we will then graph a scatter data point between the leaf expansion rate and the VPD prevailing during the experiment.

Results and discussion:

The two graph below provides preliminary data analysis of the kind of data generated in this time-serie experiment. Here, two pearl millet lines were grown in two consecutive experiments which varied for the VPD conditions prevailing. In the first experiment, the slope of the leaf area development as a function of thermal time was slightly lower in PRLT than in H77 (left graph). Under a higher VPD experiment (right graph), the results are inverted, which points to a possible VPD effect on these expansion rates. Again, these two experiments represent only two data points and a lot many more data point will be needed to infer possible VPD effects on the leaf expansion rate, possible genotypic variation within species, possible variation between species.

Feb 17th – Mar 16th Mean **VPD = 1.20 kPa**	Mar 19th – Apr 14th Mean **VPD = 1.68 kPa**
	



Activity 3: Upgrade of the LeasyScan platform

Rationale: There is now a large body of evidence that adaptation to terminal water stress in many crops is a question of ensuring that there is sufficient water available to the plant during the reproductive and grain filling processes, and this depends on how plants manage a finite water resource until then. The focus of our phenotypic efforts has then be on harnessing the genetics of traits involved in traits controlling the plant water budget, in particular dealing with the crop canopy development and the canopy conductance. In 2014, a high throughput phenotyping platform has been developed (LeasyScan – See Vadez et al JXB 2015 doi:10.1093/jxb/erv251) to measure both leaf canopy development and canopy conductance traits. Proof of concept of its relevance to assess plant transpiration response to increasing vapor pressure deficit (VPD) was demonstrated in several crops.

Methodology: The principle of operation, approach, methods of the LeasyScan platform is described at length in Vadez et al., 2015 (Journal of Experimental Botany 66(18), 5581-5593 doi: 10.1093/jxb/erv251) which is open access. In short, the platform uses 3D laser scanners that are mounted on an irrigation booms that travels on top of the plant canopy and generate 3D point clouds from the reflection of a 940nm wavelength on the canopy. Plant parameters such as the leaf area or the projected leaf area are then extracted from the 3D point cloud. The area scanned is 65x40 cm wide and contains 4 plants (to achieve a planting density of 16 plant m^{-2}, typical of pearl millet sowing densities). The scanning takes place every two hours and data are accessed from a database via 'R'-libraries that allow the filtering of data (for instance to exclude data acquired under high wind speed).

Here we simply wanted to show the transpiration rate difference in two genotypes that are known to contrast in their ability to cope with water stress: H77/833-2, a popular pearl millet inbred line in both Asia and Africa, which is considered drought sensitive and is known to have transpiration unrestricted under high VPD conditions. PRLT-2/89-33 is a terminal drought tolerant inbred line from Togo which hold a terminal drought tolerance QTL on LG2 and has transpiration restriction under high VPD conditions. Similarly R16 is a senescent sorghum line with transpiration unrestricted under high VPD conditions, whereas S35 has transpiration restriction under high VPD conditions.

Results and discussion:

Assessment of canopy conductance with load cells - We established the proof of concept that the analytical scales at the LeasyScan platform would be suitable to phenotype the capacity of genotypes to restrict transpiration under high VPD (see Vadez et al., 2015 – JXB doi: 10.1093/jxb/erv251). The capacity of the LeasyScan platform is currently 50 load cells, which was sufficient to establish the proof of concept. Based on this, the design of an

expansion to 1500 load cells has been developed. The transpiration profile below showed that the VPD-sensitive PRLT-2/89/33 had the transpiration rate restricted in the middle part of the days on most of the days displayed in the figure. The transpiration rate was otherwise very similar in both genotypes in the remaining hours of the day. The extrapolation of the transpiration rate data into a mm water loss difference, based on plant leaf area, plant density and sector size, came to values varying between 0.5 to 1 mm water saved per day in PRLT-2/89-33. It is the accumulation of these seemingly small but substantial water savings that lead to the economy of water during the vegetative stage and the high availability of water for the reproductive and grain filling stages.

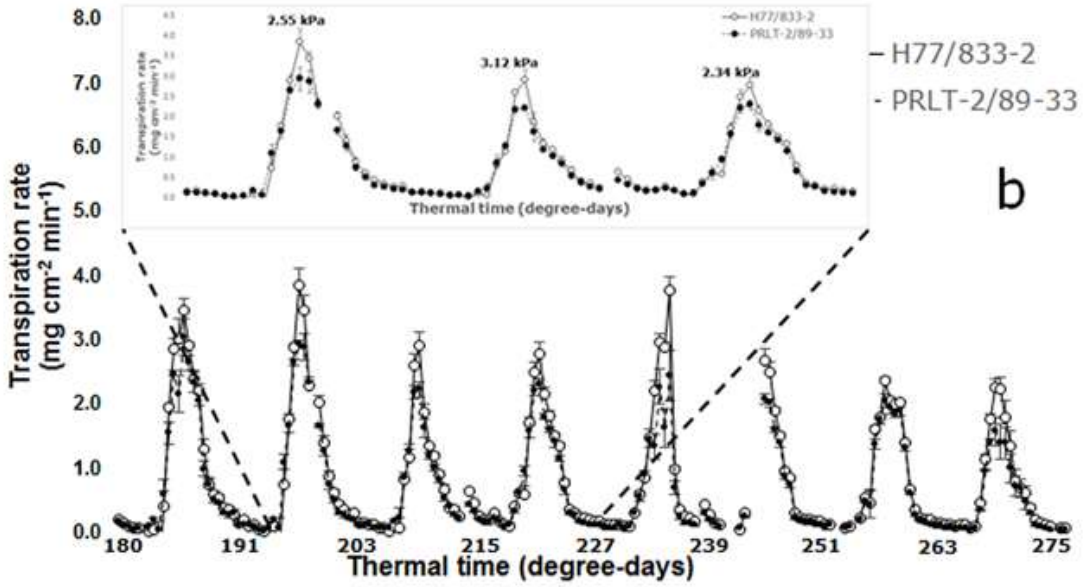


Figure 4-3 Transpiration rate (mg water cm^{-2} min^{-1}) profile over a view days, chronological time being here converted to thermal time, in two genotypes of pearl millet.

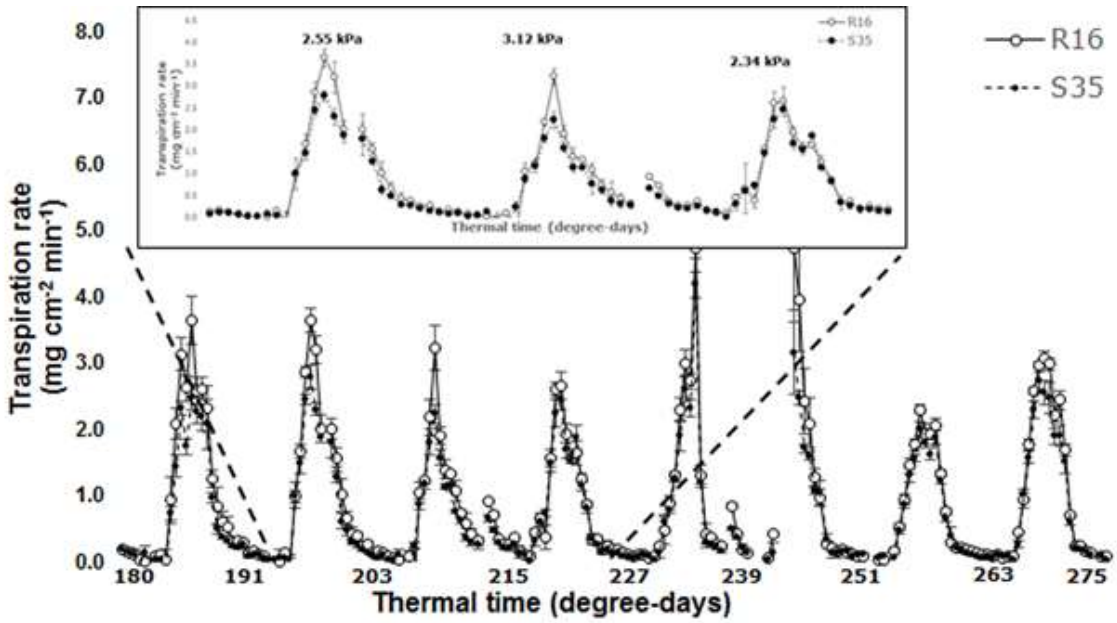


Figure 4-4 Transpiration rate (mg water cm^{-2} min^{-1}) profile over a view days, chronological time being here converted to thermal time, in two genotypes of sorghum.

As can be seen in Figure 4-3 and Figure 4-4 above, VPD-sensitive lines PRLT-2/89-33 and S35 had lower transpiration rate in the midday hours when the VPD was above 2 kPa, whereas VPD-insensitive lines H77/833-2 and R16 had high TR in those hours. These TR restrictions under high VPD conditions do contribute to major water savings.

Summary and conclusions: We have now a solid piece of evidence that the platform is suitable to pinpoint genotypic differences in the capacity to restrict transpiration under high evaporative demand. The current expansion (2016) of the load cell capacity to 1500 of them simply opens up a new and exciting field in the development of climate smart crop cultivars.

4.3 Task 4.2a: Characterization and response of maize, energy-dedicated sweet sorghum and pearl millet isogenic lines to water deficits (Lead Institute: ICRISAT, Lead scientist: Vincent Vadez)

Activity 1: Characterize leaf area development (LeasyScan) in different materials of sorghum (Stg Introgression lines, BCNAM parent) and pearl millet ("triplets" (hybrids, B-line, R-line) adapted to different zones or selected high resolution lines) of pearl millet
This work parallels other investigations on physiological traits related to water fluxes in plants. Here we report only on the pearl millet hybrids adapted to the A1, A, and B zones of pearl millet.

The graph below shows that the leaf area development pattern of F1 hybrids bred for the A1 zone was dramatically different from those of F1 hybrids developed for either the A or the B zone. There were no significant difference between the leaf area development pattern of A and B zones. The range of variation, proxied by the size of the variation from the mean, in the A1 hybrids also shows that the variation among hybrids bred for the same zone was larger than for hybrids bred for the A and B zone. Even larger zone differences were found in the leaf area development pattern between B-lines bred for the A1 zone and those bred for the A and B zones. Similar but less striking variation was found for the R-lines (data not shown). Therefore, it appears clearly that materials bred for the A1 zones developed smaller leaf area as earlier discussed (van Oosterom *et al.*, 2003), in the order of 15% less for the F1 hybrids and in the order of 40% less for the B-lines. Early maturing hybrids (65-70 days maturity) targeted for drought prone environments of A1 zone indeed produce lower biomass in comparison to medium to late maturing hybrids (75-85 days maturity) bred for relatively wetter A and B zones, hence lesser leaf area in A1 hybrids was as expected.

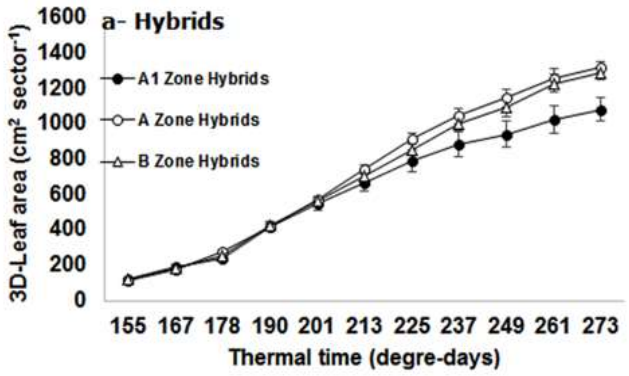


Figure 4-5 Leaf area development as a function of thermal time in hybrids of pearl millet bred for three adaptation zones of pearl millet in India (A1, rainfall < 400mm; A and B, rainfall > 400mm). Data are means +/- SE for 12 to 14 hybrids in each rainfall group.

Test the transpiration efficiency in 10 entries of pearl millet, sorghum and maize where roots have been section at an early stage (**Task 4.2**) – A small exploratory experiment was carried out with 6 genotypes of pearl millet, sorghum and maize, grown under well-watered (WW) conditions, or terminal water stress (WS). Each water treatment was again divided in sub-treatment in which a part of the crown root system was cut on one half of the plant with a knife. The purpose of trimming the root system was to potentially affect the water fluxes in the plant system and possibly provoke a transpiration restriction under high VPD, which would have been caused by a limited water transport capacity. It was then hypothesized that if such phenomenon happened, it would lead to TE differences. We could only find a slight TE increase in maize under WS conditions, but not in pearl millet and sorghum. Similarly, there was no TE differences under WW conditions between the root treatments.

Activity 2: Test staygreen QTL introgression lines of sorghum for traits putatively underlying these QTL, towards refining the QTL interval responsible for the trait

This has been done and reported in the previous year. We now know that the transpiration restriction under high VPD is one key trait contributing to the staygreen expression and carried by Stg QTL Stg3A and Stg3B.

Repeat Lysimetric assessment of ILs of B73 background

Rationale – This genetic material is a set of introgression of segment of Gaspe Flint, a landrace with good root system in the background of elite B73. The proposal was to test these introgressions for the capacity to extract water from the soil profile. A transpiration efficiency assessment was also performed.

Material and methods – TE measurements were carried out in a lysimetric system, which has been described in many papers by the group (see Vadez et al., 2014). In short, pearl millet plants were grown in 2.0-m length and 25-cm diameter PVC tubes filled with Alfisol, with one plant per tube, with tube arrangement giving a spacing of 10 plant m^{-2}. At three weeks after sowing, plant water use monitoring started by regularly weighing the cylinders. Prior to that, cylinders are brought to field capacity and soil surface covered with a plastic sheet and a 2-cm layer of bead to limit soil evaporation. Five replicated tubes were used for each genotype-by-treatment combination. Plant water use monitoring took place until maturity, when harvest was done and agronomic parameters were collected

Results and discussion - These were tested during the rainy season 2014 for the first time (reported in Year 2). A repeated assessment was carried out in the post-rainy season 2014-15.

This trial was carried out in the post rainy season, under high high evaporative demand. The variation for TE was larger (3.50-5.20 g kg-1) than in the previous trial carried out in the rainy season (3.00-4.50 g kg-1, i.e. about 1.5 fold variation) (Figure above, left panel). In this trial again, the range of variation for the water extraction was really large (about 2-folds, from 6 to more than 11 L plant-1) (Figure above, right panel). On the one hand, the amount of water extracted from the profile was small compare to other experiments with maize in a similar system. This owed in large part to the fact that these ILs were of temperate

background (B73), and indeed suffered the tropical temperature conditions of South India. On the other hand, it was expected to find large differences in the water extraction since these introgression vary for the introgression of segments responsible for root attribute differences (depth / angle).

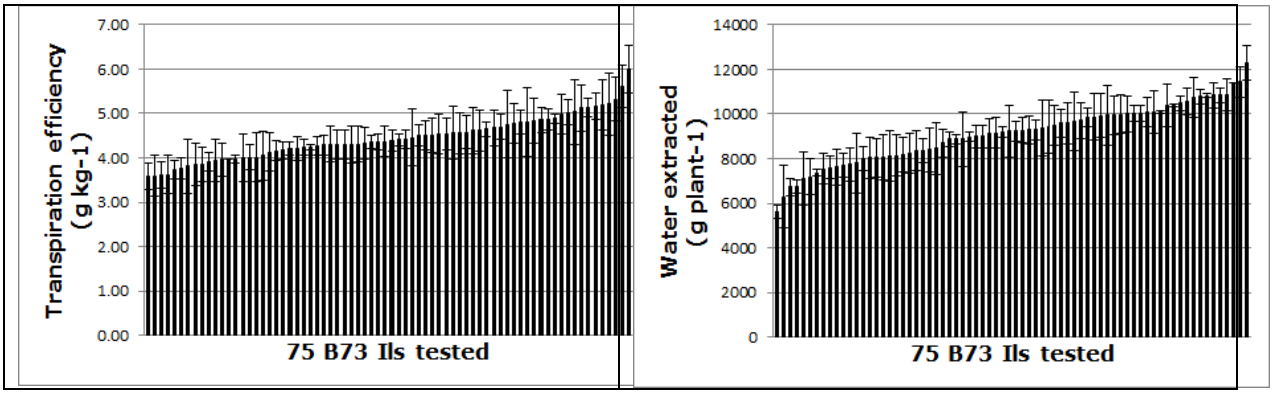


Figure 4-6 Transpiration efficiency (TE, in g biomass kg-1 water transpired) in 72 introgression lines in B73 background (Gaspe Flint as donor parent).

Re-assessment of TE in the pearl millet germplasm

Summary - In short, 234 inbred lines of the PMiGAP were again tested in 2015, along with 42 hybrids that were specifically bred for zones differing for rainfall representing Northern and Southern states of India. Twenty (20) inbred were selected having high TE across the two repeated years of experiment (2014 and 2015). In addition, differences in TE were found among groups of hybrids bred for different rainfall zones, hybrids bred for dryer zones having surprisingly lower TE than hybrids bred for wetter zones.

Rationale – There is large variation for transpiration efficiency (TE) in cereal like sorghum, variation this is being used in breeding more water efficient cultivars, which is a central target for crops that live in water limited conditions. Earlier screening of pearl millet hybrids have also shown large variation for TE and the objective here was to identified germplasm that could be directly into breeding, targeting especially those area of breeding where there are large TE gains to make.

Methodology:

TE measurements were carried out in a lysimetric system, which has been described in many papers by the group (see Vadez et al., 2014). In short, pearl millet plants were grown in 2.0-m length and 25-cm diameter PVC tubes filled with Alfisol, with one plant per tube, with tube arrangement giving a spacing of 10 plant m^{-2}. At three weeks after sowing, plant water use monitoring started by regularly weighing the cylinders. Prior to that, cylinders are brought to field capacity and soil surface covered with a plastic sheet and a 2-cm layer of bead to limit soil evaporation. Five replicated tubes were used for each genotype-by-treatment combination. Plant water use monitoring took place until maturity, when harvest was done and agronomic parameters were collected.

Results and discussion:

Like in 2014, there was a large variation in TE among the inbred germplasm, i.e. a variation between 2 and 4 g kg^{-1}, which was similar to the range of TE found in 2014 (Figure 1 below). The data were combined with the 2014 data and we came up with a list of superior lines for

TE, which could be directly used in breeding for the development of breeding populations such as backcross nested associated mapping populations (BCNAM), which are a breeding stock of choice both for deriving promising pre-breeding lines and mapping genetic regions involved in the TE differences (see list below). In making that list, attempt was made to select only entries in which the within-genotype variation was small. A set of lowest TE lines was also selected for parallel comparison of potential traits contrasting between high and low TE lines. The seed yield, total plant water extracted from the soil profile, or total plant water use did not differ between these two groups, indicating that high TE was not linked to poor vigor characteristics.

Very exciting was the finding of variation in TE among the groups of hybrids bred for pearl millet agro-ecological regions differing in rainfall conditions (A1 rainfall below 400 mm – A and B rainfall above 400 mm), and in particular that TE in A1 zone hybrids (2.75 g kg^{-1}) was lower than in A zone hybrids (2.97 g kg^{-1}) and B zone hybrids (3.16 g kg^{-1}). The average TE among inbred was 2.87 g kg^{-1}, however the TE of the top 15 inbred was 3.72, therefore providing a large scope for improvement of TE in the hybrids.

Table 4-2 List of germplasm with high TE value, and TE value (g kg-1)

Serial	Genotype	TE	Serial	Genotype	TE
1	IP 18412	**3.39**	11	IP 9532	**3.52**
2	ICMB 90111-P6	**3.39**	12	IP 9301	**3.53**
3	IP 3389	**3.40**	13	IP 11218	**3.53**
4	IP 8426	**3.41**	14	IP 10964	**3.55**
5	IP 10339	**3.42**	15	IP 17150	**3.56**
6	IP 13459	**3.43**	16	IP 15872	**3.70**
7	IP 3110	**3.46**	17	IP 9651	**3.89**
8	IP 18389	**3.46**	18	IP 2058	**3.90**
9	IP 11346	**3.46**	19	IP 7941	**3.92**
10	IP 10543	**3.47**	20	PT 732B-P2	**4.52**

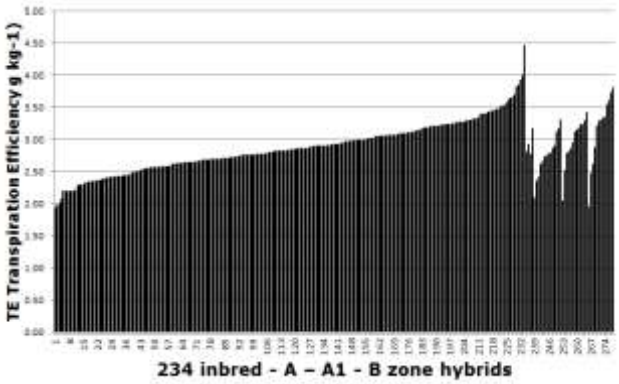


Figure 4-7 Transpiration efficiency (TE, in g biomass kg-1 water transpired) in inbred pearl millet germplasm and hybrids bred for different rainfall zones of India (A1, rain< 400 mm – A and B, rain > 400 mm, A corresponds to Northern states, B corresponds to Southern states).

Summary and conclusions: A set of germplasm is available with large TE across two repeated trials under high VPD conditions and these should be used in the development of BCNAM population in pearl millet. The hybrids bred for different agro-ecological zones had TE differences, which gives scope to increase TE in the cultivars targeted to the A1 zone.

4.4 Task 4.2b: Mapping of genomic regions controlling traits related to drought tolerance/WUE in tomato (Lead Institute: UAS-B; Task Leader: DL Savithramma)

Tomato (*Solanum lycopersicon*), which belongs to the family *solanaceae*, is one of the most popular and widely grown vegetables in the world. Ripe tomato fruit is consumed fresh as salads or cooking and utilized in the preparation of wide range of processed products such as puree, paste, powder, ketchup, sauce, soup and canned whole fruits *etc.* (Jat *et al*., 2012). Tomatoes are the important source of lycopene, ascorbic acid and b-carotene and valued for its colour and flavour.

As it is short duration crop and gives high yield, it is important from economic point of view and hence area under its cultivation is increasing day by day. Tomato ranks third in priority after potato and onion in India but ranks second after potato in the world where India stands second in term of area and production. The major tomato producing countries include China, India, USA, Turkey, Egypt, Iran, Italy, Spain, Brazil, Mexico and Netherlands. Total area harvested under tomato is 47,25,417 thousand hectares (ha) with a production of 16,39,63,770 thousand metric tons and with productivity of 34.698 metric tons/ha in the year of 2013 (FAOSTAT, 2015).

In India, there is a sizeable increase in acreage from 596.0 thousand ha in 2006-07 to 882.0 thousand ha in 2013-14, while in terms of production it has increased from 10,055 to 18,735.91 thousand tons. The leading producing states are Andhra Pradesh, Karnataka, Madhya Pradesh, Telangana, Odisha, Gujarat, Maharashtra, West Bengal and Bihar. In Karnataka, total area under tomato is 61.4 thousand ha with a production of 2,068.38 thousand metric tons with a productivity of 33.9 metric tons/ha (IHD, 2014).

Water, food and energy securities are emerging as important and vital issues for India and the world. Most of the river basins in India and elsewhere are closing or closed and experiencing moderate to severe water shortages, brought on by the simultaneous effects of agricultural growth, industrialization and urbanization. In India *per capita* water availability has decreased from 5177 m^3 in 1951 to 1869 m^3 in 2001 due to increase in population from 361 million in 1951 to 1.02 billion in 2001 which is expected to rise to 1.39 billion by 2025 and 1.64 billion by 2050 with associated decrease in *per capita* water availability of 1341 m^3 in 2025 and 1140 m^3 by 2050, respectively. There is an urgent need to manage water resource efficiently through enhancing water use efficiency and demand management (Odeh, 2003; Al-Hamaiedeh and Bino, 2010).

Climate change is regarded as one of the greatest challenges for future food production. With climate change, the importance of drought in conjunction with high temperature and radiation, and the area of irrigated land with saline soils are expected to increase significantly. It is broadly accepted that breeding for drought and salinity tolerance has proven to be difficult due to very complex and till date sometimes poorly understood tolerance mechanisms (van Bueren *et al*., 2011). In tomato, drought is one of the most important abiotic stresses reducing crop growth and yield (Jones, 1999). Breeding for resistance to drought in tomato is complicated by the lack of fast and reproducible screening techniques. Although a large number of different traits during vegetative and reproductive growth phases have been employed to characterize the physiological and

genetic basis of drought tolerance in tomato, it is still difficult to identify drought tolerant genotypes (Foolad, 2005). To differentiate the degree of drought resistance between different genotypes, several drought tolerant indices (DTIs) have been suggested in wheat (Farshadfar and Elyasi, 2012; Farshadfar *et al*., 2012), safflower (Bahrami *et al*., 2014) and so on while in tomato few reports are found. So, there is a need to attempt for identifying drought tolerant genotypes by using DTIs based on fruit yield under normal and stress conditions that can be used in large-scale screening of tomato germplasm.

Stress avoidance characters such as water use efficiency (WUE), leaf characteristics to conserve tissue water, stomatal and cuticular characteristics, root characteristics their extraction efficiency, which favours maintenance of higher tissue water content under receding moisture stress, only postpone the immediate effect of moisture stress. Therefore, under severe moisture stress conditions, the intrinsic tolerance mechanism becomes more relevant. Under rainfed situations, where the crop is subjected to cycles of stress, survival at the end of stress and recovery on alleviation is important.The earlier researchers found that there is limited tolerance and variability for drought stress in cultivated tomato (Foolad *et al*., 2003; Nahar and Ullah, 2011, 2012). Genetic variation is available in wild species of *S. pimpinellifolium*, *S. pennellii*, *S. habrochaites*, *S. chmielewskii*, and *S. cheesmaniae,S. chilense*, *S. sitiens* and some count *S. lycopersicum* (Ram, 2005; Rai and Rai, 2006; Swarup, 2006; Chavan, 2007; O'Connell *et al*., 2007; Singh, 2010; Symonds *et al*., 2010 and Rai *et al*., 2011). Breeding of promising genotypes against abiotic stress would be judged by imposing stress environments and their stability under such environments, using various biometrical approaches (Blum, 1988).

Development of molecular genetic markers and their use in QTL analysis has become a powerful approach for studying the inheritance of complex traits and helps for improving drought resistance in crop plants (Suji *et al*., 2012). Selection of drought resistance traits by phenotyping is difficult and labour-intensive, where the use of molecular markers serves as an alternate tool for selection of such complex traits in breeding. Several QTLs have been identified using traditional linkage mapping and positional cloning. However, linkage mapping is limited to the analysis of traits differing between two lines and the impact of the genetic background on QTL effect has been underlined.

In view of this, the present investigation was planned to identify variability in different drought adoptive mechanisms among different tomato genotypes with the following objectives.

Specific objectives

1. To screen the tomato germplasm for drought tolerance and to assess genetic diversity among wild and cultivated tomato germplasm for traits related to WUE and fruit yield.
2. To assess the variability among root traits for identification of water use efficient genotypes.
3. Standardization of sampling day for root traits among different species of tomato.
4. Development of mapping population and Identification of DNA markers polymorphic to parents sof mapping population.

5. Genotyping F_2 and Phenotyping F_3 mapping population for traits related to WUE and yield and Mapping of genomic regions controlling traits related to Water Use Efficiency and fruit yield.
6. Phenotyping and Genotyping of cultivated and wild germplasm accessions with informative markers to establish association with traits related to WUE and fruit yield.
7.

Objectives for the 3rd year (2015-16)

1. Phenotyping of Germplasm lines for traits related to WUE and fruit yield.
2. Phenotyping and Genotyping of cultivated and wild germplasm accessions with informative markers to establish association with traits related to WUE and fruit yield.
3. Phenotyping of F_2 and F_3 population of inter-specific cross for traits related to WUE and fruit yield
4. Parental polymorphism and Genotyping of F_2 mapping population with SSR markers linked to the traits related to WUE and fruit yield

EXPERIMENT 1: Phenotyping of germplasm lines for traits related to WUE and fruit yield.

Materials and method:

The material comprised of one-hundred germplasm accessions of six species (*Solanum* spp.) along with three check varieties (Arka Abha, Arka Vikas and Arka Meghali) procured from India including NBPGR, IIHR and IIVR, from United State (TGRC, UC-Davis) and Taiwan (AVRDC) as listed in Table 4-3

Table 4-3 103 Germplasm accessions and Check varieties from six cultivated tomato and related species (*Solanum* spp.)

Sl. No.	Code	Name	Species	Origin
1	2*	LA 1255	*Solanum habrochaites*	TGRC (UC-Davis, USA)
2	3*	LA 1353	*S. habrochaites*	TGRC (UC-Davis, USA)
3	4	LA 2976	*S. habrochaites*	TGRC (UC-Davis, USA)
4	8*	L 00673	*S. peruvianum*	AVRDC, Taiwan
5	9*	L 00882	*S. peruvianum*	AVRDC, Taiwan
6	10*	L 00671	*S. peruvianum*	AVRDC, Taiwan
7	11	L 00887	*S. peruvianum*	AVRDC, Taiwan
8	13*	EC 771608	*S. peruvianum*	NBPGR, India
9	15*	EC 771609	*S. peruvianum*	NBPGR, India
10	16	EC 771607	*S. peruvianum*	NBPGR, India
11	17*	EC 771603	*S. peruvianum*	NBPGR, India
12	18*	EC 520044	*S. cheesmanii*	NBPGR, India
13	19*	WIR 3969	*S. cheesmanii*	IIVR, India
14	20*	EC 54109	*S. pimpinellifolium*	NBPGR, India
15	21*	EC 541101	*S. pimpinellifolium*	NBPGR, India
16	22	LA 1246	*S. pimpinellifolium*	TGRC (UC-Davis, USA)
17	23*	LA 1245	*S. pimpinellifolium*	TGRC (UC-Davis, USA)
18	25*	LA 1478	*S. pimpinellifolium*	TGRC (UC-Davis, USA)
19	26*	EC 541109	*S. pimpinellifolium*	NBPGR, India

Sl. No.	Code	Name	Species	Origin
20	27*	EC 677049	*S. pimpinellifolium*	NBPGR, India
21	28	LA 0114	*S. pimpinellifolium*	TGRC (UC- Davis, USA)
22	29*	LA 0121	*S. pimpinellifolium*	TGRC (UC- Davis, USA)
23	30*	LA 0400	*S. pimpinellifolium*	TGRC (UC- Davis, USA)
24	31	LA 0369	*S. pimpinellifolium*	TGRC (UC- Davis, USA)
25	32*	LA 0373	*S. pimpinellifolium*	TGRC (UC- Davis,USA)
26	33*	LA 1468	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis, USA)
27	34*	LA 1206	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis, USA)
28	35*	LA 1632	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis, USA)
29	36*	EC 676790	*S. esculentum* var. *cerasiforme*	NBPGR, India
30	37*	EC 771615	*S. esculentum* var. *cerasiforme*	NBPGR, India
31	38*	LA 0475	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis, USA)
32	39*	LA 0168	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis, USA)
33	40*	HAT- 121	*S. esculentum* var. *cerasiforme*	IIVR, India
34	41*	LA 0292	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis, USA)
35	42*	LA 1311-19	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis,USA)
36	43*	LA 1311-16	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis, USA)
37	44*	EC 514100	*S. esculentum* var. *cerasiforme*	NBPGR, India
38	45*	LA 1545	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis, USA)
39	46*	EC 771590	*S. esculentum* var. *cerasiforme*	NBPGR, India
40	47*	LA 2205 B	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis,USA)
41	48	WIR 13706	*S. esculentum* var. *cerasiforme*	IIVR, India
42	49*	LA 1311-18	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis, USA)
43	50*	EC 25265	*S. esculentum* var. *cerasiforme*	NBPGR, India
44	51*	EC 771613	*S. esculentum* var. *cerasiforme*	NBPGR, India
45	52*	LA 0384	*S. esculentum* var.	TGRC (UC- Davis,USA)

Sl. No.	Code	Name	Species	Origin
			cerasiforme	
46	53*	LA 1479	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis,USA)
47	54*	EC 771616	*S. esculentum* var. *cerasiforme*	NBPGR, India
48	55*	LA 2138 B	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis,USA)
49	56*	LA 2138 A	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis, USA)
50	57*	LA 1713	*S. esculentum* var. *cerasiforme*	TGRC (UC- Davis,USA)
51	58*	WIR 13708	*S. esculentum* var. *cerasiforme*	IIVR, India
52	59*	WIR 3957	*S. esculentum* var. *cerasiforme*	IIVR, India
53	60*	EC 771588	*S. esculentum* var. *cerasiforme*	NBPGR, India
54	61*	IC 45	*S. esculentum* var. *cerasiforme*	IIVR, India
55	63*	EC 677191	*S. esculentum* var. *cerasiforme*	NBPGR, India
56	64*	EC 608394	*S. esculentum* var. *cerasiforme*	NBPGR, India
57	65	H 7996	*S. esculentum* var. *cerasiforme*	IIVR, India
58	66*	EC 676732	*S. lycopersicum*	NBPGR, India
59	70	EC 68687	*S. lycopersicum*	NBPGR, India
60	71	EC 677034	*S. lycopersicum*	NBPGR, India
61	73	EC 686531	*S. lycopersicum*	NBPGR, India
62	74*	EC 608275	*S. lycopersicum*	NBPGR, India
63	75*	EC 676819	*S. lycopersicum*	NBPGR, India
64	77	EC 610654	*S. lycopersicum*	NBPGR, India
65	78*	EC 676796	*S. lycopersicum*	NBPGR, India
66	80*	EC 676809	*S. lycopersicum*	NBPGR, India
67	81*	EC 109762	*S. lycopersicum*	NBPGR, India
68	83*	EC 677091	*S. lycopersicum*	NBPGR, India
69	85*	EC 676779	*S. lycopersicum*	NBPGR, India
70	86*	EC 608391	*S. lycopersicum*	NBPGR, India
71	87*	EC 677123	*S. lycopersicum*	NBPGR, India
72	88*	EC 676730	*S. lycopersicum*	NBPGR, India
73	91	EC 677076	*S. lycopersicum*	NBPGR, India
74	92*	EC 677079	*S. lycopersicum*	NBPGR, India
75	+93*	Arka Meghali	*S. lycopersicum*	IIHR, India
76	+94*	Arka Vikas	*S. lycopersicum*	IIHR, India
77	+96*	Arka Abha	*S. lycopersicum*	IIHR, India

Sl. No.	Code	Name	Species	Origin
78	97*	VRTC-17	*S. lycopersicum*	IIVR, India
79	99*	Pusa Ruby	*S. lycopersicum*	IIVR, India
80	100*	EC 676778	*S. lycopersicum*	NBPGR, India
81	101*	EC 676745	*S. lycopersicum*	NBPGR, India
82	102*	EC 676596	*S. lycopersicum*	NBPGR, India
83	103*	EC 109754	*S. lycopersicum*	NBPGR, India
84	104*	LA 4345	*S. lycopersicum*	TGRC (UC- Davis, USA)
85	105	CLN 2070 A	*S. lycopersicum*	AVRDC, Taiwan
86	106	CLN 13149	*S. lycopersicum*	AVRDC, Taiwan
87	107*	EC 771593	*S. lycopersicum*	NBPGR, India
88	108*	EC 771598	*S. lycopersicum*	NBPGR, India
89	109*	EC 771610	*S. lycopersicum*	NBPGR, India
90	110*	EC 771597	*S. lycopersicum*	NBPGR, India
91	111*	EC 771593	*S. lycopersicum*	NBPGR, India
92	112*	EC 776581	*S. lycopersicum*	NBPGR, India
93	113*	EC 771584	*S. lycopersicum*	NBPGR, India
94	114*	EC 771585	*S. lycopersicum*	NBPGR, India
95	115*	EC 771612	*S. lycopersicum*	NBPGR, India
96	116*	EC 771601	*S. lycopersicum*	NBPGR, India
97	117	EC 771580	*S. lycopersicum*	NBPGR, India
98	118	EC 771591	*S. lycopersicum*	NBPGR, India
99	119	EC 771614	*S. lycopersicum*	NBPGR, India
10	120*	EC 771594	*S. lycopersicum*	NBPGR, India
101	121*	EC 771582	*S. lycopersicum*	NBPGR, India
102	123*	EC 771589	*S. lycopersicum*	NBPGR, India
103	124*	EC 771611	*S. lycopersicum*	NBPGR, India

Note: * Germplasm accessions used for root study and +: Check varieties

The material has been planted in Augmented design (Federer, 1956) during rabi-*summer* 2015 under normal and water stress conditions in two separate but in adjacent plots of uniform soil. The design consisted of four blocks and within each block, 25 germplasm accessions with three check varieties were randomly planted. Each entry was transplanted in a single row of 5.00 m in length with a spacing of 0.75 m (between rows) x 0.50m (between plants). Drought was imposed at 60 DAT to all the germplasm by withholding irrigation in stress plot for twenty days while the normal plot was given regular irrigation twice a week using drip irrigation.

The Meteorological observation at GKVK Station, UAS, Bangalore and soil moisture was recorded during 20 days of drought treatment in *rabi-summer* 2015. No rainfall was recorded during 20 days of stress.

Field Observations for the Traits Under Normal And water Stress Conditions:

Five randomly chosen plants in each accession were labeled and used for recording fruit yield, physiological and agronomical traits, *viz*., days to 50 *per cent* flowering, days to first fruit-set, plant height, primary branches per plant, flowers per cluster, clusters per plant,

fruits per cluster, fruits per plant, fruit traits like average fruit weight, fruit volume, fruit flesh thickness (pericarp thickness), locule per fruit, total soluble solids, fruit yield per plant and five drought traits including SPAD chlorophyll meter reading, specific leaf area, stem girth, leaf rolling and relative water content.
Fifteen drought tolerant indices (DTIs) were calculated based on fruit yield under stress (Y_S) and normal (Y_P) conditions to screen for drought tolerance of accessions (Table 4-4).

Table 4-4 Fifteen drought tolerant indices (DTIs) related to WUE and Fruit Yield Traits

No	Name of DTIs	Code	Formula	References
1	Tolerance index	TOL	Y_P-Y_S	Rosielle and Hamblin, 1981
2	Mean productivity	MP	$(Y_P+Y_S)/2$	Rosielle and Hamblin, 1981
3	Geometric mean productivity	GMP	$\sqrt{(Y_s \times Y_p}$	Fernandez, 1992
4	Harmonic mean	HAM	$2*(Y_P \times Y_S)/(Y_P+Y_S)$	Kristin *et al.*, 1997
5	Stress tolerance index	STI	$(Ys \times Yp)/(\bar{Y}p^2)$	Fischer and Maurer, 1978
6	Relative drought index	RDI	$(Ys/Yp)/(\bar{Y}s / \bar{Y}p)$	Fischer and Wood, 1979
7	Abiotic tolerance index	ATI	$[(Y_P-Y_S)/(\overline{Y_P}/\overline{Y_S})] \times \sqrt{(Yp \times Ys)}$	Moosavi *et al.*, 2008
8	Stress susceptibility percentage index	SSPI	$((Yp - Ys/2(\bar{Y}p)) \times 100$	Moosavi *et al.*, 2008
9	Stress non-stress production index	SNPI	$[\sqrt[3]{(Yp+Ys)/(Yp-Ys)}] \times [\sqrt[3]{Yp \times Ys \times Ys}]$	Moosavi, 2008
10	Yield index	YI	$(Ys)/(\bar{Y}s)$	Gavuzzi, 1997
11	Yield stability index	YSI	Y_S/Y_P	Bouslama and Schapaugh, 1984
12	Modified STI	K_1STI	$Y_p^2/\bar{Y}_p^2$	Farshadfar and Sutka, 2002
13	Modified STI	K_2STI	$Y_s^2/\bar{Y}_s^2$	Farshadfar and Sutka, 2002
14	Drought resistance index	DI	$(Ys \times (Ys / Yp))/\bar{Y}s$	Lan, 1998
15	Stress susceptibility index	SSI	$(1-(Ys/Yp))/(1-(\bar{Y}s)/(\bar{Y}p))$	Fischer and Maurer, 1978

Where, $\underline{Y_P}$: Fruit yield under normal condition, Y_S: Fruit yield under stress condition,
$\underline{Y_P}$: Grand mean of fruit yield under normal condition and
$\underline{Y_S}$: Grand mean of fruit yield under stress condition

Identification of Tomato Germplasm Accessions/Species Based On Principle Component Analysis

Biplot analysis was presented by first two principle component analysis (PCA) which were computed based on the rank correlation matrix using data from fifteen drought tolerance indices along with fruit yield under normal and water stress condition by Microsoft Excel (2010) and XLSTAT 2014, Copyright Addinsoft 1995-2014 (http://www.xlstat.com) as followed by Iqbal *et al.,* (2014).

Three dimensional plots were drawn from a best suitable DTI, *viz*, STI and fruit yield (Yp and Ys) using STATISTICA ver. 10 (StatSoft, Inc.) as conducted by Khalili *et al*., (2014). The results would be showed for 103 ('All' spp), 46 ('lyco' species), 32 ('cherry' species) and 25 ('wild' species) germplasm accessions in 2015.

The most common drought tolerant and susceptible accessions from two methods were classified with respect to each of four group species.

Identification of Tomato Germplasm Accessions/Species Based On Ranking Method

Based on the value of each DTI of all the tomato accessions, a rank for each accession was classified and rank sum (RS) was calculated [RS= Rank mean (Rm) + Standard deviation of rank (SDR) as conducted by Farshadfar *et al*., (2012)]. From this, the best tolerant genotypes were identified having low R_m, SDR and RS.

Overall, the best drought tolerant as well as susceptible tomato germplasm accessions/species would be classified if they were common across methods (Biplots, three dimensional plots and ranking method).

Correlation among Drought, Fruit Yield and Yield Related Traits of Germplasm Accessions under Normal and Stress Conditions During 2015

The Pearson correlation coefficients were computed between nineteen droughts, fruit yield and yield related traits under normal and stress conditions at K_1 field condition.The results were computed for 103 ('All' spp), 46 ('lyco' species), 32 ('cherry' species) and 25 ('wild' species) germplasm accessions. Analysis was done using variance and covariance components as suggested by Al-Jibouri *et al.,* (1958) by SPSS ver 16 (SPSS Inc., 2010).

Experiment 2: Genotyping of cultivated and wild germplasm accessions with informative markers to establish association with traits related to WUE and fruit yield.

A total of 103 germplasm accessions were raised in plastic trays under net house conditions at Department of Genetics and Plant Breeding, UAS, Bangalore. Youngest leaves from each accession were collected for genomic DNA extraction uaing CTAB method (Doyle and Doyle, 1990) with some modifications. Quantification of DNA in each sample was checked by Agarose gel electrophoresis (Sambrook *et al*., 1989). The quality and quantity of genomic DNA was estimated using 0.8% agarose gel along with standard uncut lambda DNA as ladder at 50 ng (Bangalore GeNei, India).

Screening For DNA Polymorphism among 103 Tomato Germplasm Accessions/Species

A total of 165 SSR markers were screened to find ampified/polymorphism between the 103 germplasm accessions. The SSR markers were chosen from previously published by Areshchenkova and Ganal (2002), Suliman-Pollatschek *et al.,* (2002), He *et al*., (2003), Frary *et al*., (2005), Ruiz *et al*., (2005), Rajput *et al*., (2006), Benor *et al*., (2008), Mazzucato *et al*. (2008), Ranc *et al.,* (2008); Kwon *et al*., (2009), Geethanjali *et al*., (2010), Caramante *et al.,* (2011), Geethanjali *et al*., (2011), Liu *et al.,* (2011), Hu *et al*., (2012), El-Awady *et al*., (2012), Kadirvel *et al*., (2013), SolGenomics Network [(Tomato-EXPEN 2000; Tomato-EXPIMP 2008; Tomato Sun 1642 x LA1589], Kazusa Tomato Genomics Database (www.marker.kazusa.or.jp/), Yogendra and Gowda (2014), Al-Tamimi *et al*., (2015) and Zhang *et al*., (2015). List of the polymorphic markers, chromosome, forward and reverse primer sequences of SSR markers chosen for the genotyping. All these markers were designed by Eurofins Genomics, India and the dilution were done as information given enclosed for each primer.

Metaphor Agarose Gel Electrophoresis

MetaPhor agarose gel electrophoresis (MAGE) is another approach to separate alleles of microsatellite markers (Abdurakhmonov *et al*., 2007). MetaPhor agarose is an intermediate

melting temperature agarose (75°C) that provides twice the resolution capabilities of the finest sieving agarose products. Using submarine gel electrophoresis, MetaPhor agarose gives high resolution separation of 20 to 800 bp DNA fragments that differ in size by 2%, which approximates the resolution of polyacrylamide gels. MetaPhor agarose gels (2% to 4%) made in either TAE or TBE and stained with ethidium bromide are ideal for resolving SSRs (Anonymous, 2007). Asif *et al*., (2008) were successful to clearly separate SSR alleles for two cotton cultivars with a size difference of five bp by using 2% standard agarose and 2% Metaphor agarose. They suggested that MAGE is a reliable and appropriate approach for identification of small length polymorphisms while screening large number of samples. In the present study, 2% Himedia agarose and 2% Metaphor agarose (MetaPhor™ Agarose - Lonza) were adopted for gel electrophoresis.

Number of Bands & Band Sizes Recorded:
Clear and distinct bands amplified by SSR primers were recorded for band sizes by comparing them with band size known from DNA ladder (100 bp DNA ladder) among the 103 germplasm accessions. The allelic number for each SSR marker was sum of how many band sizes observed (each band at the same mobility is 1). These genotypic data were used for further analyses with suitable formats required by software programmes.

Association mapping:
A total of 165 primers were done for genotyping the 103 germplasm accessions as described earlier. Among these, 145 primers were used for further analysis whereas the remaining ones were discarded due to whether multi-bands or improbably amplified (less than 60%) across accessions.

Population Structure Analysis:
The population structure of the 103 tomato germplasm accessions was assessed using the model-based (Bayesian clustering) method implemented in STRUCTURE v2.3.3 (Pritchard *et al*., 2000).

Linkage Disequilibrium:
Linkage disequilibrium (LD) estimates and significance for each pair of SSR loci were evaluated using TASSEL v2.1 (www.maizegenetics.net/) with 1000 permutations. Prior to LD analysis 5% threshold was used to remove rare alleles in order to overcome their negative bias in LD estimation.

Phenotyping Data:
The phenotypic data from the field trials during *rabi-summer* 2015 for normal and stress conditions along with145 polymorphic SSR marker data were used to study the marker-trait associations. For marker- trait association analysis, phenotypic data was used as belows:
Among nineteen traits under field condition, Days to fifty percent flowering (DFF), Days to fifty percent fruit sett (DFFS), Plant height (PH), Branch number (BN), Fruits per plant (FPP), Clusters per plant (CPP), Avarage fruit weight (AFW), fruit yield per plant (FYPP) and five drought trait, *viz.,* Sphad cholorophyll meter reading (SCMR), Specific leaf area (SLA), Relative water content (RWC), Stem girth (STG) and Leaf rolling (LR) were computed separately under normal and stress condition during 2015. The results were computed for

103 (‘All spp.’), 46 (‘lyco’ species), 32 (‘cherry’ species) and 25 (‘wild’ species) germplasm accessions.Those traits showed non-significant phenotypic variation were also discarded.

Experiment-3: Phenotyping of F_2 and F_3 population of inter-specific cross for traits related to WUE and fruit yield

Phenotyping of F_2

The evaluations of iner-specific cross between EC-771612 × LA-2657 for traits related to water use efficiency (WUE) and fruit yield was conducted during summer 2015 at GKVK along with its parents and checks.

MATERIAL AND METHOD

The F_2 population containing 176 lines developed from the inter-specific cross between EC-771612 × LA-2657 was evaluated during 2015 summer for traits related to water use efficiency, growth parameters and fruit yield parameters.

Each seedling was transplanted in a spacing of 60cm between row to row and 45cm between two plants. The experimental area was divided into 4 blocks with each block containing 44 F_2 plants along with 4 checks (2parents + 2 checks). The recommended packages of practices were applied for raising a healthy crop.

Observations for individual plants was recorded for both WUE and fruit yield traits that includes Days to flowering (DFF), SPAD chlorophyll meter reading (SCMR), Specific leaf area (SLA), plant height (PH, cm), primary branches, flowers per cluster (FLPP), fruits per cluster (FRPP), clusters per plant (CPP), Average fruit weight (AFW), fruit number (FRT No.) and fruit yield per plant (Yld, g).

Phenotyping of F_3

The evaluations of F_3 mapping population from the cross EC-771612 × LA-2657 for traits related to water use efficiency (WUE) and fruit yield was conducted in *kharif* 2015 at GKVK in augmented design with parents and checks.

MATERIAL AND METHODS

The experimental material comprises of 112 F_3 seeds, sown in trays and transplanted along with parents and checks in augmented design. Each seedling was transplanted in a plant to progeny row with spacing of 60 cm between row to row and 45 cm between two plants. Each family consists of 12 plants. The experimental area was divided into 4 blocks with each block containing 28 families with 4 checks (2 parents + 2 checks). The recommended packages of practices were applied for raising a healthy crop.

Observations for individual plants from each family was recorded for both WUE and fruit yield traits that includes Days to flowering (DFF), SPAD chlorophyll meter reading (SCMR), Specific leaf area (SLA), plant height (PH, cm), primary branches, flowers per cluster (FLPP), fruits per cluster (FRPP), clusters per plant (CPP), Average fruit weight (AFW), fruit number (FRT No.) and fruit yield per plant (Yld).

Experiment 4: Parental polymorphism and genotyping of F_2 mapping population with SSR markers linked to the traits related to wue and fruit yield

The genotypic work was conducted with F_2 mapping population of the inter-specific cross between EC-771612 × LA-2657 based on polymorphic marker information and high contrasting phenotypic trait values **(add values)** (SLA, SCMR, $\Delta^{13}C$).

MATERIAL AND METHODS

Genomic DNA was isolated using CTAB method (Doyle and Doyle, 1990) with some modifications. Quantification of DNA in each sample was checked by Agarose gel electrophoresis (Sambrook *et al*., 1989). The quality and quantity of genomic DNA was estimated using 0.8% agarose gel along with standard uncut lambda DNA as ladder at 50 ng (Bangalore GeNei, India).

The most commonly used methodologies for quantifying the amount of nucleic acid in a sample preparation are Gel Electrophoresis and Spectrometric analysis.

Results:

Experiment 1: Phenotyping of germplasm lines for traits related to wue and fruit yield.

Analysis of variance for the data of 2014, 2015 and for 'Mean' data

Phenotypic data for nineteen quantitative traits during 2015 under normal and stress condition. The analysis of variance indicate significant variation among germplasm accessions of 'All' spp (total six species) and within each species ('lyco', 'cherry' and 'wild') for all the traits studied under normal and stress conditions given in Table 3. For DFF, BN, SCMR, LR and TSS in 2015, for 'cherry' species LR in 2015 and for 'wild' species with LR in 2015. These results indicate that 'lyco' species has narrow phenotypic variation as compared to other species because of population bottlenecks (Rick, 1976), intensive selection of a few desired traits (Williams and Clair, 1993) and narrow genetic base (Labate and Robertson, 2002 and Foolad *et al*., 2003).

Mean, range and genetic variability parameters for specific trait under field condition

Mean and range of drought,fruit yield and yield related traits for 'All' spp, 'lyco', 'cherry' and 'wild' species during 2014 and 2015 are showed in Table 3. The highlight of results and discussion of mean, range and genetic variability parameters for these traits are discussed as follows:

Days to 50 *per cent* flowering (DFF)

DFF values range from 55.00 days to 114.00 days and it has mean value of 4.62 and 75.77 days ('All' spp), 79.37 and 76.77 days ('lyco'), 70.22 and 75.41 days ('cherry') and 72.08 and 74.52 days ('wild') under normal and stress conditions, respectively.

High GCV, PCV and GAM are seen for wild species followed by 'All' spp, 'cherry' and least values in 'lyco' species. High heritability (>70%) values have been recorded for all four groups. In tomato, high PCV and GCV were reported by Narolia *et al.*, (2012) and Nwosu *et al.,* (2012); high GAM was by Mohamed *et al.*, (2012) andNadeem *et al.,* (2013) and high heritability was by Patel *et al*., (2015). The low GCV and PCV were recorded by Kumar *et al,* (2013).

Table 4-5 Mean sum of squares of fruit yield, drought and yield related traits for four groups of species under normal and stress conditions during 2015

Trait	Year	Group of species (number of tomato accessions)							
		'All' spp (n=103)		**'Lyco'** (n=46)		**'Cherry'** (n=32)		**'Wild'** (n=25)	
		Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
DFF	2015	95.29**	83.04**	22.62ns	27.98*	53.25**	37.05*	221.35**	228.86**
DFFS	2015	89.46**	92.14**	25.61*	30.64**	47.00**	39.93**	203.59**	253.14**
PH	2015	2956.49**	1744.26**	279.71**	258.02**	2702.85**	1967.27**	5307.94**	3014.10**
BN	2015	21.77**	16.09**	1.64ns	1.75**	14.08**	9.73**	29.60**	21.50**
SCMR	2015	22.30**	18.16**	9.47ns	5.54*	23.58**	18.77**	27.44**	23.04**
SLA	2015	1573.31**	1219.13**	700.95**	675.60**	1025.67**	793.48**	2604.60**	2041.93**
RWC	2015	24.63*	31.67**	15.64*	24.34**	21.07*	25.54**	25.76*	28.75**
LR	2015	0.70ns	1.93**	0.71ns	1.29**	0.68ns	1.61**	0.79ns	1.77**
STG	2015	4.12**	3.29**	3.27**	2.39**	3.89**	2.77**	3.35**	2.27**
FNPC	2015	12.59**	11.40**	0.64**	0.52**	4.59**	3.71**	25.33**	23.49**
FLPC	2015	22.50**	19.46**	0.82*	0.94**	5.87**	5.24**	50.08**	42.21**
CPP	2015	2123.64**	1030.58**	31.87*	14.31**	1020.83**	746.85**	4461.83**	1891.42**
FPP	2015	246123.98**	86373.20**	305.43**	225.63**	31329.31**	24183.51**	553109.73**	177297.49**
AFW	2015	2717.25**	1653.83**	1962.17**	1102.61**	1042.72**	730.83**	1541.87**	1105.72**
FV	2015	2863.92**	1730.76**	2073.36**	1138.09**	1106.06**	765.90**	1630.63**	1158.16**
FT	2015	4.14**	3.26**	1.20**	1.03**	2.24**	1.87**	3.74**	3.25**
LN	2015	2.17**	1.49**	2.51**	1.82**	2.59**	1.71**	3.38**	2.10**
TSS	2015	2.17**	2.70**	0.19**	0.34ns	1.26**	1.45**	4.56**	5.11**
FYPP	2015	0.20**	0.18**	0.20**	0.19*	0.16**	0.12*	0.11*	0.09*

Note: *and ** Significant @ 0.05 and 0.01 probability levels, respectively and ns-nonsignificance.

'Lyco'- *S. lycopersicum,* 'Cherry'- *S. lycopersicum* var. *cerasiforme,* 'All'spp.- Total six species'Wild'- *S. habrochaites, S. peruvianum, S. pimpinellifolium, S.***s***cheemaniae*

Table 4-6 Overview of genetic variability among 'All' spp, 'lyco', 'cherry' and 'wild' species under normal (N) and water stress (S) conditions during 2015

En.	Trait	'All' spp				'Lyco' species				'Cherry' species				'Wild' species			
		GCV	PCV	h^2	GAM	GCV	PCV	h^2	GAM	GCV	PCV	h^2	GAM	GCV	PCV	h^2	GAM
N	DFF	M	M	H	H	L	L	H	L-M	L	L-M	H	M	M	M	H	H
S		M	M	H	H	L	L	H	M	L	L	H	M	M	M	H	H
N	DFFS	M	M	H	H	L-M	L-M	H	M-H	L-M	L-M	H	M	M-H	M-H	H	H
S		M	M	H	H	L-M	L-M	H	M	L-M	L-M	H	M	M	M-H	H	H
N	PH	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
S		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
N	BN	H	H	H	H	M-H	M-H	H	H	H	H	H	H	H	H	H	H
S		H	H	H	H	M-H	H	H	H	H	H	H	H	H	H	H	H
N	SCMR	L	L	H	M	L	L	H	L-M	L	L	H	M	L-M	L-M	H	M
S		L	L	H	M	L	L	H	L	L	L	H	M	L-M	L-M	H	M-H
N	SLA	M	M	H	H	M	M	H	H	M	M	H	H	M	M	H	H
S		M	M	H	H	M	M	H	H	M	M	H	H	H	H	H	H
N	RWC	L	L	H	L-M	L	L	H	L-M	L	L	H	L-M	L	L	H	M
S		L	L	H	M	L-M	L-M	H	M-H	L	L	H	M	L	L	H	M
N	LR	H	H	M-H	H	H	H	M-H	H	H	H	L-M	M-H	H	H	M-H	H
S		H	H	H	H	L-H	M-H	H	H	M-H	M-H	H	H	H	H	H	H
N	STG	M	M	H	H	M	M	H	M-H	L-M	L-M	M-H	M-H	M-H	M-H	H	H
S		M	M	H	H	M	M	H	H	M	M	H	H	M-H	M-H	H	H
N	FLPC	H	H	H	H	M-H	M-H	H	H	H	H	H	H	H	H	H	H
S		H	H	H	H	M	M	H	H	H	H	H	H	H	H	H	H
N	FNPC	H	H	H	H	M	M-H	M-H	M-H	H	H	H	H	H	H	H	H
S		H	H	H	H	M-H	M-H	H	H	H	H	H	H	H	H	H	H
N	CPP	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
S		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
N	FPP	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
S		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
N	AFW	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
S		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
N	FV	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
S		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
N	FT	H	H	H	H	M	M-H	M-H	H	H	H	H	H	H	H	H	H
S		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
N	TSS	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
S		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
N	LN	H	H	H	H	L	L	H	M	M-H	M-H	H	H	H	H	H	H
S		H	H	H	H	L	L	M-H	L-M	M	M	H	H	H	H	H	H
N	FYPP	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
S		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H

Note: L-Low [PCV-GCV: 0-10%, h^2: 0-30%; GAM: 0-10%]; M- Medium [PCV-GCV: 10-20%, h^2: 30-60%; GAM: 10-20%] and H- High [PCV-GCV: >20%, h^2: >60%; GAM: >10%].

To be classified as low group

To be ranked as high group

High phenotypic coefficients of variability and high genotypic coefficient of variability were observed for all the characters except SLA and RWC for all species, DFF, SCMR and LN for lyco spp, DFF, DFFS, SCMR and RWC for cherry spp and RWC for wild spp (Table 4-6). The presence of narrow gap between PCV and GCV for all the characters except SLA and RWC for all species, DFF, SCMR and LN for lyco spp, DFF, DFFS, SCMR and RWC for cherry spp and RWC for wild spp which implies that expression of these traits has low environmental influence. Further, high heritability coupled with high genetic advance as a *per cent* of mean was observed for all the characters in all the different spp indicate that these characters are under the influence of additive genes hence, selection based on these traits will be rewarding for the improvement of required plant type Liu and Chen (2002), Liu *et al*.,

(2006), del Amor *et al,* (2007), Guo *et al,* (2007), Patanè and Cosentino (2010), Patanè *et al*., (2011), Helyes *et al*., (2012), Barbagallo *et al*., (2013), Helyes *et al*., (2014), Patenè and Saita (2015), Shao *et al*., (2015), Sibomana *et al*., (2015) and Cantore *et al*., (2016).

In conclusion, genetic variability exhibited for nineteen quantitative traits of 'All' spp, 'lyco', 'cherry' and 'wild' species indicating that the 'wild' species show most of traits with highest variation followed by 'All' spp, 'cherry' species and least variability in 'lyco' species. PH, CPP, FPP, AFW, FV, TSS and FYPP show highest genetic variations for all four groups of species indicating selection of those traits would be effective in breeding programme followed by BN, FNPC, FLPC, FT (medium to high variation in 'lyco' species only) and various variation from low to high variability parameters among four groups of species for LR, SLA, STG, LN, SCMR, RWC, DFF and DFFS (Table 4-6). In addition, the effect of environmental conditions between years lead to phenotypic variation of specific quantitative traits under normal and stress conditions ('Mean' data) like DFF, DFFS, SCMR,SLA, RWC, LR, STG, TSS and FYPP. These traits in 'Mean' along with non-significant variation traits within two years in respect to each of species will not be applied for marker- trait associations.

Correlation analysis between fifteen DTIs for four groups of species

In the present study, the results are analysed and discussed in case of four groups of species denoted as 'All' spp, 'lyco', 'cherry' and 'wild' species during 2015.Correlation between fruit yield (Yp- Normal and Ys- Stress condition) and different DTIs can be a good criterion for screening the best germplasm accessions and indices used.

Correlation analysis between fifteen DTIs for 'All' spp.

Y_S is seen significant and positive correlation with MP, GMP, HAM, STI, RDI, ATI, SNPI, YI, YSI, K_1STI, K_2 STI, DI and Y_P, whereas it is negatively correlated with TOL, SSPI and SSI. Y_P is significantly and positively related with all drought indices, except RDI, YSI and SSI. Thus, ten DTIs, *viz.*, MP, GMP, HAM, STI, ATI, SNPI, YI, K_1STI, K_2STI and DI are significantly and positively associated with both Y_P and Y_S (Table 4-7).

Correlation between fifteen DTIs for 'lyco' species

Y_S is significant and positive relationship with MP, GMP, HAM, STI, RDI, ATI, SNPI, YI, YSI, K_1STI, K_2STI, DI and Y_P, whereas it hasbeen observed to be negatively correlated with SSI. Y_P is significantly and positively correlated with all drought indices, except RDI, YSI and SSI. Thus, ten DTIs, *viz.*, MP, GMP, HAM, STI, ATI, SNPI, YI, K_1STI, K_2STI and DI are found significantly and positively correlated with both Y_P and Y_S (Table 4-8).

Correlation analysis between fifteen DTIs for 'cherry' species

Y_S show significant and positive correlation with MP, GMP, HAM, STI, RDI, SNPI, YI, YSI, K_1STI, K_2STI, DI and $Y_P$but it has negatively related with SSI. $Y_P$is significantly and positively correlated with all drought indices, except RDI, SNPI, YSI, DI and SSI. Hence, seven DTIs, *viz.*, MP, GMP, HAM, STI, YI, K_1STI and K_2STI are showed significant and positive relationship with both Y_P and Y_S (Table 4-9).

Table 4-7 Pearson correlation coefficient analysis between fifteen drought tolerance indices (DTIs) and fruit yield for 'All' spp during 2014 and 2015

DTIs	TOL	MP	GMP	HAM	STI	RDI	ATI	SSPI	SNPI	YI	YSI	K_1STI	K_2STI	DI	SSI	Y_P	Y_S
TOL	**1**	0.24*	0.12	0.02	0.09	-0.84**	0.92**	1.00**	-0.46**	-0.21*	-0.84**	0.60**	-0.23*	-0.50**	0.84**	0.59**	-0.21*
MP	0.10	**1**	0.99**	0.97**	0.96**	0.23*	0.52**	0.24*	0.58**	0.90**	0.24*	0.89**	0.86**	0.71**	-0.24*	**0.93****	**0.90****
GMP	0.04	0.99 **	**1**	0.99**	0.97**	0.34**	0.42**	0.12	0.63**	0.94**	0.34**	0.84**	0.90**	0.78**	-0.34**	**0.88****	**0.94****
HAM	-0.01	0.99**	0.99**	**1**	0.97**	0.43**	0.33**	0.02	0.67**	0.97**	0.43**	0.78**	0.93**	0.83**	-0.43**	**0.82****	**0.97****
STI	0.04	0.99**	0.99**	0.99**	**1**	0.32**	0.40**	0.09	0.61**	0.93**	0.33**	0.84**	0.94**	0.77**	-0.33**	**0.83****	**0.93****
RDI	-0.86**	0.35**	0.40**	0.45**	0.38**	**1**	-0.63**	-0.84**	0.76**	0.62**	1.00**	-0.17	0.58**	0.82**	-1.00**	-0.13	0.62**
ATI	0.89**	0.49**	0.44**	0.40**	0.44**	-0.55**	**1**	0.92**	-0.25*	0.10	-0.62**	0.82**	0.067	-0.22*	0.62**	0.78**	0.10
SSPI	1.00**	0.10	0.04	-0.01	0.04	-0.86**	0.89**	**1**	-0.46**	-0.21*	-0.84**	0.60**	-0.229*	-0.50**	0.84**	**0.59****	**-0.21***
SNPI	-0.50**	0.70**	0.72**	0.74**	0.71**	0.77**	-0.18	-0.50**	**1**	0.79**	0.76**	0.26**	0.78**	0.89**	-0.76**	**0.31****	**0.79****
YI	-0.21*	0.95**	0.97**	0.98**	0.96**	0.61**	0.21*	-0.21*	0.84**	**1**	0.62**	0.62**	0.97**	0.94**	-0.62**	**0.67****	**1.00****
YSI	-0.86**	0.36**	0.41**	0.45**	0.38**	1.00**	-0.55**	-0.86**	0.77**	0.61**	**1**	-0.17	0.58**	0.82**	-1.00**	-0.12	0.63**
K_1STI	0.38**	0.95**	0.93**	0.91**	0.94**	0.07	0.72**	0.38**	0.48**	0.82**	0.08	**1**	0.62**	0.35**	0.17	**0.98****	**0.62****
K_2STI	-0.20*	0.94**	0.95**	0.96**	0.97**	0.56**	0.20*	-0.20*	0.83**	0.99**	0.57**	0.82**	**1**	0.93**	-0.58**	**0.63****	**0.97****
DI	-0.48**	0.82**	0.85**	0.87**	0.84**	0.79**	-0.08	-0.48**	0.93**	0.95**	0.80**	0.61**	0.94**	**1**	-0.82**	**0.40****	**0.94****
SSI	0.86**	-0.35**	-0.40**	-0.45**	-0.38**	-1.00**	0.55**	0.86**	-0.77**	-0.61**	-1.00**	-0.07	-0.56**	-0.79**	**1**	0.12	-0.62**
Y_P	**0.38****	**0.96****	**0.94****	**0.92****	**0.93****	0.08	**0.71****	**0.38****	**0.51****	**0.83****	0.09	**0.99****	**0.82****	**0.63****	-0.08	**1**	0.67**
Y_S	**-0.21***	**0.95****	**0.97****	**0.98****	**0.96****	0.61**	**0.21***	**-0.21***	**0.84****	**1.00****	0.61**	**0.82****	**0.99****	**0.95****	-0.61**	0.83**	**1**

Notes:* and ** Significant @ 0.05 and 0.01 probability levels respectively;

Table 4-8 Pearson correlation coefficient analysis between fifteen drought tolerant indices (DTIs) and fruit yield for 'lyco'species during 2014 and 2015

DTIs	**TOL**	**MP**	**GMP**	**HAM**	**STI**	**RDI**	**ATI**	**SSPI**	**SNPI**	**YI**	**YSI**	**K_1STI**	**K_2STI**	**DI**	**SSI**	**Yp**	**Ys**
TOL	1	0.45**	0.29	0.13	0.25	-0.82**	0.95**	1.00**	-0.35*	-0.13	-0.82**	0.81**	-0.20	-0.46**	0.82**	0.76**	-0.13
MP	0.13	1	0.98**	0.94**	0.95**	0.07	0.64**	0.45**	0.52**	0.82**	0.07	0.85**	0.77**	0.56**	-0.07	**0.93****	**0.82****
GMP	0.09	1.00**	1	0.99**	0.97**	0.23	0.50**	0.28	0.61**	0.91**	0.23	0.75**	0.86**	0.68**	-0.23	**0.84****	**0.91****
HAM	0.05	0.99**	1.00**	1	0.97**	0.36*	0.37*	0.13	0.67**	0.96**	0.37*	0.64**	0.91**	0.77**	-0.36*	**0.75****	**0.96****
STI	0.09	0.99**	0.99**	0.99**	1	0.23	0.49**	0.24	0.59**	0.89**	0.23	0.75**	0.89**	0.67**	-0.23	**0.80****	**0.89****
RDI	-0.78	0.46**	0.49**	0.52**	0.46**	1	-0.67**	-0.82**	0.72**	0.60**	1.00**	-0.39**	0.62**	0.84**	-1.00**	**-0.30***	**0.60****
ATI	0.86**	0.59**	0.56**	0.53**	0.56**	-0.38**	1	0.95**	-0.20	0.10	-0.67**	0.93**	0.05	-0.26	0.67**	0.87**	0.10
SSPI	1.00**	0.13	0.09	0.05	0.09	-0.79**	0.85**	1	-0.35*	-0.13	-0.82**	0.81**	-0.20	-0.46**	0.82**	0.76**	-0.14
SNPI	-0.38**	0.77**	0.79**	0.80**	0.77**	0.78**	0.05	-0.39**	1	0.80**	0.73**	0.14	0.83**	0.91**	-0.73**	0.24	0.81**
YI	-0.10	0.97**	0.98**	0.99**	0.97**	0.64**	0.39**	-0.10	0.87**	1	0.61**	0.43**	0.97**	0.92**	-0.60**	**0.55****	**1.00****
YSI	-0.78**	0.47**	0.50**	0.53**	0.47**	1.00**	-0.37*	-0.78**	0.79**	0.65**	1	-0.39**	0.62**	0.84**	-1.00**	**-0.29***	**0.61****
K1STI	0.35*	0.97**	0.96**	0.95**	0.96**	0.24	0.76**	0.34*	0.63**	0.89**	0.25	1	0.40**	0.10	0.39**	**0.97****	**0.43****
K2STI	-0.10	0.96**	0.97**	0.97**	0.98**	0.60**	0.38**	-0.10	0.85**	0.99**	0.61**	0.89**	1	0.93**	-0.62**	**0.48****	**0.97****
DI	-0.34*	0.89**	0.90**	0.92**	0.90**	0.80**	0.16	-0.34*	0.93**	0.97**	0.80**	0.76**	0.96**	1	-0.84**	0.21	0.92**
SSI	0.78**	-0.46**	-0.49**	-0.52**	-0.47**	-1.00**	0.38*	0.79**	-0.78**	-0.64**	-1.00**	-0.24	-0.60**	-0.80**	1	**0.30***	**-0.60****
Yp	0.34*	**0.98****	**0.97****	**0.96****	**0.96****	0.26	**0.75****	0.34*	**0.65****	**0.90****	0.27	**0.99****	**0.89****	**0.77****	-0.27	1	0.55**
Ys	-0.10	**0.97****	**0.98****	**0.99****	**0.97****	0.64**	**0.39****	-0.10	**0.87****	**1.00****	0.65**	**0.89****	**0.99****	**0.97****	-0.64**	0.90**	1

Notes:* and ** Significant @ 0.05 and 0.01 probability levels respectively; above diagonal- 2014 and below diagonal- 2015

Table 4-9 Pearson correlation coefficient analysis between fifteen drought tolerant indices (DTIs) and fruit yield for 'cherry' species during 2014 and 2015

DTIs	TOL	MP	GMP	HAM	STI	RDI	ATI	SSPI	SNPI	YI	YSI	K_1STI	K_2STI	DI	SSI	Yp	Ys
TOL	**1**	0.21	0.11	0.03	0.08	-0.87**	0.92**	1.00**	-0.49**	-0.22	-0.87**	0.56**	-0.25	-0.53**	0.87**	0.55**	-0.22
MP	0.20	**1**	1.00**	0.98**	0.98**	0.24	0.51**	0.21	0.52**	0.91**	0.24	0.91**	0.88**	0.71**	-0.24	**0.93****	**0.91****
GMP	0.13	1.00**	**1**	1.00**	0.99**	0.32	0.43*	0.11	0.56**	0.94**	0.33	0.87**	0.91**	0.77**	-0.33	**0.89****	**0.94****
HAM	0.07	0.99**	1.00**	**1**	0.98**	0.40*	0.35*	0.03	0.60**	0.97**	0.41*	0.83**	0.94**	0.82**	-0.40*	**0.85****	**0.97****
STI	0.11	0.99**	0.99**	0.99**	**1**	0.32	0.40*	0.08	0.55**	0.94**	0.33	0.87**	0.94**	0.78**	-0.33	**0.87****	**0.94****
RDI	-0.96**	0.04	0.11	0.17	0.12	**1**	-0.64**	-0.87**	0.75**	0.61**	1.00**	-0.14	0.59**	0.82**	-1.00**	-0.12	0.61**
ATI	0.96**	0.40*	0.34	0.28	0.33	-0.85**	**1**	0.92**	-0.26	0.11	-0.63**	0.80**	0.07	-0.23	0.63**	0.77**	0.11
SSPI	1.00**	0.20	0.13	0.07	0.11	-0.96**	0.96**	**1**	-0.49**	-0.22	-0.87**	0.56**	-0.25	-0.53**	0.87**	0.55**	-0.22
SNPI	-0.62**	0.51**	0.55**	0.58**	0.55**	0.77**	-0.48**	-0.62**	**1**	0.73**	0.76**	0.23	0.71**	0.84**	-0.75**	0.26	0.73**
YI	-0.24	0.91**	0.93**	0.95**	0.93**	0.46**	-0.02	-0.24	0.77**	**1**	0.61**	0.67**	0.98**	0.94**	-0.61**	**0.70****	**1.00****
YSI	-0.96**	0.05	0.11	0.17	0.13	1.00**	-0.85**	-0.95**	0.77**	0.46**	**1**	-0.13	0.59**	0.82**	-1.00**	-0.11	0.61**
K_1STI	0.55**	0.92**	0.89**	0.86**	0.89**	-0.32	0.72**	0.55**	0.18	0.67**	-0.31	**1**	0.65**	0.39*	0.13	**0.99****	**0.67****
K_2STI	-0.24	0.90**	0.92**	0.94**	0.93**	0.45**	-0.02	-0.24	0.77**	0.99**	0.46**	0.67**	**1**	0.94**	-0.59**	**0.66****	**0.98****
DI	-0.60**	0.66**	0.71**	0.75**	0.71**	0.77**	-0.41*	-0.60**	0.92**	0.92**	0.77**	0.33	0.91**	**1**	-0.82**	**0.42***	**0.94****
SSI	0.96**	-0.05	-0.11	-0.17	-0.13	-1.00**	0.85**	0.96**	-0.77**	-0.46**	-1.00**	0.32	-0.45**	-0.77**	**1**	0.12	-0.61**
Yp	0.54**	**0.93****	**0.91****	**0.88****	**0.89****	-0.32	0.70**	0.54**	0.20	**0.69****	-0.31	**0.99****	**0.68****	0.35	0.32	**1**	0.70**
Ys	-0.23	**0.91****	**0.93****	**0.95****	**0.93****	0.45**	-0.01	-0.23	0.77**	**1.00****	0.46**	**0.67****	**0.99****	0.91**	-0.45**	**0.69****	**1**

Notes:* and ** Significant @ 0.05 and 0.01 probability levels respectively; above diagonal- 2014 and below diagonal- 2015

Table 4-10 Pearson correlation coefficient analysis between fifteen drought tolerant indices (DTIs) and fruit yield for 'wild'species during 2014 and 2015

DTIs	TOL	MP	GMP	HAM	STI	RDI	ATI	SSPI	SNPI	YI	YSI	K_1STI	K_2STI	DI	SSI	Yp	Ys
TOL	1	0.13	0.06	-0.001	0.07	-0.91**	0.92**	1.00**	-0.58**	-0.26	-0.91**	0.48*	-0.22	-0.57**	0.91**	0.47*	-0.26
MP	0.11	1	1.00**	0.99**	0.99**	0.20	0.43*	0.13	0.20	0.93**	0.20	0.92**	0.92**	0.73**	-0.20	**0.94****	**0.93****
GMP	0.001	0.99**	1	1.00**	0.99**	0.27	0.37	0.06	0.23	0.95**	0.27	0.89**	0.93**	0.78**	-0.27	**0.91****	**0.95****
HAM	-0.11	0.97**	0.99**	1	0.98**	0.33	0.31	-0.01	0.26	0.97**	0.33	0.86**	0.95**	0.81**	-0.33	**0.88****	**0.97****
STI	0.01	0.98**	0.97**	0.98**	1	0.23	0.37	0.07	0.21	0.93**	0.23	0.90**	0.95**	0.76**	-0.23	**0.90****	**0.93****
RDI	-0.91**	0.25	0.35	0.45*	0.32	1	-0.68**	-0.91**	0.63**	0.54**	1.00**	-0.15	0.47*	0.77**	-1.00**	-0.14	0.54**
ATI	0.91**	0.44*	0.35	0.25	0.35	-0.68**	1	0.92**	-0.49*	0.06	-0.68**	0.73**	0.06	-0.29	0.69**	0.70**	0.06
SSPI	1.00**	0.11	0.001	-0.11	0.01	-0.91**	0.91**	1	-0.58**	-0.26	-0.91**	0.48*	-0.22	-0.57**	0.91**	0.47*	-0.26
SNPI	-0.56	0.62**	0.68**	0.72**	0.67**	0.73**	-0.35	-0.56	1	0.42*	0.63**	-0.06	0.40*	0.62**	-0.63**	-0.03	0.42*
YI	-0.39	0.87**	0.92**	0.96**	0.91**	0.68**	-0.04	-0.39	0.86**	1	0.54**	0.71**	0.98**	0.93**	-0.54**	**0.73****	**1.00****
YSI	-0.91**	0.24	0.35	0.44*	0.32	1.00**	-0.69**	-0.91**	0.73**	0.68**	1	-0.15	0.47*	0.77**	-1.00**	-0.14	0.54**
K1STI	0.54**	0.88**	0.83**	0.76**	0.84**	-0.20	0.80**	0.54**	0.25	0.55**	-0.20	1	0.73**	0.43*	0.16	**0.99****	**0.71****
K2STI	-0.36	0.87**	0.91**	0.93**	0.92**	0.62**	-0.04	-0.36	0.88**	0.98**	0.62**	0.57**	1	0.91**	-0.47*	**0.74****	**0.98****
DI	-0.67**	0.65**	0.72**	0.78**	0.71**	0.87**	-0.39	-0.67**	0.93**	0.93**	0.86**	0.23	0.92**	1	-0.77**	**0.45***	**0.93****
SSI	0.91**	-0.25	-0.35	-0.45*	-0.32	-1.00**	0.69**	0.91**	-0.73**	-0.68**	-1.00**	0.20	-0.62**	-0.87**	1	0.14	-0.54**
Yp	0.55**	**0.89****	**0.84****	**0.77****	**0.83****	-0.21	0.78**	0.55**	0.27	**0.56****	-0.21	**0.99****	**0.57****	0.24	0.21	1	0.73**
Ys	-0.39	**0.87****	**0.92****	**0.95****	**0.91****	0.68**	-0.04	-0.39	0.86**	**1.00****	0.68**	**0.55****	**0.98****	0.93**	-0.68**	0.56**	1

Notes:* and ** Significant @ 0.05 and 0.01 probability levels respectively; above diagonal- 2014 and below diagonal- 2015

Correlation analysis between fifteen DTIs for 'wild' species

Y_S is seen to be significantly positive correlation with MP, GMP, HAM, STI, RDI, SNPI, YI, YSI, K_1STI,K_2STI, DI and $Y_P$but it has negative relationship with SSI. Y_P exhibit significant and positively associated with all drought indices, except RDI, SNPI, YSI, DI and SSI. Hence, seven DTIs, *viz.*, MP, GMP, HAM, STI, YI, K_1STI and K_2STI are significantly and positively correlated with both Y_P and Y_S (Table 4-10).

To investigate suitable drought tolerant indices, a suitable index must have a significant correlation with fruit yield under both the conditions (Mitra, 2001). Thus, the above results indicated that these indices are more effective in identifying high yielding accessions under different irrigated conditions. However, among these, four drought tolerant indices MP, GMP, STI and HAM are suggested as the best indices because of they has highest Pearson correlation coefficients (>0.7) under both normal and stress conditions across four groups of species.

Similar results showing the most suitable indices for screening drought tolerant genotypes had been reported in tomato like STI and GMP by Brdar-Jokanović*et al.*, (2014) and in other crops such as GMP, MP, STI, K_1STI, K_2STI, YI, DI, SNPI, RDI and YSI in wheat (Akçura *et al.*, 2011;Farshadfar and Elyasi, 2012; Drikvand*et al.*, 2012;Farshadfar*et al.*, 2013; Noorifarjam*et al.*, 2013; Mohammadi-joo*et al.,* 2015), MP, STI and GMP in barley (Sharafi *et al.,* 2011; Subhani*et al.,* 2015),STI, GMP, MP, YI, TOL, DI, RDI, YSI, SSPI, K_1STI and K_2STI in canola (Khalili *et al.,* 2014), STI, GMP, MP, HM, YI, K_1STI and K_2STI in chickpea (Sabaghnia and Janmohammadi, 2014), GMP (Ngugi *et al.,* 2013), and STI, GMP, MP, YI, DI, RDI, YSI, SSPI, K_1STI and K_2STI (Naghavi *et al.,* 2013) in maize, MP, GMP , HAM and STI in oat (Zaheri and Bahraminejad, 2012), MP, GMP, HM and STIin rice (Rahimi *et al.,* 2013), MP, GMP and STI in safflower (Bahrami*et al.*, 2014), MP (Kharrazi and Rad, 2011) and GMP, MP, HM and STI (Menezes*et al.,* 2014) in sorghum, GMP, MP, HM and STI in sunflower (Safavi*et al.,* 2015) and MP, GMP and STI in sweet potato (Agili*et al.,* 2012).

Identification of germplasm accessions based on principle component analysis and Correlation between fifteen DTIs for 'lyco' species

Biplot method based on principle component analysis (PCA)

In general, indices in the same group distinguish drought tolerant accessions in the same manner. Many authors believed that the most suitable indices for selection of drought tolerant cultivars/genotypes are indicators, which had relatively high correlation with yield in both stress and non-stress condition (Farshadfar *et al.,* 2001; Sharafi *et al*., 2011; Zare *et al*., 2012; Amiri *et al.*, 2014; Jatav and Kandalkar, 2014 and Subhani *et al*., 2015).

Relationship between germplasm accessions and their tolerance to drought plotted in a biplot are used for identification of drought tolerant accessions. The selection of accessions with higher PCA 1 and low PCA 2 are suitable for both normal and stress conditions (group A and B were classified by Fernandez, 1992).

By viewing within species, highly drought tolerant accessions are identified as 99 ('Pusa Ruby'), 108 (EC 771598) and 123 (EC 677123) for 'lyco' species, 40 (HAT-121), 48 (WIR 13706), 58 (WIR 13708) and 59 (WIR 3957) for 'cherry' species, 21 (EC 541101) and 4 (LA 2976) for 'wild' species. Whereas the most sensitive drought accessions are depicted as 106

(CLN 13149), 113 (EC 771584), 116 (EC 771601), 117 (EC 771580), 118 (EC 771591) and 119 (EC 771614) for 'lyco' species, 37 (EC 514100), 51 (EC 771613), 57 (LA 1713) and 63 (EC 676819) for 'cherry' species, and 8 (L 00673), 10 (L 00671), 11 (L 00887), 13 (EC 771608) and 16 (EC 771607) for 'wild' species (Fig. 4-10, 4-11 and 4-12).

Thus, with the above results, a number of tomato accessions have been exploited as most drought tolerant as well as drought sensitive which are recommended for further development of hybrid varieties or for advanced studies in QTL mapping related to drought QTLs. Using biplot method to separate drought tolerant genotypes had been reported in barley (Zare, 2012), safflower (Bahrami *et al.,* 2014),sweet potato (Agili*et al.,* 2012), wheat (Moosavi*et al*., 2008; Asl *et al*., 2011; Yasir *et al*., 2013 and Ashraf *et al*., 2015), rice (Rahimi *et al*., 2013), maize (Shiri *et al*., 2010) and sorghum (Menezes *et al*., 2014).

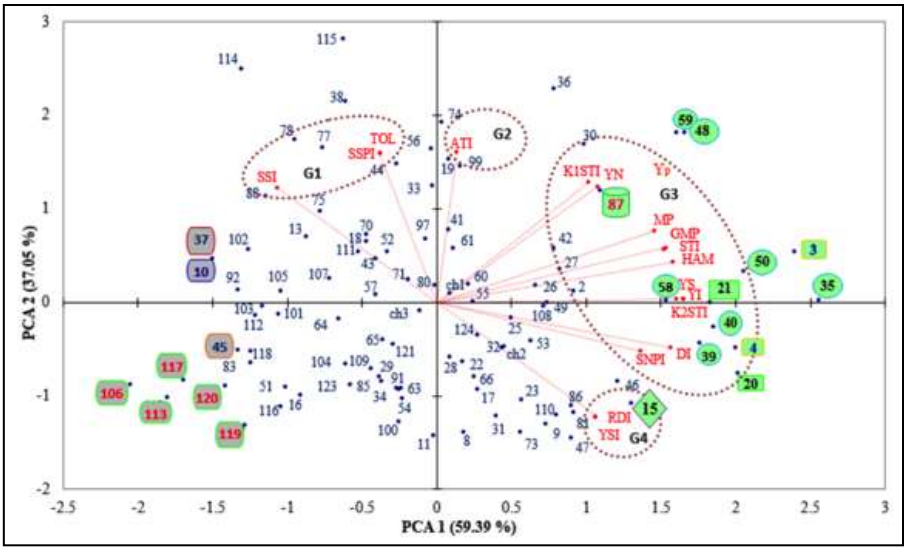


Figure 4-8 Biplot analysis based on first (PCA I) and Second (PCA-II) principle components for 'All spp durig 2014

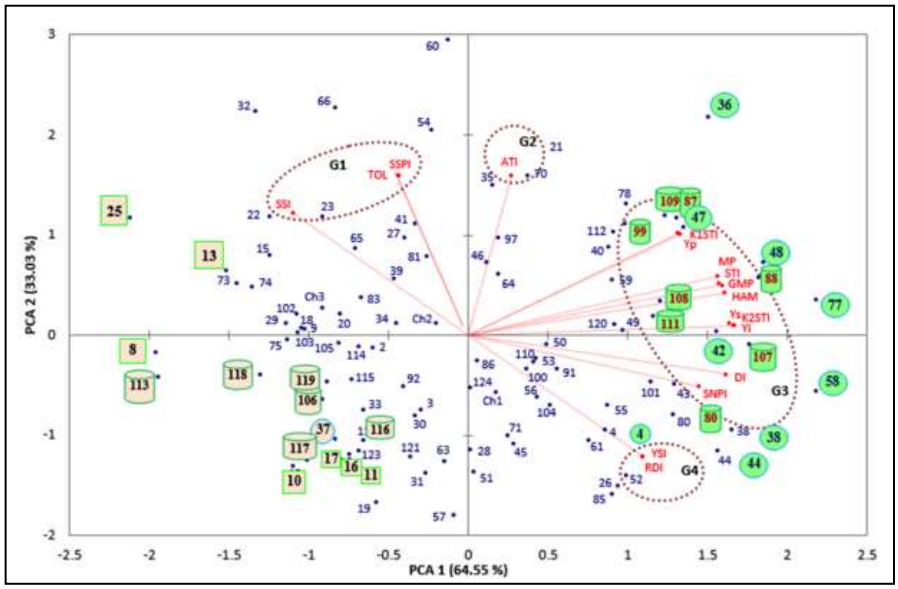


Figure 4-9 Biplot analysis based on first (PCA I) and Second (PCA 2) principle components for 'All spp during 2015

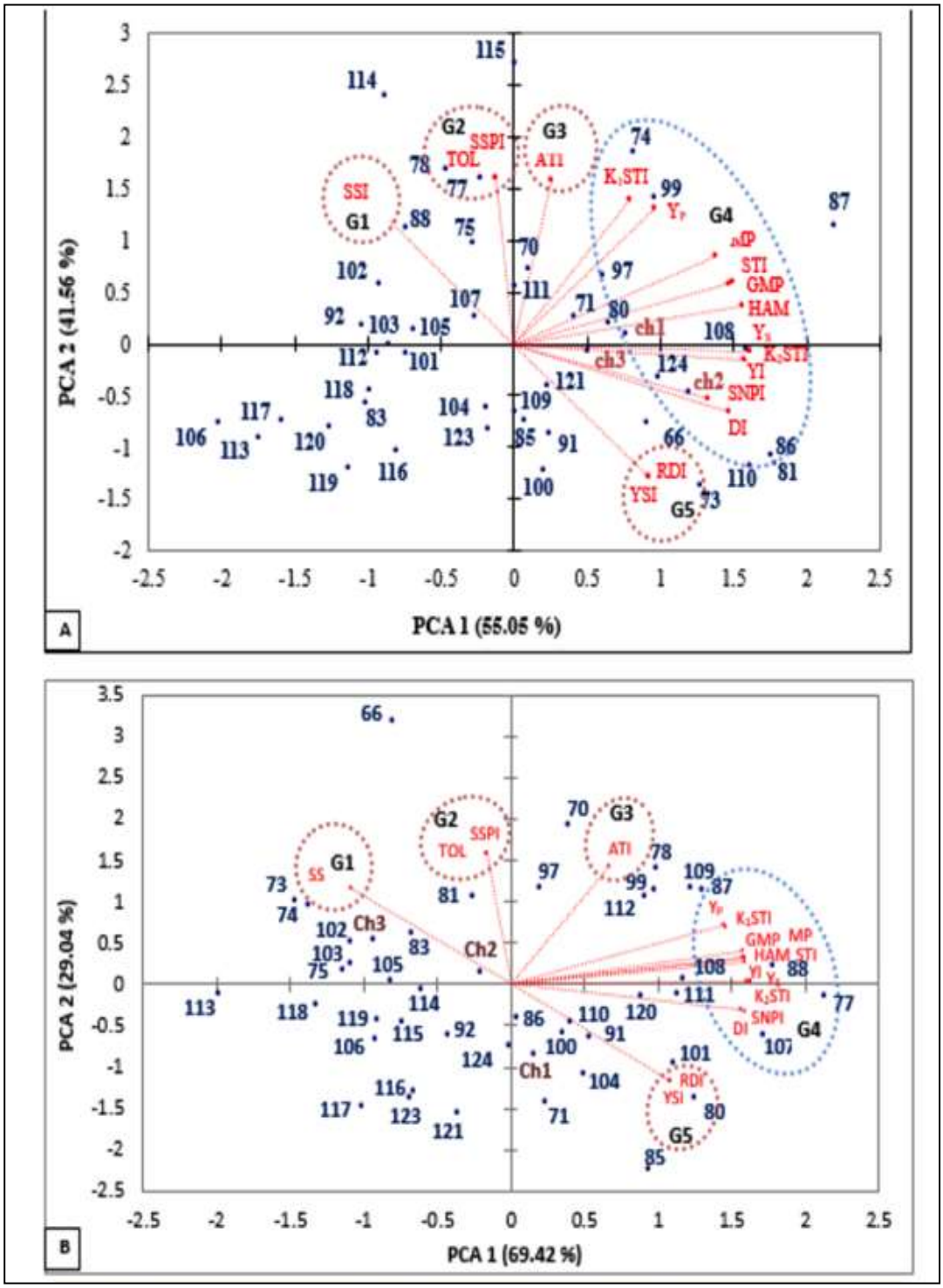


Figure 4-10 Biplot analysis based on first (PCA I) and Second (PCA 2) principle compone nts of 'Lyco species during 2014 (A) and 2015 (B)

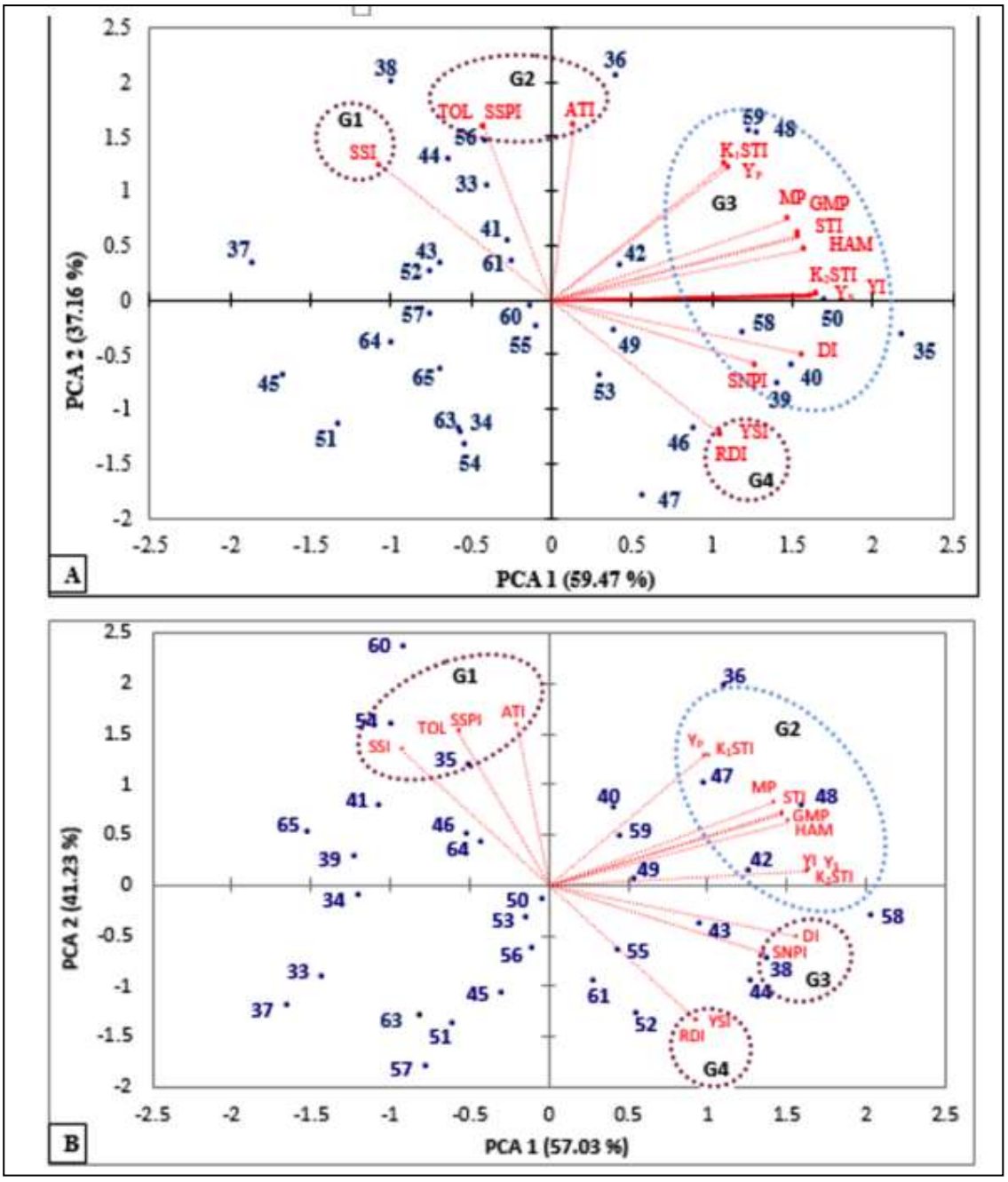


Figure 4-11 Biplot analysis on first (PCA I)and second (PCA-2) principle components of 'cherry' during 2014 (A) and 2015 (B)

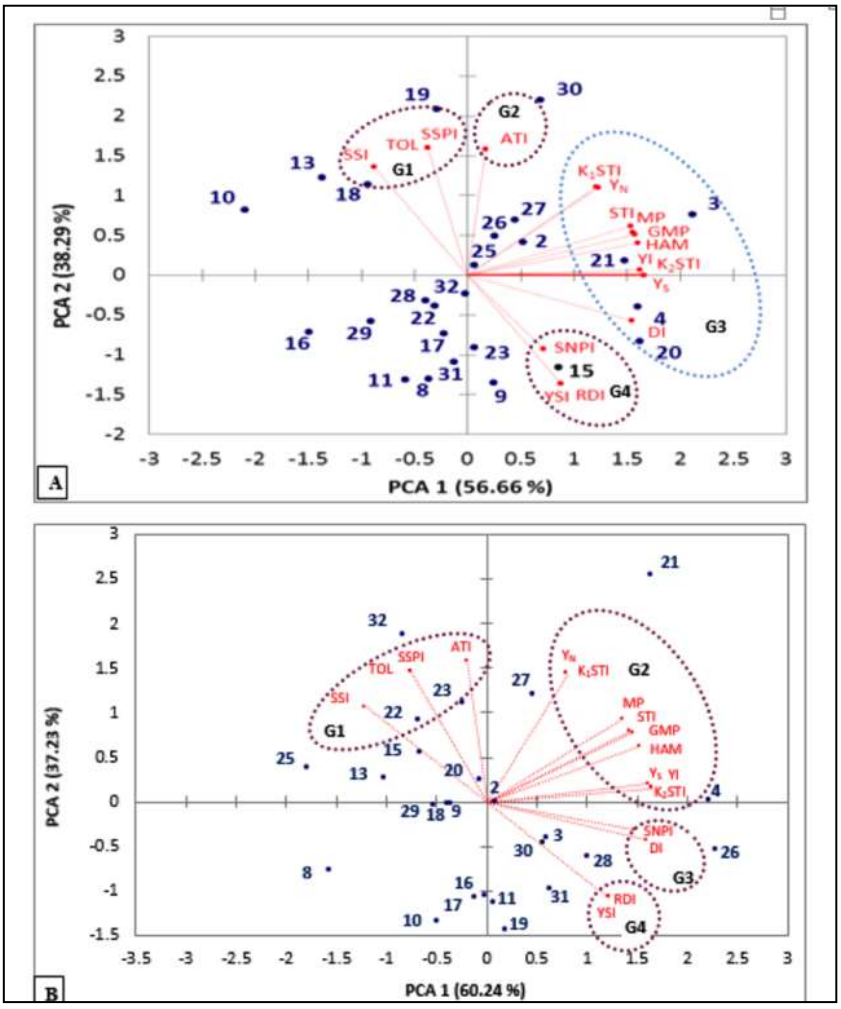


Figure 4-12 Biplot analysis on first (PCA I) and second (PCA-2) principle components of'wild species during 2014 (A) and 2015 (B)

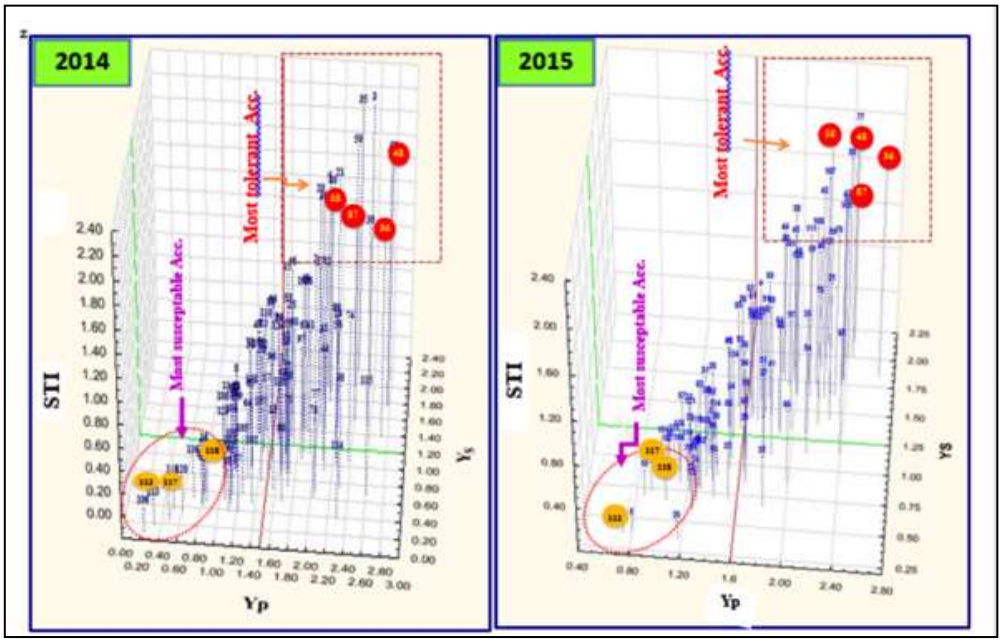


Figure 4-13 Three dimensional plots made by STI values and fruit yield of 'all spp during 2014 and 2015

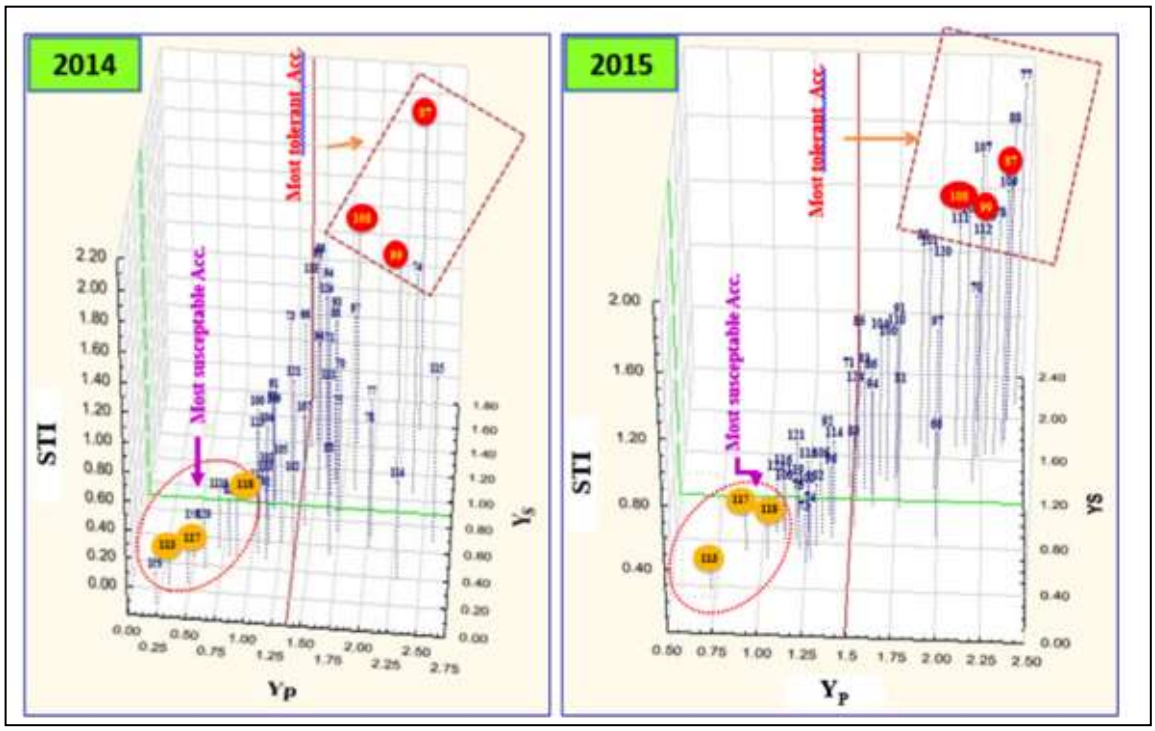


Figure 4-14 Three dimensional plots made by STI values and fruit yield of 'Lyco' species during 2014 and 2015

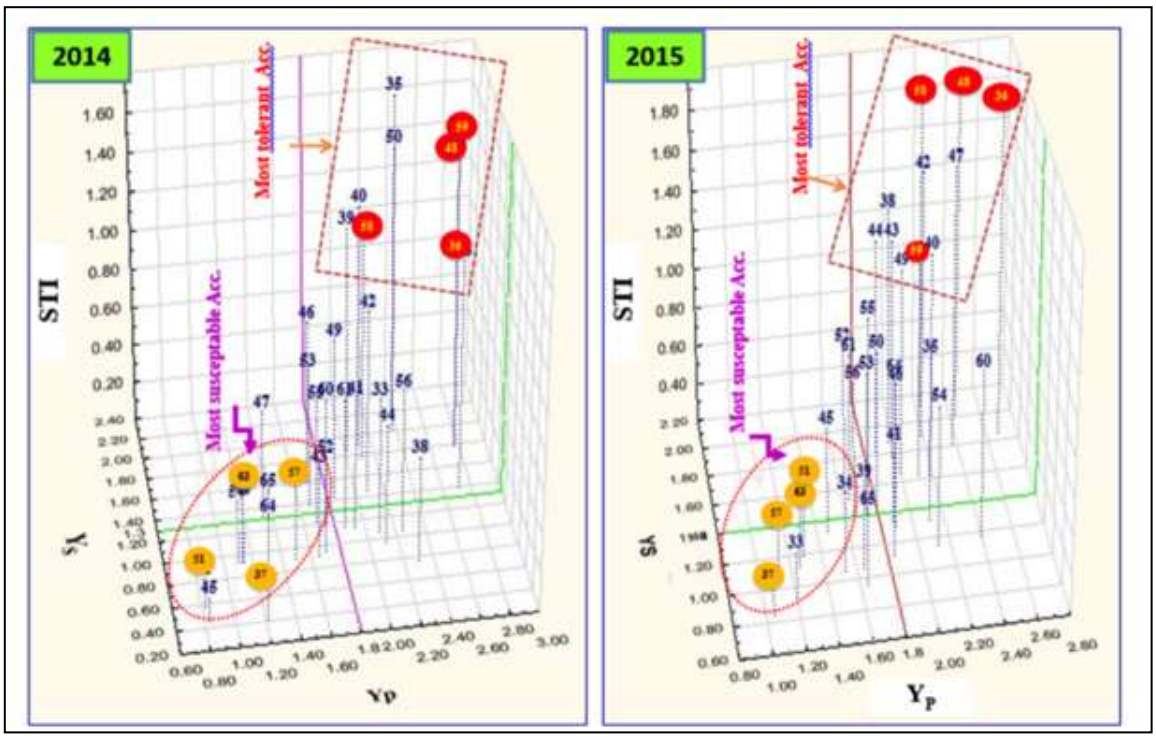


Figure 4-15 Three dimensional plots made by STI' values and fruit yield of 'cherry' species during 2014 and 2015

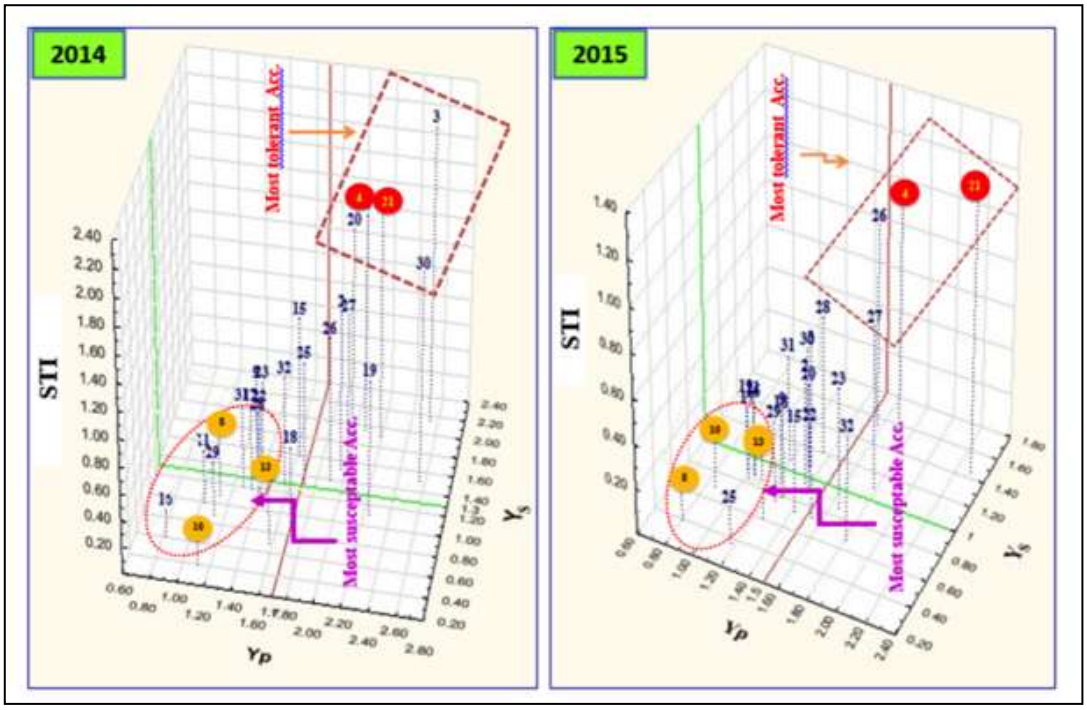


Figure 4-16 Three- dimensional plots made by STI' values and fruit yield of 'wild' species during 2014 and 2015

Three-dimensional plots based on PCA for STI and fruit yield under normal and stress conditions

Fernandez (1992) defined STI which can be used to identify genotypes that produce high yield under both stress and non-stress conditions. The above results also have been indicated that STI was highly positively correlated with both yield, Y_P and Y_S for all groups of species. Hence, STI along with Y_P and Ys are used to identify drought tolerant germplasm accessions as depicting for 'All' spp (Fig.4-13), 'lyco' species (Fig.4-14), 'cherry' species (Fig.4-15) and 'wild' species (Fig.4-16).

Across two years, the top common drought sensitive accessions for 'All' spp are 10, 16, 106, 113, 117, 118 and 119, while the drought tolerant accessions are 36, 48, 58 and 87. For within species, the most common drought sensitive accessions were 113, 117 and 118 ('lyco'), 34, 37, 51, 57 and 63('cherry'), 8, 10 and 13 ('wild'), whereas the drought tolerant accessions are 87 (Plate 8), 99 and 108 ('lyco'), 36, 40, 48, 58 and 59 ('cherry'), 4 and 21 ('wild' species).

Using STI and fruit yield for three- dimensional diagrams to discriminate drought and susceptible genotypes as the above mentioned are most similar to biplot diagram indicating this index can used confidently to separate tomato accessions. The same method was reported by Brdar-Jokanović *et al*., (2014), Brdar-Jokanović and Zdravković (2015) in tomato, Shirinzadeh *et al*., (2010) and Naghavi*et al*., (2013) in corn, Bahrami*et al*., (2014) in safflower, Anwar*et al.,* (2011), Farshadfar and Elyasi (2012), Farshadfar*et al.,* (2012), Mohammadi*et al.*,(2012) and Farshadfar*et al.,* (2013) in wheat.

Correlation between nineteen fruit yield, drought and yield related traits within each year within normal and stress conditions.

Correlation studied among 19 quantitative traits reveals that highly positive trend between DFF with DFFS, PH with BN, CPP with FPP, FLPC with FNPC, AFW with FV, FV with FT, FV with LN, while it is highly negative relation for SLA and SCMR, AFW and TSS, etc. These may help for references in marker-trait associations in case of a marker linking to more than one trait between both conditions in each year.Those traits with close relationship will be expected to be associated with common markers across years and two growing conditions in marker-trait associations.

These results were also reported in tomato for FYPP with the traits, *viz*., BN (Nwosu*et al.,* 2012 and Kumar *et al.,* 2013), CPP (Nadeem *et al.,* 2012; de Souza*et al*., 2012 and Paul *et al.,* 2014) and FPP (Prashanth, 2003; Kumar *et al*., 2006; Buckseth *et al*., 2012; Manna and Paul, 2012; Kumar *et al.,* 2013; Monamodi *et al*., 2013; Emami, 2014 and Ullah *et al.,* 2015). While the negative correlations were noticed between FYPP with the traits, *viz*., DFF (Bernousi *et al*., 2011; Monamodi *et al*., 2013 and Paul *et al.*, 2014), DFFS (Henareh *et al*., 2015), FNPC (Al-Aysh *et al.,* 2012a), FLPC (Ullah *et al.,* 2015) and TSS (Al-Aysh*et al.,* 2012b; Manna and Paul, 2012).

Correlation of nineteen fruit yield, drought and yield related traits between normal and stress conditions during 2014 and 2015

The results indicate that high variation of specific traits are seen like LR for all four groups of species in both years with range from 0.16 ('wild species- 2014) to 0.58 ('All' spp-2015), RWC in 2014 with range from 0.39 to 0.47, DFF and DFFS for cherry in 2014 with values of 0.33 and 0.44, respectively, TSS for 'lyco' species during 2014 and 2015 with values of 0.36 and 0.59, respectively and fruit yield for 'lyco' species in 2014 (0.54) and 'wild' species in 2015 (0.56). Thus, they are suggested to be effected by environmental conditions and also are expected to result in various marker-trait associations.

Table 4-11Correlation between fruit yield with drought and yield related traits of four groups of species within normal and stress conditions during 2014 and 2015

	2014								2015							
Trait	**'All' spp**		**'Lyco' species**		**'Cherry' species**		**'Wild' species**		**'All' spp**		**'Lyco' species**		**'Cherry' species**		**'Wild' species**	
	Y_P	**Y_S**	**Y_P**	**Y_S**	**Y_P**	**Y_S**	**Y_P**	**Y_S**	**Y_P**	**Y_S**	**Y_P**	**Y_S**	**Y_P**	**Y_S**	**Y_P**	**Y_S**
DFF	-0.21*	-0.29**	-0.29	-0.43**	-0.16	-0.32	-0.05	0.17	-0.09	-0.09	-0.24	-0.30*	0.04	-0.21	-0.12	0.001
DFFS	-0.22*	-0.29**	-0.36*	-0.56**	-0.13	-0.38*	-0.10	0.09	-0.15	-0.12	-0.27	-0.34*	0.00	-0.15	-0.11	0.01
PH	0.27**	0.51**	0.07	-0.02	0.15	0.21	0.38	0.66**	-0.13	-0.04	0.00	0.06	-0.08	0.15	0.36	0.46*
BN	0.38**	0.63**	0.32*	0.51**	0.30	0.26	0.43*	0.81**	-0.08	-0.03	0.31*	0.24	0.06	0.30	0.25	0.44*
SCMR	0.31**	0.42**	0.45**	0.66**	0.66**	0.74**	0.40*	0.47*	0.43**	0.35**	0.67**	0.62**	0.31	0.08	0.06	-0.08
SLA	-0.19	-0.43**	-0.43**	-0.68**	-0.51**	-0.83**	-0.19	-0.34	-0.17	-0.20*	0.04	0.01	-0.15	0.12	0.04	-0.07
RWC	0.29**	0.29**	0.48**	0.30*	0.11	0.12	0.12	0.49*	0.61**	0.67**	062**	0.77**	0.55**	0.58**	0.45*	0.20
LR	-0.27**	-0.48**	-0.25	-0.32*	0.03	-0.29	0.24	-0.55**	-0.38**	-0.33**	-0.51**	-0.63**	-0.43*	-0.56**	-0.62**	-0.29
STG	0.55**	0.52**	0.79**	0.41**	0.26	0.23	0.55**	0.71**	0.64**	0.74**	0.61**	0.71**	0.54**	0.69**	0.50*	0.55**
FNPC	0.15	0.36**	0.23	0.59**	0.21	-0.05	-0.22	0.03	-0.29**	-0.27**	0.04	0.26	0.09	0.21	-0.42*	-0.16
FLPC	0.001	0.22*	-0.04	0.02	0.15	0.20	-0.54**	-0.44*	-0.34**	-0.35**	0.02	0.13	0.05	-0.03	-0.47*	-0.24
CPP	0.26**	0.48**	0.71**	0.71**	0.34	0.50**	0.33	0.39	-0.05	-0.01	0.33*	0.32*	-0.02	0.29	0.48*	0.49*
FPP	0.20*	0.41**	0.56**	0.73**	0.26	0.25	0.37	0.43*	-0.16	-0.17	0.29	0.36*	0.00	0.24	0.45*	0.54**
AFW	-0.13	-0.37**	0.25	0.08	-0.18	-0.18	0.13	0.04	0.11	0.17	0.11	0.21	-0.04	-0.15	-0.16	-0.06
FV	-0.14	-0.36**	0.25	0.09	-0.20	-0.18	0.12	0.00	0.11	0.18	0.11	0.24	-0.04	-0.14	-0.16	-0.03
FT	-0.23*	-0.34**	0.28	0.310*	-0.29	-0.08	-0.31	-0.22	0.16	0.20*	0.13	0.20	0.06	-0.18	-0.09	0.08
TSS	0.12	0.36**	0.11	0.01	0.05	0.26	-0.26	-0.08	-0.31**	-0.35**	0.03	0.27	-0.19	-0.02	-0.41*	-0.33
LN	-0.11	-0.29**	0.10	-0.02	0.06	-0.11	0.24	-	0.18	0.08	0.15	0.02	0.24	-0.01	0.48*	-

Notes: Y_P- fruit yield under normal condition, Y_S- fruit yield under stress condition. -: data could not be analysed due to unit.

Table 4-12 Correlation coefficients between normal and stress conditions for19 drought, fruit yieldand yield related traits during 2014 and 2015

Trait	DFF	DFFS	PH	BN	SCMR	SLA	RWC	LR	STG	FNPC	FLPC	CPP	FPP	AFW	FV	FT	TSS	LN	FYPP
Species	**2014**																		
All' spp	0.76 **	0.81 **	0.95 **	0.92 **	0.77 **	0.69 **	0.47 **	0.49 **	0.78 **	0.98 **	0.98 **	0.96 **	0.96 **	0.95 **	0.94 **	0.94 **	0.89 **	0.89 **	0.66 **
Lyco'	0.70 **	0.83 **	0.89 **	0.63 **	0.70 **	0.54 **	0.52 **	0.46 **	0.46 **	0.77 **	0.91 **	0.76 **	0.90 **	0.85 **	0.83 **	0.71 **	0.36 *	0.85 **	0.54 **
Cherry'	0.33 ns	0.44 *	0.86 **	0.88 **	0.85 **	0.72 **	0.39 *	0.25 ns	0.75 **	0.76 **	0.96 **	0.81 **	0.91 **	0.96 **	0.93 **	0.94 **	0.66 **	0.84 **	0.68 **
'Wild'	0.91 **	0.92**	0.82**	0.81**	0.75 **	0.72**	0.41**	0.16 ns	0.96**	0.90 **	0.92**	0.91 **	0.85**	0.99**	0.97 **	0.86**	0.63**	1.00**	0.78**
Species	**2015**																		
All' spp	0.80 **	0.80 **	0.91 **	0.94 **	0.93 **	0.91 **	0.75 **	0.58 **	0.83 **	0.99 **	0.98 **	0.91 **	0.92 **	0.97 **	0.97 **	0.98 **	0.93 **	0.97 **	0.83 **
Lyco'	0.77 **	0.77 **	0.90 **	0.80 **	0.83 **	0.81 **	0.71 **	0.47 **	0.77 **	0.84 **	0.82 **	0.89 **	0.88 **	0.92 **	0.92 **	0.86 **	0.59 **	0.97 **	0.91 **
Cherry'	0.60 **	0.61 **	0.81 **	0.80 **	0.93 **	0.91 **	0.76 **	0.54 **	0.76 **	0.96 **	0.90 **	0.89 **	0.94 **	0.97 **	0.97 **	0.97 **	0.78 **	0.92 **	0.69 **
'Wild'	0.90 **	0.91**	0.59**	0.66**	0.82 **	0.90**	0.63**	0.39 ns	0.80**	0.95 **	0.94**	0.75**	0.70**	0.97**	0.97 **	0.72**	0.74**	1.00**	0.56**

Experiment 2: Phenotyping and genotyping of cultivated and wild germplasm accessions with informative markers to establish association with traits related to wue and fruit yield.

Molecular polymorphism of published SSR markers with cultivated tomato and related species

Molecular polymorphism of 145 published SSR markers with 103 germplasm accessions of four group species were computed by Power Marker v3.25 and the results are presented in the Appendix 13. Mean and range of molecular polymorphism from four groups of species are showed in Table 4-13.

Table 4-13 Molecular polymorphism of published SSR markers with four groups of species

Sl. No.	Group species	Mean and range	Major allelic frequency	Allele number	Genetic diversity	Heterozygosity	PIC value
1	'All' spp	Mean	0.60±0.11	2.83±2.12	0.50±0.09	0.14±0.19	0.42±0.09
		Range	0.34-0.95	2.00-5.00	0.10-0.74	0.00-0.95	0.10-0.69
2	'Lyco'	Mean	0.68±0.14	2.43±0.68	0.41±0.14	0.11±0.21	0.34±0.12
		Range	0.34-0.94	2.00-5.00	0.03-0.72	0.00-1.00	0.03-0.66
3	'Cherry'	Mean	0.66±0.14	2.50±0.65	0.43±0.13	0.12±0.13	0.36±0.11
		Range	0.42-0.94	2.00-4.00	0.03-0.68	0.00-0.97	0.03-0.62
4	'Wild'	Mean	0.62±0.16	2.68±0.71	0.48±0.14	0.21±0.23	0.40±0.13
		Range	0.25-0.94	2.00-5.00	0.12-0.80	0.00-1.00	0.11-0.76

Major allelic frequency is recorded at least mean for 'All' spp (0.60), while it is highest in 'lyco' species (0.68) and medium for 'cherry' (0.66) and 'wild' (0.62) species.

Allele numbers range from 2.00 to 5.00, except for 'cherry' (2.00-4.00), with means of 2.43, 2.50, 2.68 and 2.83 for 'lyco', 'cherry', 'wild' species and 'All' spp, respectively. The results were supported by Ranc *et al*., (2008) who also found number of alleles highest for *S. pimpinellifolium,* followed by cherry and least in *S. lycopersicum*. However, our allele numbers are lower value than that reported by Zhou *et al*., (2015) who got at 4 alleles per primer in mean for each genomic and EST-SSR markers in 29 cultivated and 14 wild tomatoes.

Genetic diversity is the total number of genetic characteristics in the genetic makeup of a species. It is distinguished from genetic variability, which describes the tendency of genetic characteristics tovary. In present study, genetic diversity is accounted in highest mean for 'All' spp (0.50) followed by'wild' (0.48), 'cherry' (0.43) and is least for 'lyco' (0.41) species. Our results are lower values than that reported by Singh *et al*., (2014) who got 0.84 in mean. The representative markers SLM11-17 (M96) with relative high (0.67) and SLM12-41 (M75) with medium (0.46) genetic diversity are presented in Plate 9 and Plate 10.

Heterozygosity with ranges is seen from zero for all groups to 1.00('lyco' and 'wild' species). Wild species is recorded at highest mean (0.21) followed by 'All' spp (0.14), 'cherry' (0.12) and lowestmean for 'lyco' (0.11) species. These indicate that low heterozygosity or low genetic variability presents in the population. High heterozygosity of wild species may be explained by high self-incompatibility of *S. habrochaites*, autogamous nature of *S. peruvianum* and *S. pimpinellifolium* while *S. lycopersicum* are more self-compatibility or

facultative allogamous leading to low heterozygousity (Peralta *et al*., 2005 and Spooner *et al*., 2005).

PIC values with highest ranges are observed for 'wild' species (0.11-0.76), whereas maximum mean value shows for 'All' spp (0.42) followed by 'wild' (0.40), 'cherry' (0.36) and least mean for 'lyco' species (0.34). They are classified as medium classes in comparison to Xie *et al.,* (2009) who defined PIC > 0.5, 0.5 > PIC > 0.25 and PIC < 0.25 as high, medium and low locus polymorphism, respectively. The results are smaller values than that reported by Zhou *et al*., (2015) who got at 0.49 and 0.45 means for genomic and EST-SSR markers in 29 cultivated and 14 wild tomatoes, respectively.

The above results indicate that 'wild' species is highest diversity by high means for allele number, genetic diversity, heterozygosity and PIC value followed by 'cherry' and least for 'lyco' species. Similar results were also reported by Mazzucato *et al*., (2008) for heterozygosity, wild tomato species were higher than landraces and cultivated cultivars.

Population structure

The marker polymorphic data with band sizes was used to compute the population structure. The STRUCTURE analysis separated the population into four clusters based on Δ K method (Evanno *et al*., 2005) as depicted in Fig. 4-16.

Using the Model-based method by structure, population structure with 0.70 membership probabilities as viewed in Fig.4-17 (derived from 'Q' matrices computed by genotypic data of 103 germplasm accessions, show total of 33 admixture accessions derived from *S. lycopersicum* for codes like 108, 109, 111, 113, 115, 119, 120 and 121, from 'cherry' for all accessions, except for codes as 34, 35, 37, 38, 44, 45, 47, 50, 55, 61, 64 and from *S. cheesmaniae* (code 18 and 19). The results are corresponding to biplot analysis (Fig. 4-18) based on first and second principle components (69.33% in total), which also exhibit four main groups (Q1, Q2, Q3 and Q4) and admixtures.

According to Neighbor-joining method based on Nei (1973) genetic distance was presented in Fig. 4-19 indicating that only two wild species, *S. peruvianum* and *S. habrochaites*, separated in the same group 1 (Q1) in model-based method by structure, while all the remaining species exhibit as admixtures like cherry accession (code 33) with *S. pimpinellifolium*, cherry accessions (codes: 44, 63, 54 and 65) with *S. lycopersicumvice versa* (codes: 71, 73, 74, 75, 77, 80 and 83) and two *S. scheemaniae* accessions are closely relations to *S. pimpinellifolium* (code 19) and *S.habrochaites* or *S.peruvianum* (code 18).

Thus, above analyses for population structure reveal that *S. habrochaites* and *S. peruvianum* are subjected to a distance separately from the others and was closely relationship to *S. pimpinellifolium* and *S. scheemaniae.* In addition, admixture populations are depicted for 'cherry' species, *S. pimpinellifolium* and *S. lycopersicum.*Those results were supported by Ranc *et al*., (2008) who reported that cherry was admixture population of*S. lycopersicum* and *S. pimpinellifolium.* Admixture results in the introduction ofchromosomes of different ancestry and allelic frequencies.

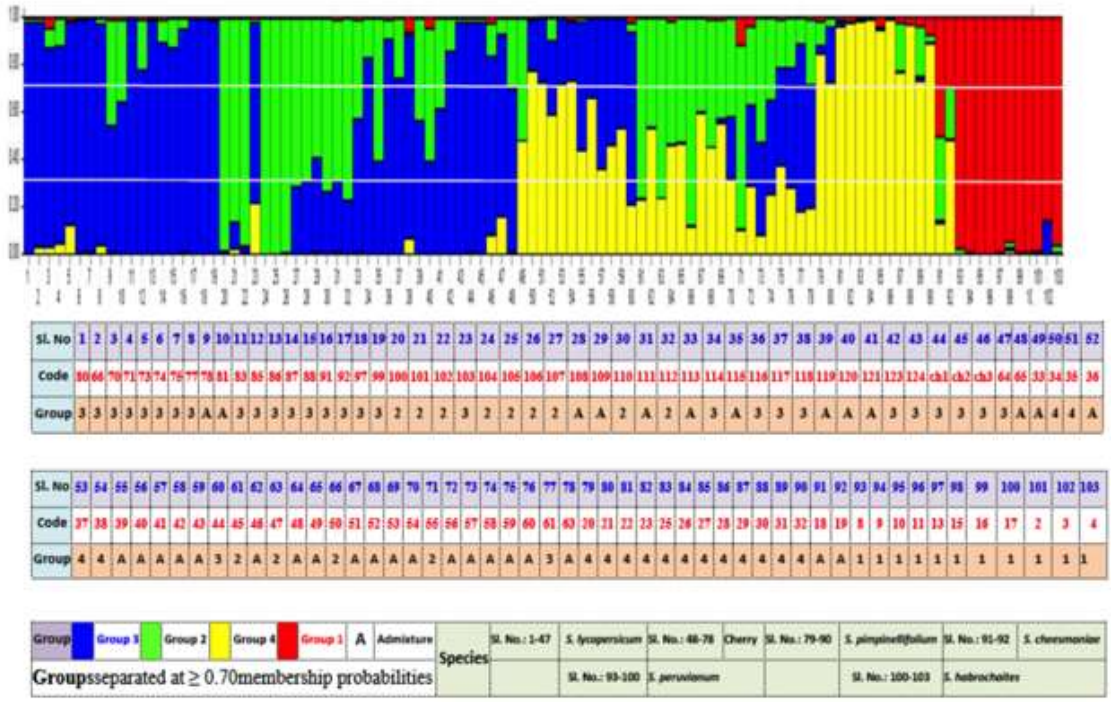


Figure 4-16: Population structure based on 145 publishes SSR markers for 103 germplasm accessions from six cultivated tomato and related species

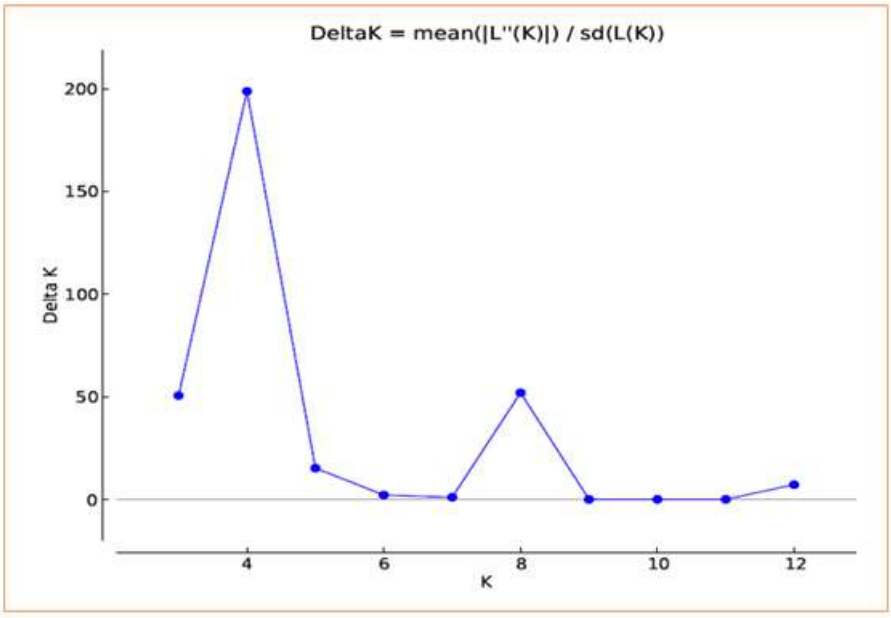


Figure 4-17 Four subgroups (K= 4) of 103 germplasm accessions from six cultivated tomato and related species according to Evanno et al., (2005), depicted from Structure Harvester-taylor0

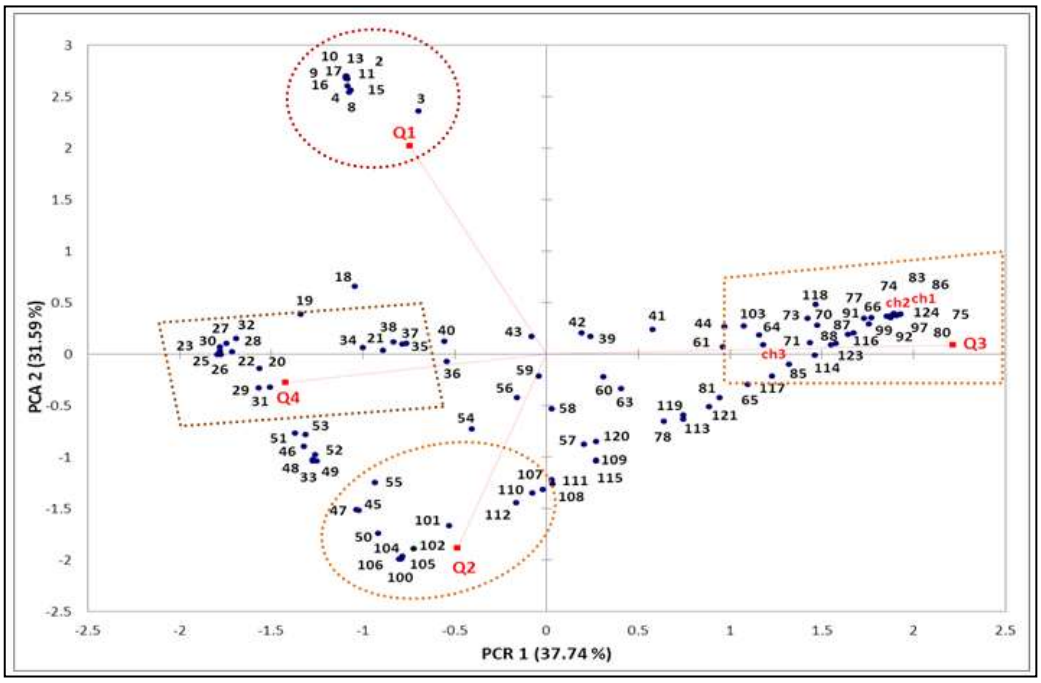


Figure 4-18 Biplot diagram for inferred clusters of 103 germpalsm accessions derived from 'Q' matrices of population structure

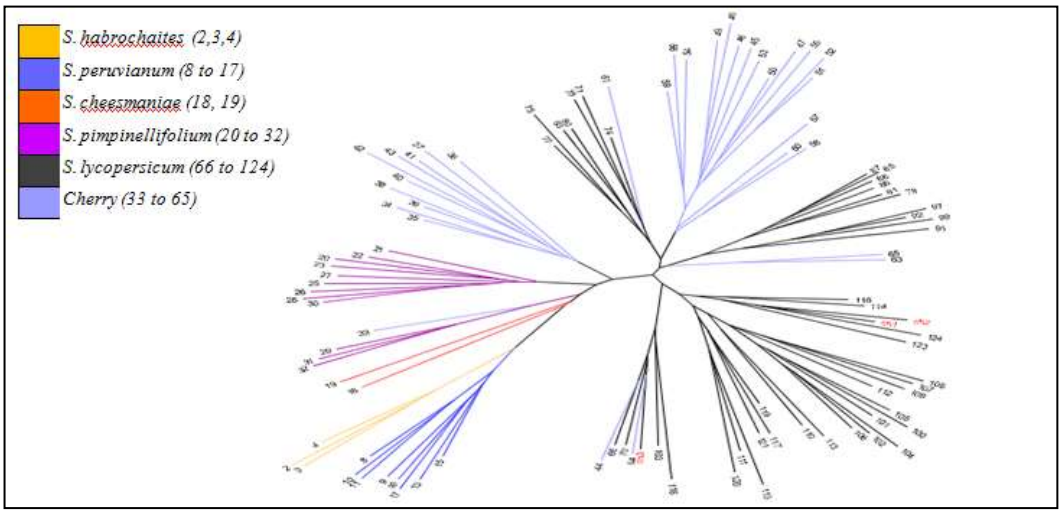


Figure 4-19 Neighbor-joining method based on Nei (1973) genetic distance of 103 germplasm accessions from six cultivated tomato and related species

Linkage disequilibrium

According to Flint-Garcia *et al*., (2003), LD decays more rapidly in outcrossing species as compared to selfing species, which is more likely to be homozygous than in outcrossingspecies. The resulting LD extends to unlinked sites, even on different chromosomes, but breaks down rapidlywith random mating (Pritchard and Rosenberg, 1999).

LD threshold in our study is lower value but higher genetic distance than that of Zhang *et al*., (2015) who reported that LDonthewholegenomefor 174 tomato accessionsof *S.l.*var. *cerasiforme* ('cherry'species) and *S. lycopersicum*with 182 SSR markers for 28 volatile traits extendedonaverageover8 cMfor r^2 = 0.2. Xu *et al,* (2013) conducted AM for quality traits in tomato using SNP markers for 44, 127 and 17 accessions of *S. lycopersicum*, *S. l*. var. *cerasiforme* and*S. Pimpinellifolium* respectively and also found that LD on thewhole genome for all accessions extended on average over18 cM for r^2 = 0.3, it is higher critical value of LD but near similar in genetic distance than that of our study.

Table 4-14 Genetic distance (D) and syntenic linkage disequilibrium (r^2) values on each of twelve chromosomes (Chr.) computed from 145 markers among the 103 germpalsm accessions

Sl. No.	Locus 1	Chr. 1	Position 1 (cM)	Locus 2	Chr. 2	Position 2 (cM)	p ≤ 0.001	D (cM)	r^2
1	M93	1	46	M51	1	35	0.00000000	11.0	0.024
2	M94	1	97.5	M52	1	22.3	0.00000000	75.2	0.046
3	M146	1	136.5	M93	1	46	0.00000000	90.5	0.054
4	M146	1	136.5	M52	1	22.3	0.00000000	114.2	0.037
5	M136	2	4.5	M36	2	25	0.00000000	20.5	0.098
6	M138	2	44	M136	2	4.5	0.00000000	39.5	0.111
7	M141	2	107	M138	2	44	0.00000000	63.0	0.113
8	M141	2	107	M66	2	77.5	0.00000000	29.5	0.187
9	M141	2	107	M67	2	53	0.00000000	54.0	0.082
10	M66	2	77.5	M5	2	53	0.00000000	24.5	0.020
11	M67	2	53	M66	2	77.5	0.00000000	24.5	0.090
12	M7	3	54	M3	3	99	0.00000000	45.0	0.136
13	M97	3	169	M7	3	54	0.00000000	115.0	0.036
14	M046	4	96	M25	4	56	0.00000000	40.0	0.110
15	M96	4	18.4	M46	4	96	0.00000000	77.6	0.038
16	M98	4	37	M25	4	56	0.00000000	19.0	0.058
17	M98	4	37	M46	4	96	0.00000000	59.0	0.028
18	M98	4	37	M96	4	18	0.00000000	19.0	0.039
19	M108	5	98.9	M1	5	28	0.00000034	70.9	0.275
20	M108	5	98.9	M64	5	106	0.00000045	7.1	0.249
21	M145	5	56	M1	5	28	0.00000000	28.0	0.126
22	M145	5	56	M108	5	98.9	0.00000000	42.9	0.093
23	M145	5	56	M64	5	106	0.00000000	50.0	0.118
24	M147	5	94.2	M1	5	28	0.00000000	66.2	0.098
25	M147	5	94.2	M108	5	98.9	0.00000000	4.7	0.053
26	M147	5	94.2	M64	5	106	0.00000000	11.8	0.201
27	M148	5	18.5	M64	5	106	0.00000000	87.5	0.167
28	M109	6	51.3	M82	6	59	0.00000000	7.7	0.045
29	M82	6	59	M76	6	25	0.00000000	34.0	0.062
30	M83	6	35.0	M76	6	25.0	0.00000000	10.0	0.149
31	M83	6	35.0	M82	6	59.0	0.00000000	24.0	0.061
32	M84	7	18.0	M34	7	3.0	0.00000000	15.0	0.057
33	M100	8	55.0	M85	8	7.0	0.00000000	48.0	0.042

Sl. No.	Locus 1	Chr. 1	Position 1 (cM)	Locus 2	Chr. 2	Position 2 (cM)	p ≤ 0.001	D (cM)	r^2
34	M113	8	71.8	M39	8	55.0	0.00000000	16.8	0.091
35	M126	8	22.5	M39	8	55.0	0.00000000	32.5	0.106
36	M126	8	22.5	M85	8	7.0	0.00000000	15.5	0.020
37	M127	8	22.7	M126	8	22.5	0.00000000	0.2	0.032
38	M116	9	99.1	M38	6	44.0	0.00000000	55.1	0.163
39	M116	9	99.1	M4	9	50.5	0.00000000	48.6	0.105
40	M116	9	99.1	M9	9	50.4	0.00000000	48.7	0.132
41	M9	9	50.4	M4	9	50.5	0.00000000	0.1	0.222
42	M129	10	33.7	M117	10	66.1	0.00000000	32.4	0.020
43	M130	10	39.0	M117	10	66.1	0.00000000	27.1	0.070
44	M130	10	39.0	M128	10	25.3	0.00000000	13.7	0.080
45	M130	10	39.0	M129	10	33.7	0.00000000	5.3	0.119
46	M118	11	3.4	M55	11	49.0	0.00000000	45.6	0.053
47	M118	11	3.4	M95	11	97.1	0.00000000	93.7	0.023
48	M134	11	40.0	M118	11	3.4	0.00000000	36.6	0.024
49	M134	11	40.0	M55	11	49.0	0.00000000	9.0	0.024
50	M134	11	40.0	M95	11	97.1	0.00000000	57.1	0.052
51	M144	11	37.5	M55	11	49.0	0.00000000	11.5	0.053
52	M144	11	37.5	M95	11	97.1	0.00000000	59.6	0.019
53	M95	11	97.1	M55	11	49.0	0.00000000	48.1	0.037
54	M063	12	14.0	M2	12	58.2	0.00000000	44.2	0.032
55	M063	12	14.0	M60	12	12.5	0.00000000	1.5	0.104
56	M063	12	14.0	M62	12	14.1	0.00000000	0.0	0.113
								Average of r^2	0.090

Notes: Position of markers extracted from TOMATO-EXPEN 2000 and others (Appendix 1)

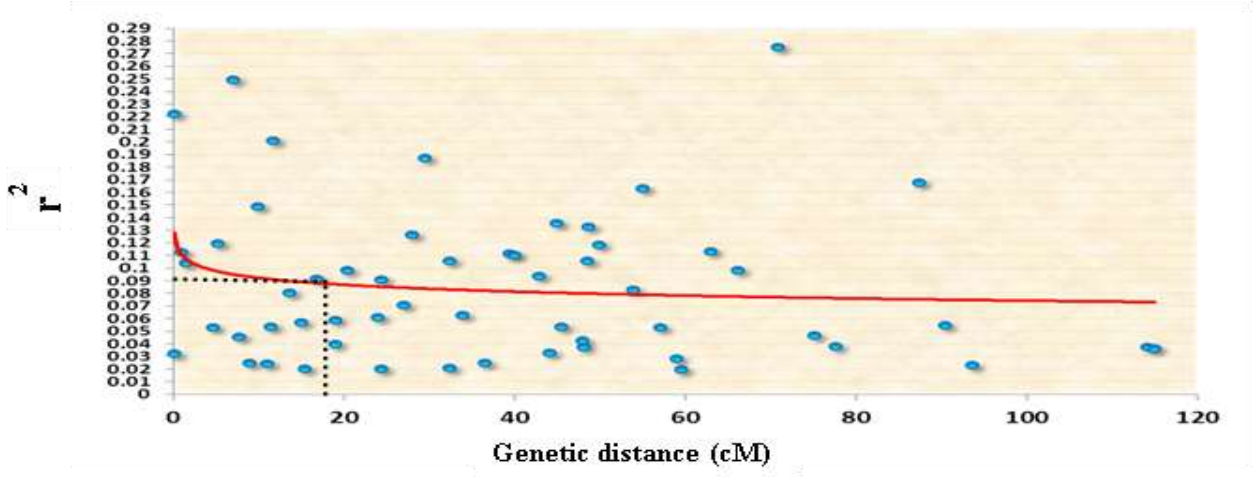


Figure 4-20 Estimates of LD (r^2) over genetic distance on 12 chromosomes of 103 germplasm accessions

Marker- trait associations

In order to reduce false positive associations, the MLM model (K+Q, kinship matrix and genetic structure) was used to detect associations between 145 SSR markers with root, shoot and other 13 quantitative traits.

Association between published SSR markers with root and shoot traits

SSR markers and root and shoot trait associations were analysed for 'All' spp (85 germplasm accessions), set 1 (45 germplasm accessions) and set 2 (40 germplasm accessions) species using the phenotypic root and shoot traits observed under normal condition in 2014.

Amount of marker-trait associations (MTAs) was obtained and varied according to the significant probability level. There are 37 (24), 12 (10) and 2 (2) for set 1, 50 (38), 11 (10) and 7 (7) for set 2 and 53 (38), 21 (18) and 14 (12) makers linked to six root and shoot traits for 'All'spp with respect to p≤ 0.05, p ≤ 0.01 and p ≤ 0.005, respectively. RDW/SHDW, RDW and RL are detected consisting of high number of marker associations, while RL/SHL is found as the least (Table 4-15).

Table 4-15 Marker-trait associations at three significant levels for root and shoot traits

Sl. No.	Trait	Species/group	Number of accessions	Number of associations		
				p ≤ 0.05	p ≤ 0.01	p ≤ 0.005
1	RL	Set 1	45	12	4	1
		Set 2	40	9	2	1
		'All' spp	85	6	0	0
2	RL/SHL	Set 1	45	4	0	0
		Set 2	40	4	2	2
		'All' spp	85	5	2	0
3	RV	Set 1	45	4	1	0
		Set 2	40	6	1	0
		'All' spp	85	8	4	4
4	RDW	Set 1	45	5	2	0
		Set 2	40	10	1	0
		'All' spp	85	15	6	3
5	SHDW	Set 1	45	7	3	0
		Set 2	40	5	2	2
		'All' spp	85	6	2	1
6	RDW/ SHDW	Set 1	45	5	2	1
		Set 2	40	16	3	2
		'All' spp	85	13	7	6
Total		Set 1	45	37 (24)	12 (10)	2 (2)
		Set 2	40	50 (38)	11 (10)	7 (7)
		'All' spp	85	53 (38)	21 (18)	14 (12)

Note: p≤ 0.05, 0.01 and 0.005- Significant @ 0.05, 0.01 and 0.005 probability levels, respectively.Total-Number in bracket denote as total of single markers linked to all the root and shoot traits.

Markers are linked to two root traits as M54 for RL (set 1) and SHDW (set 2), M147 for RL (set 1) and RL/SHL (set 1), M34 for RL (set 1) and RDW (set 1), M146 for RL ('All' spp) and RL/SHL ('All' spp), M72 for RV (set 2) and RDW (set 2), M135 for RV (set 2 and 'All' spp) and RDW ('All' spp), M19 for RDW ('All' spp) and RV (set 2 and 'All' spp), M52 for RV ('All' spp) and RDW ('All' spp), M134 for RV ('All' spp) and RDW ('All' spp), M29 for RDW (set 2) and RDW/SHDW (set 2), M95 for RDW (set 2 and 'All' spp) and RDW/SHDW ('All' spp), M51 for SHDW ('All' spp) and RDW/SHDW (set 1), M37 for SHDW (set 1) and SHDW (set 1), M146 for RL ('All' spp) and RL/SHL ('All' spp), M56 for RL/SHL (set 2) and SHDW (set 1), M83 for RDW ('All' spp) and SHDW (set 2 and 'All' spp), and M52 for RV ('All' spp) and RDW ('All' spp).

Among the root traits, some markers are identified linked with at least three root and shoot traits including M20 for RL (set 2 and 'All' spp), RV (set 2) and RDW (set 2), M1 for RL (set 2 and set 1), RV (set 1), RDW (set 1 and set 2) and RDW/SHDW (set 2 and 'All' spp), M145 for RL (set 2), RV (set 2) and RDW (set 2 and 'All' spp), M138 for RL (set 2 and 'All' spp), RDW ('All' spp) and SHDW (set 1), M37 for RL (set 2 and 'All' spp), RDW (set 1) and SHDW ('All' spp), M32 for RL (set 1), RL/SHL (set 1) and RDW/SHDW (set 1), M109 for RL (set 1), RV (set 1) and RDW (set 1), M106 for RV ('All' spp), RDW ('All' spp) and RDW/SHDW (set 2), M116 for RL (set 1), RV (set 1), RDW (set 1)and SHDW (set 1) and M34 for RL (set 1), RDW (set 1) and SHDW ('All' spp). These markers associated with more than one trait within species group may be due to the phenotypic data RL, RV, RDW and SHDW found to be highly correlated (Table 4-15 and 4-16) and pleiotropic effect of genes (Sauvage *et al*., 2014).

Many QTLs association detected between SSR marker with root and shoot traits but there are sixteen single markers has highest R^2 for each species group among various root and shoot traits (Table 35). Only marker 104 in root: shoot length is common for two species groups ('All' spp ans set 2) indicating this favourable marker for for set 2 because of high R^2(40.22%). Another marker, M145, also find common for root dry weight (set 2) and shoot dry weight ('All' spp) but it is better for root dry weight than shoot dry weight due to the former has higher R^2 value (30.80%).

Table 16 The most powerful SSR markers for root and shoot traits under normal condition during 2014

No.	Trait	Species group	Marker	Chromosome	R^2 (%)
1		'All' spp	M146	1	10.96
	RL	Set 1	M105	3	27.07
		Set 2	M22	1	28.70
2		'All' spp	M104	3	10.19
	RL/SHL	Set 1	M147	5	11.69
		Set 2	M104	3	40.22
3		'All' spp	M19	9	23.50
	RV	Set 1	M1	5	12.15
		Set 2	M12	-	28.06
4		'All' spp	M138	2	22.01
	RDW	Set 1	M116	9	8.34
		Set 2	M145	2	30.80
5		'All' spp	M145	5	17.46
	SHDW	Set 1	M141	6	19.06
		Set 2	M50	12	30.67
6		'All' spp	M144	11	30.29
	RDW/ SHDW	Set 1	M110	7	21.40
		Set 2	M46	5	31.03

Association between published SSR markers and other thirteen quantitative traits

Marker-trait association are conducted for thirteen selected quantitative traits (excluded FNPC, FLPC, FV, FT and TSS) in four groups 'All' spp, 'lyco', 'cherry' and 'wild' species. The phenotypic data in each group are taken separately during 2014, 2015 under normal and stress conditions for those traits showed significant variation (Table 4-17), while 'Mean' (pooled over years) is performed only for those traits having high Pearson correlation coefficient values (r>0.5,).

Association between published SSR markers with thirteen quantitative traits in'All' spp

Among the traits of 'All' spp, many markers are relative associations with many traits for either conditionsor years (Table 4-18) including M130 for ten traits, *viz*., DFF, DFFS, PH, BN, SCMR, RWC, LR, STG, AFW and FYPP, M42 for ten traits, *viz*., DFF, DFFS, PH, BN, RWC, LR, STG CPP, FPP and FYPP, M83 for nine traits, *viz*., DFF, DFFS, PH, BN, LR, STG, CPP, FPP and FYPP, M98 for nine traits, *viz*., DFF, DFFS, PH, BN, RWC, SLA, LR, STG and CPP, M93 for nine traits, *viz*., DFF, DFFS, PH, BN, RWC, LR, STG and AFW, M117 for eight traits, *viz*., DFF, DFFS, SCMR, SLA, RWC, STG, CPP AND FPP, M69 for seven traits, *viz*., DFF, DFFS, PH, BN, SCMR, LR and STG, M46 for seven traits, *viz*., DFF, PH, BN, SCMR, SLA, CPP AND FPP, M17 for seven traits, *viz*., DFF, DFFS, PH, BN, STG, CPP and FPP, M136 for seven traits, *viz*., DFF, DFFS, PH, STG, CPP and FPP, M32 for six traits, *viz*., DFF, BN, SCMR, STG, AFW and FYPP, M51 for six traits, *viz*., DFF, DFFS, PH, BN, LR and STG and M65 for five traits, *viz*., DFF, PH, BN, STG and AFW.

In addition, some markers also find to be frequently associated to specific traits such as M6, M56, M84 and M112 for CPP and FPP, M6, M7 and M64 for FPP, M2, M86 and M144 for DFF and DFFS, M34 and M113 for AFW, M147 for PH, M140 for BN and FPP and M148 for BN and CPP.

However, some markers are considered as the most powerful because of their highly phenotypic variation (R^2) contributed to fruit yield, drought and yield related traits like M42 for STG (39.89% and 22.87%), DFF (16.66% and 12.97%) and FYPP (13.49% and 15.50%), M46 for SLA (18.74% and 21.93%), M84 for FPP (14.69% and 12.88%), M98 for DFF (10.69% and 15.46%), DFFS (19.14% and 16.94%) and M130 for STG (41.74% and 25.03%). STG was positive associated with fruit yield across years, while DFF and DFFS showed significant and negative associations. DFF and DFFS were also found to be closely related and this may be seen common markers detected between the two traits. SLA was significant and negative relationship with fruit yield under stress condition.

Table 4-16 Marker-trait associations between published SSR markers and 13 quantitative traits in 'All' spp under normal (N) and stress (S) condition during 2014, 2015 and 'Mean'

Trait	Year	Condition	Marker- trait associations significant @ 0.05 probability level																			
DFF	2014	N	M17[1,2]	M98[1,2]	M83[1,2]	M130[1,2]	M42[1,2]	M51[1,2]	M95[1,2]	M93[1,2]	M144[1]	M48[1]	M82[1]	M100[1]	M117	M3	M101	M2	M77	M69	M67	M64
		S	M42[1,2]	M98[1,2]	M82[1]	M83[1]	M130[1]	M93	M17	M48	M3	M128	M138	M64	M134	M95	M100	M38	M117	M37	M144	
	2015	N	M83[1,2]	M98[1,2]	M130[1,2]	M17[1,2]	M93[1,2]	M86[1,2]	M129[1,2]	M42[1,2]	M67[1,2]	M117[1,2]	M82[1,2]	M51[1]	M48[1]	M136[1]	M52	M56	M76	M144	M2	M65
		S	M98[1,2]	M83[1,2]	M82[1,2]	M130[1,2]	M84[1,2]	M48[1,2]	M141[1,2]	M56	M86	M42	M129	M17	M37	M2	M136	M18	M9	M114	M25	M46
			M51	M69																		
	'Mean'	N	M98[1,2]	83[1,2]	M130[1,2]	M17[1,2]	M42[1,2]	M93[1,2]	M51[1,2]	M82[1,2]	M129[1,2]	M67[1,2]	M48[1,2]	M86[1]	M117[1]	M144[1]	M2	M136	M65	M69	M56	
		S	M98[1,2]	M83[1,2]	M82[1,2]	M42[1,2]	M130[1,2]	M48[1,2]	M86[1]	M17[1]	M37	M136	M84	M141	M129	M93	M138	M2	M3	M69	M18	M51
DFFS	2014	N	M130[1,2]	M82[1,2]	M42[1,2]	M48[1,2]	M144[1,2]	M98[1,2]	M83[1]	M117[1]	M69[1]	M100	M17	M101	M95	M2	M3	M93				
		S	M93[1,2]	M42[1,2]	M82[1,2]	M48[1,2]	M98[1,2]	M130[1]	M95[1]	M83	M134	M17	M97	M117	M128	M144	M37	M19				
	2015	N	M130[1,2]	M98[1,2]	M83[1,2]	M93[1,2]	M17[1,2]	M129[1,2]	M42[1,2]	M86[1,2]	M67[1,2]	M56[1,2]	M82[1,2]	M48[1,2]	M52[1]	M51[1]	M136	M117	M2	M114	M19	M76
		S	M83[1,2]	M98[1,2]	M130[1,2]	M82[1,2]	M136[1,2]	M42[1,2]	M48[1,2]	M84[1,2]	M17[1,2]	M129[1,2]	M86[1,2]	M51[1,2]	M56[1,2]	M69[1,2]	M141	M67	M2	M37	M18	M5
			M3																			
	'Mean'	N	M98[1,2]	M130[1,2]	M83[1,2]	M82[1,2]	M48[1,2]	M42[1,2]	M17[1,2]	M2[1,2]	M117[1,2]	M93[1]	M136[1]	M67	M69	M144	M56	M86				
		S	M98[1,2]	M83[1,2]	M42[1,2]	M130[1,2]	M82[1,2]	M48[1,2]	M136[1,2]	M17[1,2]	M37[1]	M51[1]	M93	M86	M95	M97	M144	M117	M69	M128	M3	M129
PH	2014	N	M83[1,2]	130[1,2]	M98[1,2]	M67[1,2]	M17[1,2]	M69[1,2]	M42[1,2]	M114[1,2]	M51[1]	M93[1]	M54[1]	M147	M62	M65	M140	M124	M1	M148	M121	M50
		S	M130[1,2]	M83[1,2]	M69[1,2]	M136[1,2]	M98[1,2]	M147[1,2]	M114[1,2]	M67[1,2]	M118[1,2]	M76[1,2]	M144[1]	M54[1]	M96[1]	M42	M51	M17	M46	M3	M93	
	2015	N	M46	M36	M148	M126	M47	M65	M147	M84												
		S	M136[1,2]	M11	M65	M42	M50															
	'Mean'	N	M83[1]	M130[1]	M98[1]	M67[1]	M17	M136	M46	M69	M65	M1	M50	M42	M147	M124	M148	M140	M54	M36		
		S	M130[1,2]	M136[1,2]	M69[1]	M83	M42	M76	M98	M147	M54	M65	M114	M118	M11	M96	M14					
BN	2014	N	M148[1,2]	M1	M12	M65	M72	M126	M67	M99	M130	M93										
		S	M130[1,2]	M93[1,2]	M83[1,2]	M147[1,2]	M148[1,2]	M67[1,2]	M69[1]	M99	M4	M42	M98	M114	M1	M51	M54	M46	M57			
	2015	N	M32[1]	M145[1]	M46	M148	M70	M36	M140	M89	M11	M126										
		S	M148[1,2]	M140[1]	M17	M144	M89	M145	M93	M72	M32											
	'Mean'	N	M148[1,2]	M46[1]	M126	M33	M65	M140	M1													
		S	M148[1,2]	M140[1]	M89	M69	M1	M57	M29	M145												
SCMR	2014	N	M32																			
		S	M145	M126	M138	M46																
	2015	N	M24[1,2]	M32	M95	M54	M56	M50	M69													
		S	M24[1,2]	M5	M130	M96	M98	M32	M117	M3	M29											
	'Mean'	N	M32[1,2]	M24	M5	M54	M50															
SLA	2014	N	M46[1,2]	M127	M56	M21	M112	M117	M138	M7	M142	M144	M4									
		S	M21[1]	M46[1]	M134	M101	M118	M37	M50	M127	M138	M84										
	2015	N	M98[1]	M82	M46	M140	M115	M48	M33													
		S	M110[1,2]	M132[1,2]	M40[1,2]	M33	M56	M39														

RWC	2014	N	M81[1,2]	M132[1]	M42	M34	M93	M47	M117	M130																	
		S	M31[1,2]	M56	M99	M21	M135	M117																			
	2015	N	M89[1]	M101	M29	M38	M68	M97	M81																		
		S	M3																								
LR	2014	N	M1[1,2]	M116[1,2]	M113[1,2]	M130	M33	M64																			
		S	M54[1,2]	M3[1,2]	M93[1,2]	M130[1,2]	M83[1,2]	M114[1]	M147[1]	M95[1]	M42	M129	M40	M69	M142	M96	M51	M67	M98	M52							
	2015	N	M136[1]	M108	M51	M20	M89	M143	M18	M22	M42																
		S	M70	M20	M107	M51																					
	'Mean'	S	M70	M54	M51	M52	M107	M147	M97																		
STG	2014	N	M130[1,2]	M83[1,2]	M98[1,2]	M69[1,2]	M42[1,2]	M17[1,2]	M67[1,2]	M51[1,2]	M2[1,2]	M117[1,2]	M114[1]	M136	M62	M1	M144	M129	M60	M93	M3	M32	M65				
		S	M130[1,2]	M83[1,2]	M981.2	M69[1,2]	M129[1,2]	M17[1,2]	M51[1,2]	M67[1,2]	M117[1,2]	M93[1,2]	M42[1,2]	M114[1,2]	M144[1,2]	M3[1,2]	M54[1]	M2	M86	M132	M47	M147	M60				
			M21	M5	M76	M127	M84																				
	2015	N	M136[1,2]	M76[1]	M113[1]	M51	M14	M22	M116																		
		S	M51[1,2]	M42[1,2]	M144	M112	M142	M117	M32	M136	M114	M130															
CPP	2014	N	M112[1,2]	M84[1,2]	M6[1,2]	M36[1]	M33	M140	M148	M56	M19	M52															
		S	M7[1,2]	M60[1,2]	M18[1,2]	M121[1,2]	M62[1,2]	M112[1,2]	M84[1]	M63[1]	M98[1]	M56[1]	M83[1]	M137[1]	M117[1]	M24[1]	M127[1]	M136[1]	M138[1]	M97[1]	M36[1]						
	2015	N	M18[1,2]	56[1,2]	M29[1,2]	M46[1,2]	M84	M36	M140	M42	M89	M148	M20	M64	M107	M96	M129										
		S	M112[1,2]	M18[1,2]	M36[1,2]	M46[1,2]	M56[1,2]	M83[1]	M148[1]	M7[1]	M98[1]	M72[1]	M126[1]														
	'Mean'	N	M84[1,2]	M112[1,2]	M36[1,2]	M140[1]	M6[1]	M18[1]	M89	M148	M33	M46															
		S	M18[1,2]	M7[1,2]	M112[1,2]	M83[1,2]	M60[1]	M121[1]	M56[1]	M84[1]	M36[1]	M24	M136	M63	M117	M62	M137	M17									
FPP	2014	N	M84[1,2]	M112[1,2]	M29[1,2]	M46[1,2]	M127[1,2]	M6[1]	M145[1]	M36	M140	M128	M48	M80	M57	M64	M42	M86	M21	M89							
		S	M84[1,2]	M112[1,2]	M7[1,2]	M60[1,2]	M6[1,2]	M136[1,2]	M36[1]	M62	M18	M140	M127	M98	M119	M121	M117	M137	M17	M56	M64	M52	M8				
	2015	N	M84[1,2]	M110[1,2]	M46[1,2]	M29[1,2]	M48[1,2]	M56[1,2]	M18[1]	M89[1]	M143[1]	M42[1]	M107	M117	M129	M140	M64	M120	M7	M36	M66						
		S	M112[1,2]	M84[1,2]	M18[1,2]	M56[1,2]	M46[1,2]	M36[1,2]	M7[1,2]	M83[1,2]	M19	M136	M86	M4	M17	M140	M117										
	'Mean'	N	M84[1,2]	M29[1,2]	M46[1,2]	M110[1,2]	M48[1,2]	M42[1]	M89[1]	M112	M36	M140	M64	M145	M6	M117											
		S	M84[1,2]	M112[1,2]	M18[1,2]	M7[1,2]	M136[1,2]	M36[1,2]	M117[1]	M17[1]	M6	M56	M140	M83	M60	M64	M121	M52									
AFW	2014	N	M65	M22	M108	M54																					
		S	M65	M85	M50	M144	M1	M81	M32	M131	M145																
	2015	N	M113[1,2]	M34[1,2]	M32[1]	M130	M81	M10	M93																		
		S	M113[1,2]	M32[1,2]	M81[1,2]	M10	M93	M34	M130	M65																	
	'Mean'	N	M34	M32	M81	M130	M113	M65	M108	M131																	
		S	M113[1,2]	M32[1]	M81[1]	M65	M10	M93	M34	M130	M131	M119															
FYPP	2014	N	M93[1,2]	M130[1]	M42	M32	M83	M54	M72	M86	M67																
		S	M32[1]	M130	M83	M118	M65	M69																			
	2015	N	M89[1,2]	M128[1,2]	M145[1,2]	M25	M31	M114	M72																		
		S	M32[1,2]	M42[1,2]	M77	M143	M65	M39	M132	M107	M22																

Notes: [1]and [2] Superscript denote significant @ 0.01 and 0.005 probability levels respectively

Table 4-17 List of common SSR markers associated to thirteen quantitative traits under normal and stress conditions across years and 'Mean' in "All' spp

No.	Trait	Marker code	Marker name	Chr.	Common year and condition		R^2 (%)	
					Normal	Stress	Normal	Stress
1	DFF	M2	SSR20	12	2014, 2015, 'Mean'	2014, 2015,	4.46	4.60
	DFF	M17	LEttc002	4[a]	2014, 2015, 'Mean'	2014, 2015,	18.20	9.78
	DFF	M42	SSR593	4	2014, 2015, 'Mean'	2014, 2015,	16.66	12.97
	DFF	M48	TOM8-9	9	2014, 2015, 'Mean'	2014, 2015,	11.75	9.78
	DFF	M82	SLM6-56_2	6	2014, 2015, 'Mean'	2014, 2015,	10.53	8.81
	DFF	M83	SLM6-57	6	2014, 2015, 'Mean'	2014, 2015,	13.49	10.21
	DFF	M98	SSR27	3	2014, 2015, 'Mean'	2014, 2015,	10.69	15.46
	DFF	M130	SSR218	10	2014, 2015, 'Mean'	2014, 2015,	16.00	7.91
	DFF	M17	LEttc002	4[a]	2014, 2015, 'Mean'	2014, 2015,	4.46	4.60
2	DFFS	M17	LEttc002	4[a]	2014, 2015, 'Mean'	2014, 2015,	13.25	11.27
	DFFS	M42	SSR593	4	2014, 2015, 'Mean'	2014, 2015,	14.00	14.79
	DFFS	M48	TOM 8-9	9	2014, 2015, 'Mean'	2014, 2015,	12.20	11.51
	DFFS	M82	SLM6-56_2	6	2014, 2015, 'Mean'	2014, 2015,	9.50	9.89
	DFFS	M83	SLM6-57	6	2014, 2015, 'Mean'	2014, 2015,	15.00	12.37
	DFFS	M98	SSR27	3	2014, 2015, 'Mean'	2014, 2015,	19.14	16.94
	DFFS	M130	SSR218	10	2014, 2015, 'Mean'	2014, 2015,	15.40	7.91
	DFFS	M37	SSR66	2	-	2014, 2015,	-	5.09
3	PH	M136	SSR57	2	'Mean'	2014, 2015,	3.38	5.50
	PH	M42	SSR593	4	2014, 'Mean'	2014, 2015, 'Mean'	5.39	5.37
	PH	M65	SSR49	5	2014, 2015, 'Mean'	2015, 'Mean'	0.61	1.71
	PH	M147	SSR109	5	2014, 2015, 'Mean'	2014, 'Mean'	4.49	5.50
4	BN	M148	SSR325	5	2014, 2015, 'Mean'	2014, 2015, 'Mean'	3.90	4.58
	BN	M126	TGS0939	7	2014, 2015, 'Mean'	-	7.58	-
5	SCMR	M24	LEct004	8[b]	2015, 'Mean'	2015	9.27	11.17
	SCMR	M32	SSR49	9	2014, 2015, 'Mean'	-	10.78	-
6	SLA	M46	TOM152-153	5	2014, 2015	2014	18.74	21.93
7	RWC	M81	SLM6-56_1	6	2014, 2015	-	7.31	-
	RWC	M117	TGS1305	9	2014	2014	8.75	7.92
8	LR	M51	TMS52_2	12	-	2014, 2015, 'Mean'	-	5.54
9	STG	M51	TMS52_2	12	2014, 2015	2014, 2015	18.25	18.85
	STG	M42	SSR593	4	2014	2014, 2015	39.89	22.87
	STG	M114	TGS2194	8	2014	2014, 2015	25.45	17.34
	STG	M117	TGS1305	9	2014	2014, 2015	26.20	17.45
	STG	M130	SSR218	10	2014	2014, 2015	41.74	25.03
	STG	M144	SSR76	11	2014	2014, 2015	27.55	21.01
10	CPP	M18	SSR50	2	2014, 2015, 'Mean'	2015, 'Mean'	4.19	5.68
	CPP	M112	TGS0412	7	2014, 'Mean'	2014, 2015, 'Mean'	12.40	8.49
	CPP	M84	SSR128	6	2014, 2015, 'Mean'	2014, 'Mean'	11.23	7.94
	CPP	M36	SSR150	1	2014, 2015, 'Mean'	2014, 2015, 'Mean'	1.76	2.30
	CPP	M83	SLM6-57	6	-	2014, 2015, 'Mean'	-	3.58

Table 4-18: to be continued…

No.	Trait	Marker code	Marker name	Chr.	Common year and condition		R^2 (%)	
					Normal	Stress	Normal	Stress
	CPP	M7	SSR86	3	-	2014, 2015, 'Mean'	-	4.58
	CPP	M56	TC461	11	2014, 2015	2014, 2015, 'Mean'	4.74	4.64
11	FPP	M84	SSR128	6	2014, 2015, 'Mean'	2014, 2015, 'Mean'	14.69	12.88
	FPP	M36	SSR57	1	2014, 2015, 'Mean'	2014, 2015, 'Mean'	0.93	1.77
	FPP	M112	TGS0412	7	2014	2014, 2015, 'Mean'	10.69	12.13
	FPP	M136	SSR150	2	2014	2014, 2015, 'Mean'	-	4.03
	FPP	M7	SSR86	3	2015	2014, 2015, 'Mean'	-	4.90
	FPP	M18	SSR50	2	2015	2014, 2015, 'Mean'	5.98	5.91
	FPP	M140	SSR605	2	2014, 2015, 'Mean'	2014, 2015, 'Mean'	6.51	5.56
	FPP	M56	TC461	11	2015	2014, 2015, 'Mean'	6.92	5.36
	FPP	M46	TOM152-153	5	2014, 2015, 'Mean'	2015	8.09	6.89
12	AFW	M65	SSR49	5	2014, 'Mean'	2014, 2015, 'Mean'	2.68	3.50
	AFW	M81	SLM6-56	6	2015, 'Mean'	2014, 2015, 'Mean'	3.87	4.00
	AFW	M32	SSR49	9	2015, 'Mean'	2014, 2015, 'Mean'	7.83	10.99
13	FYPP	M72	SLM12-10	12	2014, 2015	2014, 2015	11.94	-
	FYPP	M42	SSR593	4	2014	2015	13.49	15.50
	FYPP	M32	SSR49	9	2014	2015	13.72	14.80

Association between published SSR markers with thirteen quantitative traits in 'lyco' species

Among the traits of 'lyco' species, some markers are associated with many traits across conditions and years (Table 4-19) including M135 for nine traits,*viz.*,DFF, DFFS, BN, SLA, RWC, LR, STG, AFW and FYPP, M15 for eight traits, *viz*, DFF, PH, BN, SCMR, SLA, RWC, LR, STG and FYPP, M94 for six traits, *viz*, PH, RWC, LR, STG, CPP and AFW, M115 for six traits, *viz*, PH, BN, RWC, STG, CPP and FPP with intensive common tight for two last, M136 for five traits, *viz*, DFF, CPP, FPP and FYPP, and M35 for five traits, *viz*, DFFS, BN, SCMR, STG and SLA. Nevertheless, some markers are suggested as the most powerful because of their highly phenotypic variation (R^2) contributed to fruit yield, drought and yield related traits (Table 19) asM35 (SSR52) for SCMR (R^2 = 11.61% under stress condition) and M115 (TGS2002) for FPP (R^2 = 14.38% under normal and 19.89% under stress condition) and for CPP (R^2 = 14.67% under stress condition). CPP, FPP and SCMR were found to be positive significantly to fruit yield in this species. In addition, CPP and FPP were found to be most associated (Table 4-20), so it may be explained for M115 detected as common between the two traits.CPP and FPP also showed high genetic variability across years and conditions, while SCMR is high for heritability only (Table 4-21).

Table 4-18 Marker-trait associations between published SSR markers and thirteen quantitative traits in 'lyco' species under normal (N) and stress (S) conditions during 2014, 2015 and 'Mean'

Sl No.	Trait	Year	Condition	Marker- trait associations significant @ 0.05 probability level						
1	DFF	2014	N	M135	M106	M42	M15			
		2015	S	M96[1]	M135[1]	M136				
2	DFFS	2014	N	M68[1,2]						
			S	M147[1,2]	M71[1,2]	M68[1,2]	M35[1,2]	M42[1]	M29	M128
		2015	S	M135[1]	M136	M96	M71			
3	PH	2014	N	M115[1,2]	M132					
		2015	N	M15[1]	M127	M101				
			S	M94	M40	M15				
		'Mean'	N	M111	M75					
			S	M132	M52	M36				
4	BN	2014	N	M128[1,2]	M71	M70	M35			
			S	M115	M70					
		2015	S	M35	M15	M135				
		'Mean'	N	M15[1,2]	M128[1,2]					
5	SCMR	2014	N	M35	M147					
			S	M35[1,2]	M15[1,2]	SSR113				
		2015	S	M35	M147	M17				
6	SLA	2014	N	M35[1,2]	M147[1]	M135				
			S	M127	M40	M135				
		2015	N	M35[1,2]	M147	M105				
			S	M15	M35					
7	RWC	2014	N	M115[1,2]	M24	M2	M40			
			S	M94[1,2]	M15[1]	M113				
		2015	N	M94[1]	M17	M110	M135			
			S	M137	M1	M105				
		'Mean'	N	M128[1,2]	M135[1]	M33	M24			
			S	M15[1]						
8	LR	2014	S	M15[1,2]	M94[1,2]	M40				
		'Mean'	S	M135[1,2]	M94[1,2]	M41				
9	STG	2014	S	M130[1]	M115	M105	S			
		2015	N	M94[1,2]	M147[1]	M135[1]	M15	M106	M35	
			S	M123						
10	CPP	2014	N	M115[1,2]	M118	M113				
			S	M94[1,2]	M136	M47	M115	M29	M110	
		2015	N	M126[1]	M127	M55	M130	M115	M47	
			S	M127	M115					
		'Mean'	N	M138	M127					
			S	M118	M136					
11	FPP	2014	N	M110[1,2]						
			S	M136[1,2]	M115[1,2]					
		2015	N	M71[1]	M115	M138	M65	M40	M130	M47
			S	M115[1,2]	M110	M65				
		'Mean'	N	M101[1,2]	M115	M65				
			S	M115[1,2]	M118	M47				
12	AFW	2014	N	M94[1,2]	M68[1]	M65				
			S	M99[1]	M21	M124				
		2015	N	M136[1,2]	M141	M135	M79	M26		
			S	M26[1,2]	M2					
		'Mean'	S	M68	M99	M135	M65			
13	FYPP	2014	N	M15[1,2]						
			S	M136[1,2]	M30[1]	M26				
		2015	N	M89[1,2]	M47[1,2]	M72	M128	M135	M109	M145
			S	M74						

Note: [1]and[2]Superscript denote significant @ 0.01 and 0.005 probability levels respectively.

Table 4-19 List of common SSR markers associated with quantitative traits under normal and stress conditions across years and 'Mean' in 'lyco' species

No.	Trait	Marker code	Marker name	Chr.	Common year and condition		R^2 (%)	
					Normal	Stress	Normal	Stress
1	DFF	M135	SSR136	11	2014	2015	0.93	0.30
2	DFFS	M71	TOM49-50_2	5	-	2014, 2015	-	7.09
3	SCMR	**M35**	SSR52	7	2014, 2015	2015	**2.72**	**11.61**
4	SLA	M35	SSR52	7	2014, 2015	2015	0.63	2.45
5	LR	M41	SSR327	8	-	2014, 'Mean'	-	4.09
	LR	M94	SSR270	1	-	2014, 'Mean'	-	2.28
6	CPP	M115	TGS2002	8	2014, 2015	2014, 2015	7.47	14.67
7	FPP	**M115**	TGS2002	8	2015, 'Mean'	2014, 2015, 'Mean'	**14.38**	**19.89**

Association between published SSR markers with thirteen quantitative traits in 'cherry' species

Across trait and marker associations for 'cherry' species, five markers are more relative relationship with many traits across conditions and year has been summarized. Such as M92 for 11 traits, *viz.*, DFF, DFFS, PH, BN (more common under stress condition), SLA, RWC (common both conditions), LR, STG, CPP, FPP and FYPP, M34 for ten traits, *viz.,* DFF, DFFS, PH, BN, SLA, RWC, LR, SCMR, AFW and FYPP (three last were more common under both conditions), M47 for seven traits, *viz.,* DFF, DFFS, PH, BN, CPP, FPP and FYPP, M144 for seven traits, *viz.,* FF, PH, BN, SLA, STG, CPP and FYPP and M24 for six traits, *viz.,* DFFS, PH, BN, SLA, RWC, LR, STG, CPP, AFW and FYPP. The other markers are also found as more linking to specific traits like M17 for AFW, and M7 and M48 forFYPP.

Among these, some markers are suggested as the most powerful because of their highly phenotypic variation (R^2) contributed to fruit yield, droughtand yield related traits like M34 (20.99%) and M92 (19.16%) for LR under stress condition, M24 (Plate 11) for PH (20.72 and 25.06%), FYPP (20.43% and 22.23%) under normal and stress conditions, respectively and LR (19.16%) under stress condition, M17 (Plate 12) for AFW (47.54%) under stress condition. LR was correlated to fruit yield during 2015. Beside of these, this trait was also exhibited medium to high PCV and GCV parameters with high heritability and GAM indicating to be feasibly used for selection. RWC was found to be significant and positive correlations with FYPP during 2015. In addition, this trait showed high heritability and medium to high GAM under stress condition. So, the selection could be also considered this marker-trait association for further traditional or marker-assisted selection.

Association between published SSR markers and thirteen quantitative traits in'wild' species

Across trait and marker associations as above mentioned for 'wild' species, five markers are seen as more tightly associations to many traits across conditions and years including M108 for 12 traits,*viz.,* DFF, DFFS, PH, BN, SCMR, SLA, RWC, LR, STG, CPP, FPP), AFW and FYPP, M19 for 12 traits, *viz.,* DFFS, PH, BN, SCMR, SLA, RWC, LR, STG, CPP, FPP, AFW and FYPP, M63 for 12 traits, *viz.,* DFF, DFFS, PH, BN, SCMR, SLA, RWC, LR, STG, FPP, AFW and FYPP, M34 for 11 traits, *viz.,* DFF, DFFS, PH, BN, SCMR, SLA, RWC, STG, CPP, FPP and FYPP, and M1 for nine traits, *viz.,* PH, BN, SCMR, SLA, LR, STG, CPP, AFW and FYPP. The other markers were also found as more linking to specific traits like M42 for RWC, and M46 and M82 forAFW.

Among these MTAs, some makers are seen as the most powerful because of their high phenotypic variation contributed to fruit yield, drought and yield related traits (Table 4-22) like M34 for PH (27.27% and 42.47%), BN (23.05% and 29.62%), STG (25.07% and 17.13%) and FYPP (16.80% and 18.67%) under normal and stress condition, respectively. PH (stress condition), BN (stress condition) and STG (both conditions) showed significant and positive correlations with FYPP. In addition, PH,BN and STG were also correlated each other (Table 4-23). So, associations with these four traits are common by both markers, M34 (Plate 13) and M19. Four traits also exhibited high genetic variability parameters (except medium PCV and GCV for STG). Hence, these MTAs could be suitable for further study in marker- assisted selection.

Table 4-20 List of common SSR markers associated with quantitative traits under normal and stress conditions across years and 'Mean' in 'cherry' species

No.	Trait	Marker code	Marker name	Chr.	Common year and condition		R^2 (%)	
					Normal	Stress	Normal	Stress
1	DFF	M34	SSR14	3	-	2014, 2015	-	0.44
2	PH	M24	LEct004	8[b]	2014, 2015	2014, 2015, 'Mean'	20.72	25.06
3	BN	M92	LELEUZIP	8	-	2014, 2015, 'Mean'	-	2.36
4	SCMR	M34	SSR14	3	2014, 2015	2014, 2015	5.43	4.72
5	SLA	M92	LELEUZIP	8	2014, 2015	2014, 2015	2.84	5.39
6	RWC	M92	LELEUZIP	8	2014, 2015	2014, 2015	3.69	19.16
7	LR	M24	LEct004	8[b]	-	2014, 2015	-	19.16
	LR	M34	SSR14	3	-	2014, 2015	-	20.99
8	CPP	M34	SSR14	3	2015	2014, 'Mean'	6.89	3.67
	CPP	M92	LELEUZIP	8	2014, 'Mean'	2015	2.95	6.13
9	FPP	M34	SSR14	3	-	2014, 2015, 'Mean'	-	3.76
10	AFW	M34	SSR14	3	2014, 2015	2014, 'Mean'	7.64	6.80
	AFW	M17	LEttc002	4a	-	2014, 2015, 'Mean'	-	47.54
11	FYPP	M7	SSR 86	3	2014, 2015, 'Mean'	-	1.70	-
	FYPP	M24	LEct004	8[b]	2015, 'Mean'	2015	20.43	22.23
	FYPP	M34	SSR14	3	2015, 'Mean'	2014, 2015	8.48	1.07
	FYPP	M48	TOM8-9	9	2014, 'Mean'	2014	3.82	0.38

Overall, a view to specific trait, no marker is found to be common occurrence across four groups of species (except for M34 found to be common linked with CPP, FPP and FYPP between 'cherry' and 'wild' species only). Hence, we suggest that marker-trait associations depend on phenotypic data and it is essential and better to consider marker-trait associations that are within species if the material belongs to diverse of species. Those markers associate with many traits suggesting thepresence of genes with pleiotropic effects or closelylinked genes. These were also reported by Sauvage*et al*., (2014) in tomato and Zhao *et al*., (2011) in rice.

The MTAs from thirteen quantitative traits with 54 single markers recorded as highest R^2 values areshowed in Table 48. Of which, some common markers are indicated as follows: (i) M34 in 'wild' species associated with FYPP (18.39%) followed by BN (16.69%), DFF (11.17%) and DFFS (11.07%), (ii) M24 in 'cherry' species (including RWC under normal in 'lyco' species)with highest values for BN (36.65% and 49.68%) followed by PH (29.33%), RWC (18.69 and 27.57%) and FYPP (22.18%), (iii) M46 in 'wild' and 'All' spp, (iv) M42 in 'wild', 'lyco' and 'All' spp and (v) Other markers,*viz.,* M130, M147, M56, M69, M84 and M98 are associated either within or across species.

In tomato, AM had been conducted by various molecular markers such as RAPD markers (Schuelter *et al*., 2003), AFLP markers (Berloo *et al*., 2008 and Nakazato *et al*., 2012), SNP markers (Ranc *et al*., 2012; Shirasawa *et al*., 2013; Xu *et al*., 2013, Ruggieri *et al.,* 2014, Sauvage *et al.,* 2014 and Pascual *et al.,* 2016) and $(GATA)_4$ probe (García-Martínez *et al*., 2015), while for SSR markers were Ranc *et al.,* (2008), Mazzucato *et al,*. (2008), Rao *et al*., (2011), Sim *et al.,* (2011), Zhang *et al*., (2015) and Zhang *et al*., (2016).

For QTL mapping, only one report by Lin *et al.,* (2010) detected 46 QTLs including 24 and 22 QTLs under irrigated and drought conditions, respectively, for yield, fruit weight, maturity and SCMR in 92 lines of backcross inbred (BCF_4) population of *S. lycopersicum x S. pimpinellifolium*planted in pot under greenhouse condition. Among these, PH associates with M15 (SSR80_150-170) found to be similar (R^2= 0.03 and 0.01%, in 'lyco' species under normal and stress conditions during 2015, respectively), while SSR69 and SS47 with PH are not. In addition, M15 in our study has also been detected to be associated with SCMR (R^2= 4.57%, in 'lyco' species under stress condition) but Lin *et al.,* (2010) was found in both conditions for this marker at SPAD readings 1 (SCMR1).

Table 4-21 Marker-trait associations between SSR markers and thirteen quantitative traits in 'wild' species under normal and stress conditions during 2014, 2015 and 'Mean'

Sl. No.	Trait	Year	Condition	Marker- trait associations significant @ 0.05 probability level								
1	DFF	2014	N	M30[1]								
			S	M30	M108							
		Mean	N	M108[1,2]	M34[1]	M75[1]	M130	M101				
			S	M108[1,2]	M34[1]	M63	M8	M48	M74			
2	DFFS	2014	N	M108[1,2]	M82[1,2]	M34[1,2]	M39[1]	M63	M8			
			S	M34	M8							
		2015	N	M108[1,2]	M128	M19						
			S	M108[1,2]	M8	M74	M63	M19	M34			
		Mean	N	M127								
			S	M134	M78							
3	PH	2014	N	M108[1,2]	M34[1,2]	M19	M130					
			S	M108[1,2]	M34[1,2]	M19						
		2015	N	M34[1,2]	M1[1]	M63	M19					
			S	M34[1,2]	M90							
		Mean	S	M108[1]	M34[1]	M19						
4	BN	2014	N	M30[1,2]	M1[1,2]	M34	M19	M67	M140	M63		
			S	M1[1]	M34	M19	M108					
		2015	N	M108[1,2]	M34[1,2]	M1[1,2]	M19[1,2]	M42	M90			
			S	M34[1,2]	M1[1,2]	M19	M108	M30				
5	SCMR	2014	N	M1	M19							
			S	M108[1,2]	M63[1,2]	M115	M38	M1	M19			
		2015	N	M108[1,2]	M19	M34	M115					
			S	M108[1,2]	M63[1,2]	M42	M19	M147	M34			
6	SLA	2014	N	M1[1,2]	M19[1]	M34	M56	M63				
			S	M108[1,2]	M63	M30	M1					
		2015	N	M108[1,2]	M1[1,2]	M19[1,2]	M63	M34	M115	M11		
			S	M39[1,2]	M1[1,2]	M14[1,2]	M34[1]	M19	M108	M62	M48	M20
7	RWC	2014	N	M108[1,2]	M42	M63	M34	M19	M30			

Sl. No.	Trait	Year	Condition	Marker- trait associations significant @ 0.05 probability level								
			S	M108[1,2]	M34	M19						
		2015	N	M108[1,2]	M34[1]	M42	M19	M147				
			S	M108[1,2]	M34[1]	M42	M19	M102				
8	LR	2014	S	M1[1,2]	M108[1,2]	M19[1]	M8	M63				
		2015	S	M101[1]	M1	M39						
9	STG	2014	N	M5[1,2]	M108[1,2]	M69[1,2]	M1	M142	M30	M148		
			S	M1	M34	M19	M30					
		2015	N	M34[1,2]	M63[1,2]	M1[1]	M19	M51				
			S	M63[1,2]	M34[1,2]	M51[1,2]	M108[1,2]	M19	M8	M147	M21	
		Mean	S	M108[1,2]	M34[1,2]	M19	M21	M30				
10	CPP	2014	N	M82[1]	M102[1]	M65						
			S	M19[1]	M108	M34	M36					
		2015	N	M56								
			S	M51[1]	M47[1]	M36[1]	M19	M34				
		Mean	N	M19[1,2]	M34	M1						
			S	M82	M34	M102	M65					
11	FPP	2014	N	M108[1,2]	M19[1]	M34						
			S	M65	M93	M82	M108	M34				
		2015	N	M19[1,2]	M120	M30	M63	M34	M108			
			S	M36[1]	M34	M108	M68	M19				
		Mean	N	M19[1,2]	M82[1]	M34	M103	M30				
			S	M34[1]	M36[1]	M68	M62					
12	AFW	2014	N	M46[1,2]	M82[1,2]	M108	M39	M19	M93			
			S	M82[1,2]	M46[1,2]	M19	M74	M55				
		2015	N	M108								
			S	M22	M8	M59	M19					
		Mean	N	M59[1,2]	M46[1,2]	M63	M19					
			S	M82[1,2]	M108[1,2]	M1[1]	M19[1]					
13	FYPP	2014	S	M108[1,2]	M34	M19						
		2015	N	M108[1,2]	M34[1,2]	M19[1,2]	M1[1,2]	M63	M120			
			S	M19	M34	M108						
		Mean	S	M34[1,2]	M1[1,2]	M19[1]	M30					

Notes: [1]and[2]Superscript denote significant @ 0.01 and 0.005 probability levels respectively

Table 4-22 The most powerful markers linked to thirteen quantitative traits in 'All' spp, 'lyco', 'cherry' and 'wild' species under normal and stress conditions during 2014, 2015 and 'Mean' (A- 2014, B- 2015 and C- 'Mean')

No.	Trait	'All' spp				'Lyco' species				'Cherry' species				'Wild' species			
		Normal	R^2 (%)	Stress	R^2 (%)	Normal	R^2 (%)	Stress	R^2 (%)	Normal	R^2 (%)	Stress	R^2 (%)	Normal	R^2 (%)	Stress	R^2 (%)
1	**DFF**	$M98^{C}$	23.36	$M95^{B}$	17.47	$M42^{A}$	25.67	$M96^{B}$	13.11	$M24^{A}$	6.87	$M47^{B}$	12.39	$M130^{C}$	27.99	$M34^{B}$	11.17
2	**DFFS**	$M98^{B}$	24.20	$M98^{B}$	19.27	$M68^{A}$	21.96	$M42^{A}$	19.49	$M56^{B}$	21.63	$M47^{B}$	8.91	$M34^{A}$	10.35	$M34^{B}$	11.07
3	**PH**	$M98^{A}$	9.74	$M76^{C}$	6.57	$M111^{C}$	18.66	$M52^{C}$	30.26	$M75^{C}$	29.70	$M24^{B}$	29.33	$M130^{A}$	50.25	$M90^{B}$	64.06
4	**BN**	$M12^{A}$	9.88	$M46^{A}$	10.01	$M70^{A}$	28.63	$M70^{A}$	22.42	$M24^{B}$	36.65	$M24^{B}$	49.68	$M67^{A}$	61.60	$M34^{A}$	16.69
5	**SCMR**	$M32^{C}$	11.97	$M46^{A}$	18.72	$M147^{A}$	7.14	$M35^{B}$	21.51	$M38^{B}$	13.71	$M56^{B}$	34.28	$M19^{A}$	6.66	$M147^{B}$	24.55
6	**SLA**	$M46^{A}$	22.68	$M46^{A}$	21.93	$M147^{A}$	21.32	$M127^{A}$	17.01	$M44^{B}$	18.67	$M84^{A}$	24.55	$M56^{A}$	50.75	$M39^{B}$	15.84
7	**RWC**	$M42^{A}$	17.71	$M56^{A}$	12.69	$M24^{A}$	23.97	$M105^{B}$	21.27	$M24^{B}$	18.69	$M92^{A}$	27.57	$M42^{B}$	18.43	$M42^{B}$	11.52
8	**LR**	-	-	$M54^{A}$	15.35	-	-	$M40^{A}$	8.86	-	-	$M72^{B}$	51.83	-	-	$M8^{A}$	49.74
9	**STG**	$M69^{A}$	41.76	$M69^{A}$	47.46	$M106^{B}$	14.00	$M123^{B}$	9.92	$M135^{B}$	21.13	$M144^{B}$	23.35	$M130^{C}$	66.55	$M51^{B}$	37.03
10	**CPP**	$M112^{A}$	15.82	$M112^{B}$	8.83	$M126^{B}$	38.64	$M115^{B}$	19.45	$M84^{B}$	47.52	M6	43.31	$M69^{A}$	75.09	$M36^{B}$	24.60
11	**FPP**	$M84^{C}$	14.82	$M84^{A}$	15.74	$M110^{A}$	33.27	$M115^{C}$	21.41	$M10^{A}$	21.29	$M6^{C}$	46.77	$M82^{C}$	12.70	$M36^{B}$	29.03
12	**AFW**	$M69^{C}$	11.91	$M32^{C}$	17.31	$M26^{B}$	21.28	$M21^{A}$	25.82	$M6^{A}$	45.10	$M17^{A}$	53.08	$M46^{A}$	76.89	$M46^{A}$	71.88
13	**FYPP**	$M89^{C}$	50.05	$M40^{A}$	36.50	$M145^{B}$	71.85	$M26^{A}$	21.75	$M24^{B}$	22.18	$M106^{A}$	35.39	$M120^{B}$	20.09	$M34^{B}$	18.39
Mean			**21.16**		**19.07**		**27.20**		**19.41**		**25.26**		**33.88**		**39.78**		**29.66**

Experiment 3: Phenotyping of F_2 and F_3 population of inter-specific cross for traits related to wue and fruit yield

F_2 PHENOTYPIC DATA OF INERSPECIFIC CROSS BETWEEN EC- 771612 × LA 2657

Data presented in the table 4-24 clearly indicated presence of high phenotypic coefficients of variability and genotypic coefficient of variability for all the characters except for days to first flowering and SCMR. Further, presence of narrow gap between PCV and GCV for all the characters under study suggested that these traits have low environmental influence. High heritability coupled with high genetic advance as a *per cent* of mean was observed for fruits per clusters, clusters per plant, average fruit weight, and SLA indicate that these characters are governed by additive genes therefore selection based on these traits would be rewarding for the required plant type.

Data depicted in the table 4-24 reveals that fruit yield per plant (g) has a significant positive association with all the nine characters except for primary branches per plant, however it shown significant negative association with SLA. The trait SCMR exhibited significant positive association with fruit yield per plant and significant negative association with SLA. Thus the F_2 lines developed from the cross between **EC- 771612 × LA 2657** has high SCMR and low SLA values could be used as surrogates for water use efficiency.

Table 4-23 Mean performance and genetic parameters of WUE and Fruit yield characters in F_2 population

	DFF	SCMR	SLA (cm^2g-1)	PH (cm)	Branches	Flowers per cluster	Fruits per cluster	Clusters per plant	Average fruit weight (g)	Fruits No.	Fruit yield (g)
LA 2657	45.33	41.43	40.84	169.33	10.33	7.33	5.67	111.00	1.31	674.67	813.67
EC 771597	52.33	53.00	176.57	126.13	6.42	4.23	3.03	23.92	85.63	53.87	3141.33
F_2 Mean	48.49	55.32	150.69	100.90	8.24	6.90	5.72	31.46	4.74	185.7	689.6
Min.	45.00	42.40	50.54	56.00	3.00	4.00	3.00	8.00	1.00	24.0	112.3
Max.	56.00	63.90	320.27	224.00	72.00	17.00	10.00	213.00	17.00	1065.0	1870.4
PCV	6.33	6.87	22.44	33.26	68.70	26.31	24.35	64.69	50.63	65.16	55.41
GCV	6.04	6.39	20.56	29.87	63.85	25.50	23.13	57.07	48.94	60.87	50.53
h^2_{bs}	91.15	86.54	83.92	80.66	86.38	93.98	90.28	77.84	93.45	87.29	83.14
GAM	11.88	12.25	38.80	55.26	22.24	50.93	45.28	103.72	97.46	117.16	94.91

Table 4-24 Correlation Coefficients of F_2 population for traits related to WUE and fruit yield

	SCMR	SLA (cm^2g-1)	Pant height (cm)	Primary Branches	Flowers per cluster	Fruits per cluster	Clusters per plant	Fruit weight (g)	Fruits number	Fruit yield per plant (g)
Days to flowering	-0.006	-0.149*	0.866**	0.386**	0.346**	0.370**	0.442**	-0.065	0.561**	0.388**
SCMR	1.000	-0.25*	-0.011	-0.007	0.020	-0.012	-0.020	0.050	-0.012	0.498**
SLA (cm^2g-1)		1.000	-0.173*	-0.124	-0.037	-0.008	-0.101	-0.294**	-0.079	-0.328**
Pant height(cm)			1.000	0.344**	0.287**	0.280**	0.429**	-0.020	0.507**	0.376**
Primary Branches				1.000	0.129	0.078	0.334**	-0.111	0.301**	0.126
Flowers per cluster					1.000	0.880**	0.046	0.063	0.457**	0.391**
Fruits per cluster						1.000	0.053	0.010	0.520**	0.394**
Clusters per plant							1.000	-0.310**	0.858**	0.373**
Fruit weight(g)								1.000	-0.260**	0.618**
Fruits number									1.000	0.504**

Phenotyping of F_3 mapping poulation of a cross EC- 771612 × LA 2657 for traits related to water use efficiency and fruit yield:

The analysis of variance indicated presence of significant amount of variation among all the F_3 families for all the morphological, physiological and fruit parameters studied (Table4-26) which suggested that selection can be practiced both in between and within F_3 lines.

High phenotypic coefficients of variability and high genotypic coefficient of variability were observed for all the characters except for days to first flowering and SCMR (Table 4-27). The presence of narrow gap between PCV and GCV for all the characters except average fruit weight which implies that expression of these traits has low environmental influence. Further, high heritability coupled with high genetic advance as a *per cent* of mean was observed for fruits per clusters, clusters per plant, average fruit weight, and SLA indicate that these characters are under the influence of additive genes hence, selection based on these traits will be rewarding for the improvement of required plant type.

The fruit yield per plant (g) has a significant positive association with all the nine characters except for 50% days to flowering and a negative association with SLA (27). The trait SCMR exhibited positive association with fruit yield per plant while SLA showed negative association with fruit yield per plant. Thus the F_3 lines developed from the cross between **EC- 771612 × LA 2657** has high SCMR and low SLA values and thus they can be used as surrogates for water use efficiency.

Table 4-25 Analysis of Variance for Morphological, Fruit Yield and Physiological Traits of F_3 Families

		Physiological		Morphological Parameters				Fruit Parameters			
Source of Variation	DF	SCMR	SLA	DFF	PH	BRNACHES	FLPP	FRPP	CPP	AFW	FRT NO.
Blocks	3	1.823	26.943**	0.31	28.401	0.028	0.427**	0.043	13.713	2.622	792.49
Entries	115	28.639**	1141.953**	11.457**	1336.015**	6.759**	3.994**	3.563**	372.131**	457.279	16992.35**
Checks	3	142.149**	18964.45**	51.856**	648.01**	6.452**	10.224**	7.438**	8940.471**	5444.937**	323458.80**
Varieties	111	23.604**	665.384**	8.978**	1360.521**	6.374**	3.205**	2.869**	125.896**	8.947**	8862.544**
Checks vs. Varieties	1	246.96**	573.68**	165.43**	679.784**	50.431**	72.852**	69.036**	1999.145**	35259.23**	1.375
ERROR	9	1.418	2.806	0.557	22.404	0.188	0.041	0.069	19.657	1.583	847.711

Table 4-26 Genetic Parameters for Morphological, Fruit Yield and Physiological Traits of F_3 Families

	Physiological		Morphological Parameters			Fruit Parameters					
	SCMR	SLA	DFF	PH	BRNACHES	FLPP	FRPP	CPP	AFW	FRT NO.	YLD (g/p)
GCV	9.664	16.68	5.727	27.232	26.231	22.669	25.331	33.243	49.707	45.229	50.111
PCV	10.003	16.72	5.935	27.485	26.673	22.831	25.678	36.518	55.354	47.826	52.923
h^2_{bs}	93.34	99.53	93.12	98.16	96.71	98.59	97.32	82.87	80.64	89.43	89.65
GAM	19.23	34.28	11.39	55.58	53.14	46.37	51.48	62.34	91.95	88.11	97.74

SPAD chlorophyll meter reading (SCMR),
Specific leaf area (SLA)
Days to flowering (DFF)
Branches,
Plant height (cm)

Fruits per cluster (FRPP),
Clusters per plant (CPP),
Average fruit weight (AFW,g),
Fruit number (FRT No.)
Fruit yield per plant (g)

Field of F_2 segregeting population

Table 4-27 Correlation coefficient of F_3 families

	SCMR	SLA (cm^2g-1)	Pant height(cm)	Primary Branches	Flowers per cluster	Fruits per cluster	Clusters per plant	Fruit weight(g)	Fruits number	Fruit yield per plant(g)
Days to flowering	0.098	-0.095	0.121	0.194*	0.144	0.099	-0.023	0.108	0.006	0.181
SCMR	1	-0.239*	0.244*	0.274**	0.209*	0.185	0.047	0.165	0.103	0.252**
SLA (cm^2g-1)		1	-0.089	-0.022	-0.026	-0.012	0.020	-0.213*	0.078	-0.246**
Pant height(cm)			1	0.694**	0.438**	0.477**	0.194*	0.064	0.429**	0.462**
Primary Branches				1	0.424**	0.387**	0.047	0.153	0.341**	0.427**
Flowers per cluster					1	0.868**	-0.053	-0.008	0.437**	0.447**
Fruits per cluster						1	-0.023	-0.113	0.538**	0.403**
Clusters per plant							1	-0.326**	0.735**	0.486**
Fruit weight(g)									-0.327**	0.650**
Fruits number									1	0.330**

Figure 4-21 Field of F2 segregating population

Figure 4-22 Field of F3 segregeting population

Experiment 4: Parental polymorphism and genotyping of F_2 mapping population with SSR markers linked to the traits related to wue and fruit yield

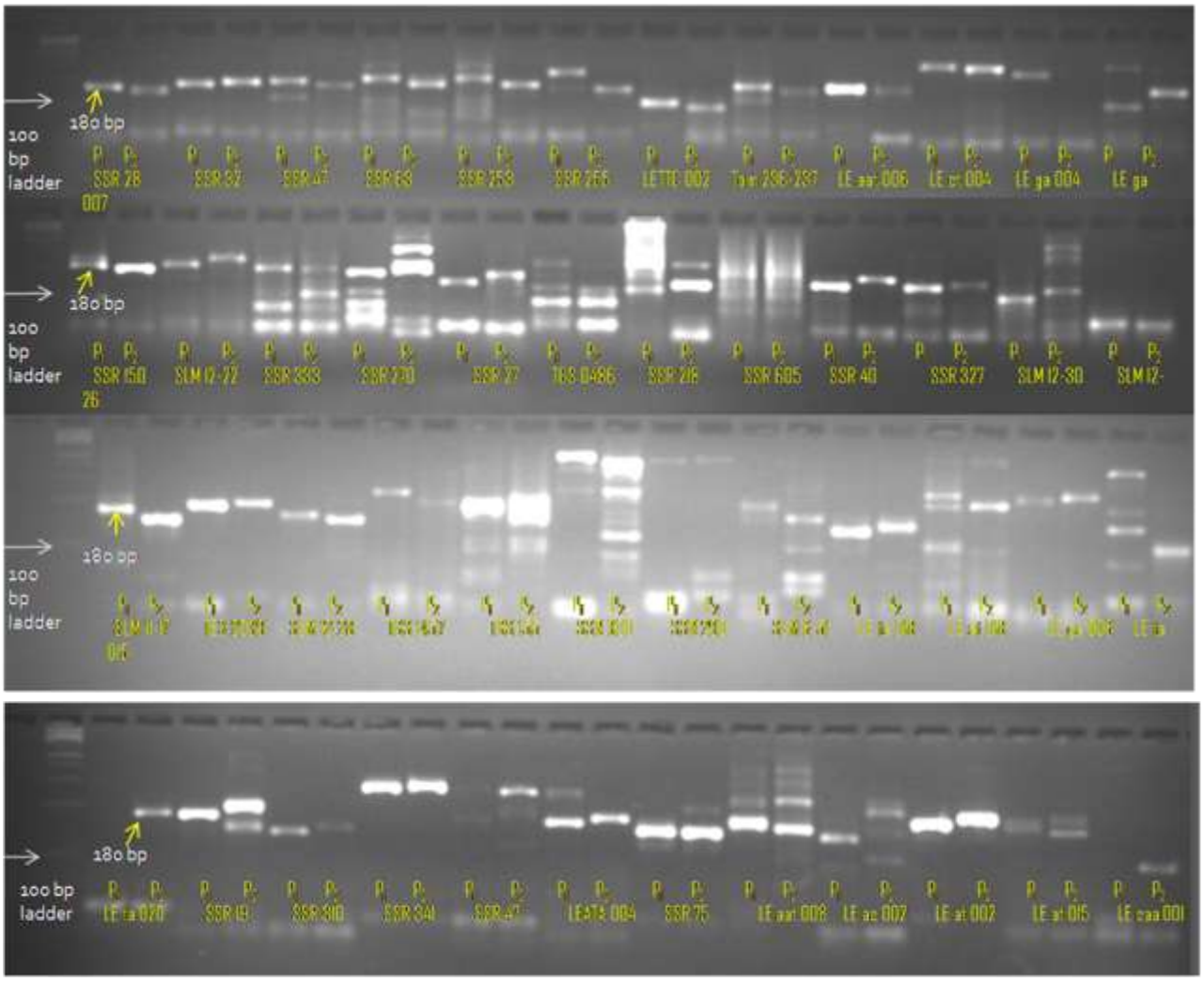
Results

DNA extraction from the leaves of parents (EC- 771612 and LA 2657 and 112 F2 plants using modified CTAB method and quantified. From among 200 primers 66 polymorphic for parents have been used in genotyping F_2 population (Table 4-29)

Table 4-28 Primers which have shown Parental Polymorphism

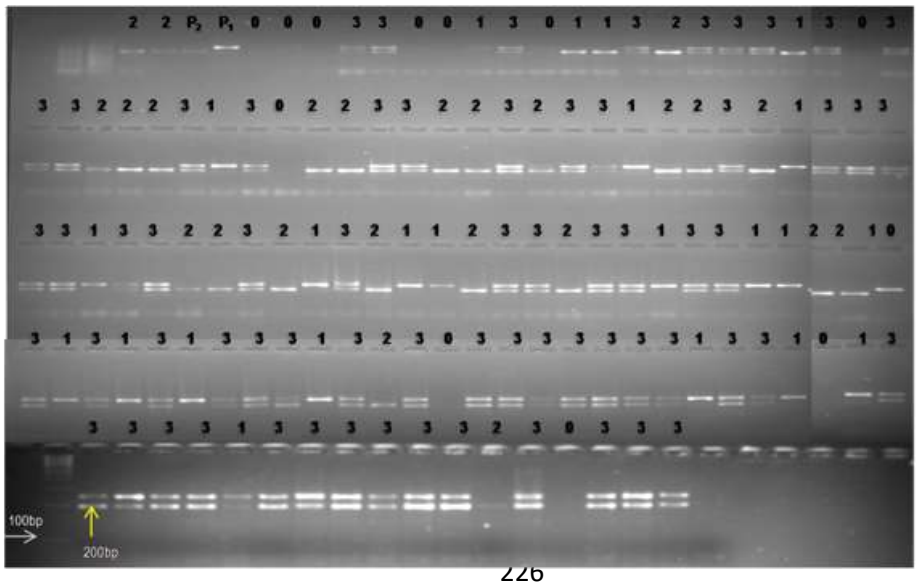
SL	PRIMER NAME	SL	PRIMER NAME	SL	PRIMER NAME	SL	PRIMER NAME
1	SSR 28	18	LE aat 006	35	SSR 270	52	TGS 0939
2	SSR 32	19	LE ct 004	36	SLM 11-17	53	SSR 327
3	SSR 47	20	LE ga 004	37	SSR 27	54	SLM 12-30
4	SSR 63	21	LE ga OO7	38	TGS 0486	55	SLM 12-28
5	SSR 253	22	SSR 150	39	TES 2039	56	SLM 12-39
6	SSR 255	23	SLM 12-22	40	SSR 218	57	SLM 12-34
7	LETTC 002	24	SLM 6-12	41	SSR 605	58	SLM 12-26
8	TOM 236-237	25	SSR 333	42	SSR 40	59	TGS 1457
9	TGS 155	26	SLM 12-23	43	SLM 6-15	60	SSR 19
10	SSR 300	27	SLM 12-1	44	SLM 6-6	61	SSR 310
11	SSR 31	28	SLM 6-39	45	LE ta 018	62	SSR 341
12	TGS 2730	29	SLM 6-28	46	LE at 018	63	SSR 47
13	SSR 146	30	SLM 6-25	47	LE ga 006	64	LEATA 004
14	TGS 2288	31	SLM 6-23	48	LE ta 015	65	SSR 75
15	SSR 135	32	SLM 6-51	49	LEta 020	66	LE aat 008
16	LE ac 002	33	LE at 002	50	LE at 005		
17	LE cgg 001	34	LE ctat 001	51	LE caa 001		

Parental Polymorphism of P_1 (Female parent: EC- 771612- *Solanum lycopersicum)* and P_2 (Male Parent*:* LA 2657- *Solanum penellii)*

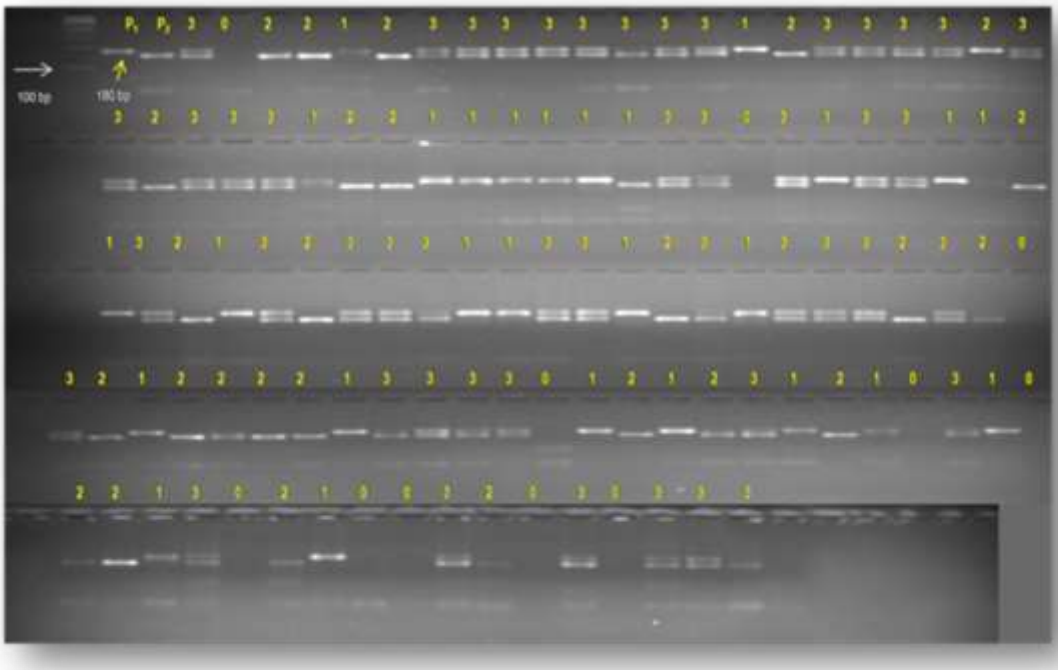


<u>GENOTYPING OF F_2 POPULATION BETWEEN CROSS (EC- 771612- *Solanum lycopersicum)* X LA 2657- *Solanum penellii*)</u>

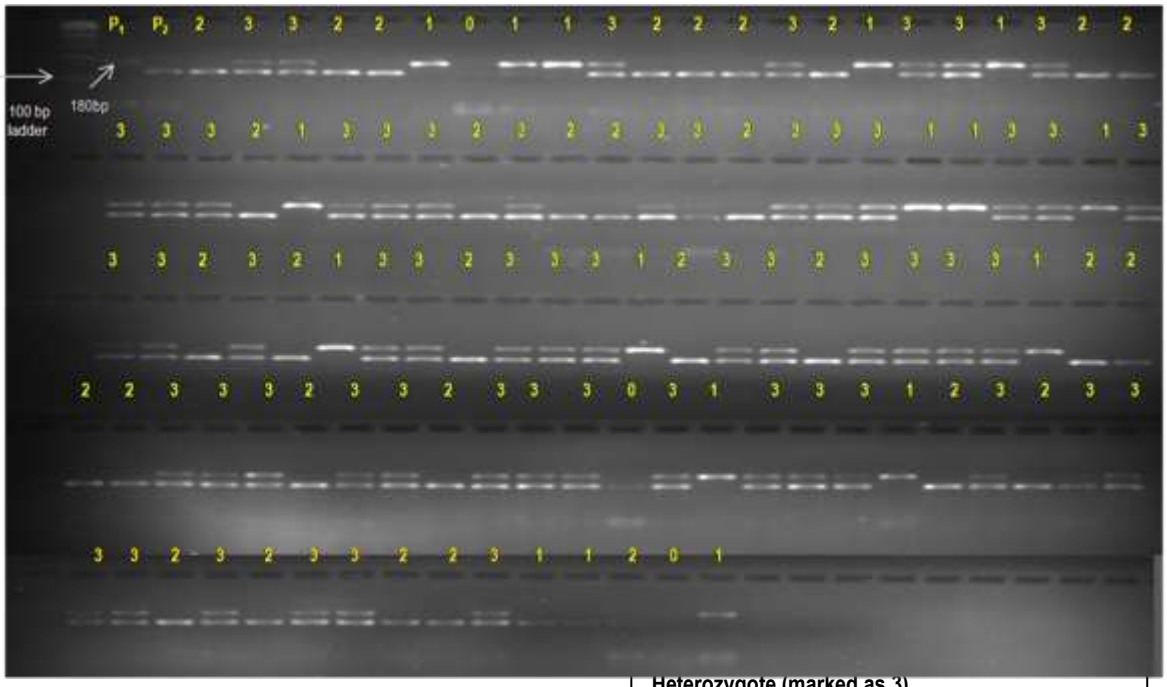
Marker Name: SSR 605



Marker Name: SLM 11-17

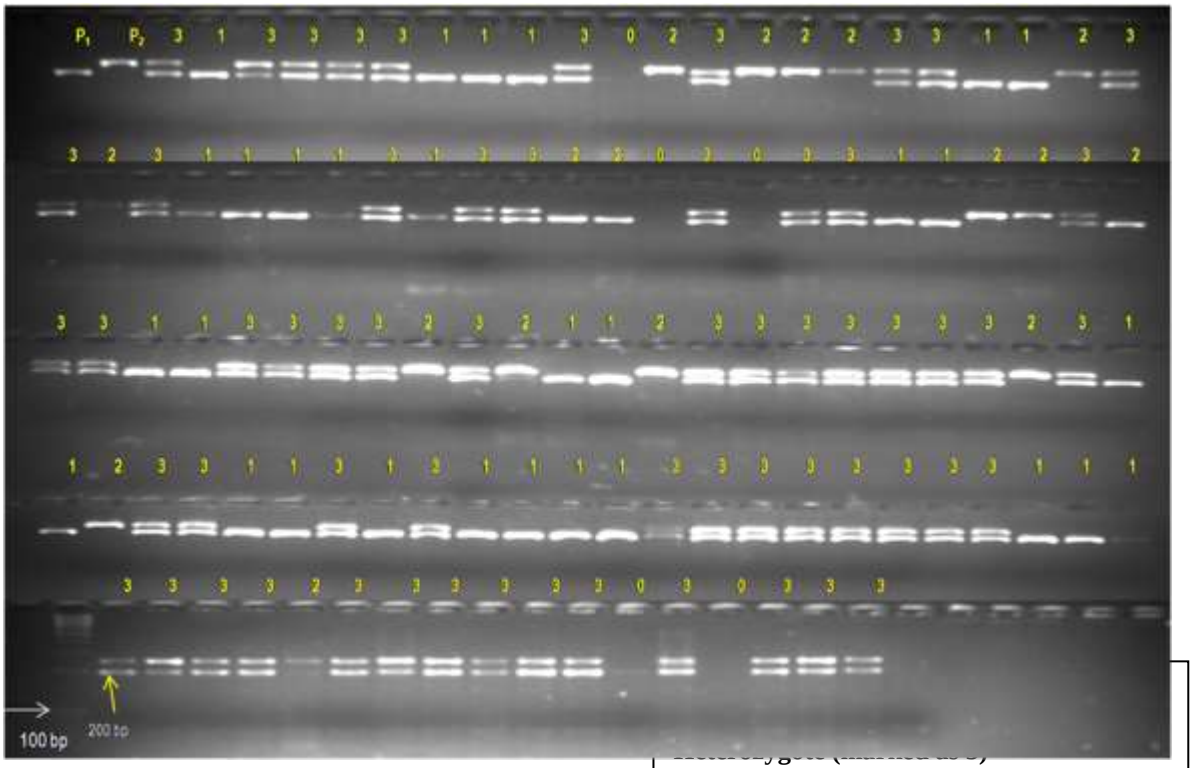


Marker name: SSR 27



Heterozygote (marked as 3)

Marker Name: TGS 2730



Marker Name: TGS 2730

- In all of the above gel pictures the primers have clearly shown the difference in the male, female and the heterozygote.
- The observed Chi square value is less than the expected (table) value, at p $_{0.05}$ so we can accept the null hypothesis and conclude that the samples have shown segregation in the ratio of 1:2:1 as expected.
- The genotyping work is still in progress.

Summary

Experiment 1: Phenotyping of Germplasm lines for traits related to WUE and fruit yield.

Genetic variability of root and shoot traits among 85 ('All' spp), 45 (set 1) and 40 (set 2) germplasm accessions showed highly significant differences for all the traits studied. Highest PCV, GCV, heritability (broad sense) and GAM were observed for RDW/SHDW followed by RL/SHL and RDW. Hence, selection could be used these traits along with other drought, fruit yield and yield related traits to develop drought tolerant and high yielding varieties in breeding program.

Identification of drought tolerant germplasm accessions based on fifteen drought tolerant indices computed from fruit yield under normal and stress conditions indicated that MP, GMP, HAM and STI are good indices to identify drought tolerant and susceptible accessions. Five germplasm accessions, LA 2976 of *S. habrochaites*, WIR 13708 of 'cherry' species, EC676809, EC677123 and EC771596 of "lyco' species were classified as high drought tolerant genotypes. Whereas, L00671 (*S. peruvianum),* EC771615 ('cherry'

species), CLN13149, EC771584 and EC771580 ('lyco' species) were the most susceptible accessions.
Across four groups of species, fruit yield showed various relationships with significant and positive associations for some traits,*viz*., STG, RWC, SCMR, CPP, FPP and BN, while it was recorded as negative associations between fruit yield withLR, SLA, DFF and DFFS.

Experiment 2: Phenotyping and Genotyping of cultivated and wild germplasm accessions with informative markers to establish association with traits related to WUE and fruit yield.

The population structure computed using 145 published SSR markers genotyped from 103 germplasm accessions of six cultivated tomato and related species revealed that four subgroups were classified along with 33 admixtures belonging to 'lyco' species, 'cherry' species and *S. pimpinellifolium*. Wild species showed more distance from other species as seen in PCA for 103 germplasm accessions. The syntenic LD r^2 = 0.09 in average *vs*. genetic distance is at 19 cM for 103 germplasm accessions.

Marker and trait associations for thirteen quantitative traits depicted that some markers detected as most powerful due to high in R^2 like M35 for SCMR (stress condition) and M115 for FPP (both conditions) and CPP (stress condition) in 'lyco'species;M34 and M92 for LR (stress condition), M24 for PH, FYPP (both conditions) and LR (stress condition), M17 for AFW (stress condition) in 'cherry' species;M34 for PH, BN, STG and FYPP (both condition) in 'wild' species;M42 for DFF, M98 for DFFS, M130 for STG, M112 for CPP, M84 for FPP, M32 and M42 for fruit yield (especially under stress condition) in'All' spp.However, some markers were found to be associated with more than one trait indicating their pleotropic effects in nature.

Experiment 3: Phenotyping of F_2 and F_3 population of inter-specific cross for traits related to WUE and fruit yield

Since SCMR is significantly positively associated with fruit yield and SLA is significantly negatively associated with fruit yield and SCMR therefore SCMR and SLA could be used as surrogative traits to select high fruit yield coupled with drought tolerant segregates

Experiment 4: Parental polymorphism and Genotyping of F_2 mapping population with SSR markers linked to the traits related to WUE and fruit yield

For the genotyping work till now, 150 SSR primes were tested for parental polymorphism out of which only 66 primers were found polymorphic. Within these 66 primers only 35SSR primers have shown polymorphism for all the 112 F_2 genotypes *viz*., they can be scored for male, female and heterozygotes and the samples have shown segregation according to Mendel's Monohybrid ratio of 1:2:1 as expected. The work is still in progress to find more polymorphic primers for genotyping to prepare the QTL map for tomato.

4.5 Task 4.3: Deep sequencing of mRNA and smRNA transcriptome of sorghum and pearl millet for identification of genes and smRNAs functioning in abiotic stress tolerance, with a focus on drought and salinity (Lead Institute: MSSRF; Task Leader: Suja George)

Introduction

Drought and salinity are the most important environmental constraints to plant survival and productivity. Research into plant responses to abiotic stresses including drought and salinity stress is becoming increasingly important under these circumstances. Despite the wealth of information on abiotic stress and stress tolerance in plants, understanding of the basic biochemical and molecular mechanisms for stress perception, transduction and tolerance is still a major challenge in biology. A better understanding of the effects of drought and salinity on plants is vital for improved management practices and breeding and transgenic efforts in agriculture and for predicting the fate of natural vegetation under climate change.

Sorghum (Sorghum bicolor L. Moench., 2n=2x=20) is a leading cereal in arid and semi-arid agriculture, ranking fifth in importance among the world's grain crops (Doggett, 1988). The crop is the dietary staple of more than 500 million people in more than 30 countries. It is grown on 42 m ha in 98 countries of Africa, Asia, Oceania and the Americas. Nigeria, India, USA, Mexico, Sudan, China and Argentina are the major producers. The Grain is mostly used for food purpose (55 %), stover is an important source of dry season maintenance rations for livestock, especially in Asia; also an important feed grain (33%), especially in the Americas.

Pearl millet [*Pennisetum glaucum* (L.) R. Br., 2n=2x=14] is an annually grown cereal on more than 29 m ha in the arid and semi-arid tropical regions of Asia, Africa and Latin America. India is the largest producer of pearl millet, both in terms of area (9.3 m ha) and production (8.3 m ton). Pearl millet is the staple food and fodder crop of millions of poor people living on the most marginal agricultural lands of sub-Saharan Africa and the Indian subcontinent. Indeed, in some of the hottest and driest regions where agriculture is possible in India and Africa, pearl millet is the only cereal that can be grown under dryland conditions and so plays a critical role in food security.

The present task focuses on Deep sequencing of mRNA and smRNA transcriptome of sorghum and pearl millet for identification of genes and smRNAs functioning in abiotic stress tolerance, with a focus on drought and salinity.

Brief description of last two year's work

In the last two years, eight genotypes of *Sorghum bicolor* were selected based on data from an irrigated field, varying in leaf temperature and grain yield potential (Mutava et al, 2011). These genotypes belonged to a collection of 300 photoperiod insensitive Sorghum genotypes. Two genotypes were selected from four categories such as 'high leaf temperature and high yield (HT_HY)', 'high leaf temperature and low yield (HT_LY)', 'low leaf temperature and high yield (LT_HY)', 'low leaf temperature and low yield (LT_LY)'.

For Pearl millet, genotypes PI586660 (drought tolerant cultivar developed for Burkina, Faso), PI591068 (drought tolerant cultivar developed for India), and PI564586 (*Pennisetum violaceum* a wild relative of *P.typhoides*) were selected.

10 day old uniform healthy seedlings were transferred to fresh Hoagland's solution with; a) milliQ water (control), b) 15% PEG-8000 (drought stress), c) 150 mM NaCl (salt stress) and leaf and root tissues of each genotype were frozen at 0h (control) and 36h of stress. To reduce plant-to-plant variability, tissue samples from six randomly selected seedlings were pooled before library construction.

Six RNAseq libraries were constructed per accession from contrl leaf tissue, drought stressed leaf tissue, salt stressed leaf tissue, control root tissue, drought stressed root tissue, and salt stressed root tissue. Three small RNA libraries were made for each species from control, drought stressed and salt stressed tissues. The RNAseq libraries were pooled together and sequenced on Illumina HiSeq 2500 platform to generate 30 million paired end reads per sample. Small RNA libraries were pooled together and ran on a single lane of SE 50. The entire sequence data generated were deposited in the National Instituted of Health (NIH) Short Read Archive database.The group completed analysis of *Sorghum bicolor* RNAseq libraries in the second year, and in the thrid year, concentrated on analysing *Pennisetum typhoides* RNAseq libraries.

Methodology

Pennisetum RNAseq libraries were further analyzed in the third year

Read trimming and mapping

The study selected 2 *Pennisetum typhoides* accessions (PI 586660, drought tolerant cultivar developed for Burkina, Faso and PI 591068, drought tolerant cultivar developed for India) and 1 *Pennisetum violaceum* accession (PI564586, wild relative of *P.typhoides*). The seedlings were grown hydroponically and leaf and root tissues were collected from control (Hoagland medium), 24 h drought (15% PEG-8000 in Hoagland medium) and 24 h salt (150mM NaCl in Hoagland medium) samples. Six samples were collected per accession, after each of these treatments: control leaf, control root, drought stressed leaf, drought stressed root, salt stressed leaf and salt stressed root.

18 bar-coded RNASeq libraries were produced, one from each treatment and accession. All 18 libraries were made using the protocol and adapter/primer combination from Kumar et al 2012 (A high throughput method for Illumina RNASeq library preparation). Library quality and integrity was confirmed with an Agilent Bioanalyzer 2100 (Agilent technologies, Palo Alto, CA) and the concentration of each individual library was calculated using qPCR .The 18 barcoded libraries were pooled in equimolar concentrations and sequenced. Using CASAVA package of Illumina HiSeq 2500 platform, 100bp paired-end raw nucleotide sequences in fastq format was generated.

Each de-multiplexed library was processed to eliminate the contamination of the adapter sequence using Scythe (https://github.com/ucdavis-bioinformatics/scythe), followed by removing low quality nucleotide bases (phred score < 25) from 3' location using sickle (https://github.com/ucdavis-bioinformatics/sickle). The reads that are less than 25nt in length after adapter trimming and base quality trimming were removed from analysis.

De novo transcriptome assembly of high quality reads of was performed using Trinity (http://trinityrnaseq.sourceforge.net/; Haas BJ, et al., 2013, *Nat. Protocols*, 8:1494) (Table 1). The raw assembled transcriptome of *P.violaceum* contains 466327 and *P.typhoides* contains 524001 predicted transcripts. BLAST search was carried for these novel transcripts, functional annotation was done using Blast2GO (http://www.blast2go.com; A. Conesa, et al., 2005, *Bioinformatics*, 21:3674; S. Götz et al. 2008, *Nucleic Acids Research*, 36:3420).

Differential expression gene profiling

Reads from all samples were aligned to the filtered transcripts using BWA's short read aligner (http://biobwa.sourceforge.net/; Li H and Durbin R 2009 Bioinformatics, 25:1754) and SAMtools (http://samtools.sourceforge.net/; Li H et al. 2009 Bioinformatics 25:2078). The raw counts were generated using HT-Seq Count and processed statistically to generate a table of normalized counts, and cluster plots to determine separation of samples.

Statistical analysis, including count normalization and differential gene expression was performed with edgeR (http://www.bioconductor.org/packages/release/bioc/html/edgeR.html; Robinson MD et al. 2010 Bioinformatics 26:139), using accession grouped leaf temperature and yield as two factors for ANOVA model (Plant Condition [CT/DT/ST] vs Tissue Type [L/R]). Differentially expressed genes using FDR (false discovery rate) <0.1 were collected into individual files for each comparison.

Gene ontology and enrichment analysis

GO enrichment analysis was carried out using GOstats R package for all differential expressed genes under drought/salt effect in leaf/root. Genes without GO terms were removed from the analysis. GO terms of all Differentially Expressed Genes (DEGs) were functionally classified into three major GO categories; molecular function (MF), biological process (BP) and cellular component (CC). Venny tool was used to generate venn diagrams showing common and differentially expressed genes between different stress/tissue/temperature and yield conditions.

Results:

Table 4-29 Assembly statistics table

Assembly	Statistics based on All transcript contigs *P.violaceum*	Statistics based on only longest isoform per component *P.violaceum*	Statistics based on All transcript contigs *P.typhoides*	Statistics based on only longest isoform per component *P.typhoides*
Contig N10	2166	1640	2080	1471
Contig N20	1548	1219	1362	1101
Contig N30	1221	965	1049	882
Contig N40	989	778	844	721
Contig N50	812	629	691	593
Median contig length	461	373	419	372
Average contig	637.69	517.79	575.36	499.42
Total assembled bases	297372148	85204556	301490049	80982155

The following Differential Gene Expression profiles were generated for Pennisetum

1. Drought upregulated genes in leaf tissue
2. Drought downregulated genes in leaf tissue
3. Drought upregulated genes in root tissue
4. Drought downregulated genes in root tissue
5. Salt upregulated genes in leaf tissue
6. Salt downregulated genes in leaf tissue
7. Salt upregulated genes in root tissue
8. Salt downregulated genes in root tissue

GO Annotations were carried out using GOstat
GO Enrichment were carried out using BINGO
KEGG Pathway Analysis carried out using KOBAS
VENNY tool was used to generate Venn diagrams to identify the following

1. Drought specific DEGs in leaf
2. Drought specific DEGs in root
3. Salt specific DEGs in leaf
4. Salt specific DEGs in root
5. DEGs in common to drought and salt in leaf
6. DEGs in common to drought and salt in root
7. DEGs common to leaf and root under drought
8. DEGs common to leaf and root under salt

Transcription factors and transporter genes among the DEGs were identified
Unique DEGs in each category was identified

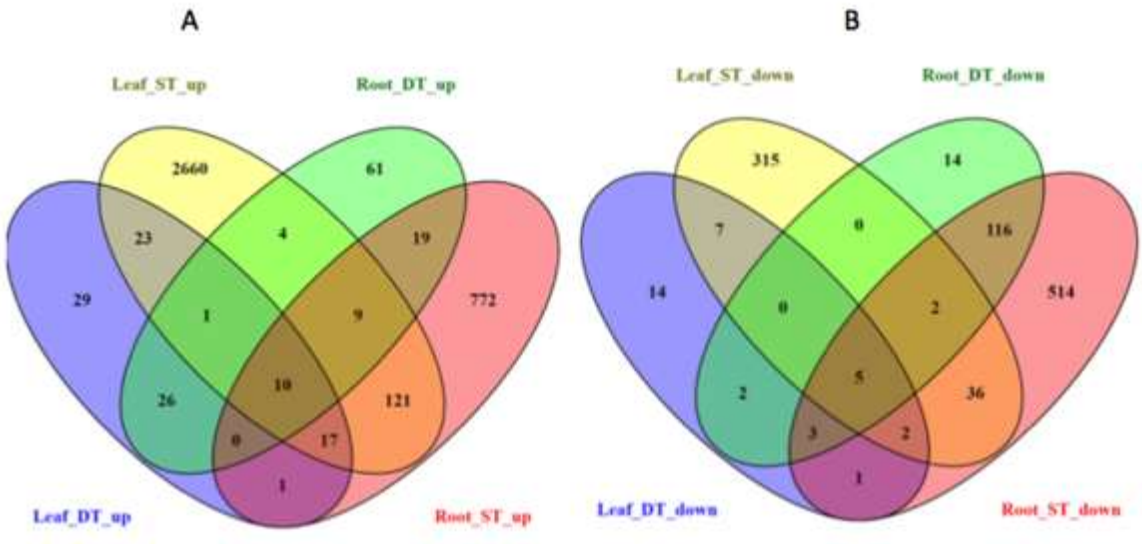


***Figure 4-23** Differential gene expression under salt and drought stress in Pennisetum typhoides: Venn diagram showing the number of common and differently expressed genes under salt and drought stress in leaf and root tissues. A) Upregulated genes,B) Downregulated genes ST – salt stress, DT – drought stress.*

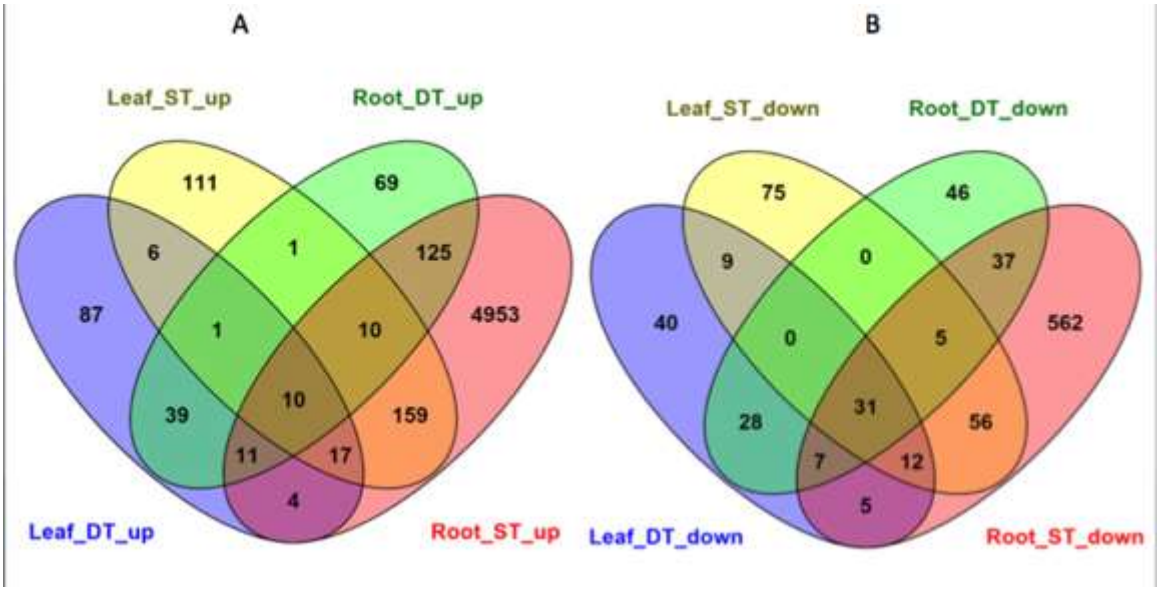


***Figure 4-24** Differential gene expression under salt and drought stress in Pennisetum violaceum: Venn diagram showing the number of common and differently expressed genes under salt and drought stress in leaf and root tissues. A) Upregulated genes,B) Downregulated genes ST – salt stress, DT – drought stress.*

Capacity building

1 research Fellows is carrying out her doctoral work under this task.

Task 4.4: Improving drought adaptation in chickpea through marker-assisted breeding and trait based selection (Lead Institute: ICRISAT, Lead Scientist: Pooran Gaur)

Activity 1: Improving drought adaptation in chickpea through marker-assisted breeding

Two popular cultivars (JAKI 9218 and JG 16) of chickpea were selected to introgress a genomic region (called "QTL-hotspot") which affects many drought tolernace related traits, including root traits, for enhancing adptaion to terminal drought stress condition. During October 2014, 444 BC3F2 seeds of JAKI 9218 x ICC 4958 cross were sown and 42 single plants selected carrying homozygous alleles for foreground markers. These plants were harvested during February 2015. Similarly in the second cross (JG 16 x ICC 4958) 629 seeds of BC3F2 were planted in the field in Nov 2014 and selected 68 single plants carrying all homozygous alleles for the foreground markers. The selected plants in both the crosses were advanced as progenies during off-season at Zonal Agriculture and Horticulture Research Station (ZAHRS), Hiriyur, Karnataka from April to July 2015. As per the work plan during 2015 post rainy season BC3F4 progenies of both crosses were evaluated in yield trials.

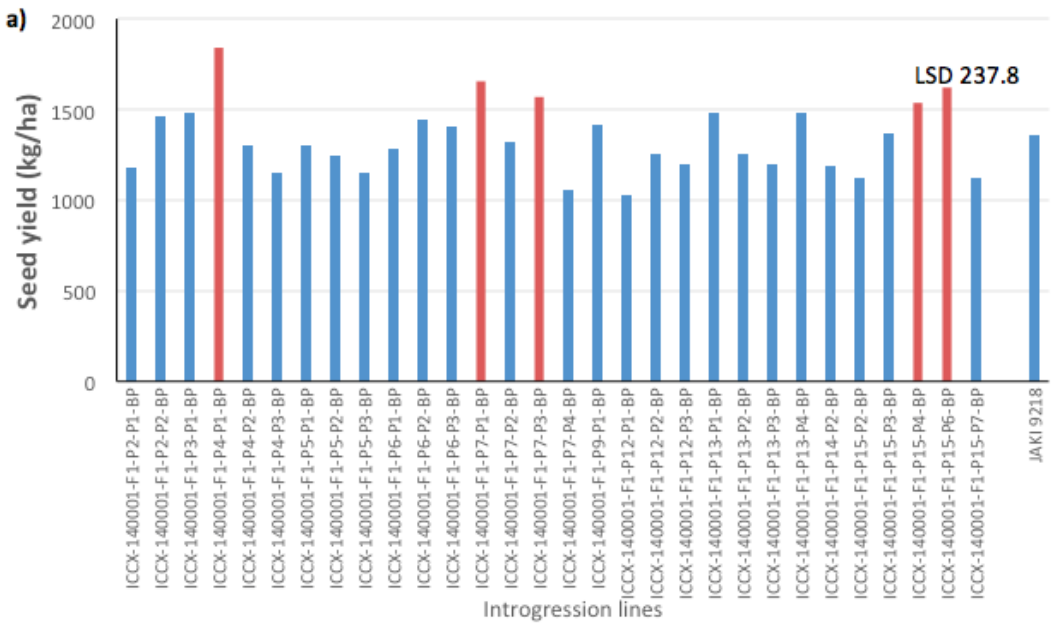
Experiment details:

No of genotypes: 30 BC3F4 lines of JAKI 9218 x ICC 4958
26 BC3F4 lines of JG 16 x ICC 4958
+

	JAKI 9218, JG 16, ICC 4958 & JG 11
Design:	Alpha lattice
Replications:	3; Blocks: 3 per rep
Spacing:	60 x 10 cm
Growing conditions:	Rainfed (no supplementary irrigation) Irrigated (one irrigation during flower initiation)
Weeding:	30 d after sowing
Chemical spray:	pesticide sprayed two times (35 d & 60 d after sowing) for protecting the experiment against helicoverpa damage

Results:

Flowering time of introgression lines (ILs) developed in the background of JAKI 9218 and JG 16 was in the range of 48-56 d under both rainfed and irrigated conditions. However, the maturity time was prolonged by one week under irrigated conditions (107 d) compared to rainfed conditions (100 d). There was no significant change of average plant height observed in ILs compared to recurrent parents (RP). Seed yield of ILs developed in JAKI 9218 was in the range of 1028-1840 kg/ha under rainfed conditions. Five (13-35%) and eleven (25-43%) ILs showed significantly higher seed yield than respective RPs JAKI 9218 and JG 16 under rainfed conditions (Figure 4-25a & 4-26a). Under irrigated conditions 18 (17-48%) and 5 (16-44%) ILs showed significantly higher seed yield than respective RPs JAKI 9218 and JG 16 (Figure 4-25b & Figure 4-26b). Interestingly the 100-seed weight of the ILs in JAKI 9218 and JG 16 increased by 8-29% (Figure 4-27a) and 50-94% (Figure 4-28a) under rainfed conditions, respectively. Similar trend was observed in irrigated conditions also. As this genomic region also also influenced seed size, most of the ILs showed 100-seed weight similar to seed weight of donor parent ICC 4958. No relationship was observed between yield under rainfed and irrigated conditions (Figure 4-29a), hence the lines show higher yield in rainfed condition could not produce higher yields under irrigated conditions. Contrary, 100-seed weight has shown a very high relationship between rainfed and irrigated conditions (Figure 4-29b)



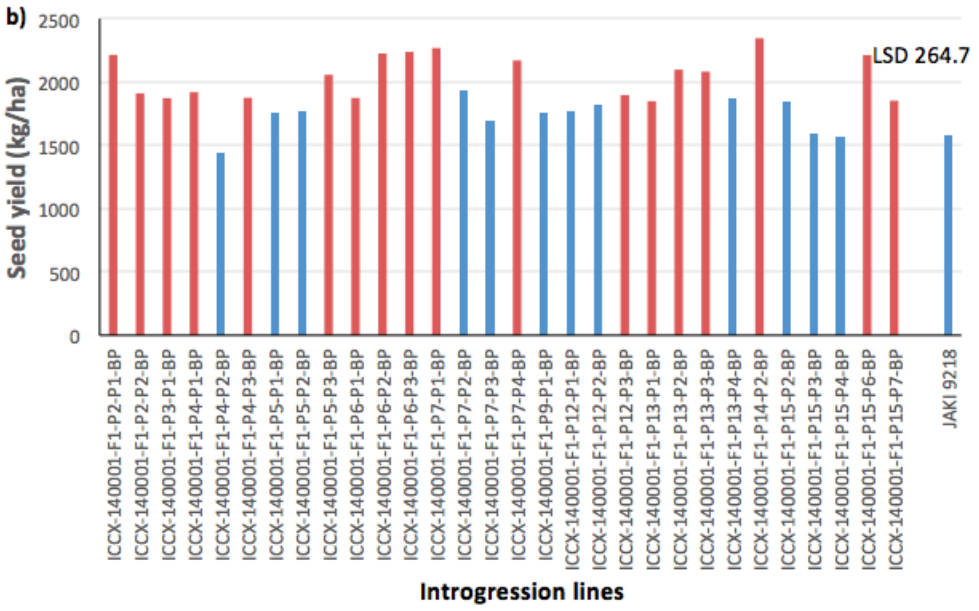
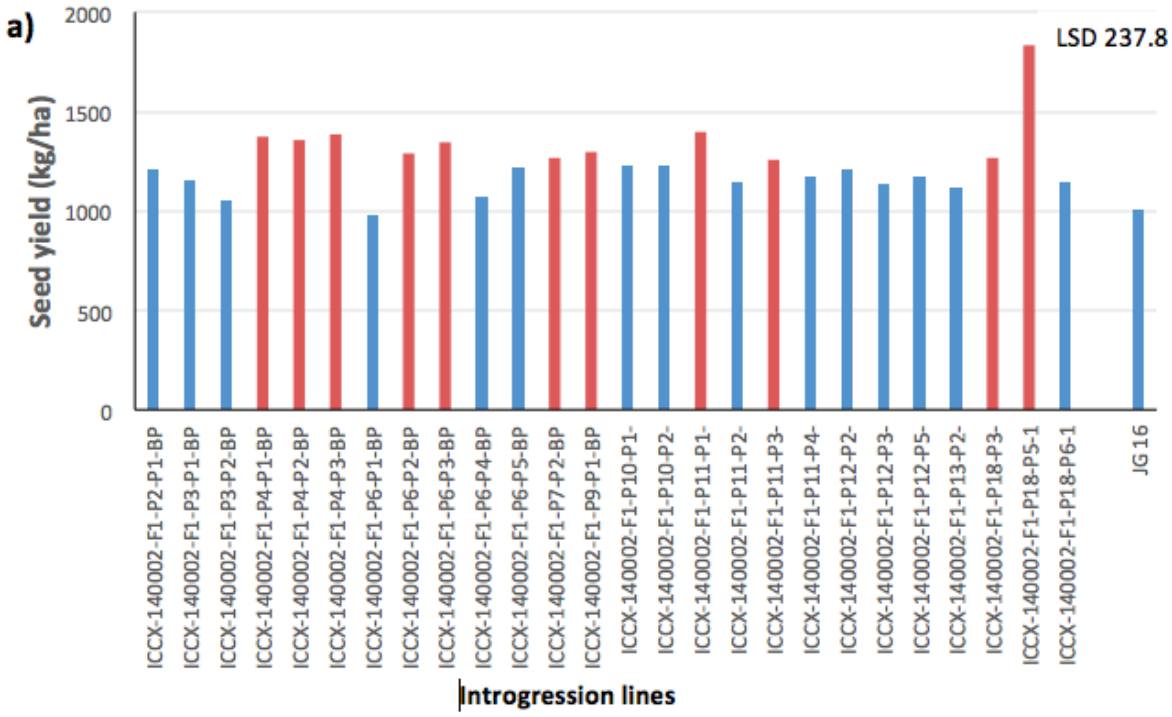


Figure 4-25 Yield performance of introgression lines developed in the background of JAKI 9218 under a) rainfed and b) irrigated conditions (*Highlighted in red are significantly higher yield than recurrent parent JAKI 9218).



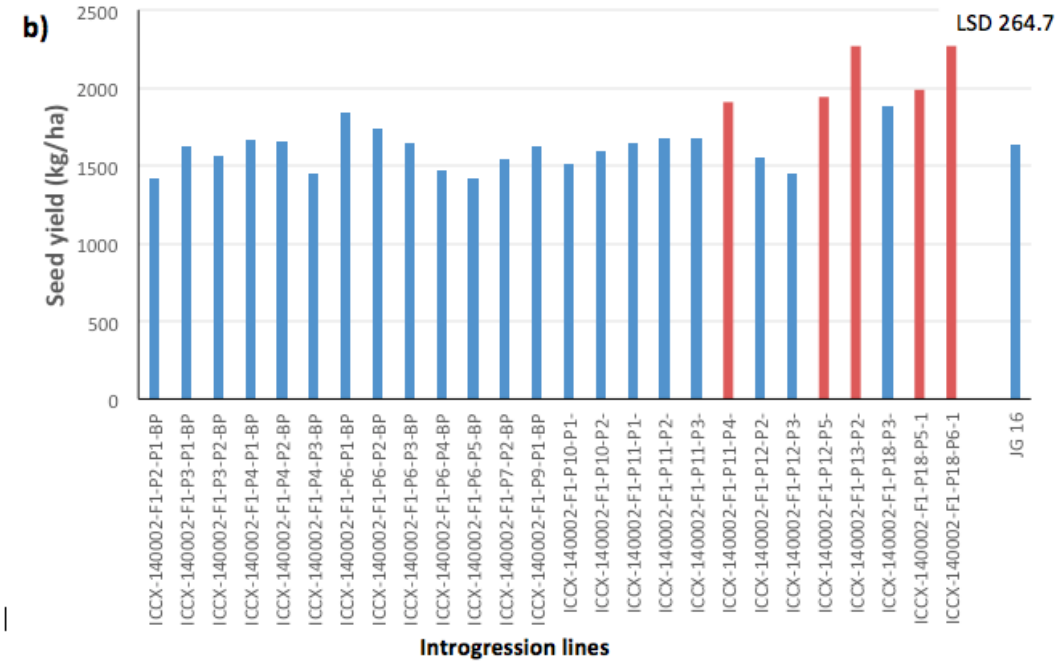
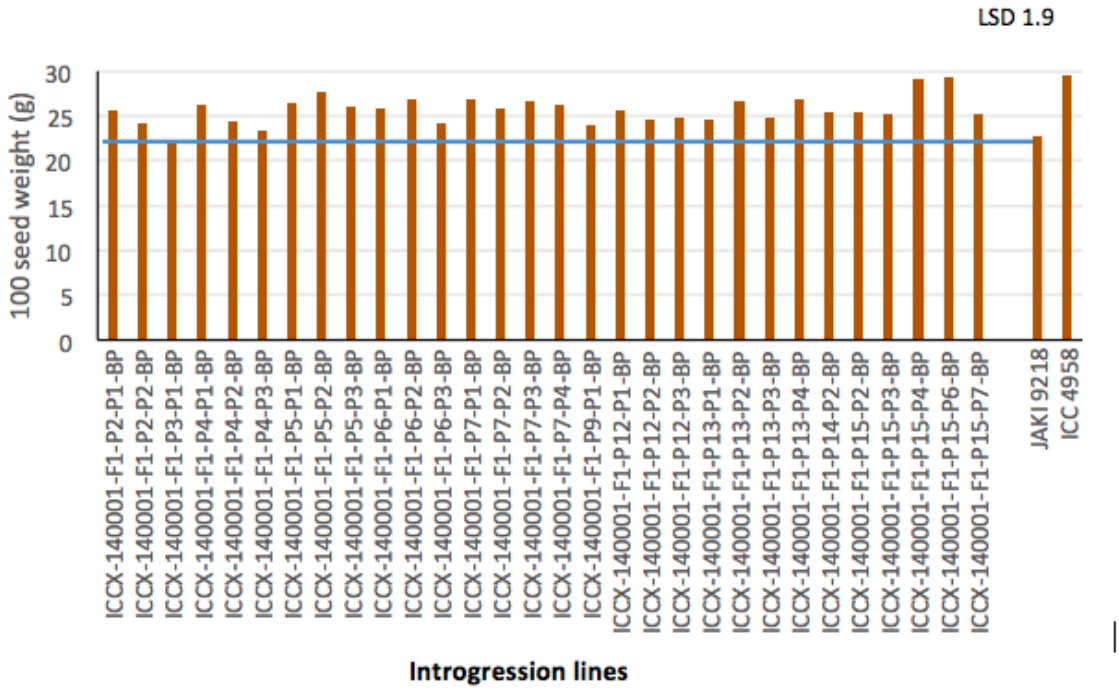


Figure 4-26 Performance of introgression lines developed in the background of JG 16 under a) Rainfed and b) irrigated conditions (*Highlighted in red are significantly higher yield than recurrent parent JG 16).



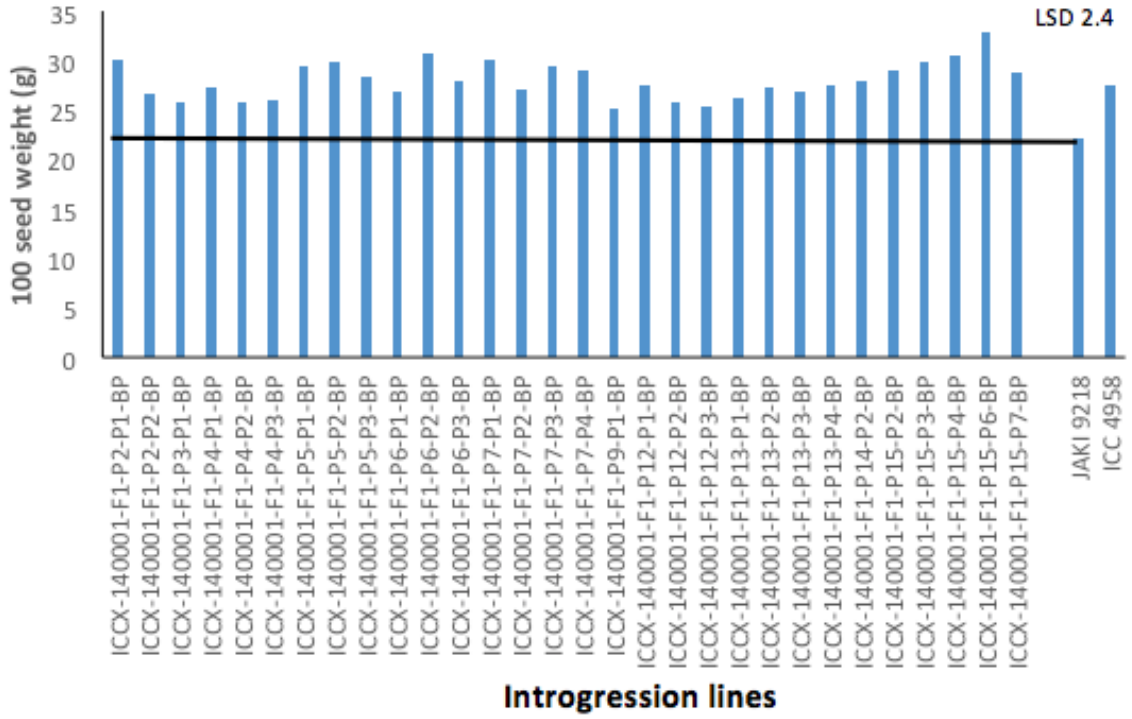
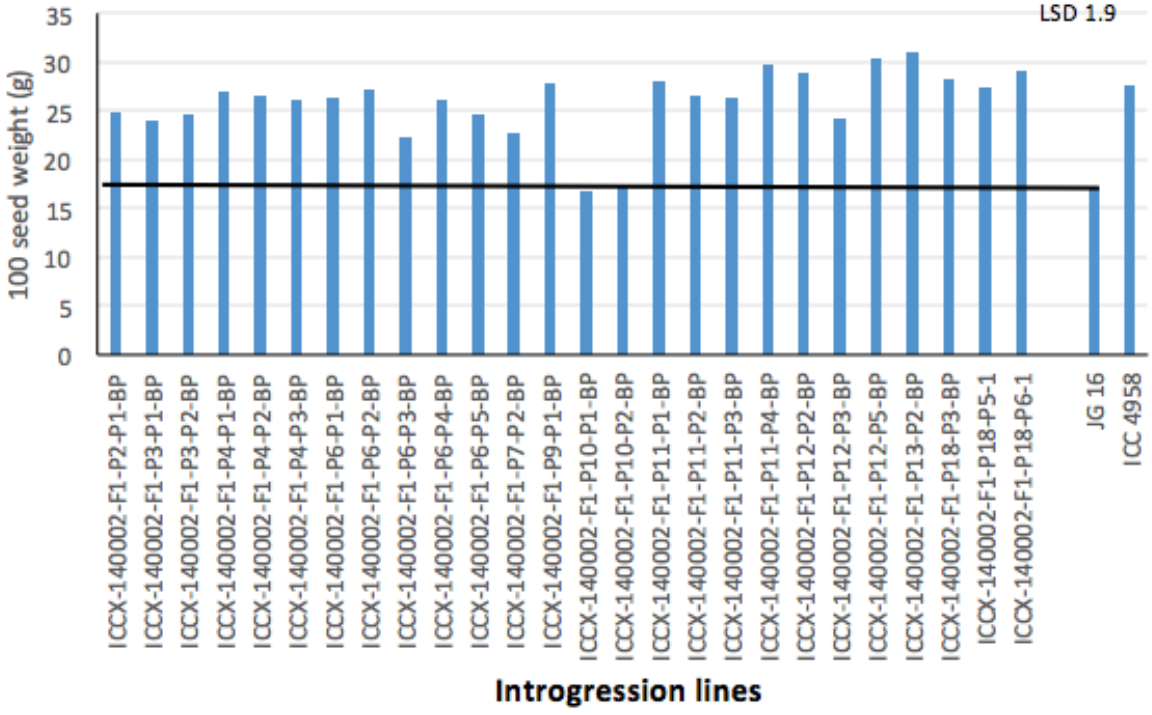


Figure *4-27 100-seed weight of introgression lines developed in the background of JAKI 9218 under a) Rainfed and b) irrigated conditions (most of the lines showed significantly higher 100 seed weight than recurrent parent JAKI 9218).*



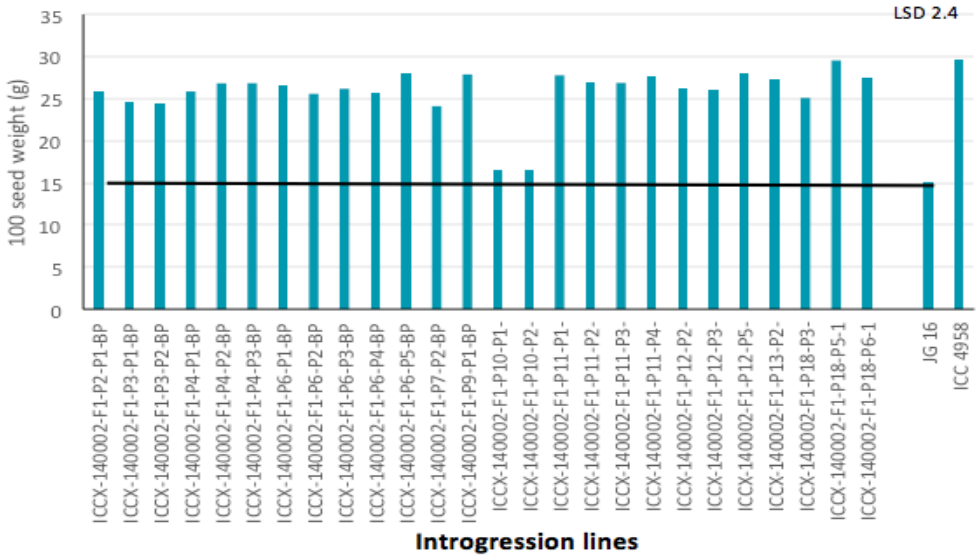


Figure 4-28 100-seed weight of introgression lines developed in the background of JG 16 under a) Rainfed and b) irrigated conditions (most of the lines showed significantly higher 100 seed weight than recurrent parent JG 16).

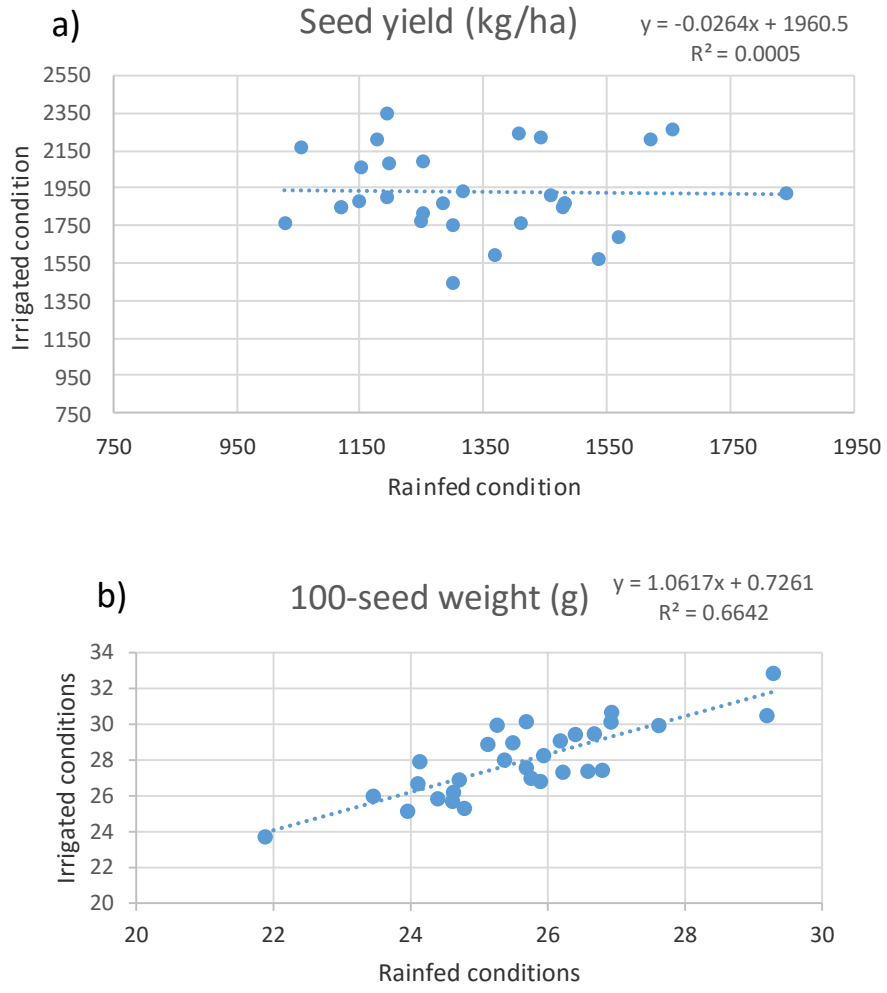


Figure 4-29 Relationship of seed yield and 100-seed weight traits between rainfed and irrigated conditions in JAKI 9218 introgression lines

(A) Improving drought adaptation in chickpea through trait based selection

Experiment 2: Year 2 evaluation of 1136 chickpea MAGIC lines under rainfed and irrigated conditions.

Background: Eight diverse chickpea genotypes were intercrossed in all possible combinations, excluding reciprocals, and generated 28 two-way, 14 four-way and 7 eight-way crosses. All F1s from 7 eight-way crosses were advanced to F2s. A total of 1200 F2 plants were advanced to F9 using single seed descent (SSD) method. A total of 1136 homozygous MAGIC (multi-parent advance generation inter-cross) lines were developed. During 2013/14, experiment was conducted in augmented design in vertisols with 1136 MAGIC lines where 8 parental lines replicated 8 times used as checks under rainfed and irrigated conditions.

Current progress: Above experiment was repeated under rainfed, irrigated and summer conditions during October 2014 to April 2015. Three experiments were conducted in augmented design. Eight parental lines used in development of MAGIC lines were used as checks. MAGIC lines were assigned randomly to each continuous plot and each check was repeated 8 times randomly in the experiment and one time in each block. Both rainfed and irrigated experiments were conducted in a single precession field block of 6 ha area. Both treatments were separated by a buffer of 20 m width. Each line was planted in 4 m row plot with 10 cm intra row and 60 cm inter row distance. Sowings of rainfed and irrigated experiments were initiated after cessation of rains on 14 October during 2014 with tractor mounted machine planter at a soil depth of 4-6 cm. planting of heat screening during summer season was done on 21 February during 2015. All lines germinated under residual soil moisture in both rainfed and irrigated experiments. Before the sowing seeds were treated with thiram and capton fungicide mixture to protect the seedlings from soil borne and seed borne diseases. 20 days after sowing (DAS) a weeding operation was done tractor drawn cultivator and manually. All the crop management practices were applied similarly in all the experiments except no supplementary irrigation to rainfed experiment. Two irrigations were given during flower initiation (45 DAS) and pod filling stage (65 DAS) for irrigated treatment. Four irrigations were provided to summer trial at 15 days interval. Plots were monitored regularly for recording various phenotypic traits. Days to 50% flowering was recorded as the date of 50% of plants starting flowering in a plot. Maturity time was recorded when more than 50% of the plants in a plot turns golden yellow and 90% leaves are dried-up. Plant height was measured from the base of stem to tip of the plants at maturity in 5 randomly selected plants in each plot. At the harvesting time, yield traits were recorded on continuous 2 m row in each plot. 100-seed weight was recorded from randomly selected seed from the bulk harvest in each plot. Whether parameters like rainfall, humidity, sunshine hours and max and min temperatures recorded during the crop growth period were presented in Table 4-31.

Table 4-30 List of different weather parameters recorded in crop season during 2014 and 2015

Date	Rain (mm)	Max Temp (°C)	Min Temp (°C)	Rel Humidity1 at 07:17 (%)	Rel Humidity2 at 14:17 (%)	Bright Sunshine (Hrs)
10-31 Oct 2014	46.8	30.8	19.1	90.3	47.9	6.7
01-30 Nov 2014	55.8	30.3	15.3	91.3	41.7	7.2
01-31 Dec 2014	0.0	28.6	12.1	89.0	40.3	7.6
01-31 Jan 2015	4.6	28.4	12.4	87.3	37.8	8.3
10-28 Feb 2015	0	32.9	14.9	77.7	28.4	9.7
01-31 Mar 2015	72.2	33.5	18.9	81.9	37.7	7.6
01-30 Apr 2015	96.8	35.3	22.1	79.5	37.8	8.7

Results: Under rainfed-2014/15, rainfed-2013/14, irrigated-2014/15, irrigated-2013/14 and Summer-2015 seasons 46, 62, 83, 50 and 61 lines showed significantly higher seed yield than the best parent, respectively. Similarly, 23 and 19 common lines were identified under rainfed and irrigated conditions over two years and no common line was identified between rainfed/irrigated and heat stress conditions (Table 4-32). The extent of yield variation observed in MAGIC lines under rainfed and irrigated conditions for two seasons are presented in Figure 4-30.

Flowering: The parents recorded little variation in flowering under both rainfed and irrigated conditions. The flowering in parents ranged from 40 days (JG 11) to 52 days (ICCV 97105) while, in MAGIC lines flowering ranged from 33-68 days.

Rainfed condition: Among the top 23 common lines, ICCML10733 had the highest seed yield (3,222 kg/ha) followed by ICCML10833 (2,989 kg/ha) and ICCML10094 (2,955 kg/ha) during 2013/14 while, ICCML10094 (3,368 kg/ha) followed by ICCML10977 (3,354 kg/ha) and ICCML10852 (2,973 kg/ha) were the top three lines in terms of seed yield during 2014/15.

Irrigated condition: Under irrigation, 19 lines were found common during the two years of study. Among these lines, ICCML10758, ICCML10209 and ICCML10740 were the top three with 4,389, 3,974 and 3,932 kg/ha seed yield, respectively, during 2013/14 whereas, ICCML10666 (5,033 kg/ha), ICCML10635 (4,479 kg/ha) and ICCML10283 (4,470 kg/ha) recorded highest seed yield in 2014/15.

Association between seed Yield and plant height:

Rainfed: One entry ICCML10383 recorded significantly higher seed yield (3,311 kg/ha) compared to the best parent/check ICCV 10 (2,041 kg/ha). Among others, five lines ICCMLs 10052, 10437, 10655, 10960 and 11014 had seed yield on par with ICCV 10 during 2013/14. During 2014/15, one line (ICCML10564) had significant seed yield of 2,817 kg/ha compared to the best check JAKI 9218 (2,023 kg/ha). Five more lines ICCMLs 10229, 10893, 10960, 10978 and 11076 recorded seed yield on par with best

parent/check (JAKI 9218). Interestingly, these selected lines from 2013/14 and 2014/15 had a plant height of 50 cm and above which makes them suitable for mechanical harvesting. The line ICCML10960 was common in both years recording on par seed yield with the best parent/check, ICCV 10 in 2013/14 and JAKI 9218 in 2014/15 besides having a plant height of 52 and 55 cm, in respective years. Plant height of ICCV 10 and JAKI 9218 was 37 cm.

Irrigated: During 2013/14, 19 lines (ICCMLs 11077, 10993, 10787, 10740, 10293, 11160, 10320, 11064, 10833, 10303, 11013, 10152 10288, 10884, 10822, 10370, 11169, 10984 and 10804) recorded significantly higher yield compared to the best parent/check JG 16 in addition to a plant height of 50 cm and above. Among these 18 lines, ICCML11077 had the highest seed yield (4,332 kg/ha) followed by ICCML10993 (4,065 kg/ha) and ICCML10787 (3,953 kg/ha).

Fifteen lines (ICCMLs 10027, 10113, 11073, 10919, 10101, 11004, 11013, 11160, 10771, 10814, 10792, 11064, 10977, 10152 and 11169) exhibited significantly higher yield compared to the best parent/check ICCV 10 (3,097 kg/ha) highest being by ICCML10027 (4,747 kg/ha) followed by ICCML10113 (4,616 kg/ha) and ICCML11073 (4,505 kg/ha). All these lines had a plant height of 50 cm and above while ICCV 10 was 40 cm tall.
Six lines (ICCMLs 10740, 11160, 11064, 11013, 11169 and 10152) were common across two years that recorded significantly higher seed yield compared to the best parent/check in both years besides a plant height of 50 cm and above.

The existing popular varieties used in the development of MAGIC lines had a plant height in the range of 41-47 cm and difficult to harvest by machine. Among the selected MAGIC lines, some lines were more than 60 cm tall which can be easily harvested by combine harvesters. The lines ICCMLs 10993, 10740, 10320, 10804, 10464 and 10359 during 2013/14; and 10027 and 10977 during 2014/15 recorded significantly higher seed yield coupled with a plant height of 60 cm and above. These potential lines can be promoted to multi-location yield trails to develop varieties suitable for mechanical harvesting. These MAGIC lines provide a useful germplasm source with diverse allelic combinations to global chickpea community.

Table 4-31 List of MAGIC lines showing significantly higher seed yield than any parent evaluated under rainfed and irrigated conditions during 2014/15 and 2013/14

			Seed yield (kg/ha)				
		Rainfed				Irrigated	
SN	MAGIC line	2014/15	2013/14		MAGIC line	2014/15	2013/14
1	ICCML10012	2479	2610		ICCML10041	4221	3595
2	ICCML10015	2402	2789		ICCML10152	3942	3716
3	ICCML10094	3368	2955		ICCML10209	4095	3974
4	ICCML10097	2865	2711		ICCML10283	4470	3734
5	ICCML10125	2691	2900		ICCML10288	4227	3706
6	ICCML10191	2699	2566		ICCML10459	3963	3799
7	ICCML10212	2682	2822		ICCML10504	3986	3782
8	ICCML10215	2496	2900		ICCML10635	4479	3764
9	ICCML10239	2416	2844		ICCML10666	5033	3683
10	ICCML10279	2412	2533		ICCML10740	4054	3932
11	ICCML10287	2709	2722		ICCML10758	4462	4389
12	ICCML10320	2592	2622		ICCML10833	4259	3773
13	ICCML10342	2645	2711		ICCML10962	4108	3814
14	ICCML10414	2471	2533		ICCML10989	4170	3796
15	ICCML10512	2763	2589		ICCML11013	4307	3734
16	ICCML10564	2817	2744		ICCML11064	3953	3824
17	ICCML10733	2626	3222		ICCML11096	4201	3807
18	ICCML10771	2521	2933		ICCML11160	4272	3867
19	ICCML10823	2612	2633		ICCML11169	3941	3677
20	ICCML10833	2849	2989				
21	ICCML10852	2973	2600				
22	ICCML10977	3354	2944				
23	ICCML11116	2441	2900				
24	ICCML11160	2635	2911				
	Parents						
	ICC 4958	1818	1456			3219	3067
	ICCV 10	1392	2041			3190	3098
	JAKI 9218	2023	1882			3125	3142
	JG 11	1396	1950			3171	2557
	JG 130	1734	1390			3097	3005
	JG 16	1771	1586			3463	2874
	ICCV 97105	1949	1733			2860	2603
	ICCV 00108	1546	1746			2626	3117
	GM	1401	1622			3073	2613
	LSD 5%	378	460			469	515
	CV %	9.8	10.3			11.4	16.0

note: underlined lines are common across four growing conditions

Table 4-32 Performance of parents for different traits and the range of variation observed in the population evaluated under rainfed and irrigated conditions during 2014/15 and 2013/14

Rainfed										
	Days to 50% flowering		Plant height (cm)		100-Seed weight (g)		Seed yield (kg/ha)		Harvest Index (%)	
	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14	2014/15	2013/14
Parents										
ICC 4958	46.5	42.6	33.0	40.4	29.6	29.7	1818	1456	0.54	0.54
ICCV 10	48.0	49.6	34.5	37.0	17.3	17.9	1392	2041	0.47	0.53
JAKI 9218	45.8	49.8	36.5	40.5	23.6	25.0	2023	1882	0.55	0.62
JG 11	43.8	40.3	34.6	36.8	22.3	22.1	1396	1950	0.53	0.67
JG 130	46.3	46.9	34.9	37.1	24.3	24.4	1734	1390	0.55	0.55
JG 16	46.5	48.5	34.9	36.1	17.8	17.5	1771	1586	0.57	0.52
ICCV 97105	48.6	50.9	40.6	40.0	26.9	25.1	1949	1733	0.54	0.52
ICCV 00108	46.5	43.9	37.3	39.3	25.8	25.3	1546	1746	0.53	0.55
MAGIC lines										
Avg	50.7	47.7	35.6	38.3	22.3	22.7	1401	1622	0.49	0.56
Range	37-56.8	33.5-63.8	23.3-58.7	24.1-53.4	10.2-43.7	10.6-41.8	179-3368	255-4400	0.20-0.83	0.11-0.88
Irrigated										
Parents										
ICC 4958	44.9	41.8	40.9	45.7	31.6	35.2	3219	3067	0.53	0.56
ICCV 10	49.6	48.9	44.8	45.7	17.0	16.8	3190	3098	0.55	0.56
JAKI 9218	47.4	46.9	44.9	45.5	24.7	25.5	3125	3042	0.51	0.58
JG 11	41.8	39.9	44.4	44.6	24.1	23.9	3171	2557	0.61	0.56
JG 130	50.8	47.8	43.5	46.1	23.3	25.6	3097	3005	0.52	0.57
JG 16	49.6	47.5	41.5	46.7	18.1	17.4	3463	2874	0.51	0.56
ICCV 97105	52.1	51.6	42.3	46.4	24.4	24.1	2860	2603	0.50	0.52
ICCV 00108	49.6	43.8	45.0	46.7	24.7	25.6	2626	3117	0.52	0.60
MAGIC lines										
Avg	50.5	48.3	42.6	46.0	23.8	23.4	3073	2613	0.54	0.54
Range	42.9-63.9	34.2-68.4	23.3-60.8	31.5-64.9	10.7-44.9	11.2-39.3	1236-5156	686-4554	0.30-0.8	0.32-0.75

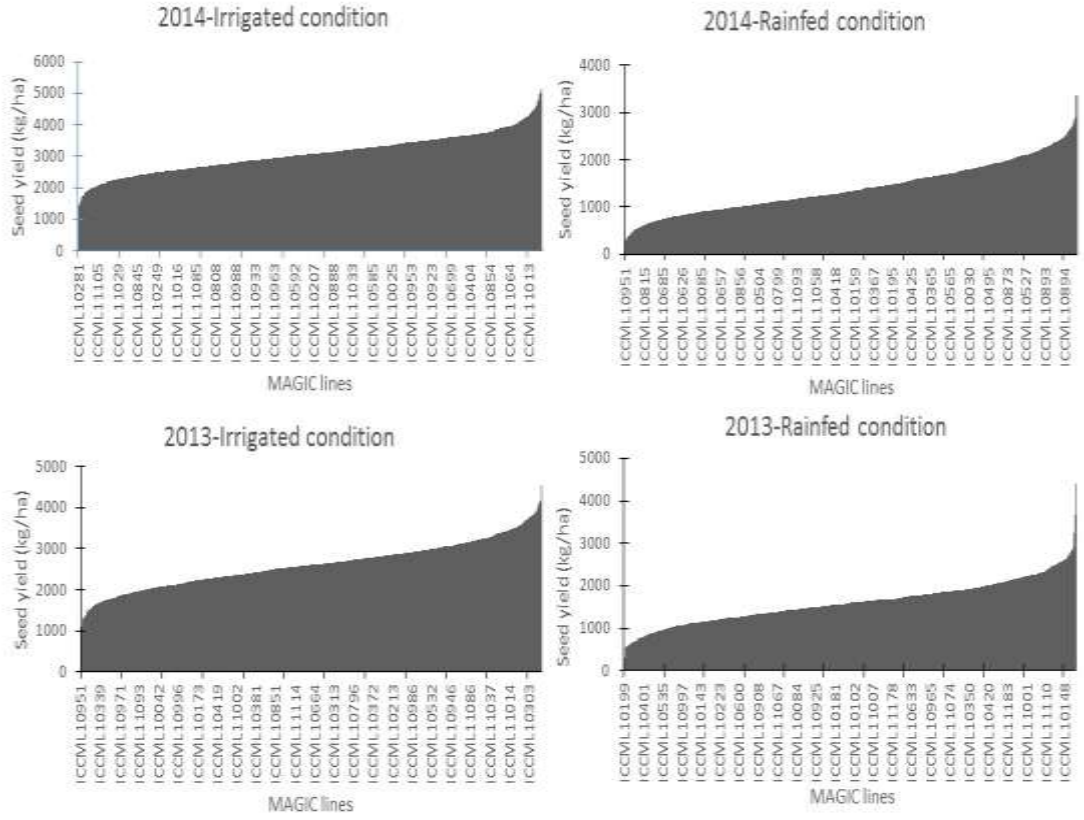


Figure 4-30 Range of yield variability observed in MAGIC lines evaluated under rainfed (a) and irrigated (b) conditions

To identify heat tolerant genotypes, MAGIC lines were screened under field condition during summer 2015. Flowering time of parents did not show much variation compared to normal season (rainfed), however MAGIC lines flowered as early as 28 d and delayed up to 67 days. All the lines matured between 63-88 days, earlier by 1-2 weeks. Plant height reduced by ~20% in both parents and MAGIC lines. Seed yield was drastically reduced compared to normal season. The reduction in seed yield ranged from a minimum 60% to a maximum of 96%. A reduction of 23% to 39% was observed in 100-seed weight due to heat stress. JG 14, heat tolerant variety suitable for late planting conditions was used as check in this experiment. Several lines with significant seed yield compared to best check were identified (Table 4-34). These lines will be further evaluated in coming season for confirmation of their performance and shared with several national and international partners.

Table 4-33 List of top 20 genotypes showing significantly higher seed yield than any parent and the best check evaluated under heat stress conditions

S n	Genotype	Days to 50% flowering	Days to maturity	Plant height (cm)	Seed yield (kg/ha)	100-seed weight
1	ICCML10088	46	79	30	1736	17.7
2	ICCML11136	38	78	30	1664	17.8
3	ICCML11138	38	74	27	1539	15.9
4	ICCML10649	43	77	36	1463	21.7
5	ICCML10070	42	78	30	1456	18.3
6	ICCML10913	43	80	32	1425	16.7
7	ICCML10657	39	78	32	1415	23.1
8	ICCML10942	45	80	32	1413	22.5
9	ICCML10043	39	80	32	1403	16.5
10	ICCML10667	45	78	34	1399	21.6
11	ICCML10302	44	78	32	1390	18.9
12	ICCML11055	45	78	28	1386	19.9
13	ICCML10363	38	77	37	1349	21.6
14	ICCML10873	39	78	35	1343	16.8
15	ICCML11013	44	80	35	1325	15.6
16	ICCML10679	38	79	35	1309	17.2
17	ICCML10905	39	74	26	1307	15.9
18	ICCML10627	39	79	30	1300	16.9
19	ICCML10521	38	79	31	1286	20.9
20	ICCML10136	42	74	43	1285	26.7
	ICC 4958	39	71	34	298	28.3
	ICCV 10	46	82	33	611	15.4
	JAKI 9218	47	78	32	621	24.4
	JG 11	37	74	32	495	19.8
	JG 130	46	77	32	601	24.6
	JG 16	44	78	29	816	15.0
	ICCV 97105	48	81	35	830	24.5
	ICCV 00108	44	71	32	744	22.7
	JG 14 (check)	38	67	37	**849**	22.0
	GM	41.14	75.46	32.01	445.89	19.62
	Range	28-67	63-88	18.5-47.5	5.8-1736	6.5-34.5
	LSD 5%	5.14	18.38	7.64	169.91	4.07
	CV %	4.46	8.83	8.60	13.42	7.38

4.6 Task 4.5: Capacity building of NARS in research on drought adaptation of crops and integrated breeding for drought adaptation (Lead Institute: ICRISAT; Lead scientist: Pooran Gaur)

- The Research Associate working in the project at ICRISAT-Patancheru was provided training on marker-assisted breeding in chickpea.
- Two PhD (Mr BP Mallikarjuna and Mr Pronob Paul) and one MSc student (Ms Prity Sundaram) completed their reserach work on chickpea at ICRISAT. The research topics included molecular mapping of early flowering genes (Mr BP Mallikarjuna), molecular mapping of heat tolerance genes (Mr Pronob Paul) and effects of earliness on seed size and seed yield in chickpea (Ms Prity Sundaram). Both Mr BP Mallikarjuna and Mr Pronob Paul have prepared research articles these are currently under internal review.
- One PhD student (Ms Karthika Guna) is carrying out research in the area of drought physiology at ICRISAT.
- One Research Fellows is carrying out her doctoral work under this task at MSSRF.

5 Work Package: Enabling Green Growth using Water Treatment and Reuse Innovations

5.1 Objectives

- To identify boundary conditions and perspectives for enabling green economy
- To facilitate a trans-disciplinary co-creation process and identification of agri-business opportunities to increase the use of bio-treatment
- To stimulate the cross-fertilization and knowledge transfer between the individual work packages and activities in Europe and India
- To evaluate and optimize the proposed combinations of bio-treatment and wastewater reuse from a perspective of supporting green growth

5.2 Database stakeholders

EIRC and WP5 EU partners ALTERRA, STEP and GIZ had an internal discussion during the 1st EU-India Joint meeting which was held from 3rd to 5th Dec in Bari, Italy. The meeting focused on developing common strategies to bring together the research and industry players of their respective consortiums in order to mobilize the transnational knowledge and technology transfers between the partners from India and Europe.

5.2.1 Establishment of Innovation Platform

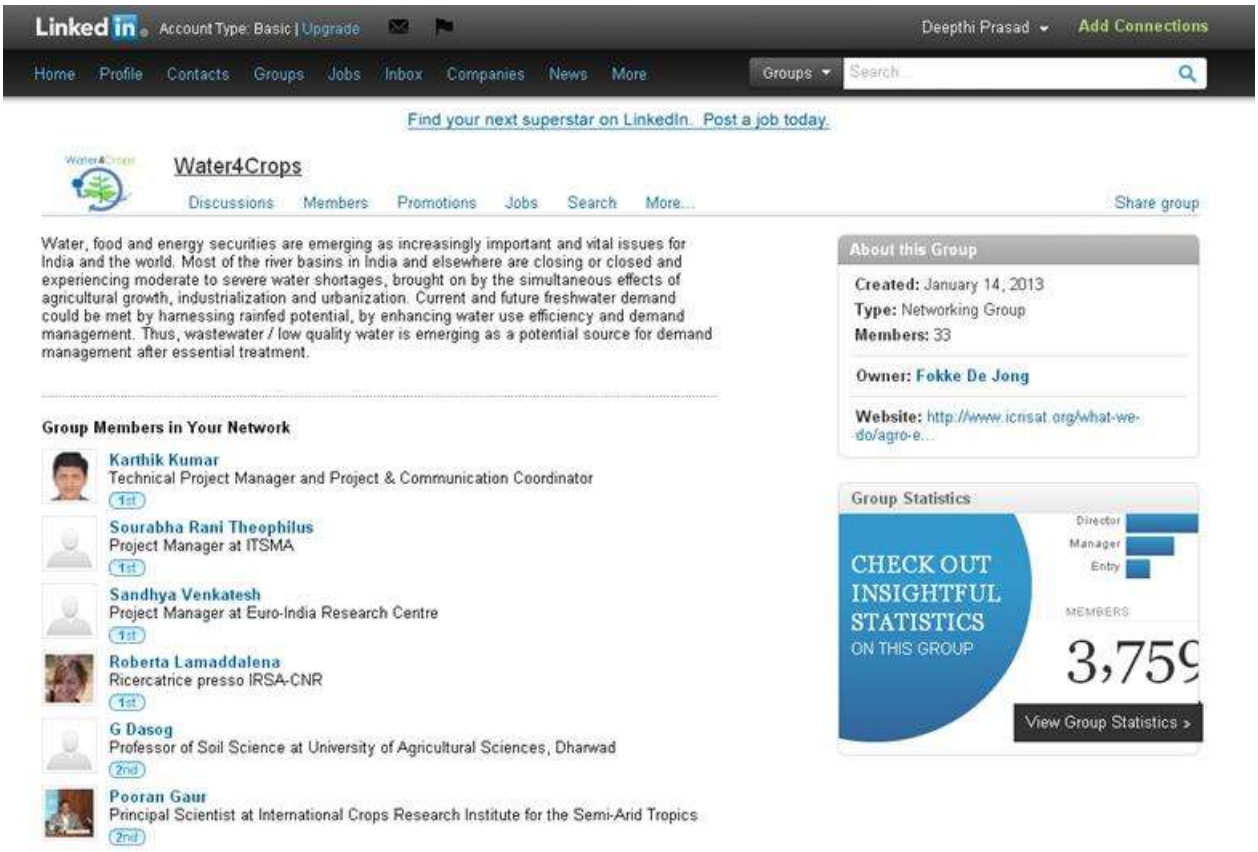
EIRC identified the key stakeholders and practitioners from knowledge (intrinsic and explicit) sector which included technology developers, researchers and industry experts. The profiles of the external experts were sent to the project Coordinator for feedback. Regular skype calls were made to discuss about organizing the Indian INNOVA meeting and the final list of experts were invited for the Meeting.

On 28th May 2014, EIRC organized the 1st Indian INNOVA Meeting at the Capital Hotel in Bengaluru, India. The meeting brought together the Industry experts from CII (Confederation of Indian Industry), EBTC (European Business Technology Center), Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) Germany and EnviroTech Water Management Pvt. Ltd. in the field of wastewater treatment and water use efficiency. These experts were challenged to explore business opportunities for the new technologies in the domains of wastewater reuse and valorization, and water use efficiency that are being developed in Water4Crops project. The meeting facilitated lively discussions between researchers and experts in which the relevance of technology for target users, economic viability and other issues related to applicability and market uptake were addressed.

5.2.2 Creation of Digiinnova Platform - LinkedIn Group

This is a common platform for both the EU and the Indian consortium to exchange and share their experiences about project activities they are undertaking. On the platform upcoming events, meetings, synthesis of specific newsletters and reports are being posted. It is also designed to host discussion on upcoming factsheets especially on the topics like legislation and cost-benefits of waste water treatment and reuse technologies. This discussion will provide inputs to the innovation process in WP5. The external stakeholders from Innova Platforms were also invited to the group. The task ahead for EIRC will be to enhance the group and encourage Indian partners and

external experts to actively participate in the discussions that have begun. EIRC will continue pursuing the Indian consortium partners to make best use of this forum to exchange ideas and share knowledge.



The Water4Crops discussion portal via Linked-in group is available under the link: http://www.linkedin.com/groups/Water4Crops-4799081

Database of stakeholders: Completed and submitted the deliverable to DBT.

5.2.3 Future trends and boundary conditions

EIRC and EU WP5 partner ALTERRA discussed and identified 5 topics on boundary conditions and trends to waste water treatment and reuse in India and EU which include – Legislation; Resource use and boundaries; Health and perceptions; Cost and benefits; and future food production. ALTERRA and EIRC together developed Factsheet Templates and were sent to all the partners to collect the "facts" and "figures", key trends and present scenario existing in both the regions in relation to waste water treatment and reuse in EU and India focused on these 5 topics. The factsheets are almost prepared and the final editing and fine tuning is under process.

5.2.4 Co-creation process of identifying innovation potentials to enable green economy

In order to prepare for the Innova platform meetings, a questionnaire was jointly prepared by the EU and Indian WP5 leaders namely Alterra and EIRC. Based on the questionnaire inputs, relevant experts (from within the consortium and also stakeholders from outside) were selected and invited for the 1st Indian INNOVA

platform meeting. The technologies and issues that have been mapped through the questionnaires were discussed during the 1st Indian INNOVA platform meeting.

5.2.5 Synthesis of results and initiation of an implementation process:

Initial "list of technical innovations" were prepared to summarize all the technologies under development in Water4Crops, with the aim to create an overview of those technologies which could lead, in one way or another, to (marketable) innovations. Further, these innovations will be discussed with the stakeholders like SMEs, farmers, local investors to define a roadmap for implementation. This activity is under progress.

5.3 5.2 Report of agribusiness opportunities

This activity is under progress. The 1st Indian INNOVA meeting inputs are recorded and analysed. Business opportunities are being identified by both the consortiums. The final short list of business opportunities will be reported in the following months.

6 Work Package: Dissemination and Technology Exchange

6.1 Objectives

- To disseminate local entrepreneur demands within the projects
- To disseminate technology offers to entrepreneurs
- To disseminate and exchange the experience between India and Europe on advancing Green Economy in cooperation with EBTC
- To disseminate project results to EBTC, the scientific and wider public community, ensuring maximum use of the project results by a broad audience (scientists, policymakers, planners)
- To provide tailor made capacity building to support the identification of green Growth solutions

6.2 Internal report on customer / entrepreneur demands and technological offer

EIRC and EU WP 6 Partners STEP, ALTERRA, IRSA and GIZ had an internal meeting on 28th May 2014 at Bangalore, India. The dissemination and communication strategies and future plans were discussed. The objective for the year 2 was to ensure effective dissemination of the project results and defining means and actions for enabling technology transfers and exchange of knowledge between India and Europe. The progress and activities undertaken under each task of WP6 are as follows.

6.2.1 Exchange of experiences and results within the Innovation Platforms (IPs) (EIRC)

At the INNOVA platform meetings, all the project partners were given a platform to share their experiences and research results to the external stakeholders. The industry experts were challenged to explore business opportunities for the new technologies in the domains of waste water reuse and valorization, and water use efficiency that are being developed in Water4Crops project.

LinkedIn Forum also acts as a common platform for both the EU and the Indian consortiums to exchange and share their experiences about project activities they are undertaking. On the platform upcoming events, meetings, synthesis of specific newsletters and reports are being posted. It is also designed to host discussion on upcoming W4C factsheets. This discussion will provide input to the innovation process in WP5. The external stakeholders from Innova Platforms were also invited to the group. The task ahead for EIRC will be to enhance the group and encourage Indian partners and external experts to actively participate in the discussions that have begun.

6.2.2 Organization of special entrepreneur and SME knowledge brokerage event (establishment of the Science Practice interface (EIRC)

A special session called “Water4Crops – SME Brokerage Discussion” was organized in the framework of IFAT India 2014 on 9th & 10th October at the Bombay Exhibition Centre, Mumbai, India. IFAT India is the country’s leading trade fair for water, waste, sewage, and recycling. The SME’s, entrepreneurs, technology producers and industry experts were invited for the event. The event focused on treatment of industrial wastewater, its

reuse and valorisation to support Green Economy in Europe and India. High-profiled researchers from India and Europe presented promising ideas and technologies, which are under development for treatment of wastewater and irrigation technologies. Besides, the event provided an opportunity to the SME's to interact and network with Indian and International experts representing premier Indian and European research organizations. The programme agenda, presentations and pictures are available under the link below:
http://www.water4crops.org/promising-research-results-water4crops-presented-ifat-india-2014-mumbai-october-9-10-2014/

6.3 Webpage and Public Dissemination material

6.3.1 Establishment and maintenance of joint project website and project document store:

The EU-India Joint water4crops website is the main dissemination tool to showcase significant results and outcomes and project events. The website is regularly updated with information from both EU and Indian side. Apart from project activities, the news, events and related articles are also posted in the website. This conveys to outsiders that W4Cs is a joint project between India & EU and both sides are working together. Moreover, it enables effective linkages between both projects partners. Every partner in this way is updated on the developments and progress on activities on both sides.

In order to measure the dissemination impact, EIRC regularly monitors the Website statistics including number of visits, duration of visits, number of downloads, download items, etc. EIRC has enabled the Awstats and Google Analytics tools to monitor the activity of the project web site and measure the progress and impact. The snapshots of the web statistics is provided at the end of the document.

Project Store: The "Project Document Store" or the "Intranet" has been developed by EIRC in the time frame of the 1st reporting period. The online store will help both the Indian and EU partners share documents and files, locally or remotely, in groups or privately in a project centric environment. Two separate accounts have been created for both EU and Indian partners and the credentials are shared with them. Partners can easily get access to all the deliverables stored in the intranet. Separate Indian and EU folders are created to avoid confusions and mishandling of documents.

Project Poster and roll ups: A common poster design was designed and developed after reviews and suggestions from both project partners. This poster was presented during the Water4Crops-India 1st Project Review and Planning Meeting which was held from 27th – 29th May 2014 at Bengaluru, India**.** The roll ups was specifically designed and developed for the 1st Indian INNOVA meeting. The snapshots of both the poster and roll up is attached in this document.

Technical posters for IFAT India 2014: Both EU and India consortium partners decided to develop technical posters to present at the IFAT India 2014 event at Mumbai. An earlier discussion was made with all the partners to know there interest and availability to develop the technical poster. A draft template was prepared and sent to all the partners

for their inputs. Later EIRC and STEP collated all the information and developed a total of 9 posters for the event. The snapshots of the posters are attached at the end of the document.

Water4Crops Booklet: EIRC and EU partner STEP together developed a booklet for the IFAT India 2014 event. This booklet consist of all the technical Innovations which were developed under water4crops. EIRC designed the booklet and it was distributed to all the SMEs and entrepreneurs who attend the SME brokerage session at IFAT India 2014 on 9th & 10th October at Mumbai.

6.3.2 Elaboration of Annual Newsletters for the wider public

EIRC and EU partner STEP together developed the common W4C Newsletter. Two annual newsletter have been published till date. The 1st newsletter contains the information on the project progress, research findings and observations made especially at its Indian case study sites. The next issue, was focused mainly on the European case studies. The 1st NL was presented and distributed to the experts at the Water4Crops-India 1st Project Review and Planning Meeting and the 2nd NL was presented at the IFAT India 2014 event. The newsletter is also widely distributed to all the stakeholders. The newsletters can be downloaded from the link below:

- Water4Crops Newsletter Issue #2
- Water4Crops Newsletter Issue #1

6.3.3 Mass media and press releases, information to social media with project progress statements (EIRC, ICRISAT and SAB Miller, All Partners)

This activity will be undertaken not only by EIRC, but also by other partners, especially ICRISAT, SAB Miller, and other partners in their respective regions. The next press release is yet to be decided.

6.3.4 YouTube Channel for Water4Crops

EIRC created a YouTube channel for Water4Crops project. All project related videos are uploaded to the Water4Crops website and is also disseminated through this social media network: http://www.youtube.com/watch?v=tOCC7z2fUdQ

6.3.5 Twitter account for Water4Crops

EIRC has also created a Twitter account for the project and all the project activities, news and events are tweeted regularly. This way the subscribed followers are informed about current activities of the project and Importantly, it lets followers communicate with the project too.
Twitter account: @water4crops

6.3.6 Input to existing information hubs:

EBTC (European Business Technology Center) was identified as one of the important initiators to promote water4crops activities and results through their EBTC web portal. As per earlier discussions, it was decided that Water4Crops will be promoted and involved in several Water Initiatives and Channels that EBTC is connected to, and EBTC expert will be invited and involved in Water4Crops events and Innova Platform meetings. EIRC was in regular contact with EBTC officials and as promised EBTC created a

separate web page for the water4crops project within their website and all the latest news and project outcomes are shared in this webpage. To view the webpage, please follow the link below:
http://ebtc.eu/index.php/sector/environment/water4crops
An article about "Water4Crops" was also published in the "EU Parliament Magazine" (pg 51, Issue 391, 26 May 2014). To read the article visit: http://www.water4crops.org/article-water4crops-eu-parliament-magazine/

6.4 Report on training course including online curricula

The demand on training and the priorities of the trainable topics are discussed and evaluated at the 1st INNOVA meeting at Bari. Using the results of this knowledge brokerage event as basis STEP and EIRC will develop a catalogue on Trainable Tools of W4Cs making it available via W4Cs internet portal. The first W4Cs tool, **SALTMED 2013**- An integrated management tool for Water, Crop, Soil and N-Fertilizers, tool is already available in the Water4crops website (http://www.water4crops.org/saltmed-2013-integrated-management-tool-water-crop-soil-n-fertilizers/). Dr. R. Ragab has developed a very useful tool which helps in agriculture resources management as well as in predicting the impact of future climate change on food production and on the environment.

The second tool called "**IHMS-Integrated Hydrological Modelling System** by Dr. R. Ragab, CEH, UK" is also available on the W4C website (http://www.water4crops.org/ihms-integrated-hydrological-modelling-system-dr-r-ragab-ceh-uk/)

STEP and EIRC also organized a one day **Workshop on SALTMED Management Tool** on 29th May 2014 at Bengaluru, India. The SALTMED Workshop was successfully carried out with 22 participants. Dr. Ragab Ragab, CEH Wellington who is the developer of the SALTMED model, gave an overview about his model and instructions for installing the SALTMED software program on the laptops of the participants. In the first part of the workshop Dr. Ragab explained the theoretical background of the SALTMED model indicating the range of its application area and its limitations. A special part of the workshop was dedicated to working with the SALTMED model in the afternoon session. The workshop flyer can be found under the link :
http://www.water4crops.org/wp-content/uploads/2014/05/SALTMED-Workshop-flyer-29th-May-2014.pdf

Publications:

1. Varkey, B.K., Dasog, G. S., S Wani., Sahrawat, K. L., Hebbara M., and Patil, C. R., 2015, Impact of long-term application of domestic sewage water on soil properties around Hubli City in Karnataka, India. *Agriculture Research* doi:10.1007/s40003-015-0171-9
2. Kamble, S.M., and Hebbara, M., 2015, Effect of long-term application of bio-methanated spentwash on sugarcane in Vertisol. *Karnataka Journal of Agricultural Sciences* 28 (2): 189-192.
3. Varkey, B.K., Dasog, G. S., Prasanna kumar, B. H., Patil, C. R., and Hebbara, M.,2015, Chemical and microbial composition of municipal Sewage water around Hubli-

Dharwad in Karnataka. *Research Journal Chemical Environment Sciences* 3 [3] : 01-05.

4. Swati, B. and Yenagi, N. B., 2015, Comparative assessment of proximate composition of sewage and fresh water irrigated vegetables. *Karnataka Journal of Agricultural Sciences* 28 (3): 398-401.
5. Kamble, S.M., Hebbara, M., Manjunatha M.V., Dasog G.S. and Veerendra Patel G. M., 2016, Long-term effect of bio-methanated spentwash irrigation on soil organic carbon and nutrient status in a Vertisol. *Research in Enivornment and Life Sciences* 9 (1): 35-38
6. Kamble, S.M., Hebbara, M., Manjunatha M.V., Dasog G.S. and Veerendra Patel G. M.,2016, Influence of long-term irrigation with bio-methanated spentwash on physical and biological properties in a Vertisol. *Research in Enivornment and Life Sciences* 9 (1):1-3.
7. Jogan, H and Dasog, G. S., 2015, Composition of domestic and industrial wastewaters and their effect on available nutrients in soils of north Karnataka. *Karnataka Journal of Agricultural Sciences* 28 (4):518-523.
8. L. Garcia-Gonzalez, F. Truzzi, A. Kaushik, H. De Wever, *Valorization of biorefinery wastewater to carboxylates*, Environmental Technology for Impact 2015 (ETEI2015), 29 - 30 April 2015, Wageningen, Netherlands (oral presentation)
9. L. Garcia-Gonzalez, F. Truzzi, A. Kaushik, H. De Wever, *The use of reactive membrane extraction for the valorization of biorefinery wastewater,* ACHEMA 2015, Frankfurt am Main, Germany (oral presentation)
10. A. Kaushik, S. Basu, V.S. Batra, M. Balakrishnan, D. Frascari, D. Pinelli, *Recovery of polyphenols from sugarcane molasses distillery wastewater using commercial resins*, Innovations in Sustainable Water and Wastewater Treatment Systems (ISWATS) Conference, April 21-23, 2016, Pune. (poster)
11. A. Kaushik, K. Singh, S. Basu, V.S. Batra, M. Balakrishnan, *Adsorptive recovery of melanoidins*, Innovations in Sustainable Water and Wastewater Treatment Systems (ISWATS) Conference, April 21-23, 2016, Pune. (poster)
12. S. Basu, S. Mukherjee, M. Balakrishnan, V. S. Batra, M. V. Deepthi, R. R. N. Sailaja, *Polysulphone/nanocomposites mixed matrix ultrafiltration membrane for the recovery of Maillard reaction products*, Membrane Water Treatment-An International Journal (under review).
13. D. Pinelli, A. E. Molina Bacca, A. Kaushik, M. Nocentini, L. Bertin, D. Frascari, *Batch and continuous flow adsorption of phenolic compounds from olive mill wastewater: a comparison between non-ionic and ion exchange resins*, International Journal of Chemical Engineering (under review)

7 Work Package: Coordination and Management

7.1 Objectives

- To co-ordinate and supervise, jointly with the Indian consortium, activities to be carried out;
- To carry out the overall administrative and financial management of the project;
- To manage the Grant Agreement with DBT and the Consortium Agreement;
- To manage the Coordination Agreement with the Indian consortium;
- To manage the foreground generated by the project and IPR;
- To manage contacts with the DBT;
- To monitor quality and timing of project deliverables;
- To establish effective internal communication procedures

7.2 Joint meeting at New Delhi

The final joint meeting was conducted during 15–17 June 2016 at Casuarina Hall, India Habitat Center (IHC), New Delhi. Following is the brief report of meeting.

7.2.1 Session 1 Inaugural Session

Dr. Wani welcomed special guest for the inaugural session Hon'ble Minister of State, Ministry of Science & Technology and Ministry of Earth Sciences, Government of India Mr YS Chowdary, Mr. Vijay Raghavan, Secretary, Department of Bio-Technology, Government of India, and H.E. Tomasz Kozlowski, the Ambassador of the European Union to India and participants from EU and India side consortiums. Dr. Antonio Lopez and Dr Wani presented brief overview of the water4crops project for highlighting the achievements of the project in terms of treatment of domestic and industrial wastewater and its safe reuse along with volarization and increased water use efficiency in agriculture.

Dr. Vijay Raghavan mentioned that seeing how good the Water4Crops project has come-up is something really satisfying and it has been a good investment. The combination of technology development, genomic development as well as emphasis on the dissemination of the knowledge gained in Water4Crops is very impressive. He also stressed on the fact that for getting high quality solutions to contemporary challenges the foreign collaborations are of paramount importance.

Mr. H.E. Tomasz Kozlowski, the Ambassador of the European Union to India has expressed that the EU has water strategy and India-EU have similar objectives in the area of research policies – in particular a focus on innovation and on common societal challenges such as health, water, climate and energy. This project is a good example of how top-level research organizations from several European countries have joined forces with their counterparts in India to develop concrete solutions that benefit both sides.

Hon'ble Mr. Y. S. Chowdary, Minister of State, Science and Technology & Earth Sciences, highlighted the importance of treated wastewater for addressing the issues of sanitation and health in rural areas as well as meeting the demand of scarce water resources for

agriculture to improve the livelihoods. Many areas of the project could be supported by government initiatives such as “Swatch Bharat” mission. He requested the Secretary to compile the key findings of the project and bring it to the notice of the government. Lastly he concluded with his strong support for collaborative projects with foreign partners as they bring more than mere capital and infrastructure a rich experience and generates human capital. Scaling-up of decentralised domestic wastewater approach is needed.

7.2.1.1 Recommendations

- Focus should be on translation of scientific and technological findings into solutions for common people (Scaling-up). The science must stay people centered and not scientists centered.
- Greater awareness about the proven technologies such as safe reuse of wastewater in agriculture is the need of the hour.
- Technologies and achievement from this project need to be compiled with detailed documentation and made available for Government and other stakeholders as some of these technologies may be supported by government initiatives such as Swatch Bharat Mission.

7.2.2 Technical Session I

7.2.2.1 Work Package 1

7.2.2.1.1 EU-Side

- It was suggested to use *in-situ* product recovery protocol for higher recovery of volatile fatty acid from biorefinery wastewater with focus on chain elongation in medium chain fatty acid.
- A modified NaOH-based polyhydroxyalkanoates extraction procedure was developed in which a recovery yield of PHAs from olive oil mill wastewaters was 84% with a purity of about 96%.
- Laboratory-Phenol Adsorption Reactor was very effective in reducing COD.

7.2.2.1.2 India-Side

- Polymeric adsorbent XAD16 was superior to activated carbon for recovery of melanoidins and polyphenols from waste media.
- Flat sheet ceramic membranes from sugarcane bagasse ash can be produced in local industries and can be potentially used for solid-liquid/solid-gas separation.
- Waste based carbon obtained from bagasse ash can be used for the recovery of residual plolyphenols from wastewater streams.

7.2.2.2 Work Package 2

7.2.2.2.1 EU-Side

- EU-side coordinator has presented an innovative system Sequencing Batch Biofilter Granular Reactor for treating wastewater produced at small settlements. This technology provides greater operational flexibility with reduced area requirement and less sludge production.
- Cascading slow sand filter with alternative filter materials and floating mat filters were tested for removal of pathogens. Halophytes are also being tested for

wastewater treatment

- Hydraulics and hydrological aspects of constructed wetland were studied to evaluate the dynamics and the effect of the evapotranspiration, and clogging's effects on the wetland.

7.2.2.2.2 India-side

- The constructed wetlands were able to reduce Chemical Oxygen Demand (COD) by 30-92%. Plant species (Canna indica, lemon grass (Cymbopogon), napier (Pennisetum perpureum X Pennisetum americarnum), para grass (Urochloa mutica), typha (Typha latifolia), water hyacinth (Eichhornia crassipes), *Agaretum Conyzoide*s and water lettuce (Pistia stratiotes) evaluated for their nutrient offtake in constructed wetland at field scale.

7.2.2.2.3 Recommendations

- A need for documenting the benefits of EU-India partnership in this project in terms of mutual learning and identifying technologies which can be transferred from EU to India and vice versa.

7.2.3 Technical Session II

7.2.3.1 EU-side

- High science tools such as COSMOS and electrical resistivity tomography for soil moisture measurement, scintillometer and eddy covariance system for measuring the actual evapotranspiration were used for measuring crop water requirement.
- The effects of suspended solid concentration and stagnation phase on the clogging frequency and biofilm development were studied and an improved pressure compensating type emitter development is in progress.

7.2.3.2 Indian-side

- The effect of treated water irrigation on the crop yield and soil quality was studied for a range of crops such as maize, wheat, sorghum, chickpea, tomato, okra, brinjal, chilly, cluster bean and gourds. Treated water usage showed an increase in yield compared to groundwater.
- Dual use of bio-treated sugar effluent in aquaculture and agriculture was found to be a good business model.
- Various pressure compensating and non-compensating emitters were tested for clogging. An improved 3-D print of an optimized emitter was developed.

7.2.3.3 Recommendations:

- Field scale study should aim on the quality of crops yield keeping in mind the health risks, to clearly establish the facts and cast aside the myths and perceptions.

7.2.4 Technical Session III

7.2.4.1 Work Package 4:

- EU-side have identified genomic regions involving the control of root architecture, water use efficiency and yield related traits in maize. Genetic variation for root architecture of tomato were first time studied.
- At India-side, sets of germplasm compared using high through put LeasyScan facility for transpiration efficiency (TE) and associated traits in crops like maize, sorghum, and millet and identified water efficient germplasm. Genomic region controlling several drought tolerance related traits identified in Chickpea can be introgress in other cultivars through MABC for improving drought tolerance. Genetic variability for different root and shoot traits in tomato germplasm accessions were studied under water stress conditions.

7.2.4.2 Work Package 5 and 6:

- Factsheets were prepared for 1) Legislation and standards, 2) Health, public perceptions, and 3) Potential contribution of wastewater to future food production.
- INNOVA platform: 9 technologies shortlisted from total 50 technologies. Further, a survey conducted for assessing status of these technologies revealed that the six of these technologies are ready for commercialization or for end use.

7.2.4.3 Recommendations

- It is decided to develop a brief document describing the technology for convincing the stakeholder/investors.
- It is suggested that the funding agency (DBT) should consider to support extension of the ongoing research work in water4crops project to maintain the continuity.

7.2.5 Technical Session IV

A dissemination workshop was organized as a part of Work Package 5 in partnership with GIZ, where important technologies developed in project were presented by the consortium partners. EU-side coordinator presented the technologies developed by EU consortium partner including advance research work in constructed wetland that addressing the clogging problem in wetland. From Indian consortium three presentation were regarding the constructed wetland one presentation related to high rate transpiration system.

7.2.6 Technical Session V

A Panel Discussion was organised on “Low cost bio-treatment technologies for improving sanitation and water reuse for irrigation in India”. The list of panellist is provided in Annexure I.

7.2.6.1 Recommendations

- Detailed documentation of the Water4Crops technologies is need to facilitate dissemination to the potential users. Information to include description of the technology, operation & management aspects, costs & economic viability aspects, benefits, applicability (replication) etc.

□ Existing government policies/plans/programmes/schemes should be mapped and reviewed for entry points for the developed technology sunder the Water4Crops project.
□ The successfully developed technologies should be got included in the compendium of wastewater treatment technologies in the CPHEEO Manual.
□ Dissemination efforts are needed on the developed technologies and their application.
  - Distribution of documents on Water4Crops technologies to the stakeholders
  - Workshops and training programs may be held with industry sectors and urban local bodies where there is a potential for application of the Water4Crops technologies.
  - Workshops with relevant stakeholders for development of policy instruments such as guidelines for application of technologies, standards for treated wastewater reuse etc.

7.2.7 General Project Discussion

- EU partners need to identify/list technologies/products developed in W4C and can be shared with India for evaluation and scaling-up.
- Indian consortium Leader to request one year no-cost extension to DBT as the money release is delayed. Those partners who will have no money left may not be able to continue during no-cost extension
- India-EU consortia should continue to interact and work jointly for exploring new project opportunities and strengthen the collaboration
- DBT would pursue with the EU for extending W4C along with India part as well as highlight the good outputs/technologies from the W4C project which need to be scaled-up in India. Both consortia need to look for the opportunities for scaling-up project

Table 1.List of Staff recruited for consortium partners

Sl. No.	Institute	RA	SRF/JRF	Project Assistant	LT/FA	Graduate Assistants and Students (M.Sc.)	Total
1	ICRISAT	3	3**		3	5	14
2	UASB						
3	JISL		2				2
4	NEERI						
5	UASD	1	1	1	2	3*	8
6	MSSRF		1				1
7	TERI	2	2	2			6
	Total						

*** These are supported out of Recurring expenses (Miscellaneous). 5 students admitted during this academic year and going to be supported from 2014-15 financial year.**

****One JRF left the job**

REFERENCES

Abbas, G., Ali, A., Saeed, A., Hussnain, H., Farooq, J., Ashraf, F. and Hussain, Z., 2007, Estimation of heritability and correlation analysis for seedling traits in various accessions of tomato. *International Symposium on Prospects of Horticultural Industry in Pakistan* 28th to 30th March, 2007, Institute of Horticultural Sciences, Univ. of Agric., Faisalabad, 257-261.

Abdurakhmonov, I.Y. and Abdukarimov, A., 2008, Application of association mapping to understanding the genetic diversity of plant germplasm resources. *Int. J. Plant Genomics***,** 574927.

Abdurakhmonov, I.Y., Buriev, Z.T., Saha, S., Pepper, A.E., Musaev, J.A., Almatov, A., Shermatov, S.E., Kushanov, F.N., Mavlonov, G.T., Reddy, U.K. , Yu, J.Z., Jenkins, J.N., Kohel R.J. and Abdukarimov, A., 2007, Microsatellite markers associated with lint percentage trait in cotton, *Gossypium hirsutum*. *Euphytica*, **156**: 141-156.

Abraha, T., Nyende, A.B., Mwangi, S.G., Kasili, R. and Araia, W., 2015, Identification of sorghum (*Sorghum bicolor* L. Moench) landraces tolerant to post flowering drought stress using drought tolerance indices. *J. Plant Breed. Crop Sci*., **7**(7): 211-218.

Abraha, T., Nyende, A.B., Mwangi, S.G., Kasili, R. and Araia, W., Identification of sorghum (*Sorghum bicolor* L. Moench) landraces tolerant to post flowering drought stress using drought tolerance indices. *J. Plant Breed. Crop Sci*., **7**(7): 211-218.

Acharya, S., Sharma, S.C., Vaghela, P.O. and Meena, R.K., 2011, Significance of root attributes for yield enhancements in cowpea under normal and water stress conditions. *In*: Book of Abstracts, National seminar on "Contemporary approaches to crop improvements", April 22-23, 2011, Univ. of Agri. Sci., Bangalore. *ISGPB*, New Delhi- 110012.

Agili, S., Nyende, B, Ngamau, K. and Masinde, P., 2012, Selection, yield evaluation, drought tolerance indices of orange-flesh sweet potato (*Ipomoea batatas* Lam) hybrid clone. *J. Nutr. Food Sci*., **2**(3): 1000138, pp: 8.

Agrama, H. and Eizenga, G., 2008, Molecular diversity and genome-wide linkage disequilibrium patterns in a worldwide collection of *Oryza sativa* and its wild relatives. *Euphytica*, **160**: 339-355.

Agrama, H.A., Eizenga, G.C. and Yan, W., 2007, Association mapping of yield and its components in rice cultivars. *Mol. Breed.*, **19**: 341-356.

Akçura, M., Partigoç, F. and Kaya, Y., 2011, Evaluating of drought stress tolerance based on selection indices in Turkish bread wheat landraces. *J. Anim. Plant Sci.,* **21**(4): 700-709.

Al-Jibouri, H.A., Miller, P.A. and Robinson, H.F., 1958, Genotypic and environmental variances in an upland cotton crop of interspecific origin. *Agron. J*., **50**: 633-634.

Allerstorfer, V., 2014, Evaluation of drought adaptation of different tomato genotypes. M.Sc. thesis submitted to Universität für Bodenkultur Wien.

Al-Maskri, A.Y., Sajjad, M. and Khan, S.H., 2012, Association mapping: a step forward to discovering new alleles for crop improvement. *Int. J. Agric. Biol.,* **14**: 153-160.

Al-Tamimi, A.J.T., Al-Saadi, A.H. and Abbass, M.C., 2015, Genetic diversity of some tomato (*Lycopersicon esculentum* Mill.) varieties in Iraq using simple sequence repeats (SSR) markers. *Magazine of Al-Kufa Univ. Biol.,* **7**(1), pp: 16.

Amiri, R., Bahraminejad, S., Sasani, S., and Ghobadi, M., 2014, Genetic evaluation of 80 irrigated bread wheat genotypes for drought tolerance indices. *Bulg. J. Agric. Sci*., **20**: 101-111.

Anitha, R., Satish, V.B., Nimai, M.P., Mukund, V., Shukla, V.D., Dwivedi, J.L., Singh, B.N, Singh, O.N., Padmini, S., Ashutosh, M.K., Robin, S., Chandrababu, R., Abhinav, J., Tilatoo, R., Shailaja, H., Stephan, H., Hans-Peter, P. and Arvind, K., 2012, Drought yield index to select high yielding rice lines under different drought stress severities. *Rice*, **5**(31): 1-12.

Anjali, I.P., Symonova, O., Mileyko, Y., Hao, Y., Belcher, H., Harer, J., Weitz, J.S. and Benfey, P.N., 2010, Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. *Plant Physiol.,* **152:** 1148-1157.

Anonymous, 2007, MetaPhor® agarose. Lonza Rockland, Inc., USA. *www.lonza.com/research*.

Anwar, J., Subhani, G.M., Hussain, M., Ahmad, J., Hussain, M. and Munir, M., 2011, Drought tolerance indices and their correlation with yield in exotic wheat genotypes. *Pak. J. Bot*., **43**(3): 1527-1530.

Asl, R.G., Arbat, H.K., Yarnia, M., Aminzade, G., Asl, L.G and Ghanifathi, T., 2011, Evaluation of drought tolerance indices and grain yield in wheat genotypes using principal components analysis. *Middle-East J. Sci. Res.,***8**(5): 880-884.

Augustina, U.A., Iwunor, O.P. and Ijeoma, O.R. 2013, Heritability and character correlation among some rice genotypes for yield and yield components. *J. Plant Breed. Genet*., **01**(02): 73-84.

Babu, R.C., Shashidhar, H.E., Lilley, J.M., Thanh, N.D., Ray, J.D., Sadasivam, S., Sarkarang, S., O'Toole, J.C. and Nguyen, H.T., 2001, Variation in root penetration ability, osmotic adjustment and dehydration tolerance among accessions of rice adapted to rain-fed lowland and upland ecosystems. *Plant Breed.,* **120:** 233-238.

Bahrami, F., Arzani, A. and Karimi, V., 2014, Evaluation of yield-based drought tolerance indices for screening safflower genotypes. *Agron. J.,* **106**(4): 1219-1224.

Barbagallo, R.N., Silvestro, I.D. and Patanè, C., 2013, Yield, physicochemical traits, antioxidant pattern, polyphenol oxidase activity and total visual quality of field-grown processing tomato cv. Brigade as affected by water stress in Mediterranean climate. *J. Sci. Food Agric*., **93**: 1449-1457.

Barrios-Masias, F. and Jackson, L., 2011, Irrigation alternatives for sustainable water use of processing tomatoes. *SARE,* UC-Davis, CA, USA, pp: 9.

Bauchet, G. and Causse, M., 2012, Genetic diversity in tomato (*Solanum lycopersicum*) and its wild relatives. *In*: Caliskan, M. (Ed.), Genetic diversity in plants. *INTECH* 978-953-51-0185-7, 133-162.

Beard, J.B. and Sifers, S.I., 1997, Genetic diversity in dehydration avoidance and drought resistance within the *Cynodon* and *Zoysia* species. *Int.Turf. Soc. Res. J*., **8**: 603-610.

Begum, F., Sultana, R. and Nessa, A., 2012, Screening of drought tolerant foxtail millet (*Setaria italica* Beauv) germplasm. *Bangladesh J. Sci. Ind. Res.,* **48**(4): 265-270.

Benor, S., Zhang, M., Wang, Z. and Zhang, H., 2008, Assessment of genetic variation in tomato (*Solanum lycopersicum* L.) inbred lines using SSR molecular markers. *J. Genet. Genomics*, **35**: 373-379.

Bera, S.D., Chandrashekar, A.P., Patel, S., Sojitra, V.K. and Maurya, A., 2014, Identification of stable sources for surrogate traits in *Arachis glabrata* and marker trait association for tolerance to water deficit stress. *Turk. J. Bot*., **38**: 309-324.

Bergougnoux, V., 2014, The history of tomato: from domestication to biopharming. *Biotech. Adv*., **32:** 170-189.

Berloo, R.V., Zhu, A., Ursem, R., Verbakel, H., Gort, G. and van Eeuwijk, F.A., 2008, Diversity and linkage disequilibrium analysis within a selected set of cultivated tomatoes. *Theor. Appl. Genet.,* **117**: 89-101.

Bernatzky, R. and Tanksley, S.D., 1986, Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. *Genetics,* **112**: 887-898.

Bernousi, I., Emami, A., Tajbakhsh, M., Darvishzadeh, R. and Henareh, M., 2011, Studies on genetic variability and correlation among the different traits in *Solanum lycopersicum* L. *Not. Bot. Hort. Agrobot. Cluj.,* **39**(1): 152-158.

Besufekad, B.Y and Bantte, K., 2013, Evaluation and association mapping for drought tolerance in sorghum [*Sorghum Bicolor* (L.) Moench]. *Global J. Sci. Front. Res.,* **13**(5): 49-54.

Birhanu, K., and Tilahun, K., 2010, Fruit yield and quality of drip-irrigated tomato under deficit irrigation. *AJFAND*, **10**(2): 2139-2151.

Bohm, W., 1979, Methods of studying root systems. *Ecological Studies No. 33*, Springer-Verlag, Berlin, Heidelberg, New York, pp: 188.

Borba, C., Oliveria, D., Brondani, R.P.V., Breseghello, F., Coelho, A.S.G., Mendonca, J.A., Rangel, P.H.N. and Brondani, C., 2010, Association mapping for yield and grain quality traits in rice (Oryza *sativa* L.). *Genet. Mol. Biol*., **33**: 515-524.

Bouslama, M. and Schapaugh, W.T., 1984, Stress tolerance in soybean. I. Evaluation of three screening techniques for heat and drought tolerance. *Crop Sci*., **24**: 933-937.

Bradbury, P.J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y. and Buckler, E.S., 2007. TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics,* **23**: 2633-2635.

Brdar-Jokanović, M. and Zdravković, J., 2014, Germination of tomatoes under PEG-induced drought stress. *Ratar. Povrt.,***52**(3): 108-113.

Brdar-Jokanović, M., Girek, Z., Pavlovic, S., Ugrinovic, M. and Zdravkovic, J., 2014, Shoot and root dry weight in drought exposed tomato populations. *Genetika*, **46**(2): 495-504.

Brdar-Jokanović, M., Pavlović, S., Girek, Z., Ugrinović, M. and Zdravković, J., 2014, Assessing tomato drought tolerance based on selection indices. *Ratar. Povrt*., **51**(1): 38-45.

Bredemeijer, G.M.M., Cooke, R.J., Ganal, M.W., Peeters, R., Isaac, P., Noordijk, Y., Rendell, S., Jackson, J., Röder, M.S., Wendehake, K., Dijcks, M., Amelaine, M., Wickaert, V., Bertrand, L. and Vosman, B., 2002, Construction and testing of a microsatellite database containing more than 500 tomato varieties. *Theor. Appl. Genet*., **105**: 1019-1026.

Breseghello, F. and Sorrells, M.E., 2006, Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. *Genetics*, **172**(2): 1165-1177.

Chavan, M.L., 2007, Drought tolerance studies in tomato (*Lycopersicon esculentum* Mill.). Ph. D. thesis submitted to Univ. of Agric. Sci., Dharwad, pp: 196.

Chavan, M.L., Mastiholi, A.B., Shashidhar, T.R. and Tirakannanavar, S., 2010, Changes in morphology and yield attributing characters of tomato genotypes due to moisture stress. *J. Ecobio*., **26**(3): 255-268.

Chavan, M.L., Tirakannanavar, S. and Munikrishanappa, P.M., 2009, Effect of drought on variation of leaf morphorlogy of tomato (*Lycopersicum esculentum*) genotypes. *J. Ecobiol.,* **25**(2): 173-183.

Chaves, M.M., Maroco, J.P. and Pereira, J.S., 2003, Understanding plant responses to drought from genes to the whole plant. *Funct. Plant Biol.,* **30**: 239-264.

CRIDA, 2012, National initiative on climate resilient agriculture- research highlight (2010-2012), Hyderabad, India.

Cuartero, J., Yeo, A.R. and Flowers, T.J., 1992, Selection of donors for salt tolerance in tomato using physiological traits. *New Phytol.,* **121**(1): 63-69.

Daljeet, S. and Dhillon, S. S., 1993, Evaluation of tomato germplasm under high temperature and water stress conditions. Adaptation of food crops to temperature and water stress. *Proc. Intern. Symp.,* Taiwan, 503-505.

Dar, R.A., Sharma, J.P., Nabi, A. and Chopra, S., 2012, Germplasm evaluation for yield and fruit quality traits in tomato (*Solanum lycopersicon* L.). *Afr. J. Agric. Res*., **7**(46): 6143-6149.

David, H., Tegstrom, C. and Ingvarsson, P.K., 2010, Using association mapping to dissect the genetic basis of complex traits in plants. *Brief Funct. Genomics.,* **9**: 157-165.

De Costa, W.A.J.M. and Ariyawansha, B.D.S.K., 1996, Effect of water stress on water use efficiency of different varieties of common bean (*Phaseolus vulgaris* L.). *J. Natn. Sci. Coun Sri Lanka,* **24**(4): 253-266.

El-Saka, Z.I.M., 2006, Genetic and biotechnological studies on drought tolerance in tomato. Ph.D. thesis submitted to Benha University, Egypt, pp: 165.

Emami, A., 2014, Effect of transplanting dates on fruit yield and related quality traits of tomato genotypes (*Lycopersicun escutentum* Mill.). *Int. J. Curr. Res. Aca. Rev*., **2**(8): 1-9.

Evanno, G.G., Regnaut, S. and Goudet, J., 2005, Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecol.*, **14**: 2611-2620.

Falush, D., Stephens M. and Pritchard, J.K., 2003, Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics***, 164**: 1567-1587.

Fang, Y. and Xiong, L., 2015, General mechanisms of drought response and their application in drought resistance improvement in plants. *Cell. Mol. Life Sci.,* **72**: 673-689.

FAOSTAT, 2015, Statistical Databases. Food and Agriculture Organization of the United Nations, Statistics Division, Rome, Italy. *http://faostat3.fao.org/download/Q/QC/E*

Farfan, I.D.B., La Fuente, G.N.D., Murray, S.C., Isakeit, T., Huang, P.C., Warburton, M., Williams, P., Windham, G.L. and Kolomiets, M., 2015, Genome wide association study for drought, aflatoxin resistance, and important agronomic traits of maize hybrids in the sub-tropics. *PLoS ONE*, **10**(2): e0117737, 1-30.

Flint-Garcia, S.A., Thornsberry, J.M. and Buckle, E.S.T., 2003, Structure of linkage disequilibrium in plants. *Annu. Rev. Plant Biol*., **54**: 357-374.

Foolad, M.R., 2005, Breeding for abiotic stress tolerances in tomato, *In*: Abiotic stresses: plant resistance through breeding and molecular approaches (Ashraf, M. and P. J. C. Harris, Eds.). The Haworth Press, New York, New York, USA, pp: 613-684.

Gavuzzi, P., Rizza, F., Palumbo, M., Campanile, R.G., Ricciardi, G.L. and Borghi, B., 1997, Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. *Can J. Plant Sci*., **77**: 523-531.

Geethanjali, S., Chen, K.Y., Pastrana, D.V. and Wang, J.F., 2010, Development and characterized of tomato SSR markers from genomic sequences of anchored BAC clones on chromosome 6. *Euphytica,* **173:** 85-97.

Geethanjali, S., Kadirvel, P., de la Pena, R., Rao, E.S. and Wang, J.F., 2011, Development of tomato SSR markers from anchored BAC clones of chromosome 12 and their application for genetic diversity analysis and linkage mapping. *Euphytica,* **178**: 283-295.

Ghebremariam, M.A.K.M., Yan, L., Zhang, M.A.Z. and Wang, M.A.Q, 2013, Effect of drought stress on physiological growth parameters of tomato inbred lines at germination stage. *Euro. Sci. J*., **9** (33): 1857-7881.

Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J. and Thompson, R., 2002, ASReml user guide release 1.0. VSN International Ltd, Hemel Hempstead.

Girdthai, T., Jogloy, S., Vorasoot, N., Akkasaeng, C., Wongkaew, S., Holbrook, C.C. and Patanothai, A., 2010, Heritability of, and genotypic correlations between, aflatoxin traits and physiological traits for drought tolerance under end of season drought in peanut (*Arachis hypogaea* L.). *Field Crops Res*., **118**: 169-176.

Glogovac, S., Takač, A., Brbaklić, L., Trkulja, D., Červenski, J., Varga, J.G. and Popović, V., 2013, Molecular evaluation of genetic variability in tomato (*Lycopersicon esculentum* Mill.) genotypes by microsatellite markers. *Ratar. Povrt.,* **50**(3), pp:7.

Hidayatullah, Jatoi, S.H., Ghafoor, A. and Mahmood, T., 2008, Path coefficient analysis of yield component in tomato (*Lycopersicon esculentum*). *Pak. J. Bot.,* **40**(2): 627-635.

Hirschhorn, J.N. and Daly, M.J., 2005, Genome-wide association studies for common diseases and complex traits. *Nat. Rev. Genet.*, **6**: 95-108.

Hu, X., Wang, H., Chen, J. and Yang, W., 2012, Genetic diversity of Argentina tomato varieties revealed by morphological traits, simple sequence repeat, and single nucleotide polymorphism markers. *Pak. J. Bot.,* **44**(2): 485-492.

Imana, C., Aguyoh, J.N. and Opiyo, A., 2010, Growth and physiological changes of tomato as influenced by soil moisture levels. Second RUFORUM Biennial Meeting 20-24 September 2010, Entebbe, Uganda, 877-886.

Indian Horticulture Database, 2014, National Horticulture Board, Ministry of Agriculture, Government of India, New Delhi, India.

Injeti, S.K., Venkataravana, P. and Rao, M.R.G., 2008, Evaluation of new germplasm and advanced breeding lines of groundnut (*Arachis hypogaea* L.) under late *kharif* situation. *Legume Res*., **31**(4): 254-258.

Kaushik, S.K., Tomar, D.S. and Dixit, A.K., 2011, Genetics of fruit yield and it's contributing characters in tomato (*Solanum lycopersicom*). *JABSD*, **3**(10): 209 -213.

Khalili, M., Naghavi, M.R., Aboughadareh, A.P. and Talebzade, S.J., 2014, Evaluating of drought stress tolerance based on selection indices in spring canola cultivars (*Brassica napus* L.). *J. Agric. Sci*., **4**(11): 78-85.

Khan, S.H., Khan, A., Litaf, U., Shah, A.S., Khan, M.A., Bilal, M. and Ali, M.U., 2015, Effect of drought stress on tomato cv. Bombino. *J. Food Process Technol.,* **6**(7), pp: 6.

Kharrazi, M.A.S. and Rad, M.R.N., 2014, Evaluation of sorghum genotypes under drought stress conditions using some stress tolerance indices. *Afr. J. Biotech.,* **10**(61): 13086-13089.

Korir, N.K., Diao, W., Tao, R., Li, X., Kayesh, E., Li, A., Zhen, W. and Wang, S., 2014, Genetic diversity and relationships among different tomato varieties revealed by EST-SSR markers. *Genet. Mol. Res.*, **13**(1): 43-53.

Kristin, A.S, Senra, R.R., Perez, F.I., Enriquez, B.C., Gallegos, J.A.A., Vallego, P.R., Wassimi, N. and Kelley, J.D., 1997, Improving common bean performance under drought stress. *Crop Sci.*, **37**: 43-50.

Long, W., Li, Y., Zhou, W., Ling, H.Q. and Zheng, S., 2013, Sequence-based SSR marker development and their application in defining the introgressions of LA0716 (*Solanum pennellii*) in the background of cv. M82 (*Solanum lycopersicum*). *PLoS ONE*, **8**(12): 1-8.

Manna, M. and Paul, A., 2012, Studies on genetic variability and characters association of fruit quality parameters in tomato. *HortFlora Res. Spect.*, **1**(2): 110-116.

Martin, B. and Thorstenson, Y., 1988, Stable carbon isotope composition (?^{13}C), water use efficiency and biomass productivity of *Lycopersicon esculentum, Lycopersicon pennellii* and the F_1 hybrid. *Plant Physiol*., **88:** 213-217.

Martin, B., Nienhuis, J., King, G. and Schaefer, A., 1989, Restriction fragment length polymorphisms associated with water use efficiency in tomato. *Science,* **243**(4899): 1725-1728.

Martinez F., Merino O., Garcia M.D. and Merino J.A., 1998, Below ground structure and production in a Mediterranean shrub community. *Plant and Soil*, **201**: 209-216.

Mazzucato, A., Papa, R., Bitocchi, E., Mosconi, P., Nanni, L., Negri, V., Picarella, M.E., Siligato, F., Soressi, G.P., Tiranti, B. and Veronesi, F., 2008, Genetic diversity, structure and marker-trait associations in a collection of Italian tomato (*Solanum lycopersicum* L.)landraces. *Theor. Appl. Genet.*, **116**: 657-669.

Mehta, N. and Asati, B.S., 2008, Genetic relationship of growth and development traits with fruit yield in tomato (*Lycopersicon esculentum* Mill). *Karnataka J. Agric. Sci*., **21**(1): 92-96.

Meitei, K.M., Bora, G.C., Singh, S.J. and Sinha, A.K., 2014, Morphology based genetic variability analysis and identification of important characters for tomato (*Solanum lycopersicum* L.) crop improvement. *Am.-Eurasian J. Agric. Environ. Sci.*, **14**(10): 1105-1111.

Nicoleta, B.A. and Nedelea, G., 2012, Research concerning drought tolerance in some tomato seedling genotypes. *J. Hort. Biotech*., **16**(2): 96-102.

Noorifarjam, S., Farshadfar, E. and Saeidi, M., 2013, Evaluation of drought tolerant genotypes in bread wheat using yield based screening techniques. *Euro. J. Exp. Bio*., **3**(1): 138-143.

Nouraein, M., Mohammadi, S.A., Aharizad, S., Moghaddam, M. and Sadeghzadeh, B., 2013, Evaluation of drought tolerance indices in wheat recombinant inbred line population. *Ann. Biol. Res*., **4**(3):113-122.

Nwosu, D.J., Onakoya, O.A., Okere, A.U., Babatunde, A.O. and Popoola, A.F., 2012, Genetic variability and correlations in rainfed tomato (*Solanum* spps.) accessions in Ibadan, Nigeria. *Greener J. Agric. Sci.,* **4**(5): 211-219.

O'Connell, M.A., Medina, A.L., Sanchez Pena, P. and Trevino, M.B., 2007, Molecular genetics of drought resistance response in tomato and related species. *In:* M. K. Razdan and A. K. Mattoo, (Eds.) Genetic improvement of *Solanaceous* Crops, Vol. 2: Tomato, Science Publishers, Enfield USA, pp: 261-283.

Ober, E.S., Werner, P., Flatman, E., Angus, P., Jack, P. and Tapsell, C., 2013, Improving water use efficiency and drought tolerance in UK winter wheat. *Project (RD-2005-3233) Report*, **476**, pp: 96.

Odeh, R.A.J., 2003, Grey water reuse: towards sustainable water management. *Desalination,* **156:** 181-192.

Ohyama, A., Asamizu, E., Negoro, S., Miyatake, K., Yamaguchi, H., Tabata, S. and Fukuoka, H., 2009, Characterization of tomato SSR markers developed using BAC-end and cDNA sequences from genome databases. *Mol. Breed.,* **23**: 685-691.

Okiyo, T., Gudu, S., Kiplagat, O. and Owuoche, J., 2011, Effect of post anthesis moisture stress on performance of stay green sorghum hybrid in eastern province of Kenya. *addis2011.ifpri.info/files/2011/10/Paper_3A_Teresa-Okiyo.pd*, pp: 14.

Peralta, I.E., Spooner, D.M. and Knapp, S., 2008, Taxonomy of wild tomatoes and their relatives (*Solanum* sect.*Lycopersicoides*, sect.*Juglandifolia*, sect.*Lycopersicon*; Solanaceae). *Syst. Bot. Monogr.,* **84**, pp: 186.

Peralta, I.E., Spooner, D.M., 2001, Granule-bound starch synthase (GBSSI) gene phylogeny of wild tomatoes (*Solanum* L. section *Lycopersicon* [Mill.] Wettst. subsection *Lycopersicon*). *Am. J. Bot.,* **88**: 1888-1902.

Peralta, I.E., Spooner, D.M., 2007, History, origin and early cultivation of tomato (Solanaceae). *In*: Razdan, M.K. amd Matto, A.K., eds. Genetic improvement of Solanaceous crops, vol. 2: Tomato. Enfield, NH: Science Publishers, pp: 1-27.

Ram, H.H., 2005. Vegetable breeding: Principles and practices. Kalyani Publishers, New Delhi, India, pp: 183-206.

Ramzan, A., Khan, T.N., Nawab, N.N., Hina, A., Noor, T. and Jillian, G., 2014, Estimation of genetic components in F_1 hybrids and their parents in determinate tomato (*Solanum lycopersicum* L.). *J. Agric. Res.,* **52**(1): 65-75.

Rathore, B.M.S., Sud, R., Saxena, V., Rathore, L.S., Rathore, T.S., Subrahmanyam, V.G. and Roy, M.M, 2013, Drought conditions and management strategies in India. Country Report prepared for the Regional Workshop for Asia-Pacific as part of the UN-Water Initiative on "Capacity Development to Support National Drought Management Policies" organized from 6-9th May 2014 in Hanoi, Vietnam.

Razdan, M.K. and Mattoo, A.K., 2007, Genetic improvement of Solanaceous crops: Tomato. Science Publishers, USA, **2**: 47.

Reddy, R.B., Reddy, S.D., Reddaiah, K. and Sunil, N., 2013, Studies on genetic variability, heritability and genetic advance for yield and quality traits in Tomato (*Solanum lycopersicum* L.). *Int. J. Curr. Microbiol. App. Sci.,* **2**(9): 238-244.

Rick, C.M., 1976, Tomato, *Lycopersicon esculentum* (Solanaceae). *In*: Simmonds, N.W. (Eds.), Evolution of crop plants. Longman Group, London, pp: 268-273.

Rick, C.M., 1979, Biosystematic studies in *Lycopersicon* and closely related species of *Solanum.In*: The Biology and Taxonomy of Solanaceae, Hawkes, J.G., Lester, R.N. and Skelding, A.D. (Eds.). Academic Press, New York, NY, USA, pp: 667-678.

Rick, C.M., 1986, Germplasm resources in the wild tomato species. *Acta Hort.,***190**: 39-47.

Rick, C.M., 1987, Genetic resources in Lycopersicon. *Pl. Bioi.,***4**: 17-26.

Rick, C.M., 1988, Tomato-like nightshades: affinities, autoecology, and breeders opportunities. *Econ. Bot.,***42**: 145-154.

Sambrook, J., Fritschi, E.F. and Maniatis, T., 1989 Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, New York.

Sánchez-Rodríguez, E., Rubio-Wilhelmi, M., Blasco, B. and Leyva, R., 2012, Antioxidant response resides in the shoot in reciprocal grafts of drought-tolerant and drought sensitive cultivars in tomato under water stress. *Plant Sci.*, **188-189:** 89-96.

Sarker, B.C., Hara, M. and Uemura, M., 2004, Comparison of response of two C_3 species to leaf water relation, proline synthesis, gas exchange and water use under periodic water stress. *J. Plant Biol*., **47**(1): 33-41.

Shashikanth, Basavaraj, N., Hosamani, R.M. and Patil, B.C., 2010, Genetic variability in tomato (*Solanum lycopersicon* [Mill].Wettsd.). *Karnataka J. Agric. Sci.*, **23**(3): 536-537.

Sherpa, P., Pandiarana, N., Shende, V.D., Seth, T., Mukherjee, S. and Chattopadhyay, A., 2014, Estimation of genetic parameters and identification of selection indices in exotic tomato genotypes. *Electro. J. Plant Breed.,* **5**(3): 552-562.

Shirasawa, K. and Hirakawa, H., 2013, DNA marker applications to molecular genetics and genomics in tomato.*Breeding Sci.*, **63**: 21-30.

Shirasawa, K., Asamizu, E., Fukuoka, H., Ohyama, A., Sato, S., Nakamura, Y., Tabata, S., Sasamoto, S., Wada, T., Kishida, Y., Tsuruoka, H., Fujishiro, T., Yamada, M. and Isobe, S., 2010a, An interspecific linkage map of SSR and intronic polymorphism markers in tomato. *Theor. Appl. Genet.,* **121:** 731-739.

Shirasawa, K., Fukuoka, H., Matsunaga, H., Kobayashi, Y., Kobayashi, I., Hirakawa, H., Isobe, S. and Tabata, S., 2013, Genome-wide association studies using single nucleotide polymorphism markers developed by re-sequencing of the genomes of cultivated tomato. *DNA Res.*, **20:** 593-603.

Shirasawa, K., Isobe, S., Tabata, S. and Hirakawa, H., 2014, Kazusa Marker DataBase: a database for genomics, genetics, and molecular breeding in plants. *Breed. Sci.,* **64**: 264-271.

Shiri, M., Choukan, R. and Aliyev, A.T., 2010, Drought tolerance evaluation of maize hybrid using biplot method. *Trend. App. Sci. Res.,* **5**(2): 129-137.

Shirinzadeh, A., Zarghami, R., Azghandi, A.V., Shiri, M.R. and Mirabdulbaghi, M., 2010, Evaluation of drought tolerance in mid and late mature corn hybrids using stress tolerant indices. *Asian J. plant Sci*., **9**(2): 67-73.

Showemimo, F.A., Olarewaju, J.D., Buah, J.N., Teteh, J.P. and Asare-Bediako, E., 2007, Genetic estimates of water stress in tomato (*Lycopersicum esculentum*). *Int. J. Plant Breed. Genet*., **1**(1): 18-23.

Shreya, R.P., Vasanthi, M., Priya, S. and Sudhakar, P., 2014, Genetic parameters for drought tolerant related traits in groundnut (*Arachis hypogaea* L.). *Electro. J. Plant Breed.*, **5**(4): 834-838.

Sibomana, C.I., Opiyo, A.M. and Aguyoh, J.N., 2015, Influence of soil moisture levels and packaging on postharvest qualities of tomato (*Solanum lycopersicum*). *Afr. J. Agric. Res*., **10**(12): 1392-1400.

Sibomana, I.C., Aguyoh, J.N. and Opiyo, A.M., 2013, Water stress affects growth and yield of container grown tomato (*Lycopersicon esculentum* Mill) plants. *GJBB*, **2**(4): 461-466.

Sim, S.C., Robbins, M.D., Van Deynze, A., Michel, A.P. and Francis, D.M., 2011, Population structure and genetic differentiation associated with breeding history and selection in tomato (*Solanum lycopersicum* L.). *Heredity*, **106**: 927-935.

Stich B., Melchinger, A.E., Frisch, M., Maurer, H.P., Heckenberger, M. and Reif, J.C., 2005, Linkage disequilibrium in European elite maize germplasm investigated with SSRs. *Theor. Appl. Genet*.**, 111**: 723-730.

Stoner, A.K., 1972, Merit, Red Rock and Potomac-tomato varieties adapted to mechanical harvesting. *USDA Prod. Res. Rep.,* 144.

Subhani, G.M., Abdullah, Ahmad, J., Anwar, J., Hussain, M. and Mahmood, A., 2015, Identification of drought tolerant genotypes of barley (*Hordeum vulgare* L.) through stress tolerance indices. *J. Anim. Plant Sci.,* **25**(3): 686-692.

Suji, K.K., Biji, K.R., Poornima, R., Prince, K.S., Amudha, K., Kavitha, S., Mankar, S. and Babu, R. C., 2012, Mapping QTLs for plant phenology and production traits using indica rice (*Oryza sativa* L.) lines adapted to rainfed environment. *Mol. Biotech***.,** **52**(2): 151-160.

Sukumaran, S. and Yu, J., 2014, Association mapping of genetic resources: achievements and future perspectives. *In*: Genomics of Plant Genetic Resources (Tuberosa, R., Graner, A. and Frison, E., (Eds.)). Springer Netherlands, pp: 207-235.

Suresh, K. and Savithramma, D.L, 2015, Genetic diversity and identification of drought tolerance analysis tomato germplasm. *Mysore J. Agric. Sci.,* **49**(2): 258-262.

Swamy, B.P.M. and Sarla, N., 2008, Yield-enhancing quantitative trait loci (QTLs) from wild species. *Biotech. Adv.,* **26**: 106-120.

Swarup, V., 2006, Vegetable science and technology in India. Kalyani Publishers, New Delhi, India, pp: 656.

Symonds, R.C., Kadirvel, P., Yen, J., Lin, J. and Peña, R.D.L., 2010, Genetic, physiological and molecular approaches to improve drought tolerance in tropical tomato. *Proc. SOL2010*, pp: 42.

Toorchi, M., Shashidhar, H. E. and Sridhara, H., 2006. Influence of the root system on grain yield and related characters in rain-fed lowland rice (*Oryza sativa* L.). *Pak. J. Bio. Sci.,* **9:** 2267-2272.

Torrecillas, A., Guillaumeb, C., Alarc, J.J. and Ruiz-Sgnchez, M.C., 1995, Water relations of two tomato species under water stress and recovery. *Plant Sci*., **105:** 169-176.

Tron, S., Bodner, G., Laio, F., Ridolfi, L. and Leitner, D., 2015, Can diversity in root architecture explain plant water use efficiency? A modeling study. *Ecological Modelling,* **312:** 200-210.

Turner, N.C., 1981, Techniques and experimental approaches for the measurement of plant water status. *Plant and Soil*, **58:** 339-366.

Udayakumar, M., Sheshshayee, M.S., Nataraj, K.N., Madhava, H.B., Devendra, R., Hussain, I.S.A. and Prasad, T.G., 1998, Why has breeding for water use efficiency not been successful? An analysis and alternate approach to exploit this trait for crop improvement. *Curr. Sci.*, **74**, 994-1000.

Ullah, M.Z., Hassan, L., Shahid, S.B. and Patwary, A.K., 2015, Variability and inter relationship studies in tomato (*Solanum lycopersicum* L.). *J. Bangladesh Agril. Univ*., **13**(1): 65-69.

Upadhyaya, H.D., 2005, Variability for drought resistance related traits in the mini core collection of peanut. *Crop Sci*., **45**: 1432-1440.

Van Berloo, R., Zhu, A., Ursem, R., Verbakel, H., Gort, G. and van Eeuwijk, F.A., 2008, Diversity and linkage disequilibrium analysis within a selected set of cultivated tomatoes. *Theor. Appl. Genet.,***117**: 89-101.

Van Bueren, L. E. T., Jones, S. S., Tamm, L., Murphy, K. M., Myers, J. R., and Messmer, M. M., 2011, The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: A review. *NJAS - Wageningen J. Life Sci.,* **58**: 193-205.

Whitt, S.R. and Buckler, E.S., 2003, Using natural allelic diversity to evaluate gene function. *In:* Plant functional genomics:methods and protocols, Grotewald, E. (Eds.), Humana Press, Clifton, NJ, USA, pp: 123-139.

Williams, C.E. and St. Clair, D.A., 1993, Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum. Genome*, **36**(3): 619-630.

Win, M.M., 2011, Evaluation on performance of chickpea (*Cicer arietinum* L.) genotypes under water stress condition. Ph. D. thesis submitted to Yezin Agric. Univ., pp: 142.

Wright, G.C., Rao R.C.N. and Farquhar, G.D., 1994, Water use efficiency and carbon isotope discrimination in peanut under water deficit conditions. *Crop Sci*., **34**: 92-97.

Xie, L., Ying, Y. and Ying, T., 2009, Classification of tomatoes with different genotypes by visible and short-wave near-infrared spectroscopy with least-squares support vector machines and other chemometrics. *J. Food Eng.*, **94**: 34-39.

Xiong, D., Chen, J., Yu, T., Gao, W., Ling, X., Li, Y., Peng, S. and Huang, J., 2015, SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics. *Scientific Reports*, **5**: 13389, doi: 10.1038/srep13389.

Xu, J., Ranc N., Munos, S., Rolland, S., Bouchet, J.B., Desplat, N., Le paslier, M.C., Liang, Y., Brunel, D. and Causse, M. 2013. Phenotypic diversity and association mapping for fruit quality traits in cultivated tomato and related species. *Theor. Appl. Genet*., **126**: 567-581.

Xu, X., Martin, B., Comstock, J.P., Vision, T.J., Tauer, C.G, Roman, B.Z., Pausch, C. and Knapp, S., 2008, Fine mapping a QTL for carbon isotope composition in tomato. *Theor. Appl. Genet*., **117**: 221-233.

Xue, Y., Warburton, M.L., Sawkins, M., Zhang, X., Setter, T., Xu, Y., Grudloyma, P., Gethi, J., Ribaut, J.M., Li, W., Zhang, X., Zheng, Y. and Yan, J., 2013, Genome wide association analysis for nine agronomic traits in maize under well watered and water stressed conditions. *Theor. Appl. Genet.*, **126**(10): 2587-2596.

Yan, S., Yan, C.J., Zheng, X.H., Yang, Y.C., Fang, Y.W., Tian, C.Y., Sun, Y.W., Cheng, Z.K. and Gu, M.H., 2008*, Rolled leaf 9*, encoding a GARP protein, regulates the leaf abaxial cell fate in rice. *Plant Mol. Biol.,* **68**: 239-250.

Zade, M.E. and Myandoab, M.P., 2012, Studying genetic diversity and selecting castor bean's most possible genotype based on drought tolerance indices. *Ann. Biol. Res.,* **3**(6): 3089-3092.

Zaheri, A. and Bahraminejad, S., 2012, Assessment of drought tolerance in oat (*Avena sativa*) genotypes. *Ann. Biol. Res.,* **3**(5): 2194-2201.

Zare, M., 2012, Evaluation of drought tolerance indices for the selection of Iranian barley (*Hordeum vulgare*) cultivars. *Afr. J. Biotech*., **11**(93): 15975-15981.

Zdravkovic, J., Jovanovic, Z., Djordjevic, M., Girek, Z., Zdravkovic, M. and Stikic, R., 2013, Application of stress susceptibility index for drought tolerance screening of tomato populations. *Genetika*, **45**(3): 679-689.

Zhang, J., Zhao, J., Liang, Y. and Zou, Z., 2016, Genome-wide association-mapping for fruit quality traits in tomato. *Euphytica*, **207**: 439-451.

Zhang, J., Zhao, J., Xu, Y., Liang, J., Chang, P., Yan, F., Li, M., Liang, Y. and Zou, Z., 2015, Genome-wide association mapping for tomato volatiles positively contributing to tomato flavor. *Front. Plant Sci.,* **6**(1042): 1-13.

Zhang, L., Cao, W., Zhang, S. and Zhou, Z., 2005, Characterizing root growth and spatial distribution in cotton. *Acta phytoecological Sci.,* **29**: 266-273.

Zhang, Z., Ersoz, E., Lai, C.Q., Todhunter, R.J., Tiwari, H.K., Gore, M.A., Bradbury, P.J., Yu, J., Arnett, D.K., Ordovas, J.M. and Buckler, E.S., 2010b, Mixed linear model approach adapted for genome-wide association studies. *Nat. Genet.,* **42**: 355-360.

Zhao, K., Tung, C.W., Eizenga, G.C., Wright, M.H., Ali, M.L., Price, A.H., Norton, G.J., Islam, M.R., Reynolds, A., Mezey, J., McClung, A.M., Bustamante, C.D. and McCouch, S.R., 2011, Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. NATURE COMMUNICATIONS, **2**(467), pp: 10.

Zhou, R., Wu, Z., Cao X. and Jiang, F.L., 2015, Genetic diversity of cultivated and wild tomatoes revealed by morphological traits and SSR markers.*Genet.Mol. Res.*, **14**(4): 13868-13879.

Zhou, X. and Stephens, M., 2012, Genome-wide efficient mixed model analysis for association studies. *Nat. Genet*., **44**: 821-824.

Zhu, C. and Yu, J., 2009, Nonmetric multidimensional scaling corrects for population structure in association mapping with different sample types. *Genetics*, **182**:875-88.

Zhu, C., Gore, M., Buckler, E.S. and Yu, J., 2008, Status and prospects of association mapping in plants. *Plant Genome*., **1**(1): 5-20.

Zhu, J., Ingram, P.A, Benfey, P.N. and Elich, T., 2011, From lab to field, new approaches to phenotyping root system architecture. *Curr. Opin. Plant Biol.,* **14**: 310-317.

Zondervan, K.T. and Cardon, L.R., 2004, The complex interplay among factors that influence allelic association. *Nat. Rev. Genet.,* **5:** 89-100.