# An assessment of potential feedstock crops for Bio-diesel production in the United Arab Emirates

**THESIS**

Submitted in partial fulfilment
of the requirements for the degree of
**DOCTOR OF PHILOSOPHY**

by

**KIRAN MENON**

ID. No. 2011PHXF003U

Under the Supervision of

**Prof. Neeru Sood**
**&**
**Dr. Nanduri. K. Rao**


**BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE**
**PILANI- DUBAI CAMPUS**
**2016**

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# CERTIFICATE

This is to certify that the thesis entitled **"An assessment of potential feedstock crops for Bio-diesel production in the United Arab Emirates"** was submitted by **KIRAN MENON** ID No **2011PHXF003U** for award of Ph. D. Degree of the Institute embodies original work done by him/her under my supervision.

Signature in full of the Supervisor: _____

Name in capital block letters: NEERU SOOD

Designation: Professor, Associate Dean- Academic Research Division,

BITS Pilani- Dubai Campus

Signature in full of the Co- Supervisor: _____

Name in capital block letters: NANDURI. K. RAO

Designation: Genetic Resources Scientist, International Center for Biosaline Agriculture, Dubai, UAE

Date: _____

# Acknowledgements

*I would like to thank my supervisor Prof. Neeru Sood for being my guide, mentor and unfailing champion through the duration of this thesis. Thank you for helping me laugh through the toughest times and stay grounded through the best, I wouldn't have made it through with my sanity if it weren't for you. My gratitude towards my co-supervisor Dr. Nanduri. K. Rao cannot be summed up in a few words, but for his patience with my shortcomings, knowledge of the subject, dedication to my cause and guidance in my work I will be eternally grateful. Both my supervisors I thank for being the best kind of leader/mentor, never feeling the need to criticize or disparage, but guiding through example and encouragement. I thank my doctoral advisor Dr. Ramachandran for his support, for always appreciating my efforts and for always prioritizing my interests and having faith in my capabilities. I thank my advisor Dr. Trupti Gokhale for her excellent technical guidance, for keeping me on my toes and making sure I dot my i's and cross my t's. Thank you for making me strive to be a better researcher and student. I take this opportunity to also express my sincere gratitude to the laboratory staff for their support in my work, the project students who assisted me in the laboratory, and the other PhD Scholars in the institute for their companionship and encouragement through these past years. My thanks also to the directors of BITS Pilani-Dubai Campus, Dr. Mittal and Dr. Saha for facilitating and supporting my work.*

*I thank the management of the International Center for Biosaline Agriculture, Dubai, UAE for providing the funds and facilities for the field trials and some of the analysis carried out in this work.*

*Thank you Amma and Achan for a lifetime of love and nurture, for your blind faith in me, and for never complaining when I don't thank you enough. Unni and Mya, for having the kind of high opinion of me that I do not truly deserve, and which inspires me to be better. And speaking of that which I do not deserve, thank you Santosh, for your unconditional support through this work. If not for you, these pages would never have been written.*

*Kiran Menon*

## Abstract

With increasingly obvious effects of global warming, nations across the world, including the UAE are looking for green energy sources to supplement or replace their fuel needs. In this thesis we compared three candidate bio-diesel feedstock crops, *Ricinus communis*, *Citrullus colocynthis* and *Brassica juncea* in terms of their salinity tolerance, oil yield and oil quality when cultivated in the arid and saline conditions of the UAE. Eleven accessions of *Ricinus communis*, thirty-seven accessions of *Citrullus colocynthis* and five accessions of *Brassica juncea* were studied in field trials with triplicates conducted at the International Center for Biosaline Agriculture (ICBA) research station in Dubai over a single season each. Three salinity treatments, at 5, 10 and 15 dS $m^{-1}$ were applied and response to salinity was observed by analyzing $K^+/Na^+$ ratios in leaf tissue using ICP-OES. Seed and oil yield was recorded and solvent (n-hexane) extracted oil was analyzed for free fatty acid content, saponification value and kinematic viscosity in order to determine suitability of oil for bio-diesel production. The data obtained was statistically analyzed using methods such as ANOVA, Pearson correlation matrices, Principal Components Analysis and Heirarchical Clustering. Among the crops studied, no significantly salt tolerant accession was found. Extrapolated seed yield of *Ricinus communis* was between 1.5 and 3 tonnes/ha in the control treatment, and between 1.8 and 2.3 tonnes/ha in the 5 dS $m^{-1}$ treatment, which was not different to a statistically significant degree. The lack of significant effect on inflorescence characteristics and seed yield at 5 dS $m^{-1}$ in spite of sodium ion accumulation was an interesting observation. Seed yield and plant growth were both severely effected in the higher salinity treatments. *Ricinus communis* was found to be a suitable feedstock oilseed crop for the region when irrigated with water at low levels of salinity on the basis of oil yield (up to 1 tonne/ha), and established agricultural practices. The viscosity of the oil from this crop was however very high, and this is a drawback for its' use as bio-diesel feedstock without blending. *Citrullus colocynthis* accessions studied for salinity tolerance were extremely sensitive to salinity. The crop had very high seed yields per plant (up to 374 gms) under irrigated conditions, but has to undergo selection and improvement before commercial-scale cultivation is feasible. Quality of oil from most of the studied accessions of *Citrullus colocynthis* was found suitable for bio-diesel production, and the plant extracts have medicinal properties that can make its cultivation economically feasible. Dormancy in *Citrullus colocynthis* seeds was also studied and a relatively effective pre-treatment was identified. The *Brassica juncea* accessions studied were not tolerant to salinity at 15 dS $m^{-1}$, and were lower yielding (0.19 to 0.62 tonnes/ha, extrapolated), but yield increased by almost two-fold in the Treated Waste Water (TWW) treatment (0.57 to 1.1 tonnes/ha). *Brassica juncea* seed oil from all five accessions was found suitable for bio-diesel production. The results of this study sets a platform for exploring the potential of bio-diesel feedstock in the region.

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## Abbreviations & Symbols

| | |
|---|---|
| UAE | United Arab Emirates |
| ICBA | International Center for Biosaline Agriculture |
| GHG | Greenhouse gas |
| MENA | Middle East and North Africa |
| UK | United Kingdom |
| FFA | Free fatty acid |
| CFPP | Cold filter plugging point |
| FAO | Food and Agriculture Organization of the United Nations |
| US/ USA | United States of America |
| EOECD | European Organization for Economic Cooperation and Development |
| GCC | Gulf Cooperation Council |
| UV | Ultraviolet |
| FDA | Food and Drug Administration |
| EC | Electrical conductivity |
| ECe | Electrical conductivity of soil |
| ECw | Electrical conductivity of water |
| SSI | Stress susceptibility index |
| STI | Stress tolerance index |
| GMP | Geometric Mean Productivity |
| TOL | Stress Tolerance |
| SOD | Superoxide dismutase |
| CAT | Catalase |
| GR/ GSR | Glutathione reductase |

| | |
|---|---|
| APX | Ascorbate peroxidase |
| DNA | Deoxy ribonucleic acid |
| ROS | Reactive oxygen species |
| ID | Identity |
| Fig. | Figure |
| GRIN | Genetic Resources Information Network |
| USDA | United States Department of Agriculture |
| ASH | Ascorbate |
| MDA | Monodehydroascorbate |
| MDAR | Monodehydroascorbate reductase |
| mRNA | messenger ribonucleic acid |
| GSSG | Glutathione disulphide |
| GSH | Glutathione |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| GPS | Global positioning system |
| RCBD | Randomized Complete Block Design |
| TWW | Treated waste water |
| NIH | National Institutes of Health, US |
| SLW | Specific Leaf weight |
| FYM | Farmyard manure |
| ICP- OES | Inductively coupled plasma optical emission spectrometry |
| $CO_2$ | Carbon di oxide |
| CO | Carbon monoxide |
| HC | Hydrocarbon |
| N | Nitrogen |
| Ca | Calcium |
| Cd | Cadmium |

| | |
|---|---|
| Fe | Iron |
| NaCl | Sodium Chloride |
| $Na^+$ | Sodium cation |
| $K^+$ | Potassium cation |
| P | Phosphorous |
| Zn | Zinc |
| Cl | Chloride |
| $H_2O_2$ | Hydrogen peroxide |
| $H_2O$ | Water |
| $O_2$ | Oxygen |
| $O_2^-$ | Superoxide |
| $Cu^+$ | cuprous ion |
| $Cu^{2+}$ | cupric ion |
| $CaCO_3$ | Calcium carbonate |
| EDTA | Ethylene diamine tetra acetic acid |
| Mg | Magnesium |
| $HNO_3$ | Nitric acid |
| KOH | Potassium hydroxide |
| PVPP | Polyvinylpolypyrrolidone |
| HCl | Hydrochloric acid |
| Tris | Tris(hydroxymethyl)aminomethane base |
| % | Percentage |
| °C | degrees Celsius |
| Km | Kilometer |
| M | Meter |
| Cm | Centimeter |
| Mm | Millimeter |

| | |
|---|---|
| Nm | Nanometer |
| Dm | Decimeter |
| Mg | Megagrams |
| Kg | Kilogram |
| Mg | Milligram |
| Ha | Hectare |
| dS | deci-siemens |
| MPa | Mega pascal |
| pH | potential of hydrogen |
| M | Molar |
| mM | Millimolar |
| µM | Micromolar |
| °N | degrees North |
| °E | degrees East |
| dS $m^{-1}$ | deci - siemens/ meter |
| Meq | Milliequivalent |
| L | Liter |
| Ml | Milliliter |
| µl | Microliter |
| Ppm | parts per million |
| N | Normality |
| EU | Enzyme unit |
| © | Copyright |
| ™ | Trademark |

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# CHAPTER 1
# INTRODUCTION

As of 2012, according to the World Resources Institute, the UAE emits 216.04 metric tonnes of $CO_2$ equivalent annually. The UAE's per capita GHG emission in 2012 (latest available data) is approximately 20.4 metric tonnes, which is about four times the global average and greater than the United States of America, UK, India and China (World Bank). This highlights the need to shift to alternative, greener fuel sources. UAE is currently exploring cleaner and greener energy alternatives with various government sponsored initiatives as part of the global movement to mitigate climate change due to fuel emissions. Biofuels have the added advantage of being plant derived, and thus an indirect result of carbon fixation. This can help mitigate the effects of carbon di oxide emissions, if production and use of these fuels is planned and managed appropriately. Bioenergy in the form of bio-ethanol from ligno-cellulosic biomasses is being investigated by research organizations such as the Masdar Institute in Abu Dhabi. There has however been little research on bio-diesel and potential feedstock crops for this region in spite of the advantage that bio-diesel and bio-diesel blends can be utilized in existing car engines without modifications. The high levels of soil and irrigation water salinity, in addition to the harsh temperatures, limit the options for candidate crops which can be successfully grown for bio-diesel feedstock production. Edible oils used as bio-diesel feedstock were not a focus of this study as they could contribute to the food vs fuel conflict. This project was thus designed to identify high yielding, non-food oilseed crops that can be cultivated in the region to provide bio-diesel feedstock. With this objective in mind, an extensive literature survey was carried out to identify candidate species for the study, to frame research methodology and to reach logical inferences from the data collected during the study with respect to the suitability of each candidate crop as bio-diesel feedstock to be cultivated in the region.

The research project detailed and described in this thesis was carried out in collaboration with the International Center for Biosaline Agriculture (ICBA), Dubai, United Arab Emirates (UAE), which is an international, non-profit organization that aims to strengthen agricultural productivity in marginal and saline environments through identifying, testing and facilitating access to sustainable solutions for food, nutrition and income security. ICBA focuses on identifying crops with the potential for cultivation in the saline soils and arid environment in the UAE and several other countries of the Middle East and North Africa (MENA) region. The following section is a comprehensive overview of the survey of literature carried out for the purpose of this thesis.

## 1.1. Why Bio-diesel?

To be a viable alternative, a biofuel should provide a net energy gain, provide environmental benefits, be economically competitive to produce and sell, and be producible in large quantities without effecting food supplies. Whether or not alternative fuels provide benefits over the fossil fuels they will replace can be determined by a thorough accounting of the direct and indirect inputs and outputs in terms of yields, commodity and fuel prices, farm energy and agrichemical inputs, production plant efficiencies, coproduct production, greenhouse gas (GHG) emissions, and other environmental effects for their full production and use life cycles. An extensive study and comparison of corn grain ethanol and soybean bio-diesel in this manner was carried out by Hill *et al.* [1]. They found that compared to the energy invested in its production, ethanol yields 25% more energy, whereas bio-diesel yields 93% more. Bio-diesel releases just 1.0%, 8.3%, and 13% of the agricultural nitrogen, phosphorus, and pesticide pollutants, respectively, per net energy gain when compared with the effluents from Ethanol production. Relative to the fossil fuels they displace, greenhouse gas emissions are reduced 12% by the production and combustion of ethanol and 41% by bio-diesel. Bio-diesel also releases less air pollutants per net energy gain than ethanol. The advantages of bio-diesel production over ethanol can be attributed to lower agricultural inputs and more efficient conversion of feedstocks to fuel. In their study, Hill *et al.* also state the following example: if all U.S. corn and soybean production were dedicated to biofuel production, it would meet only 12% of gasoline demand and 6% of diesel demand of the country, indicating that neither biofuel can replace much petroleum without impacting food supplies. High production costs makes biofuels unprofitable without subsidies, but the environmental advantages are sufficient to merit subsidy from Governments looking to enhance use of Green energy.

## 1.2. Bio-diesel production and characteristics

Bio-diesel is monoalkyl esters of long chain fatty acids derived from renewable feed stock like vegetable oils and animal fats [2]. It is produced by a transesterification reaction in which, oil or fat is reacted with a monohydric alcohol such as methanol in presence of a catalyst. The process of transesterification is affected by factors such as reaction conditions, molar ratio of alcohol to oil, type of alcohol, type and amount of catalysts, reaction time and temperature and purity of reactants [3].

Transesterification or alcoholysis is the displacement of one alcohol (glycerol from fats in this case) from an ester by another alcohol in a process similar to hydrolysis, except that alcohol is used instead of water [4]. This process has been widely used to reduce the high viscosity of triglycerides.

$$RCOOR^1 + R^2OH \xleftarrow{\text{Catalyst}} RCOOR^2 + R^1OH$$

If methanol is used in this process it is called methanolysis. Transesterification is a reversible reactions and proceeds essentially by mixing the reactants. However, the presence of a catalyst (a strong acid or base) accelerates the conversion.

Transesterification of triglycerides produce fatty acid alkyl esters and glycerol. The glycerol layer settles down at the bottom of the reaction vessel. Diglycerides and monoglycerides are the intermediates in this process. These reactions are reversible and a little excess of alcohol is used to shift the equilibrium towards the formation of esters. In alkali-catalyzed transesterification, the first step involves the attack of the alkoxide ion on the carbonyl carbon of the triglyceride molecule, which results in the formation of a tetrahedral intermediate. The reaction of this intermediate with an alcohol produces the alkoxide ion in the second step. In the last step the rearrangement of the tetrahedral intermediate gives rise to an ester and a diglyceride [5].

Transesterification can also be catalyzed by Brownsted acids, preferably by sulfonic and sulfuric acids. These catalysts give very high yields in alkyl esters but these reactions are slow, typically needing temperatures above 100 °C and more than three hours to complete the conversion [6]. The protonation of carbonyl group of the ester leads to the carbocation, which after a nucleophilic attack of the alcohol produces a tetrahedral intermediate. This intermediate eliminates glycerol to form a new ester and to regenerate the catalyst.

The free fatty acid and moisture content are key parameters for determining the viability of the vegetable oil transesterification process. To carry the base catalyzed reaction to completion a free fatty acid (FFA) value lower than 3% is needed. The higher the acidity of the oil, smaller is the conversion efficiency [7]. The addition of more sodium hydroxide catalyst can compensate for higher acidity, but the resulting soap causes an increase in viscosity or formation of gels that interfere in the reaction as well as with separation of glycerol [8]. The methoxide and hydroxide of sodium or potassium should be maintained in anhydrous state. Prolonged contact with air will diminish the effectiveness of these catalysts through interaction with moisture and carbon dioxide.

A two-step esterification process is required for feed stocks with high free fatty acid content. Initially the FFA of these is converted to fatty acid methyl esters by an acid catalyzed pretreatment and in the second step transesterification is completed by using alkaline catalyst to complete the reaction [9].

Specifications for bio-diesel quality vary between countries and have been elaborated by Meher *et al*. [3] as described in this section. Among the general parameters for bio-diesel, the viscosity controls the characteristics of the diesel injection. The viscosity of fatty acid methyl esters has to be within an acceptable level to avoid negative impacts on fuel injector system performance. Therefore, the viscosity specifications proposed are nearly same as that of the diesel fuel. Flash point of a fuel is the temperature at which it will ignite when exposed to a flame or spark. The flash point of bio-diesel is higher than petrodiesel, which makes it safer for transportation. Cold filter plugging point (CFPP) of a fuel reflects its cold weather performance. At low operating temperature fuel may thicken and might not flow properly affecting the performance of fuel lines, fuel pumps and injectors. CFPP defines the fuels limit of filterability, having a better correlation than cloud point for bio-diesel as well as petrodiesel. Normally either pour point or CFFP are specified. Pour point is the lowest temperature at which the oil specimen can still be moved. French and Italian bio-diesel specifications specify pour point where as others specify CFPP. Cetane number is indicative of its ignition characteristics. The cetane number measures how easily ignition occurs and the smoothness of combustion. Higher the cetane number, better it is in its ignition properties. Cetane number affects a number of engine performance parameters like combustion, stability, driveability, white smoke, noise and emissions of CO and HC. Bio-diesel has higher cetane number than conventional diesel fuel, which results in higher combustion efficiency. Neutralization number is specified to ensure proper ageing properties of the fuel and/or a good manufacturing process. It reflects the presence of free fatty acids or acids used in manufacture of bio-diesel and also the degradation of bio-diesel due to thermal effects. Carbon residue of the fuel is indicative of carbon depositing tendencies of the fuel. Conradsons Carbon Residue for bio-diesel is more important than that in diesel fuel because it shows a high correlation with presence of free fatty acids, glycerides, soaps, polymers, higher unsaturated fatty acids and inorganic impurities. The presence of high level of alcohol in bio-diesel cause accelerated deterioration of natural rubber seals and gaskets. Therefore, control of alcohol content is required. Bio-diesel fuel mainly consists of fatty acid alkyl esters and its quantities are specified according to the specifications of various countries. The presence of mono- di- and tri-glycerides cause engine problems like fuel filter plugging affecting the fuel properties and are specified in most of the bio-diesel standards. Saponification value is the milligrams of KOH required to saponify one

gram of fat, and depends entirely on the fatty acid composition of the oil. Variation in saponification value can thus indicate differences in fatty acid composition between oils from different sources. The saponification value is a reliable enough indicator that it can be predicted from the fatty acid composition of an oil [10]. $K^+$

From the specifications mentioned above, it is apparent that in terms of feedstock oil quality, free fatty acid content and viscosity are very important characteristics to be considered when determining the suitability of an oil for bio-diesel production.

With increasing interest and use, it is important to set standards for fuel properties and quality for the successful commercialization of bio-diesel. Accordingly, bio-diesel standards have been established or are being developed in various countries and regions around the world, including the United States ASTM D 6751 (Table 1), Europe EN 14214 (Table 2, Brazil, South Africa, Australia and elsewhere. It must be noted that these standards are for the finished bio-diesel and not for the feedstock oil used. ASTM International is an international standards organization that develops and publishes voluntary consensus technical standards for a wide range of materials, products, systems, and services. European Standards (ENs) are documents that have been ratified by one of the three European Standardization Organizations (ESOs), CEN (European committee for standardization), CENELEC or ETSI; recognized as competent in the area of voluntary technical standardization as for the EU Regulation 1025/2012. Bio-diesel is often used blended with petro-diesel and these blends are designated by notations such as B100 (100% bio-diesel), B90 (90% bio-diesel, 10 percent petro-diesel), and so on [11].

**Table 1.** ASTM Bio-diesel Standards D6751 from Knothe, 2006 **[12]**

**ASTM Biodiesel Standard D 6751[a]**

| Property | Test method | Limits | Units |
|---|---|---|---|
| Flash point (closed cup) | D 93 | 130.0 min | °C |
| Water and sediment | D 2709 | 0.050 max | % volume |
| Kinematic viscosity, 40°C | D 445 | 1.9–6.0 | $mm^2/s$ |
| Sulfated ash | D 874 | 0.020 max | % mass |
| Sulfur | D 5453 | 0.0015 max (S15) | |
| | | 0.05 max (S500) | % mass |
| (ppm) | | | |
| Copper strip corrosion | D 130 | No. 3 max | |
| Cetane number | D 613 | 47 min | |
| Cloud point | D 2500 | Report | °C |
| Carbon residue | D 4530 | 0.050 max | % mass |
| Acid number | D 664 | 0.50 max | mg KOH/g |
| Free glycerin | D 6584 | 0.020 | % mass |
| Total glycerin | D 6584 | 0.240 | % mass |
| Phosphorus content | D 4951 | 0.001 max | % mass |
| Sodium/potassium | UOP 391 | 5 max. combined | ppm |
| Distillation temperature, atmospheric equivalent temperature, 90% recovered | D 1160 | 360 max | °C |

[a]The limits are for Grade S15 and Grade S500 biodiesel, with S15 and S500 referring to maximum sulfur specifications (in ppm).

**Table 2.** European bio-diesel standards EN 14214 from Knothe, 2006 **[12]**

**European Biodiesel Standards EN 14214 for Vehicle Use and EN 14213 for Heating Oil Use**

| Property | Test method | Limits EN 14214 | Limits EN 14213 | Unit |
|---|---|---|---|---|
| Ester content | EN 14103 | 96.5 min | 96.5 min | % (mol/mol) |
| Density; 15°C | EN ISO 3675, EN ISO 12185 | 860–900 | 860–900 | $kg/m^3$ |
| Viscosity; 40°C | EN ISO 3104, ISO 3105 | 3.5–5.0 | 3.5–5.0 | $mm^2/s$ |
| Flash point | EN ISO 3679 | 120 min | 120 min | °C |
| Sulfur content | EN ISO 20846; EN ISO 20884 | 10.0 max | 10.0 max | mg/kg |
| Carbon residue (10% distillation residue) | EN ISO 10370 | 0.30 max | 0.30 max | % (mol/mol) |
| Cetane number | EN ISO 5165 | 51 min | — | |
| Sulfated ash | ISO 3987 | 0.02 max | 0.02 max | % (mol/mol) |
| Water content | EN ISO 12937 | 500 max | 500 max | mg/kg |
| Total contamination | EN 12662 | 24 max | 24 max | mg/kg |
| Copper strip corrosion (3h, 50°C) | EN ISO 2160 | 1 | — | degree of corrosion |
| Oxidative stability, 110°C | EN 14112 | 6.0 min | 4.0 h | h |
| Acid value | EN 14104 | 0.50 max | 0.50 max | mg KOH/g |
| Iodine value | EN 14111 | 120 max | 130 max | g $I_2$/100 g |
| Linolenic acid content | EN 14103 | 12.0 max | — | % (mol/mol) |
| Content of FAME with ≥4 double bonds | | 1 max | 1 max | % (mol/mol) |
| Methanol content | EN 14110 | 0.20 max | — | % (mol/mol) |
| MAG content | EN 14105 | 0.80 max | 0.80 max | % (mol/mol) |
| DAG content | EN 14105 | 0.20 max | 0.20 max | % (mol/mol) |
| TAG content | EN 14105 | 0.20 max | 0.20 max | % (mol/mol) |
| Free glycerine | EN 14105, EN 14106 | 0.020 max | 0.02 max | % (mol/mol) |
| Total glycerine | EN 14105 | 0.25 max | — | % (mol/mol) |
| Group I metals (Na + K) | EN 14108, EN 14109 | 5.0 max | — | mg/kg |
| Group II (Ca + Mg) | prEN 14538 | 5.0 max | — | mg/kg |
| Phosphorus content | EN 14107 | 10.0 max | — | mg/kg |
| Cold filter plugging point | EN 116 | — | | °C |
| Pour point | ISO 3016 | — | 0 max | °C |
| Heating value | DIN 51900-1<br>DIN 51900-2 | | | |
| | DIN 51900-2 | — | 35 min | MJ/kg |

## 1.3. Agricultural conditions in the UAE

The climate in the United Arab Emirates (UAE), which is part of the Arabian Peninsula, is categorized as hyper-arid, with <100 mm annual rainfall and relatively higher mean precipitation rates in the north-eastern regions [13]. Salinity is a concern that effects many irrigated crops and is attributable to salt water intrusion from the Gulf, and due to abstraction and use of ground water [14]. Rainfall in the Arabian Peninsula is scarce and erratic at best with annual precipitation ranging from 50 to 100 mm. Temperatures are high, reaching 50˚C in peak summers, and relative humidity is also high. Rate of surface water evaporation far exceeds rainfall at 2000 mm/ year. Excessive use of underground water has resulted in increased salinity [15]. Because of the problem with soil salinity, according to FAO statistics, less than 4 million hectares of land was cultivated with irrigated crops in the entire region in 2002, where in and the Kingdom of Saudi Arabia and Oman accounts for most of this cultivation [16].

According to the last survey conducted in 2011, 105257 hectares of land was cultivated in the UAE [17]. Water quality and scarcity are major limitations with respect to agriculture in the UAE. Increasing population, industrial activities and irrigation is putting greater stress on already scant water resources as aquifers are not recharged at the same rate as they are drawn upon. Irrigation

accounts for 70% of groundwater use [18]. Only about 3% of total groundwater is fresh, and very little of this is accessible. Desalination plants have been established to meet the shortage from conventional water resources. In addition, wastewater treatment plants have been set up to further reduce the use of groundwater and as an alternative to the high energy and fiscal costs of desalination. Desalination, in addition to being expensive, may also contribute to the groundwater salinity problem due to the disposal of waste brine in unlined ponds, which in turn reaches groundwater and increases concentration of salts and heavy metals in aquifers [19]. As of 2009, groundwater supplied 51% of water needs (including irrigation), desalinated water contributed 40% (including most potable water), and treated waste water accounted for about 9% (mainly for landscaping and industrial uses). Most waste water treatment plants in the UAE use activated sludge processes with tertiary treatment consisting of sand filtration and chlorination. To ensure water security for the future, a National Water Conservation Strategy has been launched in 2010 focused largely on water demand management (Ministry of Environment and Water, UAE) [17].

There is very limited published information regarding the growth characteristics of crops under conditions such as those of the UAE. In addition to stresses such as heat and salinity, the absence of improved cultivars and trained manpower are also factors that contribute to the lack of agricultural progress in this region. The soils here are sandy and are short of organic matter necessary for the growth of most crops on a commercial scale. All these factors result in a need to find crops suited to local conditions and marginal lands that can be cultivated in the UAE.

### 1.4. Cultivation on marginal lands

Increasing world populations, along with food and water scarcity encourage us to reserve the fertile arable lands across the planet for the production of food. In the past, in order to produce more crop the simple solution was to cultivate more land. This is no longer possible due to the increased demand of land for other purposes. For example, while grain production has doubled in the last 50 years, arable agricultural land has only increased by approximately 9% across the world [20]. In recent decades, agricultural land that was formerly productive has been lost to urbanization and other human uses, as well as to desertification, salinization, soil erosion, and other consequences of unsustainable land management. It is likely that there will be further losses due to climate change [21]. Not all areas that have the same kind of conditions and climate have the same productivity. The difference between ideally achievable yields and the realized productivity due to poor genetic resources, lack of irrigation facilities, soil fertility and agricultural management is called the yield gap. It is essential to develop strategies to improve productivity from currently

arable lands. But in the long run, it could be even more important to develop the ability to grow crops in areas that are currently unsuitable.

More and more agricultural research is now focused on the use of marginal lands for the cultivation of non-essential food crops and non-food crops, mainly bioenergy crops [22]. Inedible oilseed crops fall under this category. Vegetable oil is not just required for cooking, but for many other industrial applications such as the production of bio-diesel, pharmaceuticals, soaps, cosmetics and synthetics [23-25]. The United States of America has taken initiative and is one of the frontrunners in the effort to put more marginal lands into the production of bioenergy crops [23]. Using land cultivated with food crops for bio-energy production could decrease the production and increase the prices of food commodities. Clearing forest land for new bioenergy crops on the other hand, could result in $CO_2$ emissions from terrestrial carbon pools that are much greater than any greenhouse gas benefits provided by biofuels [24]. The use of non-cultivated, arid lands does not contribute to either of these problems. It could also have added advantages such as carbon sequestration, which could mitigate the effects of fossil fuel emission in the region to a small extent [25]. If not beneficial, cultivation, harvesting and oil production could at least be carried out in such a way that carbon neutrality is achieved. This approach is however, not without fault, as it could result in increased polluted run-off, adding nitrates from fertilizers to groundwater and have other ecological impacts that are as yet unidentified [26]. Researchers are currently exploring the possible merits and de-merits of this approach and trying to determine in which direction the scale tips [27].

### 1.5.**Bio-diesel feedstock crops for the UAE**

Fossil fuel is a non-renewable resource but the worldwide demand for fossil fuel energy constantly increases with increasing population and industrial development. The harmful effects of greenhouse gas emissions resulting from the combustion of fossil fuels have also been brought to light and the changes in the environment and climate have become all too obvious and cannot be ignored any longer. Bio-diesel is more prone to oxidation than petroleum based fuels but is the lowest emission diesel fuel [28]. It is derived from renewable resources, is biodegradable and non-toxic [29]. The idea of using vegetable oil to run diesel engines is not a novel one and has been under debate from the time the first diesel engine was invented in the 1890s. Bio-diesel by definition is a diesel fuel derived from vegetable oils or animal fats by the trans-esterification of fatty acids into alkyl esters and glycerol using an alcohol (usually methanol or ethanol) for use in compression ignition engines [30]. Bio-diesel can be used to run most cars manufactured post-1992, which do not contain plastic parts that may be affected by the bio-diesel. Blends (2%-99%)

of bio-diesel with petro-diesel and 100% bio-diesel are widely used in cars (mainly in countries of the European Union). Virgin oil feedstock such as rapeseed (in Europe) and soya (in the US) are most widely used, in addition to other plant oils such as mustard, sunflower, safflower and palm. According to data projected by the European Organization for Economic Cooperation and Development (EOECD), vegetable oils are the main source of oil feedstock in the bio-diesel industry [31]. It will most likely remain so in spite of the development of strategies and technology for the use of cellulosic biomass and non-food crops, as production of these has not yet been established as viable or sustainable on an industrial scale. The use of arable land and vegetable oils for bio-diesel production increases the already significant threat to global food security, hence the focus has shifted to non-food crops such as *Jatropha curcas.* Jatropha's main selling point is that it does not compete with food crops because it yields well on marginal land. But a review of the published literature suggests that there is still relatively little convincing data that Jatropha can produce economically consistent yields even on good land with fertilizers and irrigation [32]. Hence, there is a need for identifying plants which can be cultivated in non-arable lands (such as those of the UAE).

The UAE is rich in fossil fuel resources and is a major exporter of petroleum fuels [33]. There is an increasing demand for petroleum fuels to meet worldwide electricity and transportation needs. These resources are however non-renewable in nature and there is a growing necessity to make a shift to alternative fuels such as bio-diesel. Bio-diesel blends are used commercially in some countries such as those of the European Union [34] and this has set an example to the rest of the world to decrease the pressure on nature's resources, and to decrease pollution. Saudi Arabia accounts for about 13 per cent of global oil output, and the conventional wisdom has been that the country's proven reserves would last at least a century [33]. However, a new research note from Citibank says Saudi Arabia risks becoming an oil importer within 20 years [38-39]. This could be true for other GCC countries, including the UAE.

Bio-diesel feedstock crops are being cultivated in many regions of the world, with the USA and countries of the European Union and Latin America (mainly Brazil) leading the way in this area [31]. The most common bio-diesel feedstock crops currently in use like soy and corn are edible vegetable oils. Bio-diesel produced from these crops is used as fuel (mostly as blends with petroleum diesel) in internal combustion engines. The result of using these crops for bio-diesel production is that the prices of edible oils increase in the market, putting a strain on the already fragile food security issue [30]. Another factor that affects food security is the use of arable lands to grow these crops instead of necessary food crops. For example, if all the arable land in the United States of America were used for growing food crops the harvests would still not be

sufficient to meet just that country's food needs. It is thus important to use cultivable land to produce food [35]. Due to these reasons there is now a shift in emphasis from the use of first generation biofuel crops (food crops) to second generation biofuels produced from ligno-cellulosic feedstocks [36]. The use of non-food crops, grown on marginal lands is a solution intermediate to these two alternatives. Most of the world depends on petroleum from the Middle East for fuel. The use of bio-fuels has the added advantage of providing them with a measure of fuel security. Another important consideration is the decrease in greenhouse gas emissions as a result of using bio-fuels. This is of great importance because of the observable climatic changes and increase in UV radiation we are witness to as a result of greenhouse gas emissions over the past decades [37].

While the UAE's economic growth in the past decades has been significant there has also been no check on the ecological and environmental impacts of this growth. With growing concerns regarding these issues there are now a few initiatives on the part of some government bodies and private institutes like Masdar in Abu Dhabi to address these environmental problems [38]. The possibility of growing bio-diesel feedstock crops or even the use of bio-diesel blends as fuel has however not been studied in the UAE in spite of the resulting benefits to the environment. This is mainly due to the abundance of fossil fuel reserves in the region. Fossil fuels however, being non-renewable, the time has now come to look to the future and seriously consider the use of alternative fuels, as discussed above (e.g. Saudi Arabia) [37].

General information regarding cultivation of bioenergy species in the arid climate, dry soils and saline irrigation waters is also scarce, and practically non-existent. The lack of such information can be remedied by research in this field, as proposed in this work. The soils in the region are not used for commercial cultivation, with the exception of the date palm, which adapts well in the hot and dry conditions of the region [15]. The countries of the Middle East such as Qatar, UAE, Bahrain and Kuwait have the worst per capita carbon emission figures in relation to per capita income [39]. The UAE alone emits 30-35 tonnes of $CO_2$ per capita annually, around 7 times the global average [40]. There is however little concern about the consequences of these figures within the region. By growing crops that require minimal water and that can survive the arid conditions of the region, the effect of a fraction of these emissions could possibly be remedied.

Since the land cannot be used for the economic production of conventional food crops due to the various abiotic stresses, it would be ideal if it can be used to grow non-food crops with economic and environmental benefits. If such plants can be cultivated on a commercial scale in this region it can supplement the already thriving fuel export industry while simultaneously improving

environmental quality and reducing desertification/erosion which is a matter of serious concern in this region.

### 1.6.Oilseed crop candidates

Keeping the above points in mind we have propose the assessment of the potential of growing oilseed plants as bio-diesel feedstock in this region. The candidate crops had to meet the criteria of being high yielding, inedible, salt-tolerant/resistant, have a growing period suitable for the scale of this study, and be suited to growth in marginal lands. As mentioned in the previous section, corn, rapeseed and soybean oil are the most popular feedstocks for bio-diesel production currently, but were all ruled out for our study in lieu of being food-crops. Recycling waste oils from restaurants and food industries is another option, but involves expensive additional pre-processing steps due to the variable composition of feedstock when compared to pure vegetable oils of consistent quality [41]. Oil yielding microalgae are another feedstock source that has gained popularity in recent times due to their high yield, possible less land requirement and low cultivation times when compared to terrestrial crops. These results were however, based on pilot-scale/ laboratory studies and it has proven difficult to replicate these results upon large scale cultivation. The biggest challenges with respect to large scale cultivation of microalgae as bio-diesel feedstock were found to be: difficulty in maintaining a desirable strain in culture, low yield, and high cost of harvesting oil [42]. Jojoba or *Simmondsia chinensis* is an alternative feedstock crop that met many of the requirements for our study such as low water requirement and high oil yield [43] but was discarded as a candidate as the plant is dioecious and the gender of plants can only be determined at flowering, which occurs 3-4 years after seedling [44]. The halophyte *Salicornia bigelovii* was an attractive candidate due to its' suitability for cultivation with seawater but was not chosen for this study as its cultivation in the region has been investigated previously [45], and its' low oilseed yield and high lingo-cellulosic biomass makes it more attractive as a bioethanol feedstock rather than a bio-diesel feedstock [46]. *Jatropha curcas* is a non-food oil crop with high oil content in seeds and tolerance to marginal lands and salinity [47]. This shrub/tree has already been studied extensively for its' potential as a bio-diesel feedstock crop, mainly for marginal lands in India [48]. However, previous studies at ICBA showed that the species is not well adapted to the UAE environment, possibly because of its tropical origin (personal communication) and thus was not chosen for the current study. Nevertheless, findings from literature based on studies of *Jatropha curcas* and *Salicornia bigelovii* have been used for comparison to discuss our results. Based on the available literature and because of the high oil content of the seeds and tolerance of the species to marginal growing conditions, three species

were selected for the study: castor (*Ricinus communis* L.), desert gourd [*Citrullus colocynthis (L.) Schrad*] and mustard [*Brassica juncea (L.) Czern*]. While castor is already known for its suitability for bio-diesel production [49], desert gourd is native to the UAE and expected to tolerate the harsh growing conditions and mustard is a conventional edible oil crop, but reported to tolerate high levels of salinity.

### 1.6.1. Castor (*Ricinus communis* L.)

Castor is a member of the Euphorbiaceae family. It is a perennial plant and probably native to Africa. The fruit is a globose capsule 2.5 cm in diameter, usually containing 3 seeds. Yields of up to 5000 kg/ha have been reported, but can be less without adequate moisture [50]. In a study of improved cultivars in Greece, yields between 2.5 and 5 Mg (megagrams) per hectare were reported [51]. In India, castor is cultivated in marginal lands and under rain- fed conditions, limiting productivity to around 1200 kg/ha. In the wild this plant is able to adapt to arid conditions and withstand long periods of drought [52]. The oil from the seeds of this plant is inedible. The presence of ricinoleic acid and its derivatives makes the oil indigestible and it also has high hygroscopicity (capable of attracting and holding water molecules), solubility in alcohol and viscosity (due to C-12 hydroxyl groups) compared to other vegetable oils [53]. Viscosity of castor oil is approximately 100 times more than that of diesel fuel and 5-10 times higher than that of other vegetable oils. Ricinoleic acid from castor oil is used in cosmetics, paints, lubricants and other products. There are conflicting reports regarding the effect of temperature on bio-diesel synthesis from castor oil due to its solubility in ethanol at room temperature and high viscosity [54]. Bio-diesel from castor oil has properties such as very low cloud point and pour point which make it suitable for use in extreme winter temperatures. However, a single reaction step is required for the transesterification process of castor oil because of its favorable acidity level. Therefore, in a large-scale process it would be less costly to produce bio-diesel from castor seeds than other oils with a higher acidity level. The properties of the B100 (100%) bio-diesel and its B10 (10% bio-diesel, 90% petro-diesel) and B20 (20% bio-diesel, 80% petro-diesel) blends are comparable to those of petroleum diesel and acceptable within international bio-diesel standards (ASTM D 6751) with the exception of viscosity and humidity of B100 [52].

### 1.6.2. Desert gourd [*Citrullus colocynthis (L.)* Schrad.]:

Desert gourd (also known as Handhal, and Thumba in Arabic) is a member of the Cucurbitaceae family. It is not to be confused with *Colocynthis citrullus* or Egusi, an edible seed-bearing crop known for its high protein (35% in seeds) and oil (50% in seeds) content [55] which is cultivated

mainly in African countries for the seeds [56]. *Citrullus colocynthis* grows in the wild in many regions of the world such as Western India, the Middle East and North Africa. It is a perennial creeper that grows on the ground, covering large areas and one of the few native species of plants that grow in the arid conditions of the UAE. It bears a yellow-green inedible fruit, the size of a small watermelon at maturity [57]. The plant is said to be highly xerophytic, and tolerates annual precipitation between 25 and 37 cm, and thrives at temperatures between 23-27˚C. It thrives in sandy loam, sub-desert soils and sandy sea coasts [58]. According to certain sources, this plant is listed as a medicinal halophyte that grows in coastal areas with salty or brackish waters [59]. A study of this plant in Saudi Arabia indicates that it compensates for the high temperatures in deserts by an increased transcription rate which cools the leaf temperatures to less than ambient temperature to prevent tissue damage. In natural habitats, its distribution is determined by the level of the underground water table, which the tap root needs to reach [60].

There were a few previous studies exploring the potential of *C. colocynthis* for biofuel production [32.66]. The oil from *C. colocynthis* has a high unsaturated fatty acid content which suggests a possible hypo-cholestronic effect [57]. It also has a high free fatty acid content, which was lowered by up to 63% by alkali refining [61]. Raw oil from the seeds of this plant is used in the soap industry in India. Compared to Jatropha it has a shorter crop cycle (6 months), can grow in dry desert soils and the oil has lower viscosity [62] . Bio-diesel from *C. colocynthis* seed oil was found to conform to European (EN 14214) and international (ASTM D6751) standards and has lower kinematic viscosity than most bio-diesels but it has low oxidative stability due to its high polyunsaturated fatty acid content [29]. Bio-diesel from *C. colocynthis* also exhibits performance parameters similar to that of bio-diesel from Jatropha when blended with petroleum diesel. The blends also have lower smoke opacity [62]. Up to 30% of raw oil blend with diesel has been reported to exhibit no change in engine performance and even reduced tail pipe emissions. A 20% blend however corresponded with optimum engine performance data [63]. The low flash point of bio-diesel from this oil also makes it safer to handle and store than diesel [64].

In addition to its potential as a bio-diesel feedstock the root and callus extracts of *C. colocynthis* have been reported to have anti-microbial [65], anti-cancer [66], anti-inflammatory [67], anti-diabetic [68] and anti-oxidant [69] effects. From the 14$^{th}$ century this plant has been cultivated and exported from the island of Cyprus for use in traditional medicines, mainly as a treatment of chronic constipation [70]. Anti-cancer properties of the fruit have been studied for decades now and in a study as early as 1958, the toxic and potent resinous constituents of the fruit have been seen to cause damage to tumors in mice [71]. The rise of drug resistance and opportunistic infections has lead researchers back to traditional medicines in search of solutions. Extracts of the

*C. colocynthis* plant, which was used in folk medicine in Tunisia was found to have antibacterial and anticandidal properties [72]. In India, in addition to the fruits, root extracts have been indicated for jaundice, ascites, liver problems, rheumatism, fever, urinary disease and stomach pains in traditional medicine [73].

In spite of the seed tar being used as medicine in Sahelian countries, an old study from 1984 reports that condensate from *C. colocynthis* seeds contain a large number of polycyclic aromatic hydrocarbons, including known carcinogens like benzo(a)pyrene [74]. The manufacture and use of the seed tar may thus be hazardous to health. This study has not been followed up by others and requires further investigation. The use of this plant as a bio-indicator of available nutrients (N, Ca) and contaminating heavy metals (Cd, Fe) in soil has also been suggested by researchers in Saudi Arabia [75]. These additional characteristics of *C. colocynthis* adds to the interest in studying its cultivation in the soil and climate conditions of the Middle East. If a medicinal extract can be obtained simultaneously with its use as a bio-diesel feedstock and a contamination and nutrient indicator the commercial viability of its cultivation in this region could be envisaged.

1.6.3. Mustard [*Brassica juncea (L.)* Czern.]:

Mustard, commonly known as brown or black mustard is a cool season annual of the Brassicaceae family. It is commonly grown as for its' variable, glabrous, thin leaves which are edible and cooked like a vegetable in some parts of the world [76]. In India, the crop is grown mostly for its seeds and the seed oil is used as a cooking oil in some Indian cuisines. Mustard seed has between 40-45% oil content. Seed yield of mustard under drought conditions is reportedly higher than that of canola [77], which is a more popularly cultivated close relative of mustard. Mustard (*B. juncea)* is also more suited to semi-arid conditions and with higher yields and better characteristics with respect to use of the seed meal as animal feed than its closely related species canola (*B. napus)* and rapeseed *(B. rapa*) [78]. Mustard oil, like oil from other members of the Brassicaceae family is suitable for bio-diesel production and conforms to ASTM standards for bio-diesel feedstock [79]. Most Brassica species are moderately salt tolerant [80]. There are varieties of mustard with high erucic acid content that have been deemed inedible due to reported toxic effects on the heart in animal studies. Erucic acid is monounsaturated omega 9 fatty acid. Limits for human consumption of erucic acid have been set by various food regulatory bodies such as the Food and Drug Administration (FDA) in the US [81], and Food Standards, Australia [82]. This makes some varieties of mustard oil unsuitable for human consumption [83]. These high erucic acid varieties can therefore be suitable as bio-diesel feedstock [79]. Owing to its' popularity as an oilseed crop, well established agricultural practice, reported drought and salinity tolerance and high yields and

superior adaptability to regional conditions in a pilot study [76], mustard, though an edible crop, was chosen for the study.

### 1.7. Salinity tolerance

Some 20% of global agricultural land and more than 6% of all land is affected by salinity [84]. Salts in soil are common and often essential for plant growth, such as nitrates and potassium. Salts originate from human actions such as use of inorganic fertilizers, soil treatments such as composts and manures, and irrigation waters. Lands that were not previously saline are being affected by salinity due to land clearing and irrigation practices, raising the groundwater table, which leads to concentration of salts around the plant's root zone. Salts also accumulate in soil over a period of time due to the weathering of parental rocks, which releases soluble salts of various types, mainly chlorides of sodium, calcium, and magnesium, and to a lesser extent, sulfates and carbonates [85]. Sodium chloride is the most soluble and abundant salt released. Another cause of accumulation is the deposition of oceanic salts carried in wind and rain. Rainwater contains 6–50 mg/kg of sodium chloride; the concentration of which decreases with distance from the coast [86]. Rain containing 10 mg/kg of sodium chloride would deposit 10 kg/ha of salt for each 100 mm of rainfall per year. When certain salts are present in relatively high amounts, plant growth is adversely affected. In the UAE, soil salinity is mainly due to the use of saline groundwater for irrigation.

Salinity is determined by measuring the electrical conductivity (EC) of solution extracted from a water-saturated soil paste, or of the irrigation water itself. Soil salinity is abbreviated as ECe with units of deci-siemens per meter (dS/m) or millimhos per centimeter (mmhos/cm), both of which are equivalent units [87]. Soils are categorized as saline when the ECe values exceed 4 dS/m [88], which is equivalent to approximately 40mM NaCl and generates an osmotic pressure of approximately 0.2 MPa.

Saline soils are categorized into two types: sodic (or alkali) and saline. A third type can be referred to as saline-sodic soils. Saline soils are predominant in arid regions and are dominated by sodium cations, but predominant anions are chloride and sulphate. These soils have lower pH and exchangeable sodium percentage than sodic soils. Sodic soils are widely distributed in arid and semi-arid regions. Sodic soils have high concentrations of free carbonate and bicarbonate and excess of sodium. They are deficient in nitrogen, phosphorus and zinc and have high pH (8.5 - 10.7). Clay fraction and organic matter are dispersed, thus soils are sticky when wet and hard when dry, resulting in poor water permeability, and high impedance to root growth [89].

Plants sensitive to salinity exhibit various symptoms such as white leaf tip, leaf browning and death, stunted growth, spikelet sterility, low harvest, low tillering, less yield, lower 1000 seed

weight and overall decrease in plant health. Different crops and different cultivars within a crop differ for their inherent capability to modify various physiological and biochemical processes in response to the salt stress. These changes control the solute and water balance and their distribution on whole plant and tissue basis and determines whether the species is sensitive or tolerant to salinity. In most plants there is high $Na^+$ transport to the shoot and accumulation of $Na^+$ in older leaves, in addition to high $Cl^-$ uptake and lower $K^+$ uptake [90]. Low P and Zn uptake and increase in organic compatible solutes and increased level of reactive oxygen species (ROS) is also observed [91].

Soil salinity stresses plants in two main ways, high salt concentrations in the soil make it harder for roots to extract water (osmotic stress), and high concentrations of salts within the plant can be toxic to the plant itself (ionic stress). Salts on the outside of roots have an immediate effect on cell growth and associated metabolism while toxic concentrations of salts inside plants takes time to accumulate before they affect plant function. Most plant species have developed mechanisms to exclude $Na^+$ and $Cl^-$ ions from the roots and tolerate osmotic stress because NaCl is the most universally widespread salt [92]. Certain plants which can exclude these ions at a higher level than glycophytes (plants growing in non-saline soils) are called halophytes. The variation in salinity tolerance in dicotyledonous species is even greater than in monocotyledonous species [86]. For most species, $Na^+$ appears to reach a toxic concentration before $Cl^-$ does, and so most studies have concentrated on $Na^+$ exclusion and the control of $Na^+$ transport within the plant. However, for some species such as soybean, citrus, and grapevine, $Cl^-$ is considered to be the more toxic ion [99-100]. It is possible that these species are better at excluding $Na^+$ from the leaf blades than $Cl^-$. For example, $Na^+$ does not increase in the leaf blade of grapevines until after several years of exposure to saline soil, then the exclusion within the root, stem, and petiole breaks down, and $Na^+$ starts to accumulate in the leaf blade, whereas leaf blade $Cl^-$ concentrations increase progressively [93].

Osmotic stress reduces cell expansion in root tips and young leaves, and causes stomatal closure [94]. A reduced response to the osmotic stress would result in greater leaf growth and stomatal conductance, but the resulting increased leaf area would benefit only plants that have sufficient soil water like in an irrigated system, but could be undesirable in water-limited systems, causing soil water to be used up. Tissue tolerance to accumulated $Na^+$ or $Cl^-$ is observed in some species. This involves compartmentalization of $Na^+$ and $Cl^-$ at the cellular and intracellular level to avoid

toxic concentrations within the cytoplasm, especially in mesophyll cells of the leaf. Toxicity occurs with time, after leaf $Na^+$ increases to high concentrations in the older leaves [95].

There are ways to differentiate between osmotic and ionic stress. In osmotic stress the onset of symptoms is rapid, and the most common symptom is decreased new shoot growth. Ionic stress on the other hand is indicated by increased senescence of older leaves and the onset is relatively slower. In case of osmotic stress the leaf surface area also reduces. This may be because reduction in leaf area relative to root growth would decrease the water use by the plant. The second, ion-specific, phase of plant response to salinity starts when salt accumulates to toxic concentrations in the old leaves (which are no longer expanding and so no longer diluting the salt arriving in them as younger growing leaves do), and they die. If the rate at which the leaves die is greater than the rate at which new leaves are produced, the photosynthetic capacity of the plant will no longer be able to supply the carbohydrate requirement of the young leaves, which further reduces their growth rate. Leaf Na+ concentration is best measured in a defined leaf of a defined age, if the plant was exposed to Na+ at around the time of the emergence of that leaf to study response to ionic stress [96]. Increased osmotic tolerance is indicated when there is an increased ability to continue production of new leaves, whereas tissue tolerance is evident by the increased survival of older leaves.

The main site of $Na^+$ toxicity for most plants is the leaf blade, where $Na^+$ accumulates after being deposited in the transpiration stream, rather than in the roots [97]. A plant transpires 50 times more water than it retains in leaves [98], so excluding $Na^+$ from the leaf blades is important, even more so for perennial than for annual species, because the leaves of perennials live and transpire for longer. Most $Na^+$ that is delivered to the shoot remains in the shoot, because for most plants, the movement of $Na^+$ from the shoot to the roots in the phloem can likely recirculate only a small proportion of the $Na^+$ that is delivered to the shoot. The concentration of $K^+$ in the cytoplasm relative to that of $Na^+$ may be a contributing factor to salinity tolerance. In *Arabidopsis,* an additional supply of $K^+$ alleviated the phenotype of the salt sensitive mutants [99], which may be due to an increase in cytoplasmic $K^+$ concentrations. Potassium is an essential nutrient for plants, and required for various metabolic processes. One of the main reasons sodium accumulation is toxic is because it results in less uptake of potassium, decreasing its' availability for essential functions. Because of this, the $K^+/Na^+$ ratio in plant tissue is used as an indicator of salinity stress in plants [100].

Salinity stress tolerance of plants can be studied using a number of indices based on morphological parameter observations such as yield and plant height. Stress susceptibility index (SSI), Stress Tolerance Index (STI), Geometric Mean Productivity (GMP) and Stress Tolerance (TOL) are some of these parameters.

Calculation of tolerance indices [101]:

$$SSI = \frac{1-(\frac{Ys}{Yp})}{SI} \quad \text{where } SI = 1 - \frac{\overline{Ys}}{\overline{Yp}}$$

Where Ys is the yield of lines under stress, Yp the yield of lines under normal conditions, $\overline{Ys}$ and $\overline{Yp}$ are the mean yields of all genotypes in stress and non-stress conditions, respectively.

$TOL = Yp - Ys$

$$GMP = \sqrt{Yp.Ys}$$

$$STI = \frac{Yp-Ys}{Yp^2}$$

Cultivars with low values for SSI and TOL and high values for GMP and STI can be said to be relatively more tolerant to salinity stress.

### 1.8.Objectives of the thesis

Based on the research gaps identified in the literature survey, the following thesis objectives were coined:

- Evaluate the growth characteristics of *Citrullus colocynthis* (L.) Schrad.**,** *Ricinus communis* (L.) and *Brassica juncea* (L.) Czern. in the arid climate and with saline waters of the UAE
- Assess the potential of these plants as bio-diesel feedstock
- Identify the best cultivars for the region on the basis of the following parameters:
  - optimum growth and tolerance to heat and salinity
  - productivity and convenient propagation
  - suitability of oil for bio-diesel production
  - cost-effectiveness
  - potential for cultivation on a commercial scale

# CHAPTER 2
# GERMPLASM COLLECTION

The first phase of the study involved sourcing suitable germplasm for the field trials of all three crops. Accessions to be studied had to be chosen carefully on the basis of their chances of performing well in the local conditions. The details of germplasm sources are given in this section.

### 2.1. *Ricinus communis* germplasm

Eleven hybrid accessions of *Ricinus communis* were obtained from Vibha Seeds™, Hyderabad, India (Table 3).

**Table 3.** Accession IDs of *Ricinus communis* germplasm

| S.No. | Accession ID | S.No. | Accession ID |
|---|---|---|---|
| 1. | VBC 777 | 7. | VBC 1115 |
| 2. | VBC 999 | 8. | VBC 1116 |
| 3. | VBC 1109 | 9. | VBC 1121 |
| 4. | VBC 1111 | 10. | VBC 1122 |
| 5. | VBC 1112 | 11. | VBC 1123 |
| 6. | VBC 1114 | | |

### 2.2. *Citrullus colocynthis* germplasm

Mature fruits of *Citrullus colocynthis (L.)* Schrad. were collected from thirty different locations in the UAE (Fig.1.), with a distance of at least one km between sites. The geographical coordinates (Table 4), morphological characteristics and features of the plants were recorded. In addition to these, seven accessions were obtained from the United States Department of Agriculture (USDA)'s National Plants Germplasm system. These accessions were requested as they were sourced from arid/semi-arid regions, according to the information on the Genetic Resources Information Network (GRIN). Accessions obtained from the USDA are listed in Table 5.


**Figure 1.** Collection of *Citrullus colocynthis* germplasm from local off-road sites across the UAE (right). *Citrullus colocynthis* germplasm collection site map generated using GPS coordinates on Tableau® software (Left)

**Table 4.** Geographical co-ordinates of local *Citrullus colocynthis (L.)* Schrad. germplasm collection sites

| S.No. | Date | Collector's No | Latitude | Longitude | Elevation (feet) |
|---|---|---|---|---|---|
| 1 | 13.6.2013 | RMS-215 | N25°09.52 | E55°25.52 | 49 |
| 2 | 13.6.2013 | RMS-220 | N25°04.30 | E55°33.45 | 127 |
| 3 | 16.6.2013 | RMS-227 | N24°41.48 | E55°37.48 | 230 |
| 4 | 16.6.2013 | RMS-228 | N24°36.00 | E55°56.00 | 300 |
| 5 | 16.6.2013 | RMS-231 | N24°20.30 | E55°46.28 | 291 |
| 6 | 19.6.2013 | RMS-232 | N24°52.36 | E55°34.15 | 164 |
| 7 | 19.6.2013 | RMS-234 | N24°54.36 | E55°47.30 | 192 |
| 8 | 19.6.2013 | RMS-236 | N24°46.00 | E55°46.52 | 200 |
| 9 | 23.06.2013 | RMS-237 | N24°29.12 | E55°47.01 | 311 |
| 10 | 23.06.2013 | RMS-238 | N24°24.53 | E55°40.00 | 160 |
| 11 | 23.06.2013 | RMS-239 | N24°24.55 | E55°27.04 | 107 |
| 12 | 23.06.2013 | RMS-240 | N25°14.48 | E55°34.08 | 57 |
| 13 | 23.06.2013 | RMS-241 | N25°24.20 | E55°38.45 | 31 |
| 14 | 25.06.2013 | RMS-244 | N25°16.40 | E55°41.20 | 62 |
| 15 | 25.06.2013 | RMS-245 | N25°31.28 | E55°44.08 | 22 |
| 16 | 25.06.2013 | RMS-246 | N25°39.20 | E55°51.40 | 45 |
| 17 | 26.06.2013 | RMS-247 | N24°40.28 | E55°34.28 | 130 |
| 18 | 26.06.2013 | RMS-248 | N24°30.24 | E55°34.20 | 233 |

| | | | | | |
|---|---|---|---|---|---|
| 19 | 26.06.2013 | RMS-249 | N24°24.32 | E55°42.20 | 274 |
| 20 | 08.07.2013 | RMS-250 | N25°16.43 | E55°49.51 | 100 |
| 21 | 09.07.2013 | RMS-253 | N25°22.06 | E55°33.24 | 43 |
| 22 | 09.07.2013 | RMS-254 | N25°28.17 | E55°38.22 | 19 |
| 23 | 09.07.2013 | RMS-255 | N25°33.40 | E55°45.02 | 26 |
| 24 | 09.07.2013 | RMS-256 | N25°39.08 | E55°51.06 | 41 |
| 25 | 09.07.2013 | RMS-257 | N25°32.02 | E55°40.49 | 23 |
| 26 | 09.07.2013 | RMS-258 | N25°05.50 | E55°23.07 | 39 |
| 27 | 09.07.2014 | RMS-259 | N25°01.36 | E55°20.03 | 27 |
| 28 | 04.05.2013 | KMK-1 | N25°09.22 | E55°38.13 | 52 |
| 29 | 04.05.2014 | KMK-2 | N25°05.01 | E55°74.06 | 67 |
| 30 | 04.05.2015 | KMK-3 | N25°06.03 | E55°76.10 | 36 |

**Table 5.** Details of *Citrullus colocynthis (L.)* Schrad. germplasm obtained from USDA

| S.No. | Accession ID | Origin |
|---|---|---|
| 1. | PI 525080 | Egypt |
| 2. | PI 386024 | Iran |
| 3. | PI 525082 | Egypt |
| 4. | PI 537277 | Punjab, Pakistan |
| 5. | PI 652554 | Rajasthan, India |
| 6. | PI 388770 | Morocco |
| 7. | PI 386014 | Iran |

### 2.3. *Brassica juncea* germplasm

Seeds for *Brassica juncea (L.)* Czern. was obtained from the gene bank at the International Center for Biosaline Agriculture (ICBA) in Dubai, United Arab Emirates. The seeds were originally sourced from the Australian Temperate Field Crops Collection, Horsham, Australia. Five high yielding accessions were selected for the study (Table 6) based on the results of a pilot scale study by ICBA where 100 accessions of mustard were compared for seed yield [76].

**Table 6.** Accession IDs of *Brassica juncea (L.)* Czern. germplasm

| S.No. | Accession ID | Origin |
|---|---|---|
| 1 | ATC-90783 | Turkey |
| 2 | ATC-93142 | India |
| 3 | ATC-93161 | India |
| 4 | ATC-93358 | Pakistan |
| 5 | ATC-93402 | India |

# CHAPTER 3
# ESTABLISHMENT OF TRIALS

Field trials were designed using randomized complete block design (RCBD) with replicates with appropriate spacing within and between plots depending on the crop being studied. Fields for all treatments were prepared uniformly in order to control any variations due to soil quality. Seeds were sown at the beginning of the cropping season of the year mentioned. The details of design and treatments are given in this section.

### 3.1. *Ricinus communis* salinity field trial

The study was conducted at ICBA research station (25.09 ˚N, 55.38 ˚E) during the cropping season 2012-13. The soils at research station are sandy in texture (sand 98%, silt 1%, and clay 1%), calcareous (50–60% $CaCO_3$ equivalents), porous (45% porosity) and moderately alkaline (pH 8.22) with very low in organic matter (<1%). Prior to planting, the soil fertility of the experimental site was improved by incorporating farmyard manure (FYM) at the rate of 40 tonnes/haa. A Randomized Complete Block Design (RCBD) [102] with three replications was used to evaluate the performance of the eleven hybrids (Table A1. 1). The seeds were sown in field plots, each of 4 rows of 3 meters, with a distance of 50 cm both between the rows as well as between the plants within the row. The spacing between plots was 1 meter. Three salinity treatments with electrical conductivities ($EC_w$) of 5 dS $m^{-1}$, 10 dS $m^{-1}$, 15 dS $m^{-1}$ were established by mixing saline ground water (22-25 dS $m^{-1}$) with low quality municipal water having an electrical conductivity ($EC_w$) of 0.3-0.5 dS $m^{-}1$. An additional treatment, having irrigation solely with the municipal water ($EC_w$ 0.3-0.5 dS $m^{-1}$) served as the control (Figs. A1.1 and A1.2). The plants were irrigated twice daily by drip irrigation at the flow-rate of 4 liters/hour per dripper.

**Figure 2.** *Ricinus communis* field trials at ICBA. Clockwise from top left: Control, 5 dS $m^{-1}$, 10 dS $m^{-1}$ and 15 dS $m^{-1}$treatments

*Ricinus communis* salinity field trials were established successfully for the study as designed and data was collected periodically (Figure 2).

### 3.2. *Citrullus colocynthis (L.)* Schrad. diversity study

The field trial was established in mid-November 2013 using a Randomized Complete Block Design (RCBD) with three replicates at the International Center for Biosaline Agriculture (ICBA) research station (25.09˚N, 55.38˚E) during the cropping season 2013-14 (Table A1.2, Fig. A1.3). Soil characteristics and cultural practices were the same as described for the *Ricinus communis* field trial except for a spacing of 1.5 m between plots (Figures 3 & 4).

**Figure 3.** *Citrullus colocynthis* germplasm diversity field trial

**Figure 4.** *Citrullus colocynthis* germplasm diversity field trial: fruits at maturity

### 3.3. *Citrullus colocynthis* (L.) Schrad. salinity tolerance study

This study was conducted at ICBA research station (25.09 ˚N, 55.38 ˚E) during the cropping season 2013-14. The soil of the experimental site was treated with Farm Yard Manure (FYM) added at the rate of 40 tonnes/ha. to improve the fertility. A Randomized Complete Block Design (RCBD) with triplicates was used to evaluate the performance of 5 accessions selected on the basis of *in vitro* screening of all 37 accessions for salinity tolerance during germination (Table A1.3). The accessions chosen were RMS 244, RMS 253, RMS 227, RMS 215 and RMS 237. Of these, according to the *in vitro* study, RMS 227 was sensitive to salinity while the other 4 were relatively tolerant. The seeds were sown in field plots, each of 4 rows of 2 meters, with a distance of 50 cm both between the rows as well as between the plants within the row. Three salinity treatments with electrical conductivities ($EC_w$) of 5 dS $m^{-1}$, 10 dS $m^{-1}$, 15 dS $m^{-1}$ were established by mixing saline ground water (22-25 dS $m^{-1}$) with municipal water (0.3-0.5 dS $m^{-1}$), in addition to the control treatment, irrigated with low quality municipal water having an electrical conductivity of 0.3-0.5

dS $m^{-1}$ (Fig. A1.4). The plants were irrigated twice daily by drip irrigation at the flow-rate of 4 liters/hour per dripper.

It was expected that *Citrullus colocynthis* may be tolerant to salinity due to its xerophytic nature, and a few reports of its growth in coastal habitats. Due to constraints in conducting a field trial for salinity tolerance in triplicates for all thirty-seven accessions, it was decided to screen the various accessions *in vitro* (described in next section) and five accessions were chosen for the field trial on the basis of their higher percentage germination following dormancy breaking treatments. Among these, four accessions were relatively tolerant and one was sensitive to salinity during germination in *in vitro* studies. In the field trial with four treatments (Control, 5 dS $m^{-1}$, 10 dS $m^{-1}$ and 15 dS $m^{-1}$), chosen to test for tolerance at the low and moderate salinity range, salinity treatment commenced two weeks after germination in all accessions. From the very beginning however, it was observed that the plants started to wilt and die. No diseases or infections could be detected, so the most likely explanation was sensitivity to salinity, especially since the plants in the control treatment were healthy after germination.

In order to rule out any other factors, the trial was repeated by growing the plants in pots under controlled environment of the greenhouse, using two levels of salinity. The pot trial was established (Figure 5) in the ICBA greenhouse during the cropping season 2013-14 in order to study salinity tolerance in *Citrullus colocynthis (L.)* Schrad. The same five accessions from the field trial were selected for this study and three replicates of each treatment were used in a complete randomized design. Each replicate consisted of five pots of 2.5-gallon capacity, in each of which, three seeds were sown at a depth of 2-3 cm. Sand mixed with potting soil was used in the pots. Two salinity treatments with electrical conductivities ($EC_w$) of 2 dS $m^{-1}$ and 4 dS $m^{-1}$ were established by mixing saline ground water (22-25 dS $m^{-1}$) with municipal water (0.3-0.5 dS $m^{-1}$), in addition to the control treatment, irrigated with low quality municipal water having an electrical conductivity of 0.3-0.5 dS $m^{-1}$ served as the control. Again, most of the plants died out in the salinity treatments a few weeks after commencement of the treatment and in one or two plants that persisted from each accession, leaf tissue samples were harvested to check for ion accumulation. Plants in the freshwater irrigated control treatment were healthy, but a steady and drastic decline in K/Na ratios in leaf tissue was observed even at salinity levels as low as 2 and 4 dS $m^{-1}$. It would thus appear that the five accessions chosen for the salinity trial were all highly sensitive to salinity, and the results of the *in vitro* studies apply only to the germination stage.

**Figure 5.** *Citrullus colocynthis* green house pot trial

### 3.4. *Brassica juncea (L.)* Czern field trials

The study was conducted at ICBA research station (25.09 ˚N, 55.38 ˚E) during the cropping season 2014-15. A Completely Randomized Block Design (CRBD) with three replications was used to evaluate the performance of five accessions (Table A1.4). The seeds were sown in field plots, each of five rows of 3 meters, with a distance of 50 cm both between the rows as well as between the plants within the row. One salinity treatment with electrical conductivity ($EC_w$) of 10 dS $m^{-1}$ was established by mixing saline ground water (22-25 dS $m^{-1}$) with sweet water (0.3-0.5 dS $m^{-1}$), in addition to the control treatment, irrigated with low quality municipal water having an electrical conductivity of 0.3-0.5 dS $m^{-1}$ (Fig. A1.5). A separate trial with Treated Waste Water (TWW) was also set up. The plants were irrigated twice daily by drip irrigation at the flow-rate of 4 liters/hour per dripper.

The field trials were established as designed and all seeds germinated successfully. Data was collected from the field periodically (Figure 6).

**Figure 6**. *Brassica juncea* field trials. Top: TWW treatment, Bottom: Control (Right) and 15 dS $m^{-1}$ treatments (Left)

The experimental design schemes and field layouts for all field trials are detailed in Appendix 1.

# CHAPTER 4

# GERMINATION STUDIES

Seed dormancy was encountered when attempting to establish the *Citrullus colocynthis* field trial. This necessitated the identification of effective pre-treatment methods to break dormancy in this crop. For uniformity, *Ricinus communis* and *Brassica juncea* germination was also studied. In addition, the response of each crop to salinity at germination stage was also recorded.

**4.1. Germination and seed dormancy**

*Ricinus communis* and *Brassica Juncea* seeds of all accessions showed 100% germination *in vitro*. In all locally collected accessions of *Citrullus colocynthis*, no germination was observed without any pre-treatment (control). Seeds of all *Citrullus colocynthis* accessions were tested for germination efficiency *in vitro* on 0.8% agar plates [103] due to observed dormancy of nearly 100% upon planting.

Seven different pre-treatment methods such as chemical treatment, mechanical seed coat removal, warm and cold stratification (T1-T7), modified or replicated from literature were compared for their efficacy to overcome seed dormancy.

- T1- Manual scarification using sandpaper followed by soaking for 48 hours and incubation at room temperature [104]
- T2- Manual scarification using sandpaper followed by soaking for 48 hours and incubation at 30˚C [105]
- T3- Manual scarification using sandpaper followed by soaking for 48 hours and incubation at 30˚C on 0.8% agar containing 0.2% potassium nitrate [106]
- T4- Incubate at 4˚C for 48 hours, followed by manual scarification using sandpaper and incubation at room temperature [107]
- T5- Manual scarification followed by incubation at 30˚C [108]
- T6- Incubation at 40˚C for 48 hours and manual scarification using sandpaper followed by incubation at 30 ˚C [109]
- T7- Soaking for 48 hours followed by incubation at 30˚C [110]

Triplicates of 10 seeds each were tested in each accession. The treated seeds were placed on 0.8% agar in petri plates and observed daily for germination. As test control, seeds were placed on 0.8% agar plates at room temperature without any pre-treatments.

Among the different dormancy breaking treatments studied, manual scarification followed by soaking for 48 hours and incubation at 30˚C gave the best results, with almost 100% germination occurring within 48 hours (Table 7). Manual scarification or soaking alone did not have the same effect. Neither did scarification and soaking, followed by incubation at room temperature.

**Table 7.** Effect of pre-treatment methods on germination of *Citrullus colocynthis* (X represents the individual pre-treatments applied in each treatment, T1-T7)

| | Heat treatment @ 40˚C | Manual Scarification | Soaking (48 h) | 0.2% KNO3 | Incubation @ 4˚C (48h) | Incubation @RT | Incubation @ 30˚C | Germination |
|---|---|---|---|---|---|---|---|---|
| **T1** | | **X** | **X** | | | **X** | | **-** |
| **T2** | | **X** | **X** | | | | **X** | **Within 48h** |
| **T3** | | **X** | **X** | **X** | | | **X** | **Within 62 h** |
| **T4** | | **X** | | | **X** | **X** | | **-** |
| **T5** | | **X** | | | | | **X** | **-** |
| **T6** | **X** | **X** | | | | | **X** | **-** |
| **T7** | | | **X** | | | | | **-** |

### 4.1.1. Percentage germination after pre-treatment

Twenty seeds each of the thirty locally collected *Citrullus colocynthis* accessions were manually scarified using sandpaper, soaked for 48 hours and incubated on 0.8% agar at 30˚C and germination (radicle emergence) was observed and data recorded daily.

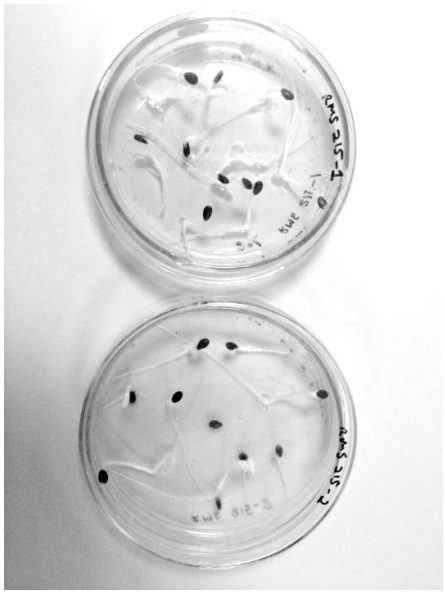

**Figure 7.** *In vitro* germination of *Citrullus colocynthis* seeds on 0.8% agar plates after pre-treatment

Of the thirty accessions of *Citrullus colocynthis* studied, percentage germination varied between 0 and 100% after the pre-treatment described above (Figure 7). Ten accessions had germination efficiency greater than 80% *in vitro* (Figure 8).

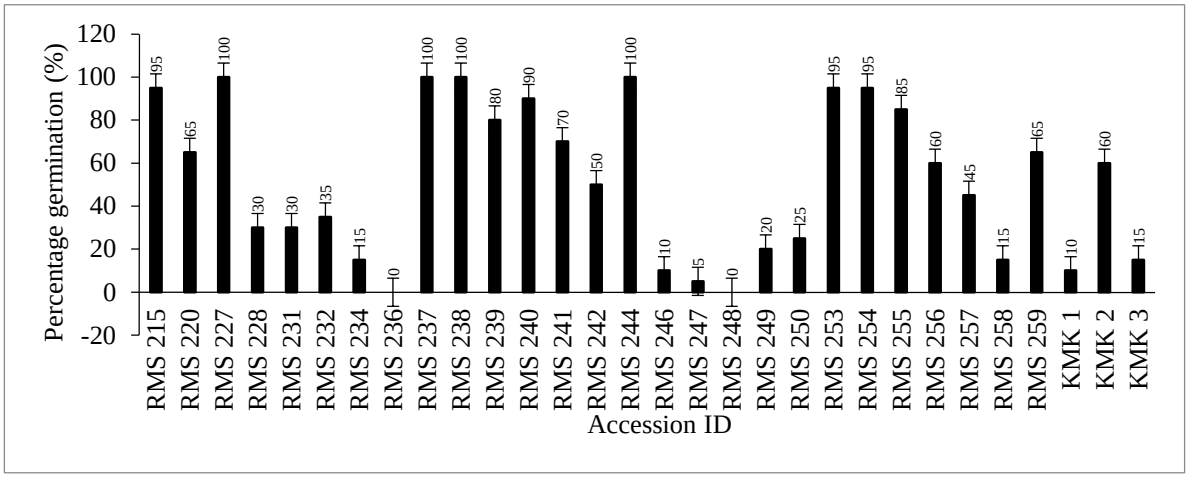


**Figure 8.** Mean percentage germination of thirty *Citrullus colocynthis* accessions after pre-treatment T2 (Manual scarification followed by 48 hours soaking and incubation at 30 °C). Error bars represent standard error between mean values of each accession

Accessions of *Ricinus communis* and *Brassica juncea* included in this study did not exhibit any dormancy and did not require pre-treatment before sowing to enhance germination. Surface sterilization with a fungicide was the only treatment carried out. Dormancy is of concern from the agricultural point of view if it is either short or too long. Seeds usually stay dormant as an adaptive mechanism, in order to wait for the climate or conditions suitable for the species [111]. If dormancy duration is too short, there is the risk of pre-harvest sprouting, while if too long it may not be possible to achieve germination in the field and optimal plant stand. It was thus important to study dormancy in *Citrullus colocynthis* seeds once it was observed. Manual scarification

followed by soaking was the most effective pre-treatment. This suggests that seed dormancy in *C. colocynthis* could be attributed to both mechanical and bio-chemical factors. It is possible that the seed coat prevents absorption of water. Alternatively, or in combination, the seed may contain certain inhibitory factors that need to leach out (for which the seed coat breach and soaking are necessary) in order for germination to occur [112]. Temperature is an important factor with respect to germination, and the temperature requirement of *Citrullus colocynthis* is in keeping with the plants natural environment. The dormancy observed in these seeds may be a mechanism of seed persistence, which is necessary for a species to survive in the extremely harsh and xerophytic conditions characteristic of its in natural habitat [113]. Even after pre-treatment, percentage germination was low in most accessions. Germination efficiency did not improve up on long storage like it does for some species [114]. It may be worthwhile to try other pre-treatments such as Gibberellic acid, or *in vitro* propagation from shoot explants [115] because dormancy was an issue in the field trials as well, and could be a real hurdle to the domestication of this species.

### 4.2. Response to salinity during germination

Seeds of ten accessions of *Citrullus colocynthis* with percentage germination greater than 80% after pre-treatment, eleven accessions of *Ricinus communis* and five accessions of *Brassica juncea* (ten seeds per plate) were germinated on 0.8% agar (control), 0.8% agar supplemented with 50 mM NaCl which corresponded with an $EC_w$ of 5 dS $m^{-1}$, and 0.8% agar supplemented with 100 mM NaCl [116] which corresponded with an $EC_w$ of 10 dS $m^{-1}$ after manual scarification and soaking. Triplicates were maintained for each treatment and germination (radicle emergence) was recorded for one week.

The *in vitro* tests for salinity tolerance showed that *Citrullus colocynthis* is highly sensitive to salinity during germination (Figure 9). At 5 dS $m^{-1}$ germination decreases by between 10 and 100% in comparison with the control, depending on the accession. In the 10 dS $m^{-1}$ medium, germination was very low, varying between 0 and 5%. There was significant variability in salinity response between different accessions at the p=0.037 level. The decrease in germination efficiency in the salinity treatments is also highly significant (p= 1.98E-09). On the basis of germination in the 5 dS $m^{-1}$ treatment, accessions RMS 215, 237, 244 and 253 were found to be relatively tolerant and RMS 227 sensitive to salinity during germination. These 5 accessions were chosen for further salinity tolerance studies. Seeds of *Ricinus communis* and *Brassica juncea* showed no response to salinity during germination. 100% germination was observed in all treatments (Tables 8, 9).

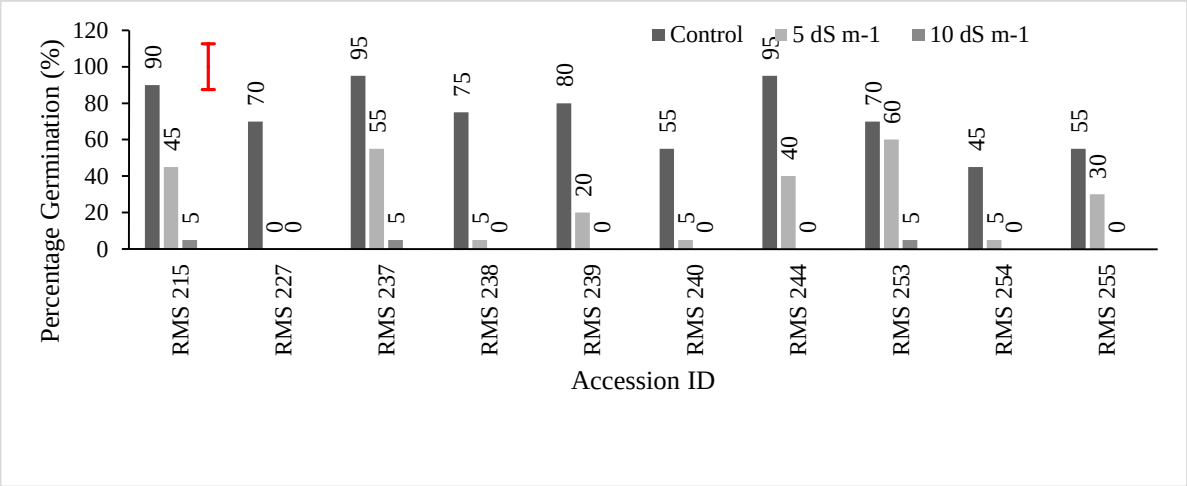


**Figure 9.** Effect of salinity on germination of *Citrullus colocynthis* accessions *in vitro*- Mean percentage germination of ten accessions. Error bars indicate standard errors within treatments

**Table 8.** Effect of salinity on germination/ radicle emergence of *Ricinus communis* accessions *in vitro*- Mean percentage germination of eleven accessions

| | | Percentage Germination (%) | | |
|---|---|---|---|---|
| S.No. | Accession ID | Control | 5 dS $m^{-1}$ | 10 dS $m^{-1}$ |
| 1. | VBC 777 | 100 | 100 | 100 |
| 2. | VBC 999 | 100 | 100 | 100 |
| 3. | VBC 1109 | 100 | 100 | 100 |
| 4. | VBC 1111 | 100 | 100 | 100 |
| 5. | VBC 1112 | 100 | 100 | 100 |
| 6. | VBC 1114 | 100 | 100 | 100 |
| 7. | VBC 1115 | 100 | 100 | 100 |
| 8. | VBC 1116 | 100 | 100 | 100 |
| 9. | VBC 1121 | 100 | 100 | 100 |
| 10. | VBC 1122 | 100 | 100 | 100 |
| 11. | VBC 1123 | 100 | 100 | 100 |

**Table 9.** Effect of salinity on germination/ radicle emergence of *Brassica juncea* accessions *in vitro*- Mean percentage germination of five accessions

| | | Percentage Germination (%) | | |
|---|---|---|---|---|
| S.No. | Accession ID | Control | 5 dS $m^{-1}$ | 10 dS $m^{-1}$ |
| 1. | ATC-93161 | 100 | 100 | 100 |
| 2. | ATC-93358 | 100 | 100 | 100 |
| 3. | ATC-90783 | 100 | 100 | 100 |
| 4. | ATC-93402 | 100 | 100 | 100 |
| 5. | ATC-93142 | 100 | 100 | 100 |

Different species respond differently to salinity at different growth stages. A species that is sensitive to salinity during germination may exhibit tolerance at maturity, and vice versa [125-126]. This is why, in parallel to the field trials, effect of salinity on germination was also studied. *Ricinus communis* and *Brassica juncea* seeds of all accessions studied were tolerant to salinity during germination. *C. colocynthis* on the other hand was highly sensitive to salinity during germination. Ten accessions that had more than 80% germination efficiency after pre-treatment were chosen for this study in order to discount the possibility of dormancy for low percentage germination. There was significant decrease in germination with increase in salinity of the medium. There was also variability in salinity response between different genotypes, germination of RMS 253 for instance was not drastically decreased in the 5 dS $m^{-1}$ treatment, while RMS 227 did not germinate at all in the salinity trial. Since salinity tolerance/sensitivity varies depending on developmental stage, further studies were necessary under field conditions to assess the effect of salinity on growth performance and yield potential. Five accessions from the *in vitro* test were selected to study the response to salinity during germination and growth under field conditions, even though a correlation between sensitivity/tolerance during germination and in field trials is not necessary.

# CHAPTER 5

# MORPHO-AGRONOMIC EVALUATION OF CASTOR (*Ricinus communis*)

To study the crop's response to salinity treatment through the growing season, various morpho-agronomic parameters were recorded. Five plants from each plot were selected randomly and tagged for identification so that the following physical growth measurements could be taken from the same plants periodically. Visual observation of the physical descriptors of plants in the trial plots was used to categorize the physical/morphological characteristics of the different accessions. Recorded characteristics were based on Castor descriptors described by Mahajan *et al*. [117].

**5.1. Morphological characteristics**

- Growth habit
    1. Semi-erect
    2. Erect
    3. Other
- Stem color
    1. Green
    2. Lightly pigmented
    3. Deeply pigmented
- Leaf color
    1. Green
    2. Dark green
    3. Other
- Spike type
    1. Mostly female
    2. Mostly male
    3. Mixed with male : female flower ratio
        1. 25M : 75F
        2. 50M : 50F
        3. 75M : 25F
- Spike compactness
    1. Compact
    2. Loose
    3. Very loose
- Waxy coating
    1. None
    2. Only on stems
    3. Stems, fruits and lower side of leaf

4. All parts

- Fruit surface:
  1. Spiny
  2. Non-spiny
- Fruit dehiscence:
  1. Dehiscent
  2. Non- dehiscent

Morphological traits of all accessions were recorded across treatments (Table 10). These characteristics remain constant across treatments for each accession except for spike compactness, which increased in the salinity treatment, and an increase in the male:female flower ratio in the 10 and 15 dS $m^{-1}$ treatments for some accessions (VBC 1123 and VBC1116 in both 10 and 15 dS $m^{-1}$ treatments, and VBC 1114 in the 15 dS $m^{-1}$ treatment alone).

**Table 10.** Range of variation in morphological traits of *Ricinus communis*

| Accession ID | Treatment | Growth Habit | Stem Color | Leaf color | Spike Type | Spike compactness | Waxy coating | Fruit surface | Fruit dehiscence |
|---|---|---|---|---|---|---|---|---|---|
| VBC 1123 | Control | 2 | 2 | 2 | 3.1 | 2 | 2 | 1 | 2 |
| VBC 1116 | Control | 2 | 2 | 1 | 3.1 | 2 | 2 | 1 | 2 |
| VBC 1112 | Control | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 2 |
| VBC 1114 | Control | 2 | 3 | 1 | 1 | 2 | 1 | 1 | 2 |
| VBC 1121 | Control | 2 | 2 | 2 | 3.1 | 2 | 2 | 1 | 2 |
| VBC 777 | Control | 2 | 2 | 1 | 3.1 | 2 | 2 | 1 | 2 |
| VBC 1122 | Control | 2 | 3 | 1 | 1 | 2 | 1 | 1 | 2 |
| VBC 1115 | Control | 2 | 3 | 1 | 3.1 | 2 | 1 | 1 | 2 |
| VBC 999 | Control | 2 | 2 | 1 | 3.1 | 2 | 2 | 1 | 2 |
| VBC 1111 | Control | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 2 |
| VBC 1109 | Control | 2 | 1 | 1 | 3.1 | 2 | 3 | 1 | 2 |
| VBC 1123 | 5 dS $m^{-1}$ | 2 | 2 | 2 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 1116 | 5 dS $m^{-1}$ | 2 | 2 | 1 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 1112 | 5 dS $m^{-1}$ | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 2 |
| VBC 1114 | 5 dS $m^{-1}$ | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 2 |
| VBC 1121 | 5 dS $m^{-1}$ | 2 | 2 | 2 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 777 | 5 dS $m^{-1}$ | 2 | 2 | 1 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 1122 | 5 dS $m^{-1}$ | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 2 |
| VBC 1115 | 5 dS $m^{-1}$ | 2 | 3 | 1 | 3.1 | 1 | 1 | 1 | 2 |
| VBC 999 | 5 dS $m^{-1}$ | 2 | 2 | 1 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 1111 | 5 dS $m^{-1}$ | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 2 |
| VBC 1109 | 5 dS $m^{-1}$ | 2 | 1 | 1 | 3.1 | 1 | 3 | 1 | 2 |
| VBC 1123 | 10 dS $m^{-1}$ | 2 | 2 | 2 | 3.2 | 1 | 2 | 1 | 2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| VBC 1116 | 10 dS $m^{-1}$ | 2 | 2 | 1 | 3.2 | 1 | 2 | 1 | 2 |
| VBC 1112 | 10 dS $m^{-1}$ | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 2 |
| VBC 1114 | 10 dS $m^{-1}$ | 2 | 3 | 1 | 3.1 | 1 | 1 | 1 | 2 |
| VBC 1121 | 10 dS $m^{-1}$ | 2 | 2 | 2 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 777 | 10 dS $m^{-1}$ | 2 | 2 | 1 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 1122 | 10 dS $m^{-1}$ | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 2 |
| VBC 1115 | 10 dS $m^{-1}$ | 2 | 3 | 1 | 3.1 | 1 | 1 | 1 | 2 |
| VBC 999 | 10 dS $m^{-1}$ | 2 | 2 | 1 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 1111 | 10 dS $m^{-1}$ | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 2 |
| VBC 1109 | 10 dS $m^{-1}$ | 2 | 1 | 1 | 3.1 | 1 | 3 | 1 | 2 |
| VBC 1123 | 15 dS $m^{-1}$ | 2 | 2 | 2 | 3.2 | 1 | 2 | 1 | 2 |
| VBC 1116 | 15 dS $m^{-1}$ | 2 | 2 | 1 | 3.2 | 1 | 2 | 1 | 2 |
| VBC 1112 | 15 dS $m^{-1}$ | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 2 |
| VBC 1114 | 15 dS $m^{-1}$ | 2 | 3 | 1 | 3.2 | 1 | 1 | 1 | 2 |
| VBC 1121 | 15 dS $m^{-1}$ | 2 | 2 | 2 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 777 | 15 dS $m^{-1}$ | 2 | 2 | 1 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 1122 | 15 dS $m^{-1}$ | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 2 |
| VBC 1115 | 15 dS $m^{-1}$ | 2 | 3 | 1 | 3.1 | 1 | 1 | 1 | 2 |
| VBC 999 | 15 dS $m^{-1}$ | 2 | 2 | 1 | 3.1 | 1 | 2 | 1 | 2 |
| VBC 1111 | 15 dS $m^{-1}$ | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 2 |
| VBC 1109 | 15 dS $m^{-1}$ | 2 | 1 | 1 | 3.1 | 1 | 3 | 1 | 2 |

Qualitative characteristic traits such as growth habit, stem and leaf color waxy coating and fruit surface are characteristic of the genotype and it is expected that they will not change because of increase or decrease in salinity. The inflorescences in all three salinity treatments were markedly more compact than in the control treatment, with significantly smaller peduncles attaching the fruits to the main stalk. This could possibly be because the plant is directing all resources towards survival under stress, instead of increase in biomass [118]. The increase in the male to female flower ratio in accessions VBC 1123, VBC 1116 and VBC 1114, suggests greater sensitivity to salinity. Sex ratios have been reported to be altered in response to stresses, largely due to hormonal changes [119]. While various theories exist as to the mechanism and causes of these changes, there is no consensus in this respect [120]. Whatever the mechanisms, since the fruit and seed are of interest in cultivation, this increase in male: female flower ratio is undesirable for commercial scale cultivation in stress environments.

### 5.2. Plant height

In salinity treatments, plant height was measured at maturity using a meter scale from ground level to the shoot tip.

**Figure 10.** Variation in *Ricinus communis* plant height and number of primary branches across treatments. Clockwise from top left: Control, 5 dS $m^{-1}$, 10 dS $m^{-1}$ and 15 dS $m^{-1}$

A significant decrease in plant height (Figure 10) at maturity was observed between treatments at and between accessions (Figure 11), as interpreted from the results of the two-way analysis of variance (Table 11). There was no significant interaction between the variance due to salinity and accession (p= 0.074), suggesting that all accessions responded similarly to salinity treatments. Average plant height in the control treatment was 164.5 cm (ranging from 133 to 194 cm for different accessions). Height decreased by 39 and 68% in the 5 dS $m^{-1}$ treatment with an average of 74 cm, ranging from 52- 92 cm depending on accession. VBC 1123 showed the least decrease, at about 39% and accession VBC 1122 the most decrease in plant height, approximately 68%, in the 5 dS $m^{-1}$ salinity treatment. Average plant height in the 10 dS $m^{-1}$ was 40 cm, ranging between

32 and 49 for various accessions. In the 15 dS $m^{-1}$ treatment average height was 27.3 cm, ranging between 21 and 31 cm for different accessions. Accessions VBC 1123, VBC 777 and VBC 1112 show maximum decrease in plant height in the 15 dS $m^{-1}$ treatment, with height being approximately 86% less compared to control. Accession VBC 1123 exhibited least decrease in plant height at maximum salinity as well, with 79%.

**Table 11.** *Ricinus communis* plant height: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 3051.358 | 10 | 305.1358 | 3.410618 | 0.004415 | 2.16458 |
| Treatments | 126431.8 | 3 | 42143.92 | 471.0584 | 2.59E-25 | 2.922277 |
| Interactions | 7970.7 | 30 | 265.7 | - | 0.074 | - |
| Error | 2683.993 | 30 | 89.46645 | | | |
| Total | 132167.1 | 43 | | | | |

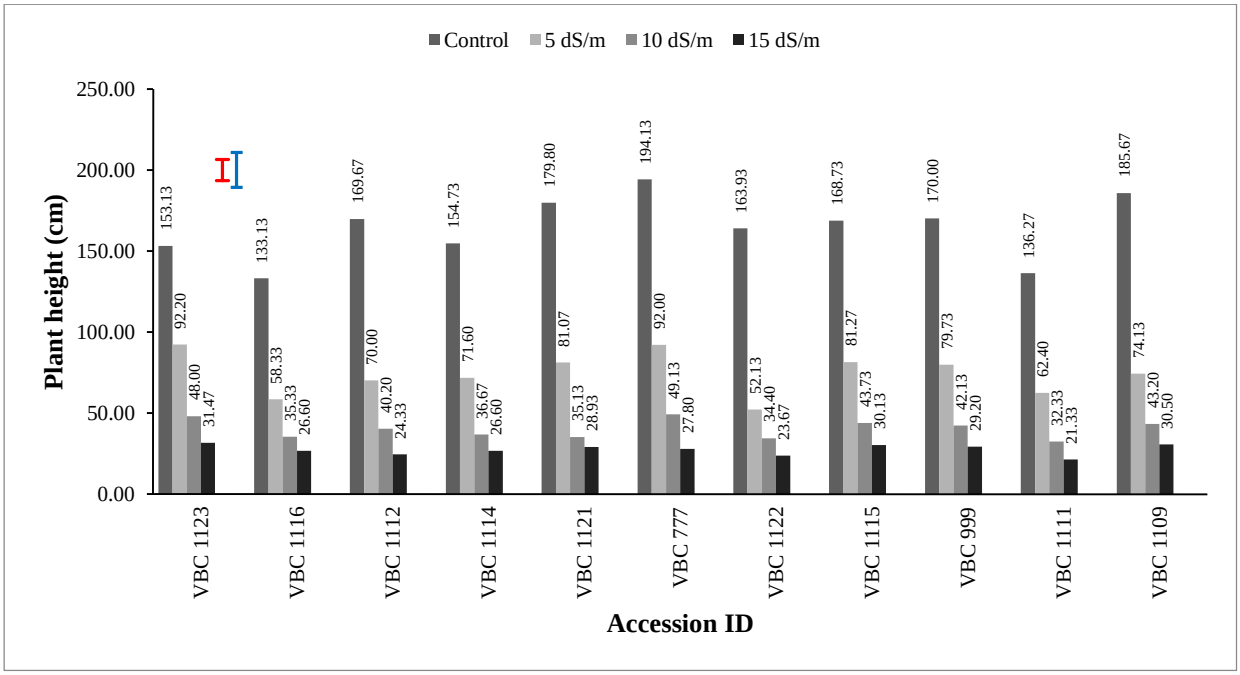


**Figure 11.** *Ricinus communis*: Mean plant height at maturity in the different levels of irrigation water salinity. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

### 5.3. Number of primary branches

Number of primary branches was counted and recorded at maturity. The average number of primary branches among accessions in the control treatment was between 5 and 7. In the 5 dS $m^{-1}$ treatment average number of primary branches decreased to between 1 and 3. In the 10 and 15 dS $m^{-1}$ no more than one primary branch was observed.

### 5.4. Stem diameter

Measured at the first node from the ground using digital vernier calipers (Mitutoyo), after four months of growth. Stem thickness of the primary shoot (Figure 12) decreased significantly for all accessions with increase in salinity and varied significantly among accessions, as indicated by the results of a two-way analysis of variance (Table 12). There was no significant interaction between the salinity and genotype factors. The average stem thickness in the control treatment was 18.5 mm (ranged between 17 and 20.6 mm), which decreased to an average of 13.6 mm in the 5 dS $m^{-1}$ treatment (ranged between 11 and 15.8 mm). Average stem thickness in the 10 dS $m^{-1}$ treatment was 8.36 mm and in the 15 dS $m^{-1}$ treatment was 6.4 mm, ranging between 7.3 and 9.8 mm and 5.6 and 7.9 mm respectively for different accessions in the two treatments.

**Table 12.** *Ricinus communis* stem diameter: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 26.7565 | 10 | 2.67565 | 4.927337 | 0.000316 | 2.16458 |
| Treatments | 984.4808 | 3 | 328.1603 | 604.3228 | 6.63E-27 | 2.922277 |
| Interaction | 50.636 | 30 | 1.688 | - | 0.0828 | - |
| Error | 16.29064 | 30 | 0.543021 | | | |
| Total | 1027.528 | 43 | | | | |

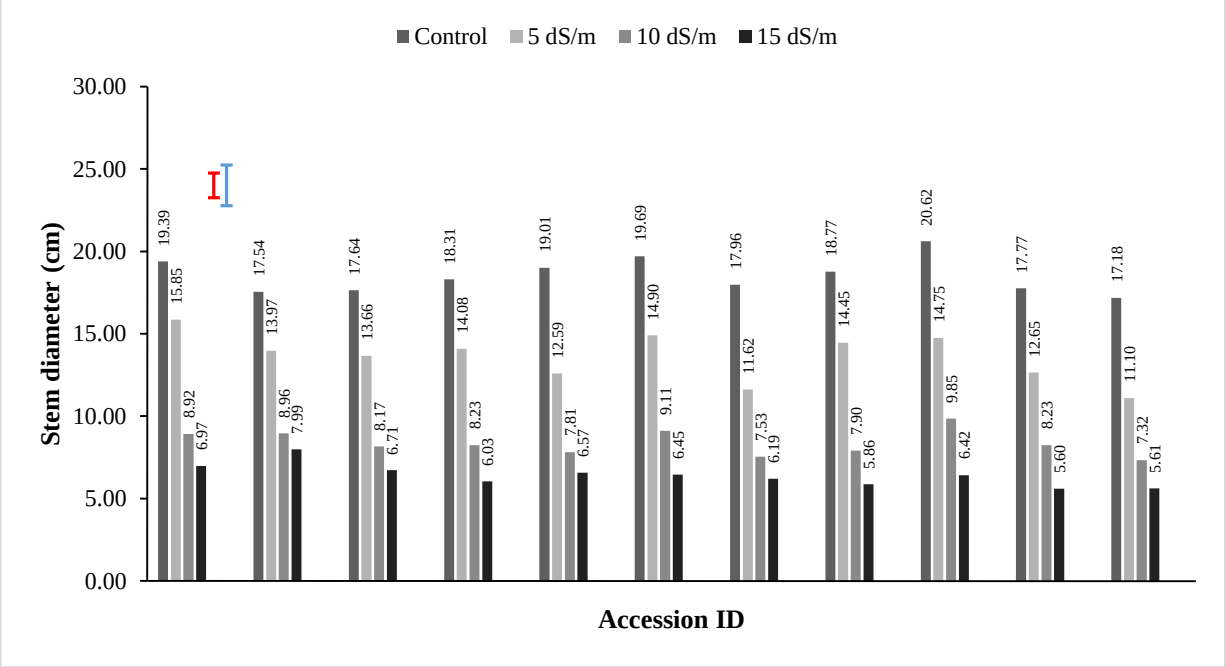


**Figure 12.** *Ricinus communis*: stem diameter at different levels of irrigation water salinity. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

### 5.5. Leaf size

The leaf originating at the node of the primary inflorescence was chosen as a standard for all leaf measurements. Leaves from 5 plants in each plot were harvested from the control treatment and images were captured against a marked scale. Leaf surface area of salinity treatments was determined non-destructively so as to not affect plant growth by plucking fresh leaves. Leaf image was captured against a white board with a marked scale.

Surface area was determined in both instances using the ImageJ Image processing and analysis software provided as freeware by the National Institutes of Health (NIH), US, which is a benchmark tool for image analysis and area measurements and has been used for leaf surface area measurements by researchers [131-133]. The scale for the image was set against the marked scale using the 'Set scale' option in the 'Analyze' menu. The outline of the leaf was traced using the freehand selection tool, and the area was measured using the 'Measure' option in the 'Analyze' menu (Figure 13). Length and breadth of leaves and the length of petiole was also measured.

The surface area of the leaf at the node of the primary inflorescence (Figure 14) was observed to decrease significantly with increase in salinity for all accessions at but did not vary greatly among accessions (Table 13). The average leaf surface area in control treatment was 524 $mm^2$ and ranged between 459 and 597 $mm^2$ for different accessions. Average leaf surface area in the 5 dS $m^{-1}$ treatment decreased to 211.7 $mm^2$ ranging from 145.5 to 289.8 $mm^2$ among accessions. Average leaf surface area in the 10 dS $m^{-1}$ and 15 dS $m^{-1}$ treatments were 100.4 and 62 $mm^2$ respectively and ranged between 77 and 150 $mm^2$ and 49 and 76 $mm^2$ among accessions in the two treatments. There was no significant interaction between factors.

**Figure 13.** *Ricinus communis* leaf surface area measurements. Clockwise from top left: Control, 5 dS $m^{-1}$, 10 dS $m^{-1}$ and 15 dS $m^{-1}$

**Table 13.** *Ricinus communis* leaf surface area: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 17978.03 | 10 | 1797.803 | 1.479765 | 0.195441 | 2.16458 |
| Treatments | 1445443 | 3 | 481814.2 | 396.5795 | 3.23E-24 | 2.922277 |
| Interaction | 109343 | 30 | 3645 | - | 0.605 | - |
| Error | 36447.74 | 30 | 1214.925 | | | |
| Total | 1499868 | 43 | | | | |

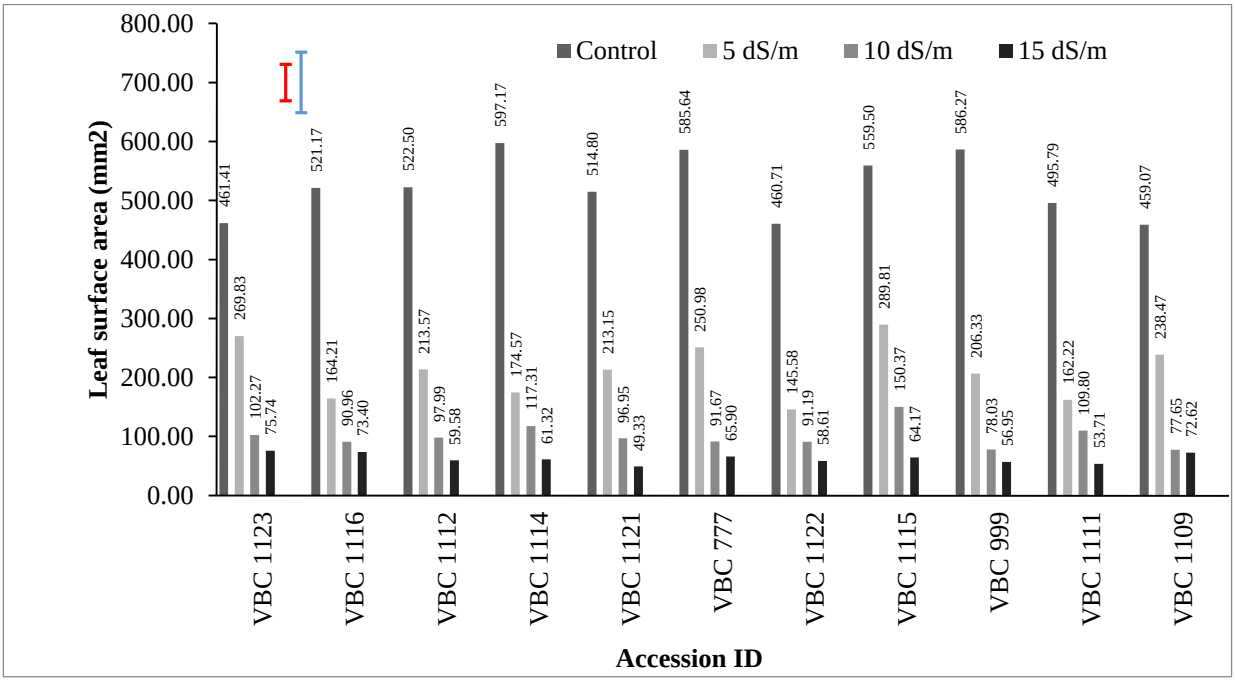


**Figure 14.** *Ricinus communis*: Mean leaf surface area at different levels of irrigation water salinity. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

## 5.6. Leaf dry weight

Dry weight was recorded after the leaves were washed in tap water to remove dirt and dried in an oven at 80˚C for up to 96 hours until a static weight was reached and all the moisture had been removed. The dried leaves were then crushed and stored in paper bags in a cool, dry place at room temperature for further analysis.

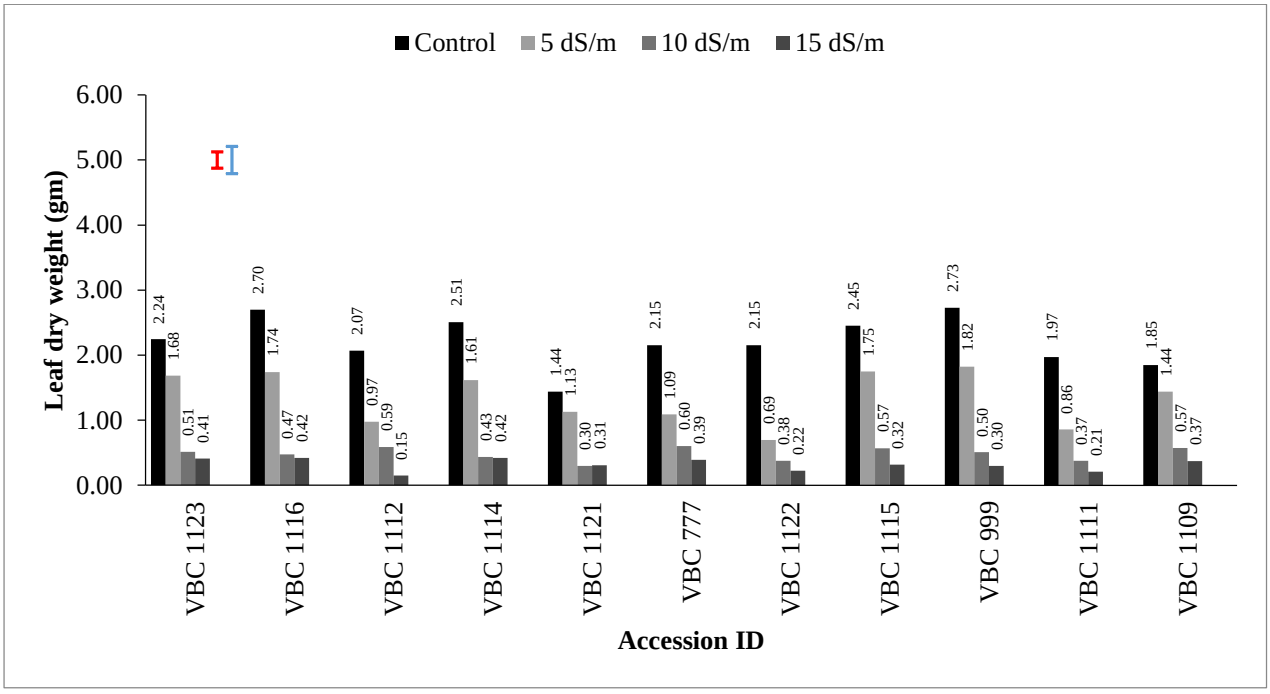


**Figure 15**. Ricinus communis: Mean leaf dry weight at different levels of irrigation water salinity. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

Leaf dry weight decreased significantly with increasing salinity for all accessions (Figure 15) and varied significantly among accessions ( Table 14). There was also a significant interaction between the two factors. The average leaf dry weight in control treatment was 2.2 gms, ranging between 1.4 and 2.7 gms among accessions. In the 5 dS $m^{-1}$ treatment leaf dry weight ranged between 0.7 and 1.8 gms, with an average of 1.34 gms. In the 10 dS $m^{-1}$ treatment, average was 0.48 gms, and ranged from 0.3 to 0.6 gms. In the 15 dS $m^{-1}$ there was a further decrease to an average of 0.32 gms, ranging from 0.14 to 0.42 gms among accessions.

**Table 14**. *Ricinus communis* leaf dry weight: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *Df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 1.647667 | 10 | 0.164767 | 2.98632 | 0.009862 | 2.16458 |
| Treatments | 25.00511 | 3 | 8.335038 | 151.0687 | 3.41E-18 | 2.922277 |
| Interaction | 6.076 | 30 | 0.2025 | - | <0.001 | - |
| Error | 1.655214 | 30 | 0.055174 | | | |
| Total | 28.308 | 43 | | | | |

### 5.7. Specific weight and moisture content

Two leaf punches of a total 0.438 $cm^2$ area were weighed for fresh-weight values. The punched sections were then dried at 60˚C till a static weight was attained to obtain dry weight. The following equations were then used to determine Specific Leaf Weight (SLW) and moisture content (%) [121]. Three sets of samples were estimated from each plot.

SLW= Dry weight of leaf disks in mg/ Area in $mm^2$

Water content (%) = (Fresh weight – Dry weight/ Fresh weight) * 100

Specific leaf weight did not increase or decrease in a consistent pattern for any accession with changes in salinity (Table 15). The differences among treatments were not statistically significant.

**Table 15.** *Ricinus communis*: Specific Leaf weight at different levels of irrigation water salinity

| Accession ID | Control | 5 dS $m^{-1}$ | 10 dS $m^{-1}$ | 15 dS $m^{-1}$ |
|---|---|---|---|---|
| VBC 1123 | 7.88 | 6.78 | 6.64 | 8.11 |
| VBC 1116 | 7.50 | 7.53 | 7.55 | 6.36 |
| VBC 1112 | 8.45 | 7.29 | 7.20 | 6.74 |
| VBC 1114 | 7.92 | 8.19 | 7.80 | 8.83 |
| VBC 1121 | 7.92 | 8.31 | 6.89 | 8.89 |
| VBC 777 | 6.17 | 7.54 | 7.54 | 7.99 |
| VBC 1122 | 8.83 | 9.12 | 10.38 | 9.55 |
| VBC 1115 | 6.89 | 7.46 | 7.77 | 9.24 |
| VBC 999 | 6.44 | 6.97 | 7.50 | 8.56 |
| VBC 1111 | 3.23 | 5.84 | 7.25 | 8.98 |
| Mean | 7.12 | 7.50 | 7.65 | 8.32 |

The differences in leaf moisture content between treatments, while statistically significant at p=0.002, did not increase or decrease in a clear pattern (Table 16) and the differences among accessions were not significant (p>0.05).

**Table 16.** *Ricinus communis*: Leaf moisture content at different levels of irrigation water salinity

| Accession ID | Control | 5 dS $m^{-1}$ | 10 dS $m^{-1}$ | 15 dS $m^{-1}$ |
|---|---|---|---|---|
| VBC 1123 | 67.09 | 69.53 | 71.90 | 68.15 |
| VBC 1116 | 68.57 | 70.24 | 67.39 | 76.67 |
| VBC 1112 | 68.14 | 72.39 | 74.53 | 74.50 |
| VBC 1114 | 64.93 | 68.50 | 71.11 | 72.84 |
| VBC 1121 | 64.50 | 66.46 | 69.29 | 69.39 |
| VBC 777 | 68.47 | 69.49 | 68.61 | 74.17 |
| VBC 1122 | 67.46 | 71.56 | 70.60 | 69.19 |
| VBC 1115 | 65.92 | 73.21 | 65.55 | 71.02 |
| VBC 999 | 68.28 | 72.89 | 76.48 | 69.46 |
| VBC 1111 | 66.20 | 73.22 | 73.38 | 67.30 |
| Mean | 66.95 | 70.75 | 70.88 | 71.23 |

## 5.8. Inflorescence length

Primary inflorescences were harvested at maturity and length was measured from node to tip. Average length was 47.55 cm in the control treatment (ranging from 37.3 to 59 cm). Length of primary inflorescences (Figure 16) decreased significantly in the 5 dS $m^{-1}$ treatment, to an average of 29.88 (ranged between 19.7 and 35.5 cm). The inflorescences were stunted and size decreased by almost half in the 10 (average 15.22 cm, range 12.6- 17.2 cm) and 15 dS $m^{-1}$ (average 9.89 cm, range 7.2- 11.8 cm) treatments. Differences among accessions were less significant (Table 17). There were no significant interactions between the two factors.

**Table 17.** *Ricinus communis* Inflorescence length: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 106.3743 | 10 | 10.63743 | 1.782412 | 0.130197 | 2.347878 |
| Treatments | 2343.153 | 2 | 1171.576 | 196.3098 | 7.16E-14 | 3.492828 |
| Interaction | 19686 | 30 | 612.382 | - | 0.073 | - |
| Error | 119.36 | 20 | 5.967998 | | | |
| Total | 2568.887 | 32 | | | | |

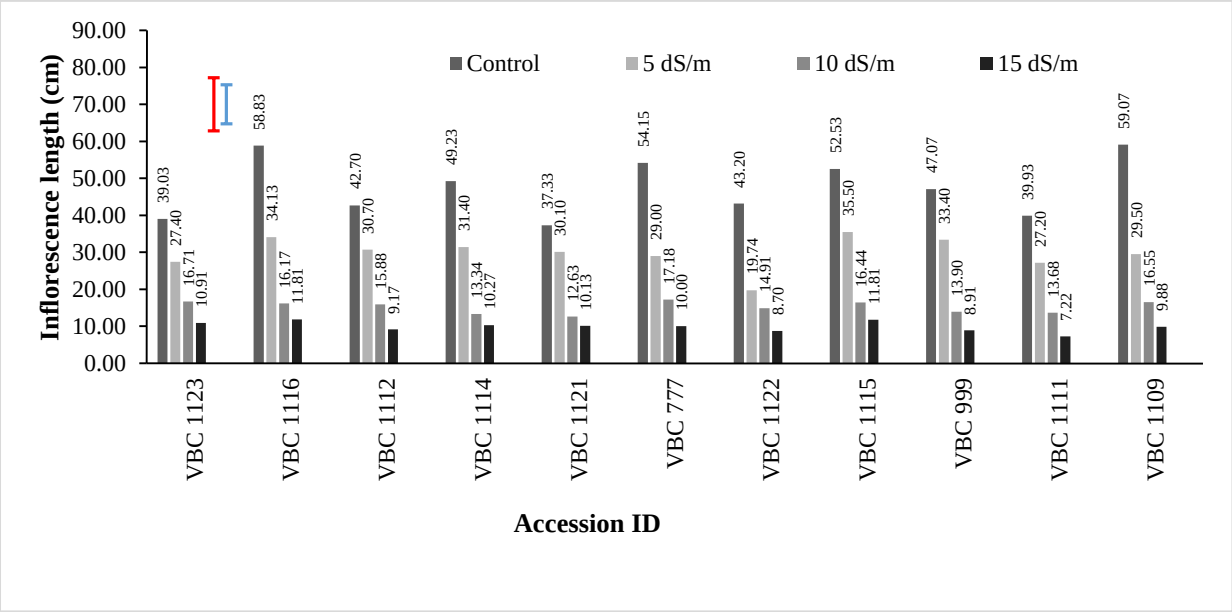


**Figure 16.** *Ricinus communis*: Inflorescence length at different levels of irrigation water salinity. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated.

### 5.9. Number of spikes per plant and fruits per spike

In the control treatment (most having more than a single inflorescence), all inflorescences were harvested and fruits counted. The number of spikes per plant was the same as the number of primary branches. The number of fruits was recorded as an average of 10 spikes per plot.

Like other characteristics, number of fruits per spike also decreased for all accessions at higher salinities (Figure 17). The average number of fruits per spike in the control treatment was 33.07 and ranged from 22.5 to 43.9 among accessions. In the 5 dS $m^{-1}$ treatment the average number was 36.03 (ranging from 21 to 54.4) and there was an increase in number of fruits per spike in accessions VBC 1116, VBC 1112, VBC 1114, VBC 777, VBC 1115, VBC 999 and VBC 1111. The difference in number of fruits per spike between just the control and 5 dS $m^{-1}$ treatments was statistically insignificant. Differences among accessions varied for this characteristic (Table 18). There was also a significant interaction between the two factors, showing that different accessions responded differently to the salinity treatments. In the higher salinity treatments average number of fruits were 16.8 9 (ranging from 8.4 to 23.4) and 8.6 (ranging from 6.5 to 9.9) in the 10 and 15 dS $m^{-1}$ treatments respectively.

**Table 18.** *Ricinus communis* Inflorescence (Number of fruits/spike): Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 705.7438 | 10 | 70.57438 | 2.460059 | 0.027675 | 2.16458 |
| Treatments | 5679.726 | 3 | 1893.242 | 65.99402 | 2.58E-13 | 2.922277 |
| Error | 860.6425 | 30 | 28.68808 | | | |
| Total | 7246.112 | 43 | | | | |

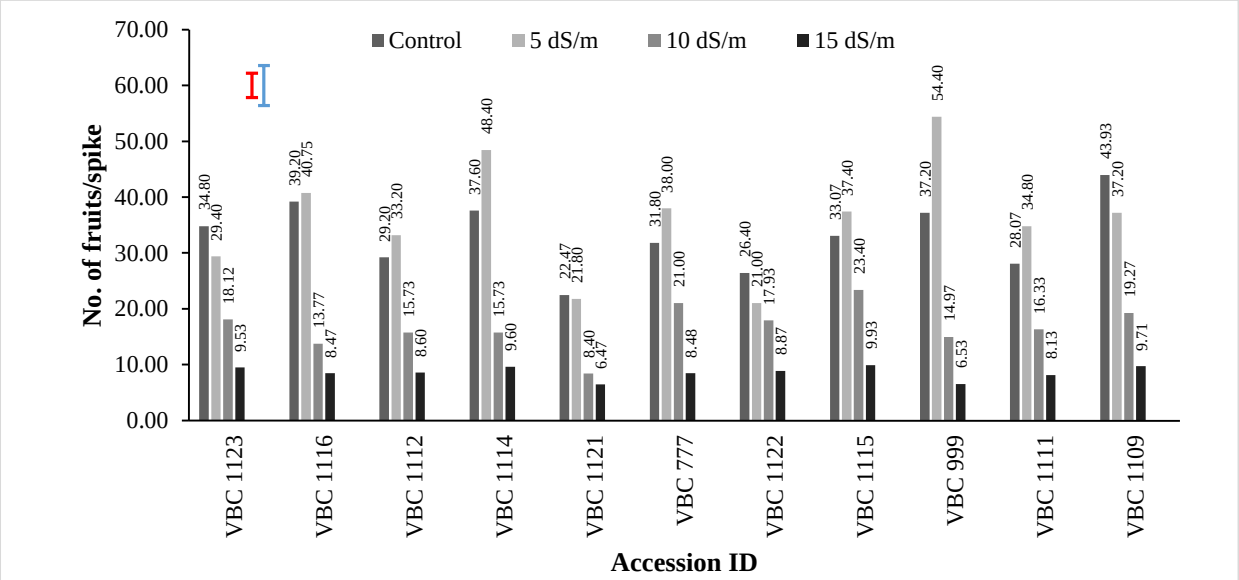


**Figure 17.** *Ricinus communis* - Number of fruits/spike at different levels of irrigation water salinity. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

## 5.10. Fruit size

The length of peduncle, axial and radial diameters of the fruits were measured as average of 10 values for each plot (Figure 18).

Average axial and radial diameters of the fruits were measured to be between 16 and 14 mm for all accessions and did not vary significantly with treatment. The peduncle length decreased significantly with increasing salinity, from an average of 5.6 cm in the control treatment to 1.8 cm in the 15 dS $m^{-1}$ treatment, as the inflorescences grew more compact with increase in salinity (Table 19). There was significant interaction between the factors.


**Figure 18.** *Ricinus communis* fruit axial and radial diameter measurements

**Table 19.** *Ricinus communis* Peduncle length: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 16.38945 | 10 | 1.638945 | 5.62411 | 0.000106 | 2.16458 |
| Treatments | 86.46595 | 3 | 28.82198 | 98.90388 | 1.19E-15 | 2.922277 |
| Interaction | 147.93 | 30 | 19.347 | - | <0.001 | - |
| Error | 8.742423 | 30 | 0.291414 | | | |
| Total | 111.5978 | 43 | | | | |

## 5.11. Seed yield

Seeds from five plants in each plot were extracted and weighed. Seed yield is expressed in tonnes per hectare based on extrapolation of the average yield of the five plants from each plot and assuming a stand of 40,000 plants per hectare, at the same density as in the current field trial.

The seed yield per plant did not differ significantly between the control (average of 60.47 gms, ranging between 42.5 and 82.6 gms) and 5 dS $m^{-1}$ treatments (average of 58.4 gms, ranging between 52 and 66.1 gms) (Table 20), but drastically reduced at the higher salinities (10 and 15 dS $m^{-1}$). Average yield per plant in the 10 dS $m^{-1}$ treatment was 9.6 gms while in the 15 dS $m^{-1}$ treatment it was 6.6 gms. There was no significant difference in seed yield among accessions within each treatment (

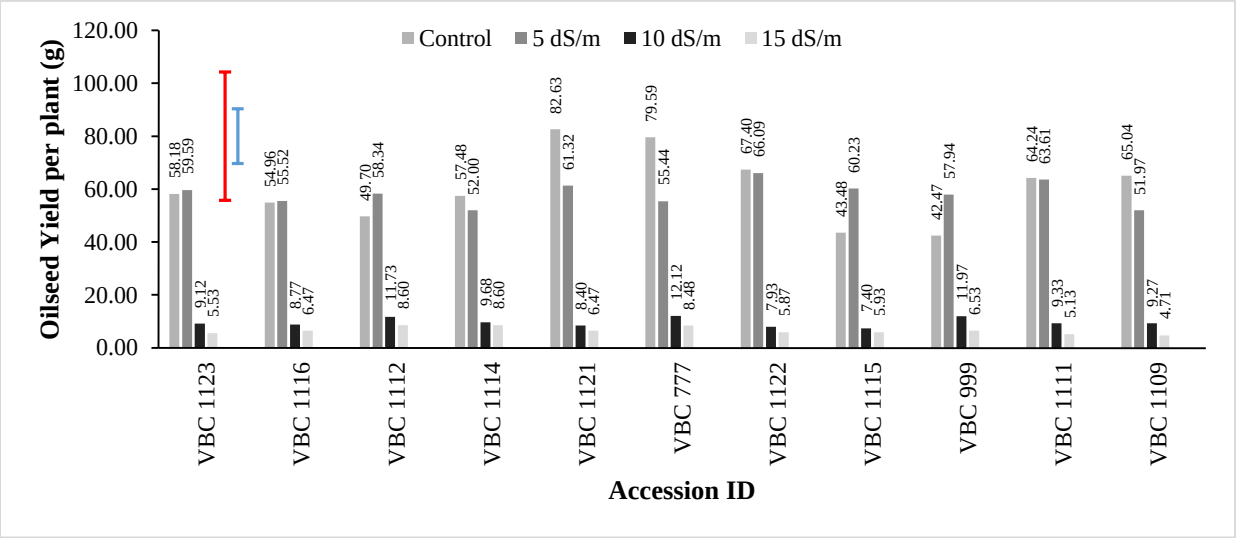


Figure 19). There was a significant interaction between the two factors.

**Table 20.** *Ricinus communis* Yield per plant: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 495.943 | 10 | 49.5943 | 1.017439 | 0.452309 | 2.16458 |
| Treatments | 29052.89 | 3 | 9684.297 | 198.6756 | 7.06E-20 | 2.922277 |
| Interaction | 72357 | 10 | 7236 | - | <0.001 | - |
| Error | 1462.328 | 30 | 48.74426 | | | |
| Total | 31011.16 | 43 | | | | |

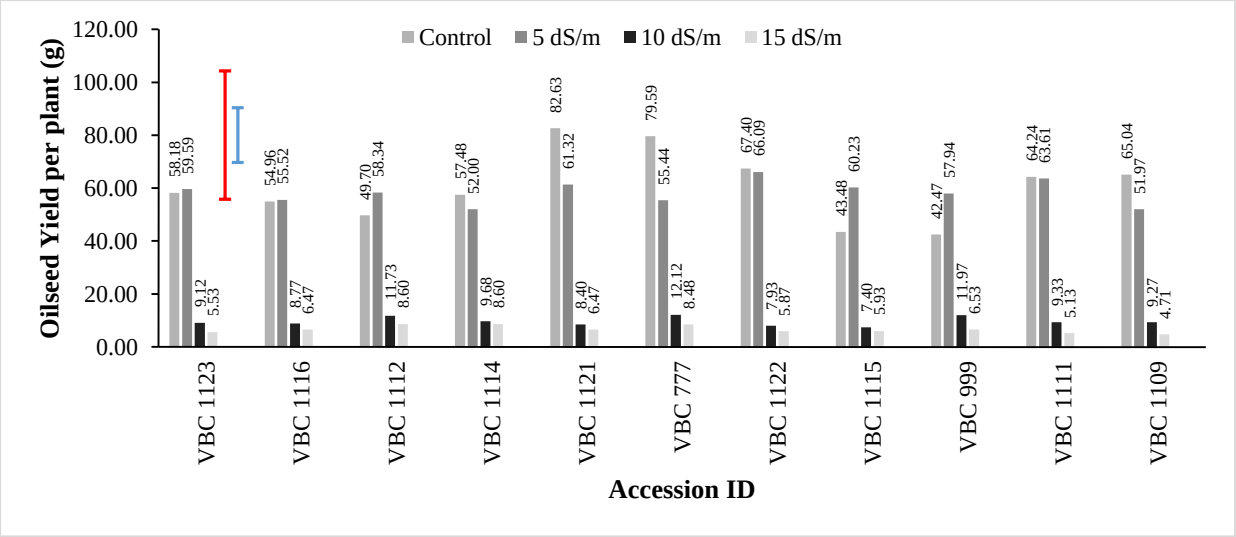


**Figure 19.** *Ricinus communis*: Seed yield per plant at different levels of irrigation water salinity. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

Assuming a stand of 40,000 plants per hectare. with the 50 cm spacing used in the study, the extrapolated yield per hectare ranged from 1.70 to 3.3 tonnes/ha. in the control with an average of 2.42 tonnes/ha., 1.87 and 2.37 tonnes/ha. in the 5 dS $m^{-1}$ treatment (average of 2.33), 0.27 and 0.43 tonnes/ha. in the 10 dS $m^{-1}$ treatment (average of 0.38) and between 0.17 and 0.31 tonnes/ha. in the 15 dS $m^{-1}$ treatment with an average of 0.26 tonnes/ha. (Figure 20). Least significant

differences are not presented as the data is extrapolated, but would be similar in scale to Figure 19.

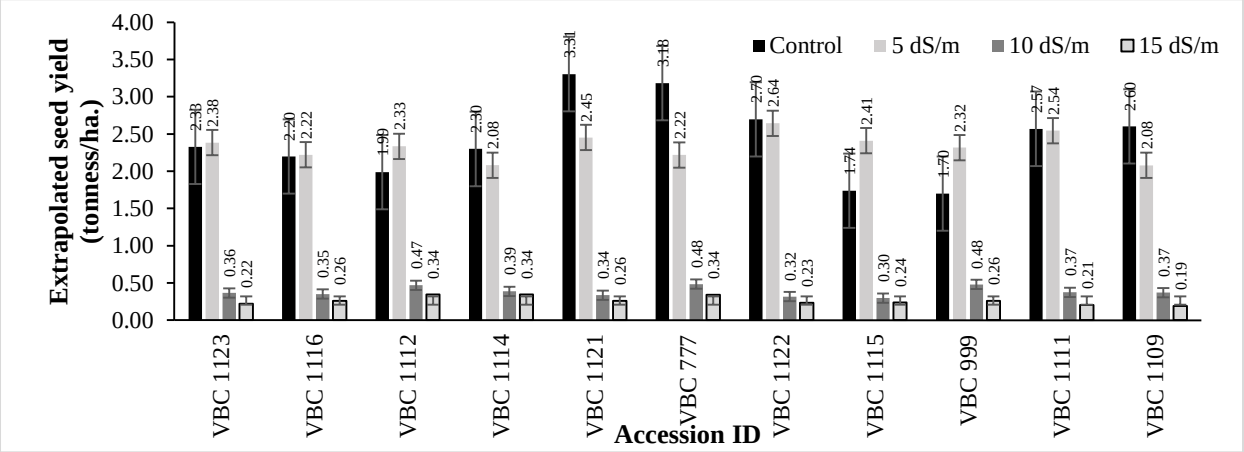


**Figure 20.** *Ricinus communis*: Extrapolated seed yield at different levels of salinity of irrigation water (Extrapolated). Error bars represent standard deviations from mean.

### 5.12. Seed oil Content

A soxhelet apparatus was used for solvent extraction of oil using n-hexane from 50 grams of seed. This was repeated in triplicates.

Seed oil content (%)= (Average weight of oil extracted/ 50) *100

There is no pattern of increase or decrease in seed oil content with increase in salinity (Figure 21) but the differences were statistically significant, as were differences among accessions (Table 21). Average seed oil content in the control treatment was 37.7% and ranged from 23.5 to 48.6%. In the 5 dS $m^{-1}$ treatment, average oil content was 36.5 and ranged between 25.7 and 47.9%. In the 10 dS $m^{-1}$ treatment average was 37.37%, and ranged between 25.2 and 49.3%. In the 15 dS $m^{-1}$ treatment the average value was 32.6% and varied between 16.5 to 50.6% in different accessions. Using these percentages and the extrapolated seed yield, the oil yield per hectare was estimated (Figure 22).

**Table 21.** *Ricinus communis* Seed Oil Content: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 1803.917 | 10 | 180.3917 | 9.906035 | 4.54E-07 | 2.16458 |
| Treatments | 184.4152 | 3 | 61.47172 | 3.375661 | 0.031134 | 2.922277 |
| Interaction | 1980 | 30 | 66.001 | - | <0.001 | - |
| Error | 546.3084 | 30 | 18.21028 | | | |
| Total | 2534.64 | 43 | | | | |

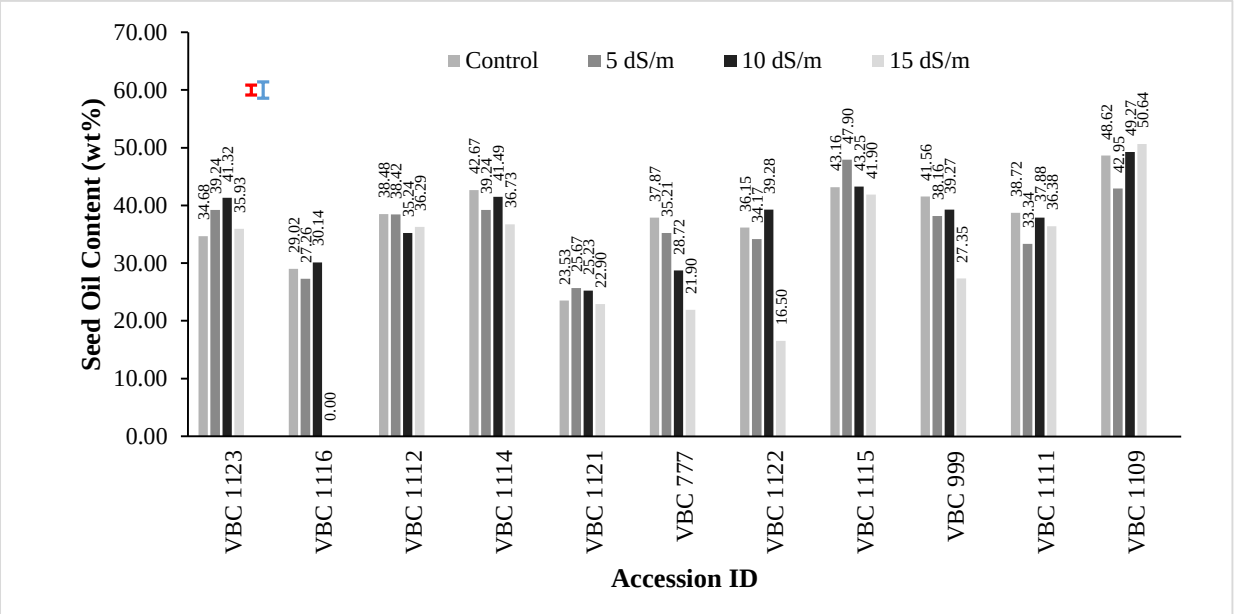


**Figure 21.** *Ricinus communis* - Seed oil content (% by weight) at different levels of irrigation water salinity. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

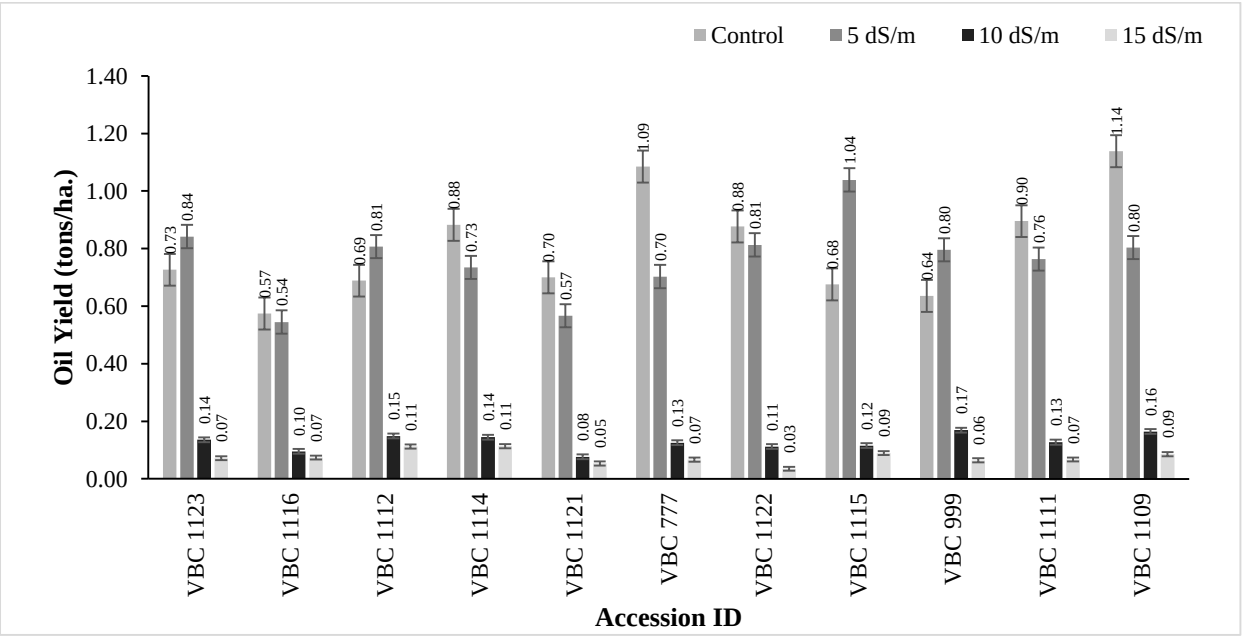


**Figure 22.** *Ricinus communis* - Oil yield per hectare at different levels of irrigation water salinity (Extrapolated).

### 5.13. 1000-seed weight

Thousand seed weight was calculated as average of three samples of 1000 seeds counted using a Contador Seed Counter from each accession. 1000-seed weight was recorded for all treatments (Figure 23) and while there was no statistically significant difference in 1000-seed weight between the control and 5 dS $m^{-1}$ treatments, differences with the other treatments were found to be highly significant (Table 22). The average 1000-seed weight in the control treatment was 307 gms, while in the 5 dS $m^{-1}$ treatment it was 342.3 gms and 307 and 299 gms in the 10 and 15 dS $m^{-1}$ treatments respectively.Within each treatment, differences among the accessions were also found to be

significant with values ranging between 227 and 440 gms in the control treatment, 222.4 and 380 gms in the 5 dS $m^{-1}$ treatment, 325 and 401.5 in the 10 dS $m^{-1}$ treatment, and 216.5 and 360.7 gms in the 15 dS $m^{-1}$ treatment.

**Table 22.** *Ricinus communis* 1000-seed weight: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 68874.73 | 10 | 6887.473 | 8.603004 | 1.97E-06 | 2.16458 |
| Treatments | 12997.26 | 3 | 4332.419 | 5.411537 | 0.00426 | 2.922277 |
| Interaction | 113875 | 30 | 3596 | - | 0.813 | - |
| Error | 24017.68 | 30 | 800.5893 | | | |
| Total | 105889.7 | 43 | | | | |

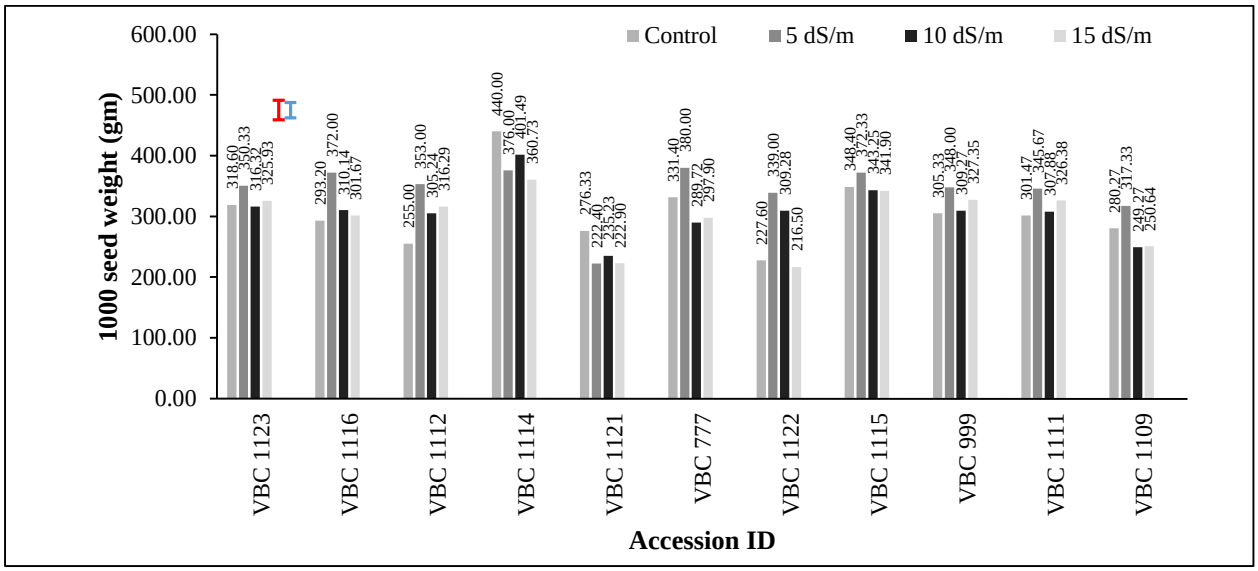


**Figure 23.** *Ricinus communis*: 1000-seed weight at different levels of irrigation water salinity. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

## 5.14. Discussion of *Ricinus communis* agronomic evaluation

*Ricinus communis* field trial was conducted to determine whether or not the hybrid accessions were tolerant to salinity. In a previously reported study of *Ricinus communis* bean emergence under salinity stress, a salinity threshold of 7.1 dS $m^{-1}$ was established [122]. In our study, in addition to the control, three treatments were set up at 5, 10 and 15 dS $m^{-1}$ to test for tolerance in the low and moderate salinity range.

The approximately 50-86% decrease in plant height and other growth parameters across accessions in the salinity treatments indicates that the species, or at least the accessions used in this study are sensitive to salinity. The reduction in leaf area reduces the amount of water used up

by the plant. The insignificant decrease in leaf water was interesting, considering the plant would be under osmotic stress at higher salinities and its ability to uptake water is compromised. This may be due to reduced height and leaf surface area, allowing water to reach all parts and water content in leaf tissue to be maintained at normal levels. As with many other abiotic stresses, plant growth is inhibited by salinity. A possible reason is stomatal closure resulting in decreased uptake of carbon dioxide and reduced photosynthesis or inhibition of cell growth and division [123]. The direct effects of salinity stress on cell expansion and division are not yet fully understood. In our study however, the rapid and extreme response of the plants in the 10 dS $m^{-1}$ and 15 dS $m^{-1}$ treatments suggests that the osmotic stress at such high salinity induces a stress response and stalling of growth in these plants. This is because high salinity in the root area decreases the plants capability to absorb water (osmotic stress) [124]. Within each treatment, the differences among the accessions were found to be insignificant. What was interesting to note is that even though biomass growth was severely reduced in the 5 dS $m^{-1}$ treatment, as indicated by decrease in height, stem thickness, leaf size, etc., there was no significant decrease in the size of the inflorescences, number of inflorescences per plant or number of fruits per inflorescence. As a result, in the 5 dS $m^{-1}$ salinity treatment, the extrapolated seed yields were between 1.8 and 2.3 tonnes/ha for different accessions while freshwater cultivation yielded between 1.5 and 3 tonnes/ha of seed, this difference not being statistically significant. In comparison with the control treatment, seed yield decreased by 66% and 82% with increase in salinity to 10 and 15 dS $m^{-1}$, respectively. It has been reported that some plants respond to drought stress by flowering and producing seed faster, while others store reserves in organs such as the stem and root which are later mobilized during the reproductive phase [125]. In our study the fruits in the salinity treatments were observed to mature almost 4 weeks before those in the control treatment, suggesting that the lack of decrease in reproductive function in the 5 dS $m^{-1}$ treatment is a survival mechanism in response to stress. Seed oil content and 1000-seed weight did not change with treatment. These seem to be inherent properties of a particular genotype. According to literature, *Ricinus communis* seeds generally contain up to 48% oil of which 42% can be extracted [126]. If irrigated with freshwater, accession VBC 1109 from this study can yield more than 1 tonne/ha. of oil from a single harvest, which is almost twice the annual yield of soybean oil [127].

# CHAPTER 6

# MORPHO-AGRONOMIC EVALUATION OF DESERT GOURD (*Citrullus colocynthis*)

Morphological characteristics and parameters of agronomic importance were recorded in all trials in order to assess the crop's response to treatment and to characterize the different accessions. Five or ten plants from each plot were selected randomly for observations, depending on the trait recorded. Visual observation of the physical descriptors of plants in the trial plots was used to categorize the physical/morphological characteristics of different accessions. Descriptors for *Citrullus colocynthis* was adapted from minimum descriptors for Cucurbita spp. described by the European Cooperative Programme for Plant Genetic Resources [128].

### 6.1. Morphological characteristics

- Fruit color at maturity:
    1. Dark green with light green striations
    2. Dark green with yellow striations
    3. Dark green with orange tinged striations
- Seed Color:
    1. Dark brown
    2. Medium brown
    3. Chestnut brown
    4. Olive green/ brown

Dark green with light green striations was the most commonly observed fruit color pattern among accessions. Only one accession had a green color with dark yellow/ orange tinged striations (Figure 24). Dark brown was the most common seed color (Table 23).

**Figure 24.** *Citrullus colocynthis* fruit color. Green with light green striations (Left), Green with yellowish-orange striations (Right)

**Table 23.** *Citrullus colocynthis* fruit and seed color

| Accession ID | Seed color | Fruit color | Accession ID | Seed color | Fruit color |
|---|---|---|---|---|---|
| RMS 246 | 2 | 1 | KMK3 | 1 | 1 |
| RMS 254 | 1 | 1 | RMS 258 | 1 | 2 |
| RMS 247 | 2 | 1 | RMS 227 | 1 | 1 |
| RMS 234 | 2 | 1 | RMS 237 | 1 | 1 |
| RMS 239 | 2 | 2 | RMS 240 | 2 | 1 |
| RMS 231 | 1 | 1 | RMS 249 | 1 | 1 |
| RMS 257 | 2 | 1 | RMS 215 | 1 | 1 |
| RMS 228 | 1 | 3 | KMK1 | 1 | 1 |
| RMS 255 | 1 | 1 | PI 525080 02 SD | 2 | 1 |
| RMS 220 | 2 | 2 | PI 386024 01 SD | 1 | 1 |
| RMS 245 | 2 | 1 | PI 525082 01 SD | 1 | 2 |
| RMS 244 | 1 | 1 | PI 537277 01 SD | 4 | 1 |
| RMS 256 | 3 | 2 | PI 388770 01 SD | 1 | 1 |
| RMS 250 | 1 | 1 | | | |

## 6.2. Number of branches

Number of vines or branches was recorded at first flowering. Average of five plants was calculated. Some accessions exhibited a more branched pattern of growth than others (Figure 25). Average number of branches ranged from 1.4 to 14 among accessions. The most highly branched accession was PI 388770. Among locally collected accessions, RMS 220 had the most number of branches. The difference among the accessions was statistically significant (Table 24).

**Table 24.** *Citrullus colocynthis* number of branches: Single factor ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Accessions | 677.3303839 | 26 | 26.05117 | 8.910939 | 1.03E-11 | 1.701636 |
| Within Accessions | 157.8692266 | 54 | 2.923504 | | | |
| Total | 835.1996105 | 80 | | | | |

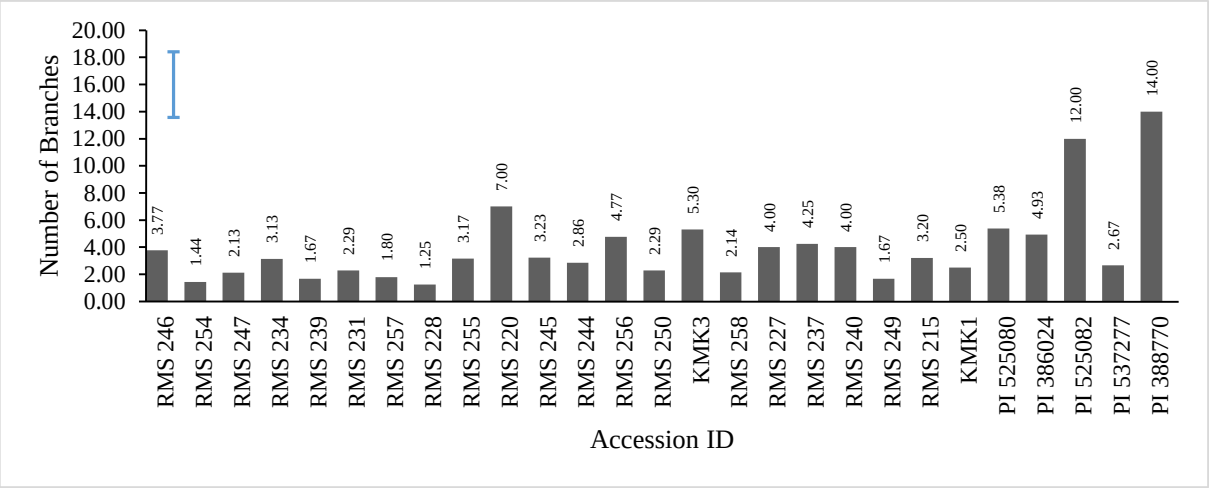


**Figure 25.** *Citrullus colocynthis:* Number of branches in the different accessions. Least significant difference among accessions (blue error bar) is indicated

### 6.3. Vine length

Vine length was recorded at first flowering using a meter scale. Average of five plants was calculated for each accession.

Average vine length was not significantly different among the accessions (Table 25), but ranged from 23 to 250 cm. Accession PI 388770 had the longest vines and among the locally collected accession RMS 256 had highest mean vine length (Figure 26).

**Table 25.** *Citrullus colocynthis* vine length: Single factor ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Accessions | 54 | 2 | 27 | 0.007483 | 0.992546 | 3.113792 |
| Within Accessions | 281454 | 78 | 3608.385 | | | |
| Total | 281508 | 80 | | | | |

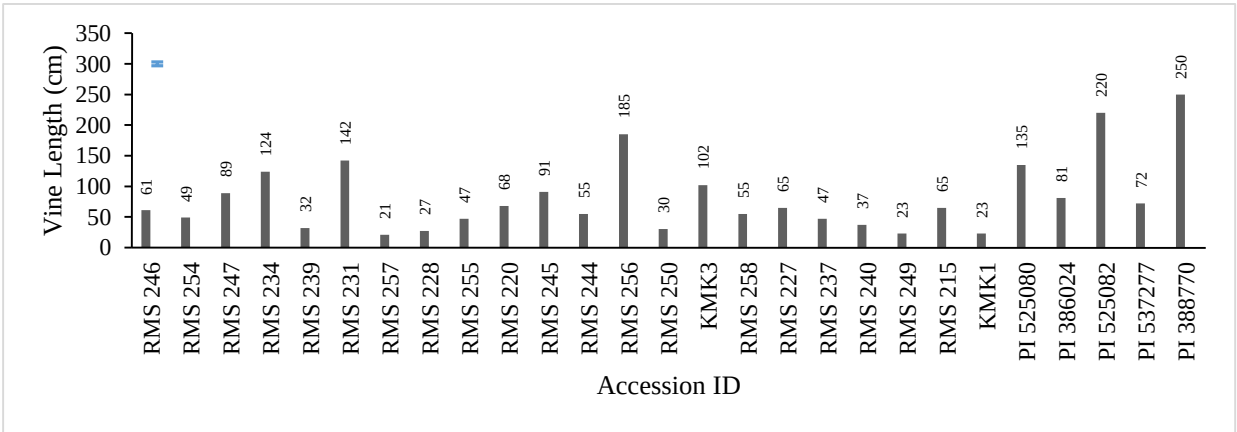


**Figure 26.** *Citrullus colocynthis*: Vine length in different accessions. Least significant difference among accessions (blue error bar) is indicated

### 6.4. Leaf size

Leaf length and breadth was noted. Average of twenty readings from each plot was calculated. The fifth leaf on a branch was considered as the standard.

Leaf length and breadth increased or decreased in proportion with each other and in keeping with the shape of the leaves, breadth is always lower than length. Leaf length and breadth varied significantly among the accessions (Leaf fresh and dry weight was noted and average moisture content was calculated for each accession (Figure 28), which ranged from 76 to 84 % and varied significantly (Table 26).

Table 27). RMS 231 and RMS 256 had the longest leaves (Figure 27).

**Table 26.** *Citrullus colocynthis* leaf length: Single factor ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Accessions | 233.2296 | 25 | 9.329183 | 2.822265 | 0.000797 | 1.718753 |
| Within Accessions | 171.8895 | 52 | 3.305567 | | | |
| Total | 405.119 | 77 | | | | |

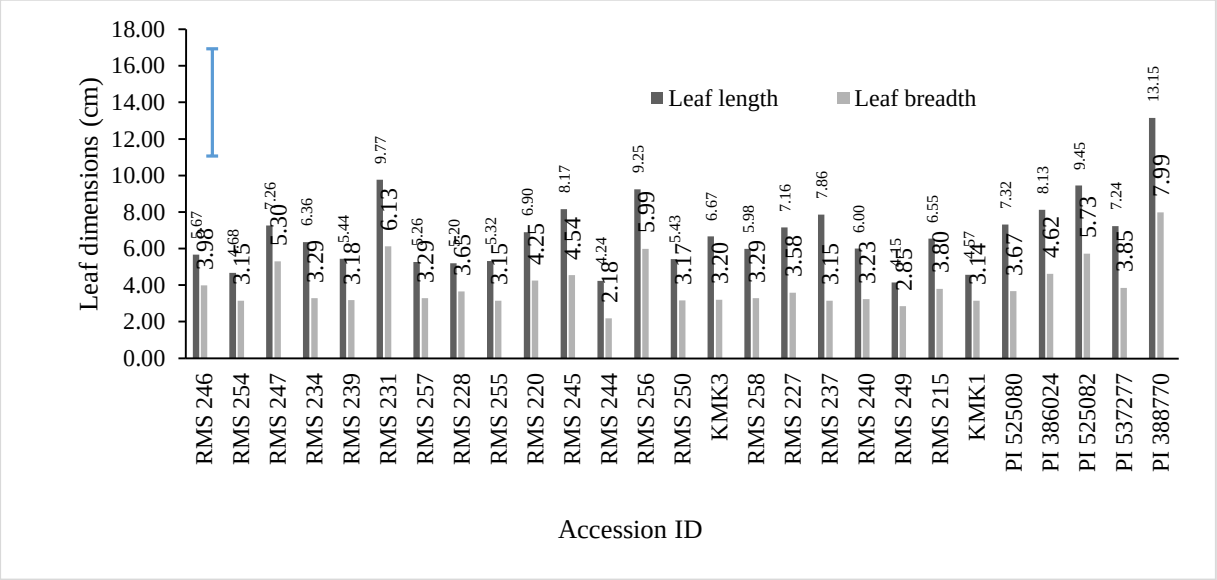


**Figure 27.** *Citrullus colocynthis*: Leaf Size. Least significant difference among accessions (blue error bar) is indicated

### 6.5. Leaf dry weight and moisture content

Twenty leaves were collected at maturity from each plot, cleaned to remove dirt, and fresh weight was recorded. Leaves were then dried at 80°C in a hot air oven till a static weight was reached, and dry weight was recorded. Moisture content was calculated as [(Fresh weight- Dry weight)/ Dry Weight] *100.

Leaf fresh and dry weight was noted and average moisture content was calculated for each accession (Figure 28), which ranged from 76 to 84 % and varied significantly (Table 26).

**Table 27.** *Citrullus colocynthis* leaf moisture: Single factor ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Accessions | 534.6887 | 25 | 21.38755 | 4.234167 | 5.62E-06 | 1.718753 |
| Within Accessions | 262.6615 | 52 | 5.051182 | | | |
| Total | 797.3501 | 77 | | | | |

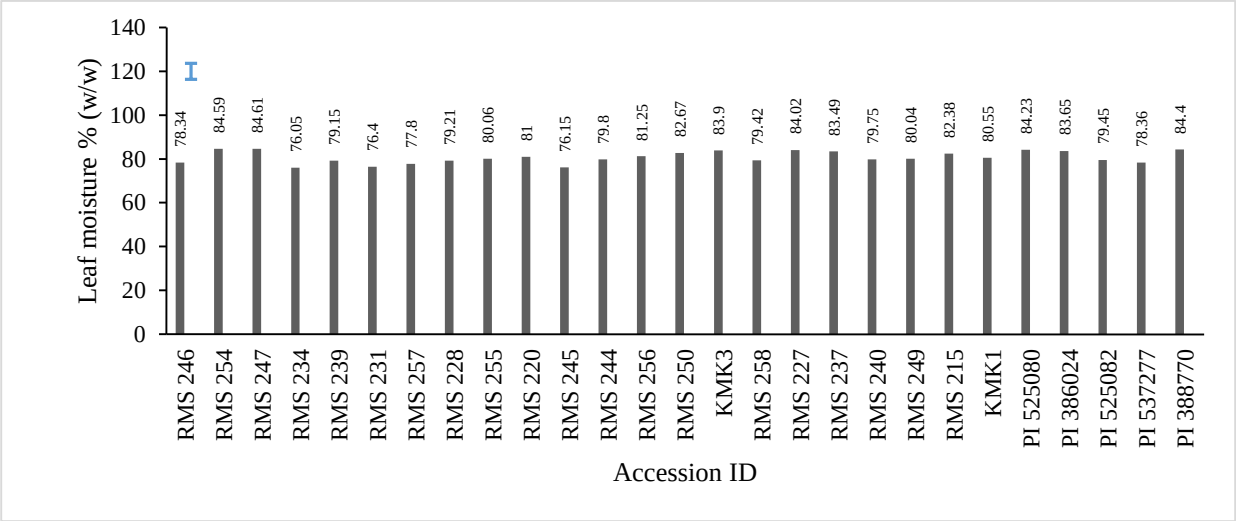


**Figure 28.** *Citrullus colocynthis*: Leaf moisture content. Least significant difference among accessions (blue error bar) is indicated

### 6.6. Number of fruits

Number of fruits per plant was recorded and collected over a period of one year. Average number of fruits per plant ranged from 2.4 to 36 and varied significantly (Table 27) between accessions. RMS 228, RMS 239 and RMS 227 bore the most number of fruits among all accessions (Figure 29).

**Table 28.** *Citrullus colocynthis* number of fruits per plant: Single factor ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Accessions | 5847.16 | 25 | 233.8864 | 5.048576 | 4.32E-07 | 1.718753 |
| Within Accessions | 2409.014 | 52 | 46.3272 | | | |
| Total | 8256.175 | 77 | | | | |

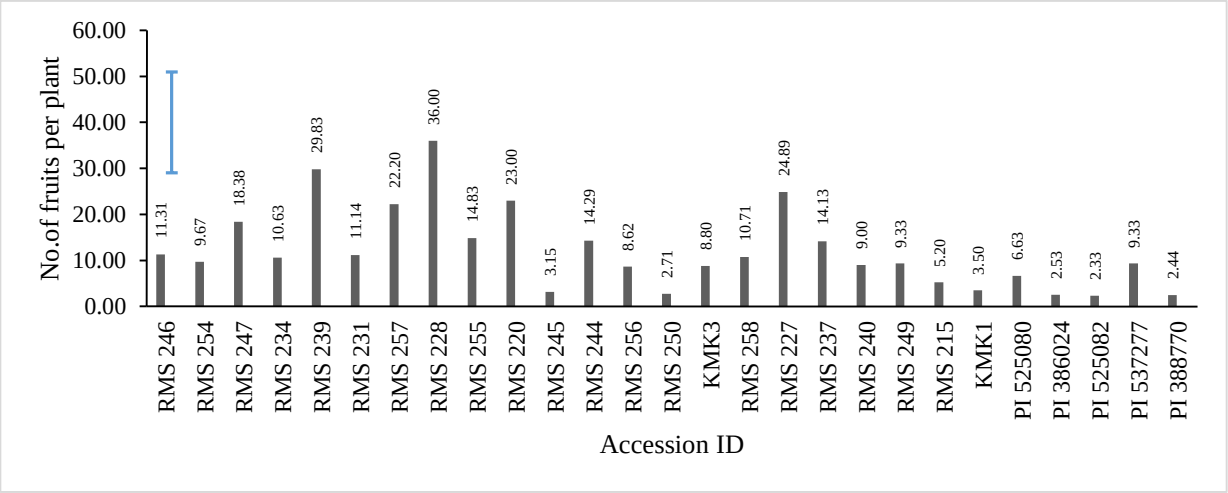


**Figure 29.** *Citrullus colocynthis*: Number of fruits per plant. Least significant difference among accessions (blue error bar) is indicated

### 6.7. Fruit size

Average fruit diameter of 10 fruits from each plot was calculated. Average fruit size ranged from 4.3 to 15.3 cm but did not vary significantly between accessions (Table 29), except for one particular accession, PI 525082 01 SD, which bore significantly larger fruits than all the other accessions (Figure 30).

**Table 29.** *Citrullus colocynthis* fruit diameter: Single factor ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Accessions | 349.896 | 25 | 13.99584 | 0.318493 | 0.998637 | 1.718753 |
| Within Accessions | 2285.087 | 52 | 43.94398 | | | |
| Total | 2634.983 | 77 | | | | |

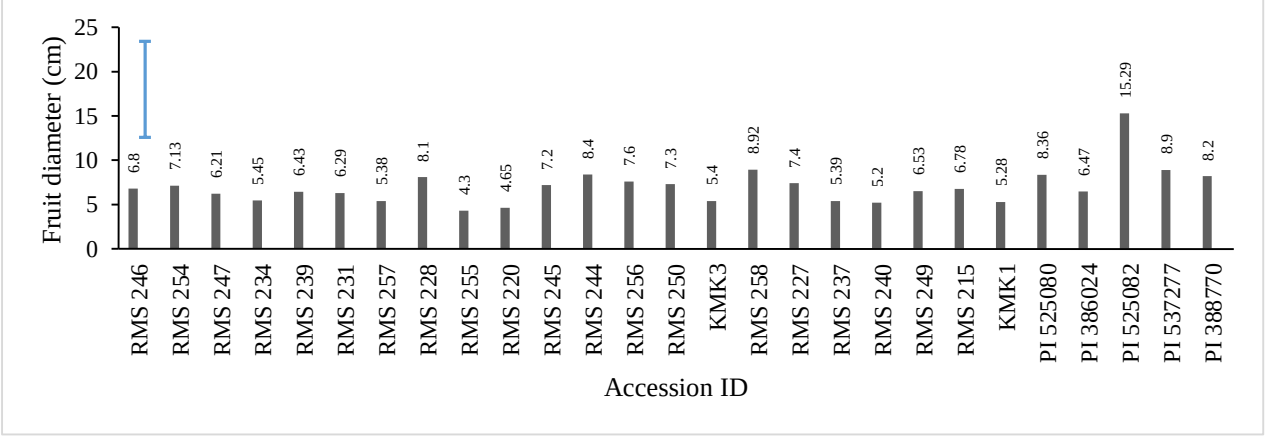


**Figure 30.** *Citrullus colocynthis*: Fruit size. Least significant difference among accessions (blue error bar) is indicated

### 6.8. Seed yield

Seeds from all fruits from each plot collected over the year were extracted and weighed (Figure 31). Yield per plant was calculated by dividing total yield by number of plants. This data was used to extrapolate yield in tonnes/ha. for a stand of 40,000 plants with the same density as that of the field trial.

Average oilseed yield per plant ranged from 12 to 374 gms and varied greatly between accessions (Table 30), as seen in Figure 32. Extrapolated oilseed yield per hectare based on this data ranged between 0.48 to nearly 15 tonnes/ha. as shown in Figure 33. KMK1, RMS 227, RMS 228, RMS 244 and RMS 239 were the highest yielding in terms of seed yield.

**Figure 31.** *Citrullus colocynthis* seed yield

**Table 30.** *Citrullus colocynthis* yield per plant: Single factor ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *Df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Accessions | 544103.9 | 25 | 21764.16 | 134.9163 | 6.71E-39 | 1.718753 |
| Within Accessions | 8388.433 | 52 | 161.316 | | | |
| Total | 552492.4 | 77 | | | | |

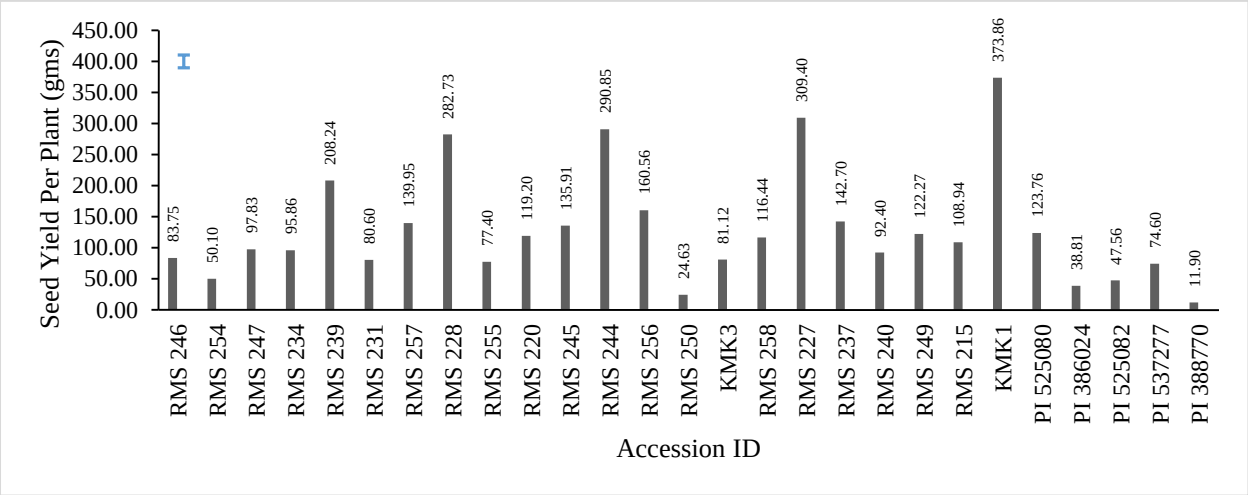


**Figure 32.** *Citrullus colocynthis*: Seed yield per plant. Least significant difference among accessions (blue error bar) is indicated

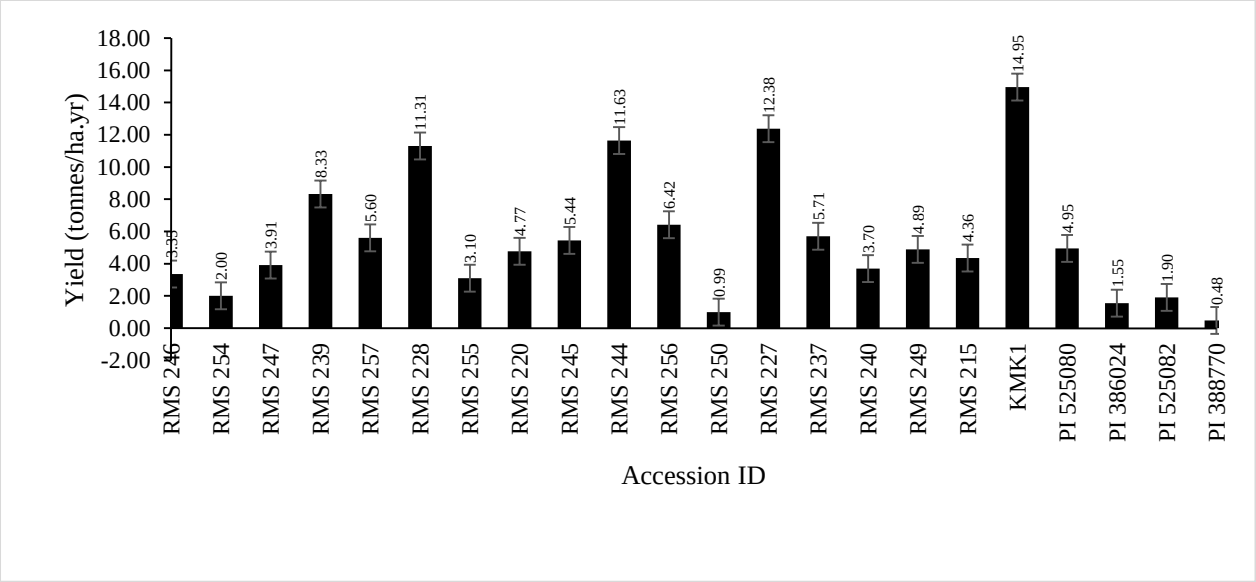


**Figure 33.** *Citrullus colocynthis*: Extrapolated oilseed yield per hectare. Error bars represent standard error

### 6.9. Seed oil Content

A soxhelet apparatus was used for solvent extraction of oil using n-hexane from fifty grams of seed. This was repeated in triplicate.

Seed oil content (%)= (Average weight of oil extracted/ 50) *100

Average oil content in seeds based on solvent extraction ranged from 9.4 to 43.8%, depending on accession (Figure 34) and varied significantly between accessions (Table 31). Based on this data, extrapolated oil yields from each accession is presented in Figure 35. It is interesting to note that in spite of the high oil content, RMS 247 is not the highest yielding in terms of total oil, due to the low oilseed yield. RMS 244 and RMS 227 on the other hand still have a high oil yield in spite of the lower oil content due to high seed yield. Extrapolated oil yields ranged from 0.04 to 3.44 tonnes/ha. RMS 228 is potentially the highest oil yielding accession in our study.

**Table 31.** *Citrullus colocynthis* seed oil content: Single factor ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Accession | 4774.753 | 25 | 190.9901 | 296.8167 | 1.14E-47 | 1.718753 |
| Within Accessions | 33.46 | 52 | 0.643462 | | | |
| Total | 4808.213 | 77 | | | | |

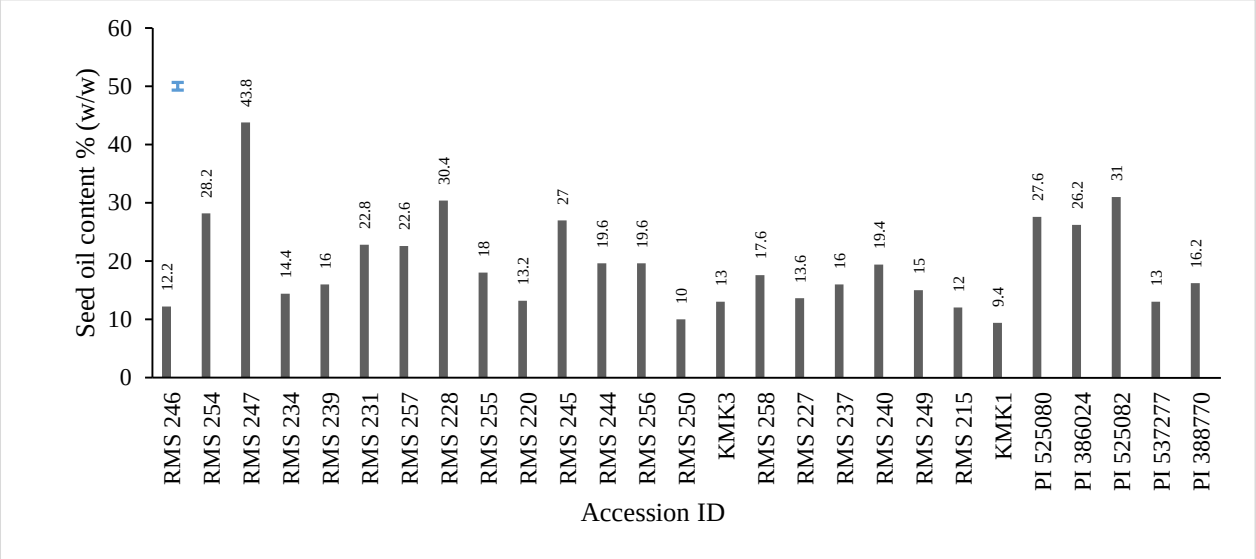


**Figure 34.** *Citrullus colocynthis*: Seed oil content. Least significant difference among accessions (blue error bar) is indicated

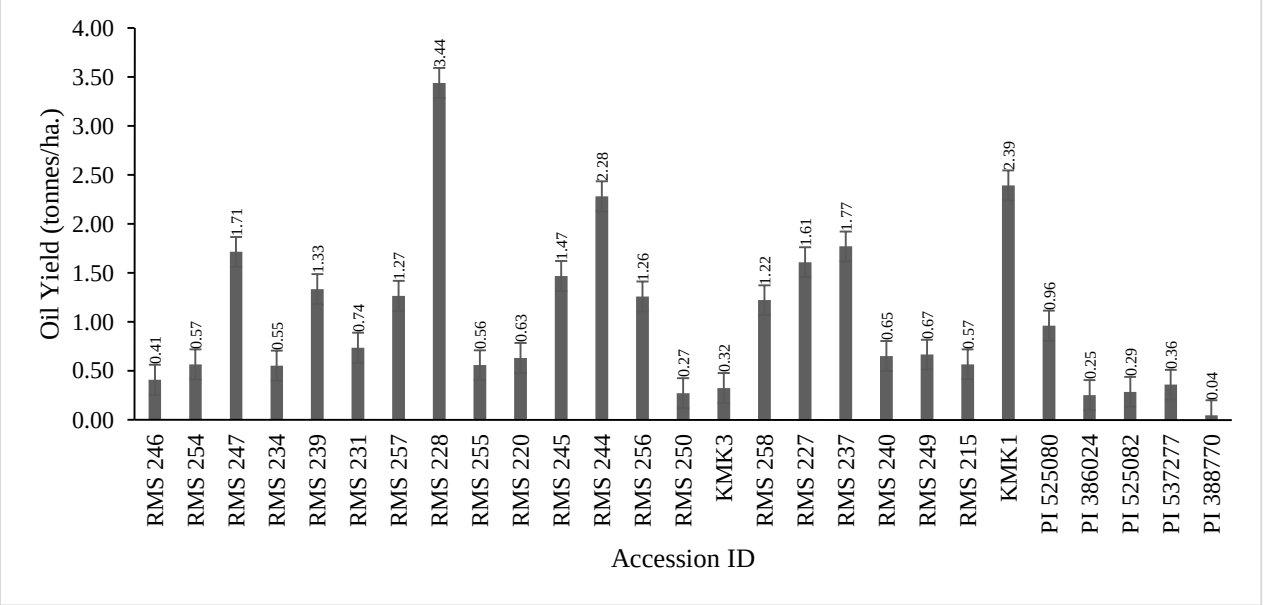


**Figure 35.** *Citrullus colocynthis*: Oil yield per hectare (Extrapolated). Error bars represent standard error

## 6.10. 1000-seed weight

Thousand seed weight was calculated as average of three samples of 1000 seeds each from each plot, for which seeds were counted using a Contador Seed Counter. Thousand seed weight was higher in the accessions obtained from GRIN, USDA, ranging between 28 and 72 gms (Figure

36). Thousand seed weight of locally collected accessions ranged between 18 and 38 gms. There was not a very significant variability in this characteristic among the locally collected accessions (Table 32).

**Table 32.** *Citrullus colocynthis* thousand seed weight: Single factor ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Accessions | 54 | 2 | 27 | 0.150994 | 0.860103 | 3.113792 |
| Within Accessions | 13947.56 | 78 | 178.8148 | | | |
| Total | 14001.56 | 80 | | | | |

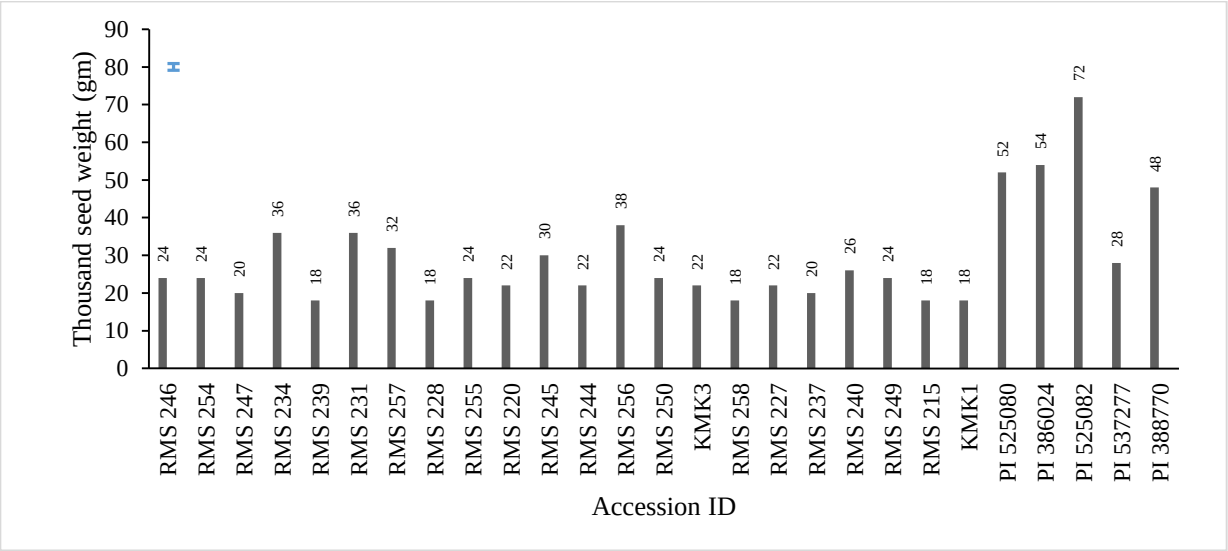


**Figure 36.** *Citrullus colocynthis*: Thousand seed weight. Least significant difference among accessions (blue error bar) is indicated

### 6.11. Seed size

Approximate seed size (surface area of the large, flat phase) was calculated from images using the ImageJ Image processing and the analysis software provided as freeware by the National Institutes of Health (NIH), US, which is a benchmark tool for image analysis and area measurements [129].

Most accessions had seeds of a similar size, ranging between 0.11 and 0.14 $cm^2$ (Figures 37 and 38), but a few such as PI 525082 had significantly larger seeds (Table 33).

**Table 33.** *Citrullus colocynthis* seed surface area: Single factor ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Accessions | 0.082404 | 21 | 0.003924 | 87.11172 | 1.47E-16 | 2.058728 |
| Within Accessions | 0.000991 | 22 | 4.5E-05 | | | |
| Total | 0.083395 | 43 | | | | |

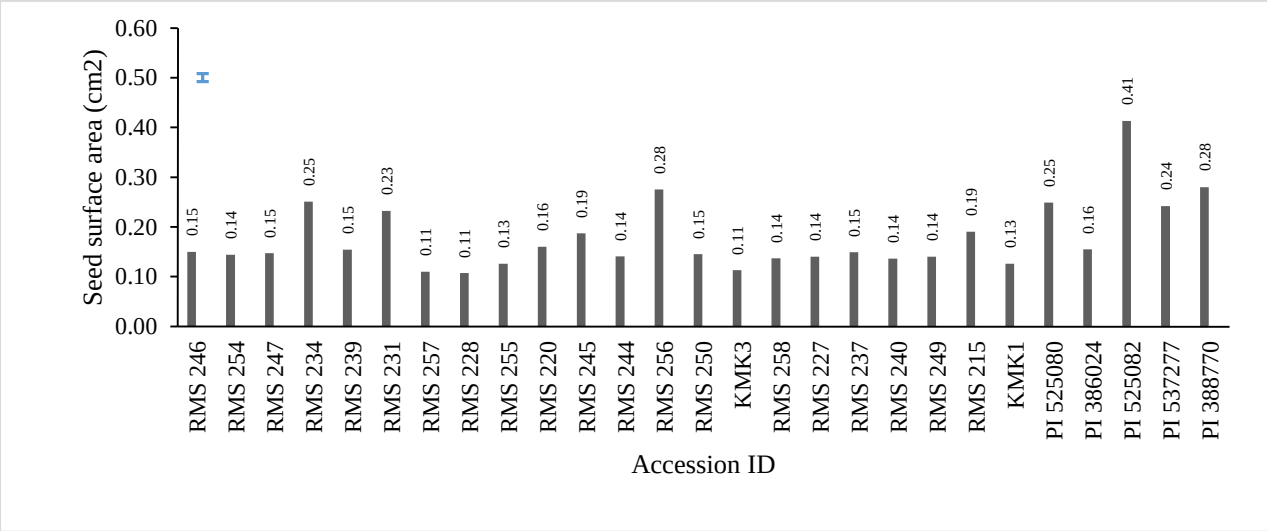


**Figure 37.** *Citrullus colocynthis*: Seed size. Least significant difference among accessions (blue error bar) is indicated

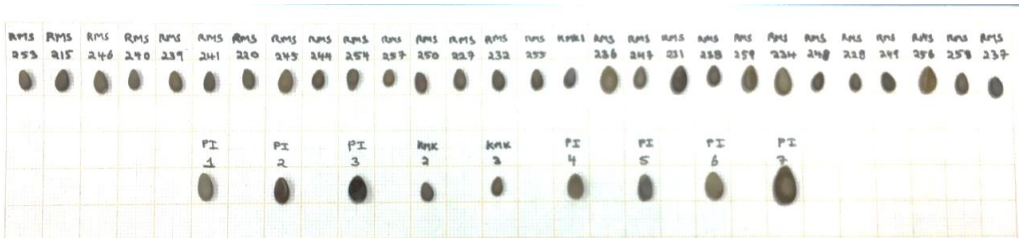

**Figure 38.** *Citrullus colocynthis* seed size measurement using ImageJ ® software

## 6.12. Discussion of *Citrullus colocynthis* agronomic evaluation

The data from the *Citrullus colocynthis* salinity trial cannot be presented due to inadequate and non-uniform sample size for all accessions, but as described earlier the five accessions chosen for the study were all extremely sensitive to salinity. The germplasm diversity assessment trial was conducted with freshwater irrigation. Of the 37 accessions, only 27 germinated and persisted in the field trial. Morphological and agronomic data was collected for these 27 accessions. There was significant variability in almost all observed and determined characteristics among the different

accessions. While most seeds are small and dark brown, there were some that were lighter/ had a green tinge or were larger. Some accessions had a highly branched pattern of growth while some others had plants with a single main stem or single branch. The stems could grow long and the mean branch length recorded at first flowering was greater than half a meter for most accessions. Because of the tendency for the plants to grow indeterminately, and the branches had to be coiled back into the plot as the plants grew, it was not possible to take branch length measurements at the end of the year's growth without cutting through tangled branches. There was no great variability in leaf size, the older leaves closer to the base were the largest, and these were measured for all accessions. Leaf moisture content also varied significantly between accessions even though all the leaves were collected at the same time and from the same position. In the wild, *Citrullus colocynthis* reportedly has two flowering seasons annually [130]. Under irrigated conditions it was observed that the plants flower almost continually through the year. This could be the reason for the very high seed and fruit yield per plant Fruits were collected from each accession over a period of one year to determine the average number of fruits per plant under irrigated conditions. While some accessions bore more fruits than others, accessions RMS 239, RMS 228, RMS 227, RMS 220, RMS 257 and RMS 247 yielded above the average. The average fruit size was more or less similar with the exception of PI 525082 01 SD, which bore very large fruits. Accessions KMK1, RMS 227, RMS 244, RMS 239 and RMS 228 had the highest oilseed yield per plant (200-375 g) and these accessions should be considered for large scale cultivation. As with *Ricinus communis*, the higher seed yielding accessions did not necessarily have the higher oil content. *Citrullus colocynthis* has a very high yield per plant in comparison with most other oilseed crops such as *Jatropha curcas* [131], extrapolation based on the spacing used in the field trial gives a stand of 40000 per hectare, for which seed yields of the aforementioned accessions would range between 8 and 15 tonnes/ha. under irrigated conditions. From the point of view of agricultural management however, it may be necessary to choose and breed the more compact varieties as the highly spread out growth habit of the plant could hinder agricultural practices such as mechanical harvesting, weeding, etc. The extrapolated annual oil yield between 1- 3.4 tonnes/ ha. for the high yielding accessions is greater than that from most oilseed crops [132].

# CHAPTER 7

# MORPHO-AGRONOMIC EVALUATION OF MUSTARD

Morphological traits and characteristics of agronomic relevance were recorded to study the response of this crop to salinity and TWW treatment. Five plants from each plot were selected randomly for observations. Visual observation of the physical descriptors of plants in the trial plots was used to categorize the physical/morphological characteristics of different accessions. Descriptors for *Brassica juncea* was adapted from descriptors for Brassica spp. described by the International Board for Plant Genetic Resources, & Commission of the European Communities [133].

### 7.1. Plant height

Height was measured at maturity using a meter scale and average of five plants per plot calculated. Average plant height was not significantly different when comparing all treatments (Table 34). There was an observed increase in plant height in the treated waste water treatment (Figure 39) at the level p= 0.004. There is also a significant decrease in plant height in the salinity treatment when compared to the control treatment (p=0.0006), which is especially obvious in the accessions ATC- 90783 and ATC- 93402. The differences in plant height between accessions was not significant when comparing the control and treated waste water treatments (p>0.05), but there was significant difference in response of individual accessions to the salinity treatment (p=0.003). There was significant interaction between the two factors. Average plant height in the control treatment was 98.31 cm, 148.8 cm in the TWW treatment and 67.6 cm in the 15 dS $m^{-1}$.

**Table 34.** *Brassica juncea* plant height: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accession | 2074.902 | 4 | 518.7255 | 0.285827311 | 0.88058 | 3.47805 |
| Treatment | 16803.1 | 2 | 8401.4 | 29.46 | <0.001 | 4.689 |
| Interaction | 1347 | 40 | 168.4 | - | <0.001 | - |
| Total | 20223.12 | 14 | | | | |

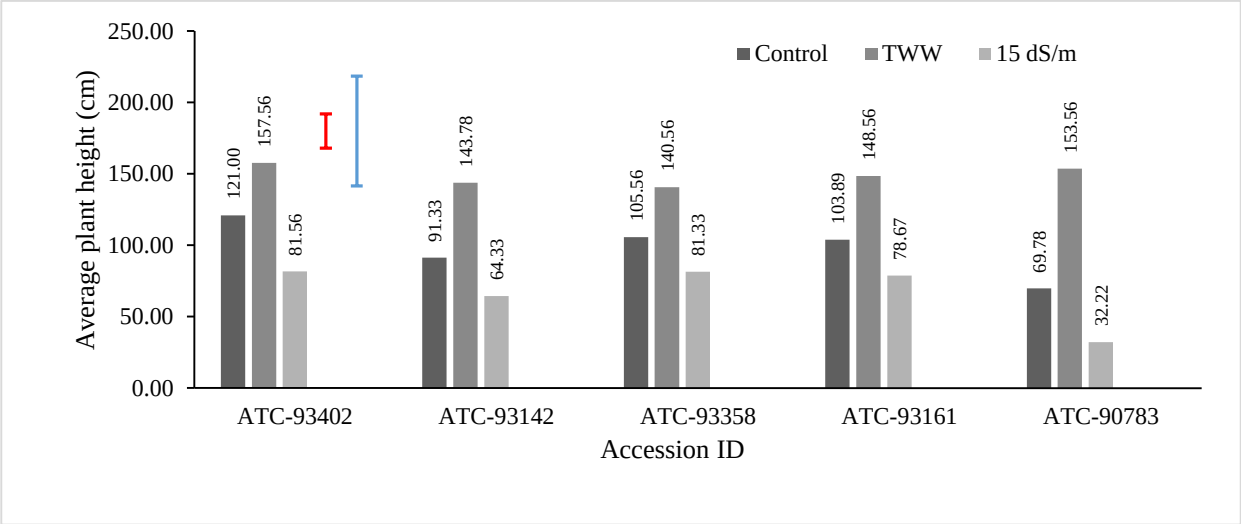


**Figure 39.** *Brassica juncea*: average plant height in treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

## 7.2. Leaf Size

Leaf length and breadth was calculated as average of 10 from each plot. Second leaf from base of plant was considered as standard. The difference among all treatments was significant while those among accessions was not (Table 35). Similar to plant height, there was an increase in all accessions in the treated waste water treatment (p=0.02), most significantly in accessions ATC-93402, ATC- 93358 and ATC- 93161 (Figure 40). The decrease in leaf length in the salinity treatment was not statistically significant (p>0.05). There was significant interaction between the factors.

**Table 35.** *Brassica juncea* leaf length: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 24.24815 | 4 | 6.062037037 | 0.4523 | 0.8 | 3.83785335 |
| Treatments | 397.437 | 2 | 198.7185185 | 14.826 | <0.001 | 4.45897011 |
| Interaction | 321.68 | 8 | 40.211 | - | <0.001 | - |
| Error | 107.2296 | 8 | 13.4037037 | | | |
| Total | 528.9148 | 14 | | | | |

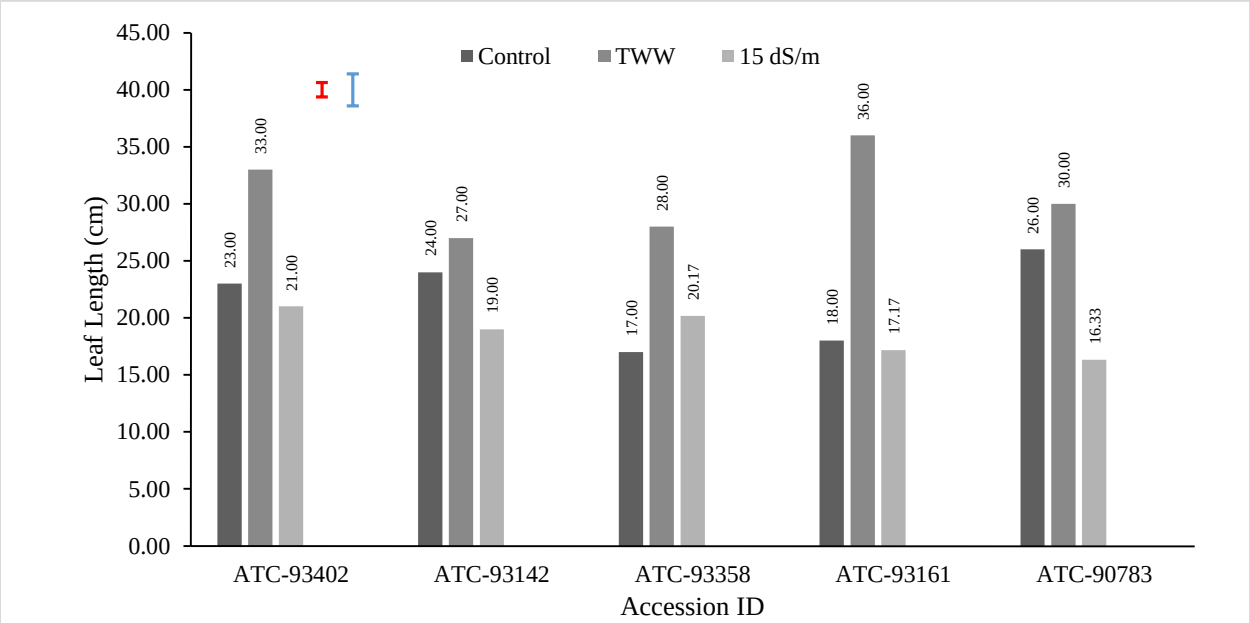


**Figure 40.** *Brassica juncea* average leaf length across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

**Table 36.** *Brassica juncea* leaf breadth: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 19.48474 | 4 | 4.871185185 | 1.0722 | 0.4 | 3.83785335 |
| Treatments | 92.24548 | 2 | 46.12274074 | 10.152 | <0.001 | 4.45897011 |
| Interaction | 109.03 | 8 | 13.629 | - | <0.001 | - |
| Error | 36.34415 | 8 | 4.543018519 | | | |
| Total | 148.0744 | 14 | | | | |

The difference in leaf breadth (Figure 41) between the control and TWW treatments and between control and salinity treatment was statistically insignificant ($p>0.05$), but significant if all treatments were considered (Table 36). There was significant interaction between the two factors.

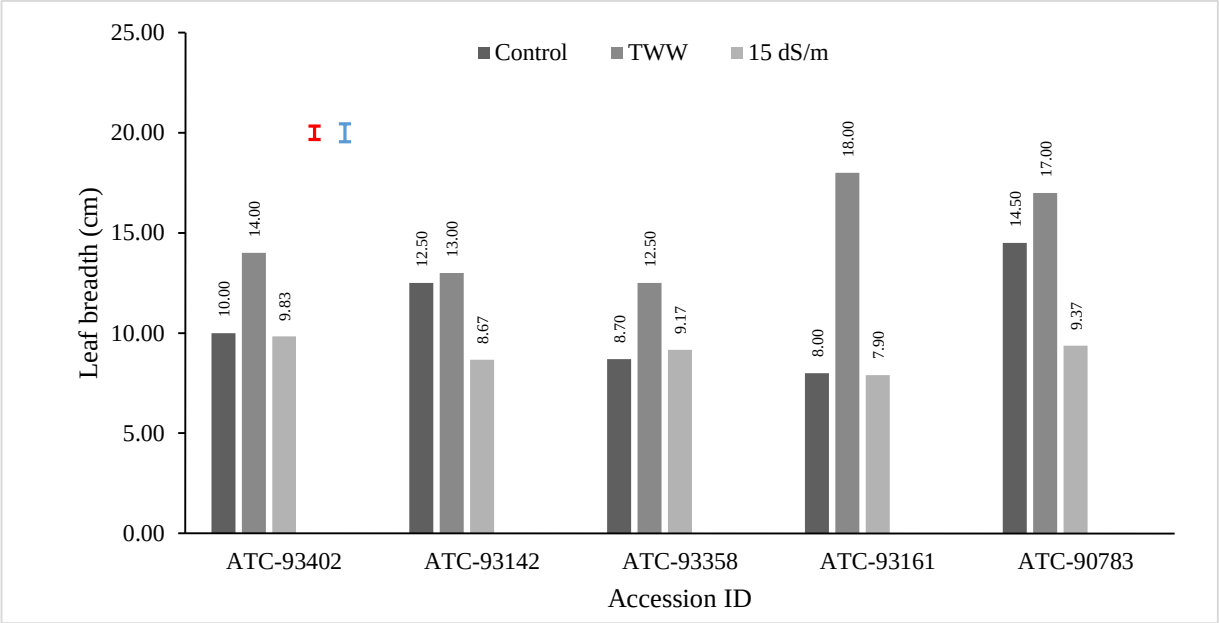


**Figure 41.** *Brassica juncea* average leaf breadth across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

### 7.3. Leaf dry weight and Moisture content

Ten leaves were collected at maturity from each plot, cleaned to remove dirt, and fresh weight was recorded. Leaves were then dried at 80°C in a hot air oven till a static weight was reached, and dry weight was recorded. Moisture content was calculated as [(Fresh weight- Dry weight)/ Dry Weight] *100.

Leaf dry weight is a measure of biomass, and a significant increase was observed in the TWW treatment (Figure 42) at the level p=0.005. In the salinity treatment that is no significant difference in dry weight (p>0.05) from control except for an increase in the leaf dry weight of Accession ATC- 93358. The differences among accessions across treatments was statistically insignificant (Table 37). There was no significant interaction between the two factors.

**Table 37.** *Brassica juncea* leaf dry weight: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 0.261432 | 4 | 0.065358 | 2.177257 | 0.162103 | 3.837853 |
| Treatments | 1.038288 | 2 | 0.519144 | 17.29413 | 0.001245 | 4.45897 |
| Interaction | 6.48 | 8 | 0.8105 | - | 0.186 | - |
| Error | 0.240148 | 8 | 0.030019 | | | |
| Total | 1.539868 | 14 | | | | |

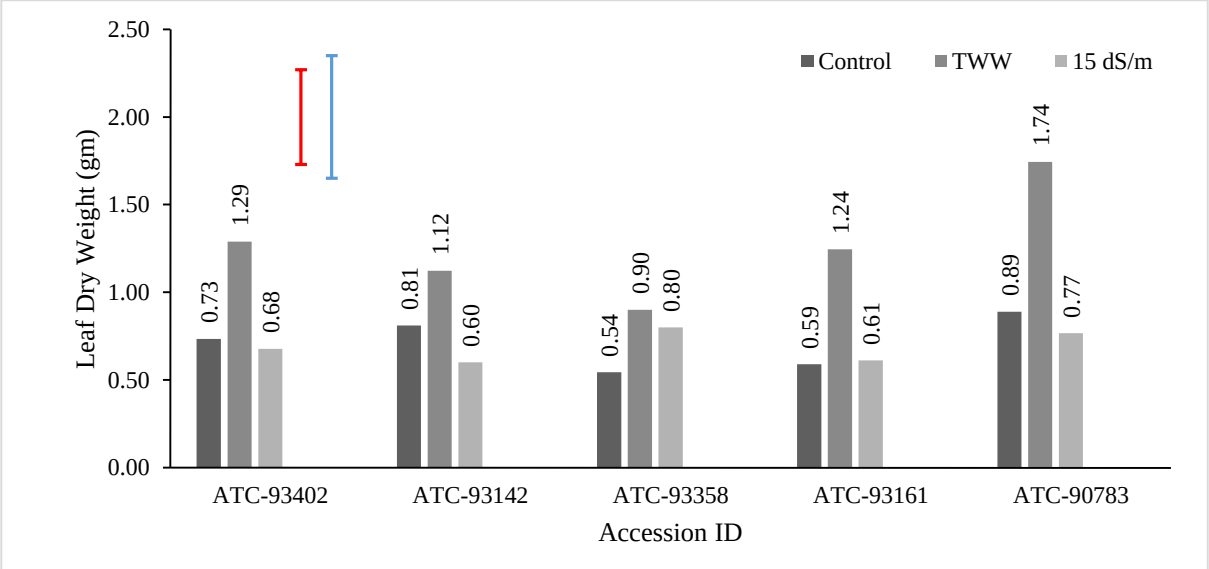


**Figure 42.** *Brassica juncea* average leaf dry weight across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

**Table 38.** *Brassica juncea* leaf moisture content: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *Df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 2.162174 | 4 | 0.540544 | 1.157811 | 0.396894 | 3.837853 |
| Treatments | 8.039551 | 2 | 4.019776 | 8.61011 | 0.010124 | 4.45897 |
| Interaction | 12.63 | 8 | 1.579 | - | 0.033 | - |
| Error | 3.734935 | 8 | 0.466867 | | | |
| Total | 13.93666 | 14 | | | | |

Leaf moisture content also increase significantly with the TWW treatment (Table 38) and decreased in the salinity treatment (Figure 43), but the decrease was not statistically significant ($p>0.05$). Different accessions did not respond differently to the treatments. There was no significant interaction between factors.

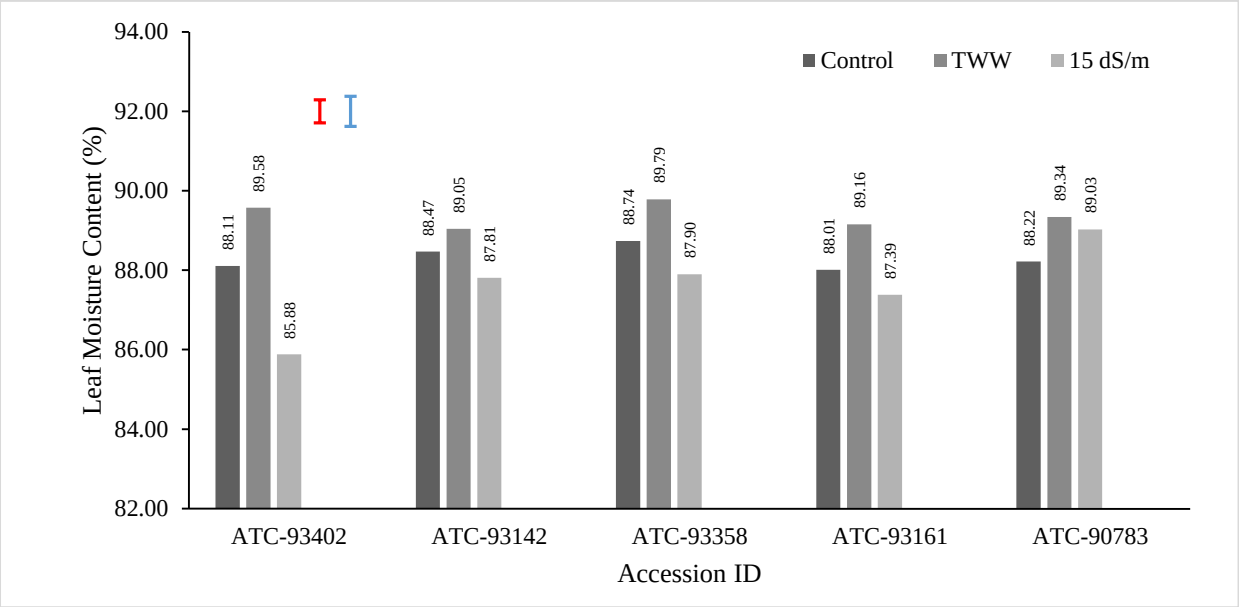


**Figure 43.** *Brassica juncea* average leaf moisture content across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

## 7.4. Pod/ Silique size

Length of ten pods from each plot noted and average was calculated.

**Figure 44.** *Brassica juncea* seed pods/ silique

**Table 39.** *Brassica juncea* pod length: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 1.718267 | 4 | 0.429567 | 8.811323 | 0.004989 | 3.837853 |
| Treatments | 0.368853 | 2 | 0.184427 | 4.45897 | 0.006968 | 3.782982 |
| Interaction | 1.15 | 8 | 0.144 | - | <0.001 | - |
| Error | 0.390013 | 8 | 0.048752 | | | |
| Total | 2.477133 | 14 | | | | |

There was not a significant difference in average pod length between the control and TWW treatments as (Figure 45). There was a significant decrease in pod length in the salinity treatment when compared to control (p=0.019). There was also a significant difference in the response of different accessions to the salinity treatment (Table 39). There was a significant interaction between the two factors.

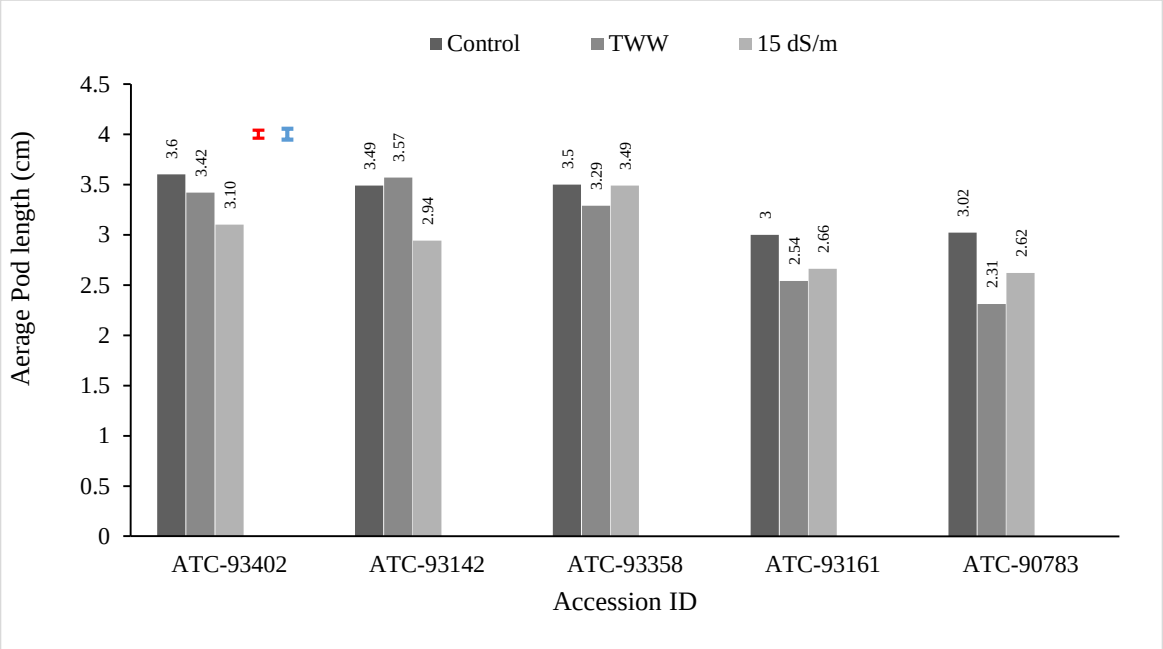


**Figure 45.** *Brassica juncea* average pod length across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

## 7.5. Seed yield

Seeds from five plants in each plot were extracted and weighed. Seed yield is expressed in tonnes per hectare based on extrapolation of the average yield of the five plants from each plot and assuming a stand of 40,000 plants per hectare, at the same density as in the current field trial.

Seed yield per plant increased in the TWW treatment (p=0.027). The increase was obvious for four accessions (approximately three-fold increase in yield), while there was no significant difference for accession ATC- 93358, which is the highest yielding accession in the control treatment, by a significant margin (Figure 46). It does not however, stay stable in the salinity treatment, and yield is drastically decreased. Average oilseed yield per plant in the control treatment was 8.3 gms and this decreased in the salinity treatment to 2.19 gms but the difference was not statistically significant in comparison to control. The average yield per plant in the TWW treatment was 23 gms. There was not significant difference between accessions across treatments (Table 40).

**Table 40.** *Brassica juncea* yield per plant: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 30.87388 | 4 | 7.718471 | 0.291065 | 0.875834 | 3.837853 |
| Treatments | 1147.292 | 2 | 573.6459 | 21.63226 | 0.000593 | 4.45897 |
| Interaction | 201.98 | 8 | 25.248 | - | 0.09 | - |
| Error | 212.1446 | 8 | 26.51807 | | | |
| Total | 1390.31 | 14 | | | | |

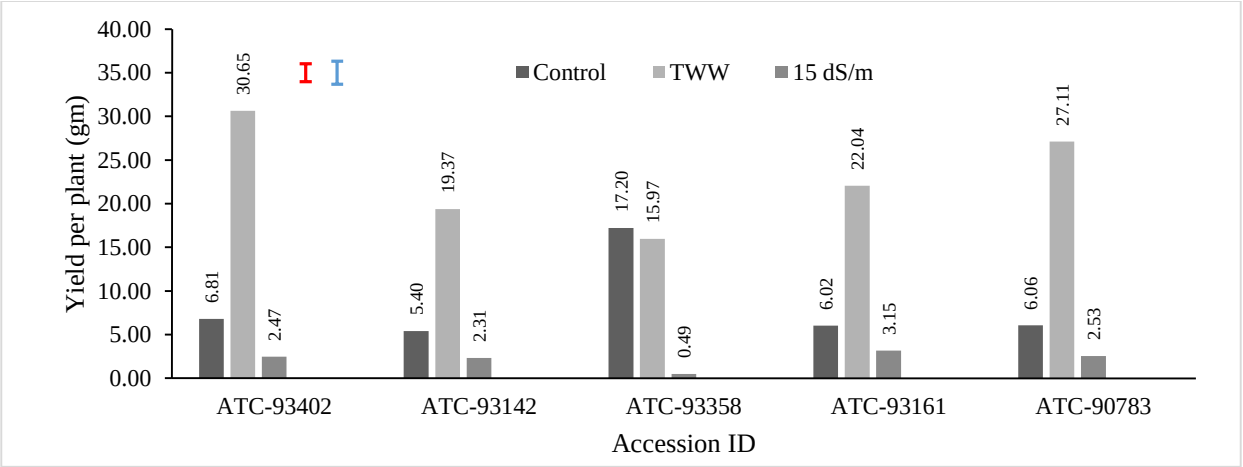


**Figure 46.** *Brassica juncea* average seed yield across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

Extrapolated data (Figure 47), assuming a stand of 40000 plants per hectare showed a potential yield of 0.57 to 1.1 tonnes per hectare depending on the accession, upon irrigation with treated waste water.

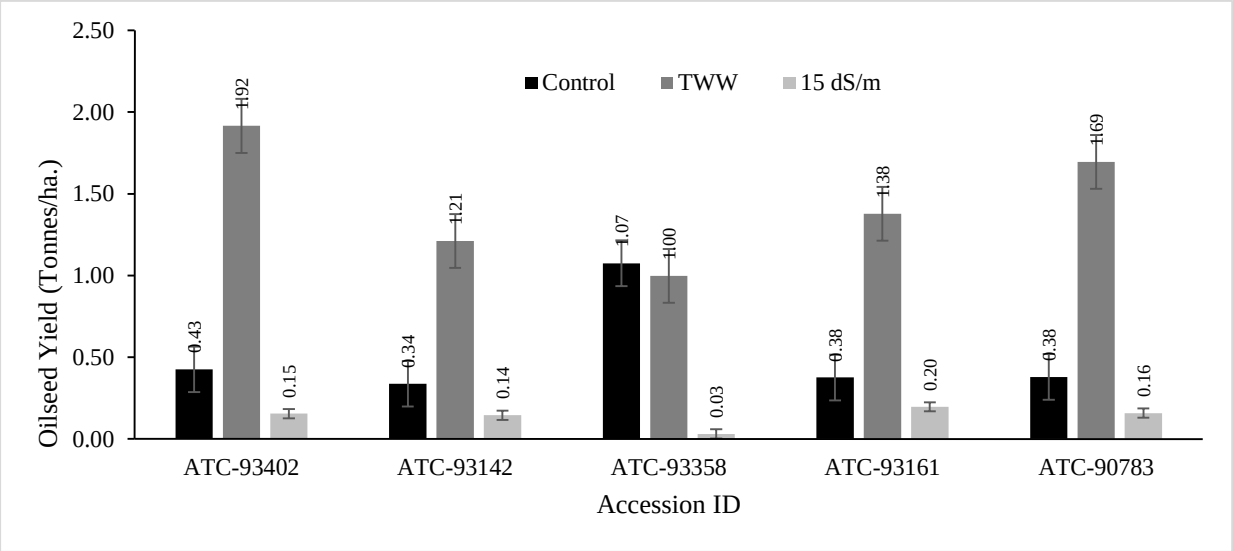


**Figure 47.** *Brassica juncea* seed yield in tonnes per hectare (Extrapolated). Error bars indicate standard error

## 7.6. Seed Oil Content

A soxhelet apparatus was used for solvent extraction of oil using n-hexane from 50 grams of seed. This was repeated in triplicates.

Seed oil content (%)= (Average weight of oil extracted/ 50) *100

Accession ATC- 93402 had the highest seed oil content among the five accessions studied, across treatments, with 17.6% in the control treatment and 24.6% in the TWW treatment (Figure 48). The increase of oil content in accessions ATC-93402, ATC-93142 and ATC- 93358 observed with TWW treatment was not significant ($p>0.05$). There was also no significant difference between control and salinity treatment or between accessions in terms of seed oil content (Table 41). There was no significant interaction between factors.

**Table 41.** *Brassica juncea* seed oil content: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 239.5334 | 4 | 59.88335 | 9.13984 | 0.004449 | 3.837853 |
| Treatments | 37.47858 | 2 | 18.73929 | 2.860129 | 0.115587 | 4.45897 |
| Interaction | 157.246 | 8 | 19.656 | - | 0.138 | - |
| Error | 52.41523 | 8 | 6.551903 | | | |
| Total | 329.4272 | 14 | | | | |

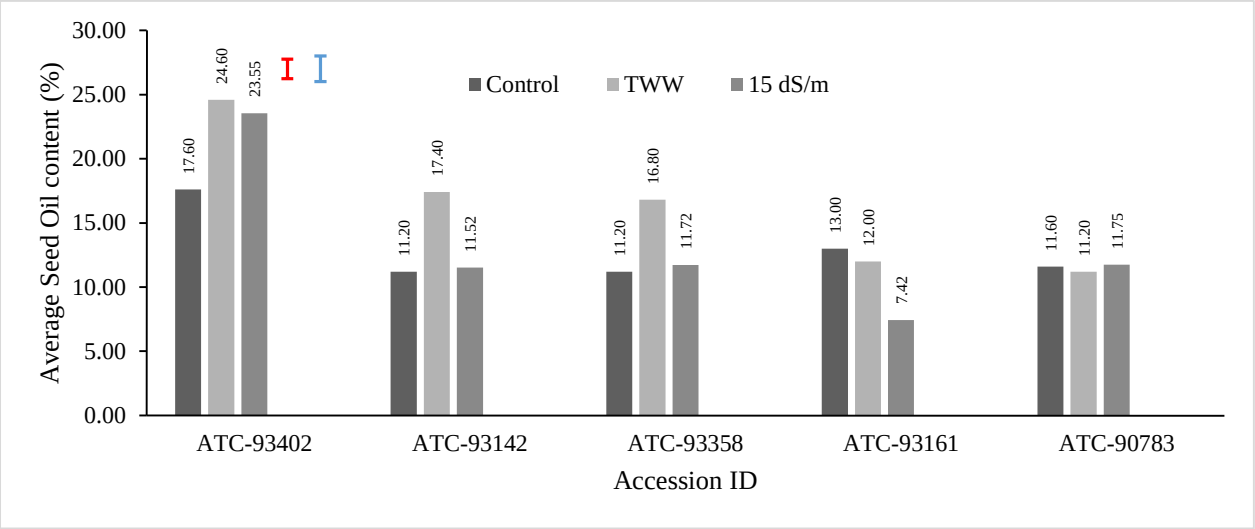


**Figure 48.** *Brassica juncea* seed oil content % w/w across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

Based on these data and extrapolated oilseed yield per hectare, projected oil yield values are given in Figure 49. In the control treatment, Accession ATC- 93358 is the highest yielding, with 0.07 tonnes/ha. With TWW irrigation, ATC-93402 can yield more than 0.2 tonnes/ha. In the TWW treatment all other accessions have a comparable yield between 0.09 and 0.12 tonnes/ha. Oil yield is highly affected by salinity due to decreased oilseed yield.

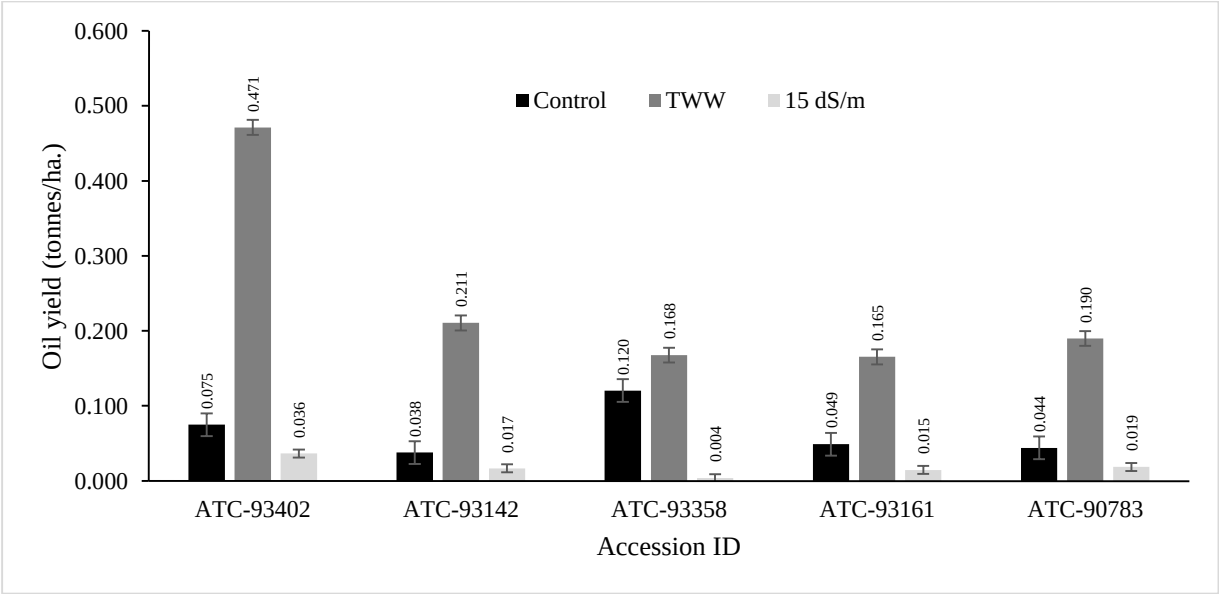


**Figure 49.** *Brassica juncea*: Oil Yield per hectare (extrapolated). Error bars indicate standard error

### 7.7. 1000-seed weight

Calculated as average of three samples per plot, seeds were counted using a Contador Seed Counter.

Thousand seed weight is a characteristic of a particular accession (Figure 50), ranged from 2 to 3.8 gms, and varied significantly among accessions across treatments (Table 42). There was no significant difference as a result of the TWW treatment ($p>0.05$) in comparison with control, but there was a significant difference between control and salinity treatment, at the level $p=0.0099$. There was no significant interaction between factors.

**Table 42.** *Brassica juncea* 1000 seed weight: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accession | 3.976909 | 4 | 0.994227 | 64.15584 | 4.08E-06 | 3.837853 |
| Treatment | 0.285248 | 2 | 0.142624 | 9.203285 | 0.008424 | 4.45897 |
| Interaction | 2.02 | 8 | 0.2527 | - | 0.206 | - |
| Error | 0.123977 | 8 | 0.015497 | | | |
| Total | 4.386134 | 14 | | | | |

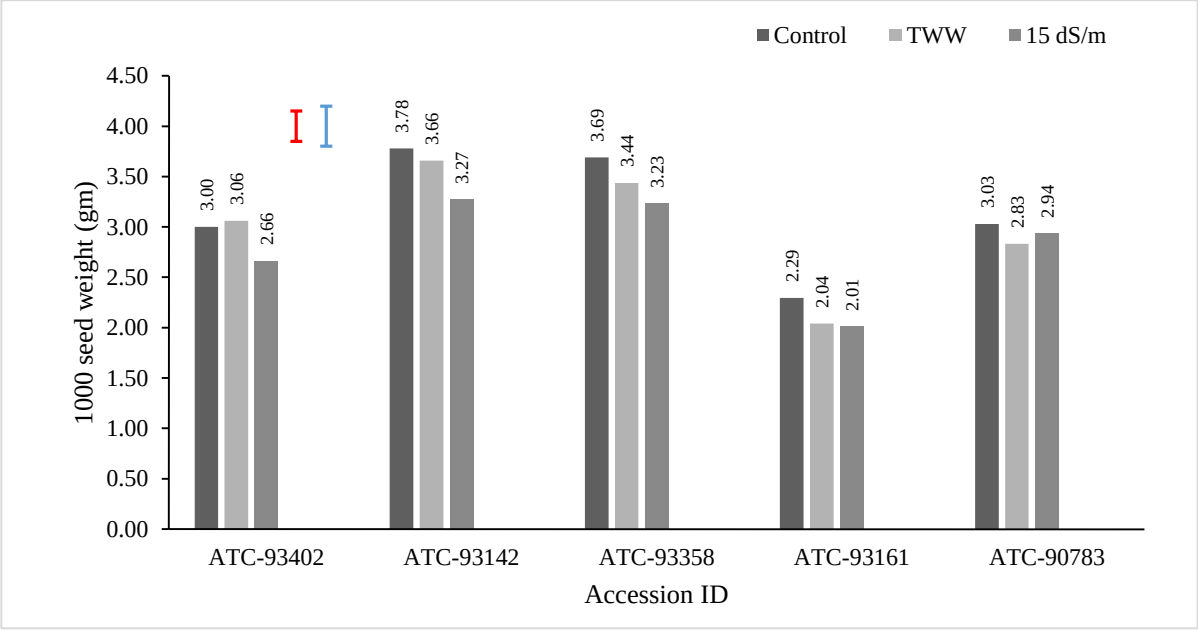


**Figure 50.** *Brassica juncea* thousand seed weight across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

## 7.8. Discussion of *Brassica juncea* agronomic evaluation

The five accessions of *Brassic juncea* studied in this trial were chosen from a previous pilot scale study at the International Center for Biosaline Agriculture due to their high reported yields and because they showed some promise of tolerance to salinity in another field trial. For this study, Treated Waste Water (TWW) was also available hence the effect of this alternative source of irrigation was studied. Thus, in addition to control and saline irrigation treatments (15 dS $m^{-1}$), one field was set up for TWW treatment dS $m^{-1}$. The salinity treatment was chosen with the expectation of salinity tolerance in the crop based on the previous studies, with the intention of

adjusting the salinity at a later stage depending on response at this level. The salinity treatment was not increased however, because of significant decrease of growth characteristics in the salinity treatment in comparison to the control, even though the decrease in yield was not statistically significant. For the first few weeks after germination, no differences were observed in the three treatments. Once salinity was introduced, there was a delay in bolting (the stage where apical dominance sets in and the main shoot grows tall and begins to flower). Across all treatments, accession ATC-90783 lagged by approximately six weeks for the onset of flowering. After this stage, which set in at the same time for the other four accessions in the control and TWW treatment, it was observed that the plants in the TWW treatment grew taller than those in the control treatment, especially for ATC-90783 which grew to a height similar to the other accessions in this treatment while it was shorter than the other accessions in the other two treatments. At maturity, all accessions were shorter by a similar margin in the salinity treatment. Leaf size increased significantly in three accessions in the TWW treatment but did not decrease significantly in the salinity treatment except for ATC-90783 which appeared to be most affected by salinity among the five accessions. Leaf dry weight and moisture also increased in the TWW treatment but did not drop in the salinity treatment. The increased height, leaf size and dry weight indicates an increase in vegetative growth in the TWW treatment (Figure 51).

**Figure 51.** *Brassica juncea* growth in TWW (left) and control (Right) treatments

While there were no significant differences among the accessions in fruit size, number of fruits and number of inflorescence bearing branches per plant, these traits were scored higher in the TWW treatment, as indicated by the 2-3 fold increase in seed yield of the accessions. Averaged over accessions, the mean seed yield in the control treatment was 0.52 tonnes/ha., which was low compared to average seed yields of 0.6 to 1.1 tonnes/ha., reported from India [145-146]. The

average yield in the TWW treatment was 1.44 tonnes/ha., which was above the Indian yields range, with ATC-93402 having an extrapolated yield of 1.92 tonnes/ha. However, this was much lower than the 3.05 tonnes/ha. maximum reported in the pilot study in a different location. Seed oil content as determined from solvent extraction was relatively low, with the highest being 17-24.6% and lowest approximately 11% in the control and TWW treatments. The maximum extrapolated oil yield was 0.47 tonnes/ha., which is low compared to soybean. Thus, in current studies, while TWW irrigation improved the yield of *Brassica juncea* compared to the control, other cultural practices may be necessary to further improve the yield of this crop.

# CHAPTER 8

# WATER AND SOIL ANALYSIS

Water used in the field and greenhouse trials was analyzed regularly to monitor salinity levels. In addition, the concentration of important ions in water samples was also analyzed to determine their concentration in irrigation water and account for possible effects of different salts. Soil was analyzed at the beginning and end of trials to study the accumulation of salts. Details of analysis, results and implications are given in this section.

### 8.1. Soil pH and salinity

Soil pH and electrical conductivity were measured using the saturated paste method [134]. Five soil samples each were collected from the root zone (30 cm depth) of all treatment plots using an auger. Soil samples were collected before planting and after completion of the crop cycle. 300 gms of soil was weighed from each sample and a saturated soil paste was made by mixing with distilled water. The quantity of distilled water required to make saturated paste of each sample was recorded. The paste was covered and left overnight. The pH and conductivity of soil paste was recorded (Scichem Tech, SCT-BEN-PH-2).

Soil pH and salinity were measured at the beginning (one month after commencement of salinity treatment) and end of *Ricinus communis* field trial (Table 43). There does not appear to be a significant change in soil salinity at the end of the six months cropping season. Soil electrical conductivity also does not change significantly in the control treatment, but increased slightly in the salinity treatment fields.

**Table 43.** *Ricinus communis* field trial average soil electrical conductivity and soil pH

| Treatment | Soil Electrical Conductivity ($EC_e$) @ the beginning of trial (dS $m^{-1}$) | Soil Electrical Conductivity ($EC_e$) @ the end of trial (dS $m^{-1}$) | pH @ beginning of Trial | pH @ the end of Trial |
|---|---|---|---|---|
| Control | 1.75 | 1.71 | 7.79 | 7.96 |
| 5 dS $m^{-1}$ | 2.53 | 3.35 | 7.63 | 7.93 |
| 10 dS $m^{-1}$ | 10.38 | 12.25 | 7.94 | 7.76 |
| 15 dS $m^{-1}$ | 16.94 | 18.59 | 7.7 | 7.77 |

The *Citrullus colocynthis* field trial for salinity tolerance lasted only for 2-3 months and soil pH and electrical conductivity (Table 44) was not altered significantly. Average soil pH in the germplasm diversity field trial was 7.83 in the beginning of the trial and 7.84 at the end of the year of trial. The same field was used for the salinity trial control plots.

**Table 44.** *Citrullus colocynthis* field trial average soil electrical conductivity and soil pH

| Treatment | Soil Electrical Conductivity ($EC_e$) @ the beginning of trial (dS $m^{-1}$) | Soil Electrical Conductivity ($EC_e$) @ the end of trial (dS $m^{-1}$) | pH @ beginning of Trial | pH @ the end of Trial |
|---|---|---|---|---|
| Control | 1.73 | 1.77 | 7.83 | 7.81 |
| 5 dS $m^{-1}$ | 3.19 | 3.38 | 7.98 | 8.11 |
| 10 dS $m^{-1}$ | 9.47 | 9.18 | 7.74 | 7.79 |
| 15 dS $m^{-1}$ | 13.82 | 13.75 | 7.71 | 7.67 |

In the greenhouse salinity trial an increase in soil salinity was observed at the end of the trial period of nine months (Table 45) but the increase was not statistically significant. There was also no significant change in soil pH.

**Table 45.** *Citrullus colocynthis* greenhouse trial soil pH and electrical conductivity

| Treatment | Soil Electrical Conductivity ($EC_e$) @ the beginning of trial (dS $m^{-1}$) | Soil Electrical Conductivity ($EC_e$) @ the end of trial (dS $m^{-1}$) | Soil pH @ beginning of Trial | Soil pH @ end of Trial |
|---|---|---|---|---|
| Control | 0.69 | 2.57 | 7.92 | 7.67 |
| 2 dS $m^{-1}$ | 2.69 | 4.11 | 7.82 | 7.72 |
| 4 dS $m^{-1}$ | 3.84 | 6.99 | 7.94 | 7.87 |

In the *Brassica juncea* field trial soil pH and electrical conductivity were recorded (Table 46). The soil in the TWW treatment had an electrical conductivity of 2.5 one month after commencement of treatment, which increased at the end of the six months cropping cycle, but not significantly.

**Table 46.** *Brassica juncea* field trial soil pH and electrical conductivity

| Treatment | Soil Electrical Conductivity ($EC_e$) @ beginning of trial (dS $m^{-1}$) | Soil Electrical Conductivity ($EC_e$) @ the end of trial (dS $m^{-1}$) | Soil pH @ beginning of Trial | Soil pH @ end of Trial |
|---|---|---|---|---|
| Control | 1.19 | 1.25 | 7.81 | 7.85 |
| 15 dS m-1 | 15.83 | 13.26 | 7.93 | 7.94 |
| TWW | 2.59 | 3.42 | 7.16 | 7.04 |

### 8.2. Water pH and salinity

Irrigation water samples were collected from all treatments (control, 5 dS $m^{-1}$, 10 dS $m^{-1}$ and 15 dS $m^{-1}$). The pH and $EC_w$ value of each sample was determined using a pH and electrical conductivity meter (Scichem Tech, SCT-BEN-PH-2) on a bi-weekly basis.

Average values for water salinity and pH in all the trials are presented in this section.

**Table 47.** *Ricinus communis* study: Average irrigation water electrical conductivity and pH

| | Treatment | | | |
|---|---|---|---|---|
| | Control | 5 dS $m^{-1}$ | 10 dS $m^{-1}$ | 15 dS $m^{-1}$ |
| Electrical Conductivity (dS $m^{-1}$) | 0.339 | 4.68 | 9.16 | 16.06 |
| pH | 8.1 | 7.92 | 7.67 | 7.44 |

**Table 48.** *Citrullus colocynthis* field trial: Average irrigation water electrical conductivity and pH

| | Treatment | | | |
|---|---|---|---|---|
| | Control | 5 dS $m^{-1}$ | 10 dS $m^{-1}$ | 15 dS $m^{-1}$ |
| Electrical Conductivity (dS $m^{-1}$) | 0.52 | 5.03 | 8.65 | 14.94 |
| pH | 8.26 | 8.91 | 7.82 | 7.97 |

**Table 49.** *Citrullus colocynthis* greenhouse trial: Average irrigation water electrical conductivity and pH

| | Treatment | | |
|---|---|---|---|
| | Control | 2 dS $m^{-1}$ | 4 dS $m^{-1}$ |
| Electrical Conductivity (dS $m^{-1}$) | 0.78 | 2.41 | 4.13 |
| pH | 8.18 | 8.96 | 8.72 |

**Table 50.** *Brassica juncea* field trial: Average irrigation water electrical conductivity and pH

| | Treatment | | |
|---|---|---|---|
| | Control | 15 dS $m^{-1}$ | TWW |
| Electrical Conductivity (dS $m^{-1}$) | 1.15 | 16.59 | 2.25 |
| pH | 8.25 | 7.93 | 7.51 |

### 8.3. Water solutes analysis

#### 8.3.1. Sodium and Potassium

Irrigation water samples from each treatment was collected on a bi-weekly basis. $Na^+$ and $K^+$ levels in the irrigation water were determined by means of flame photometry [135]. 100 ml of 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm, 12 ppm and 15 ppm Na and K standards were prepared for instrument calibration by diluting 100 ppm standard solutions with distilled water. The flame photometer filter was set to Na/K for each set of readings. Zero was adjusted using distilled water and the 100 reading was set using the 15 ppm standard. Readings were obtained for all 6 standards to determine the calibration curve. The water samples were diluted as required to obtain readings within the range of the calibration standards and the dilutions were recorded. Readings for the 5 water samples were then recorded and plotted against the calibration curve to determine the concentration of $Na^+$ and $K^+$ in the water samples.

#### 8.3.2. Soluble Chlorides

Soluble chlorides can be determined by titrating with standard silver nitrate solution in the presence of Potassium chromate indicator [136]. Potassium chromate solution (5%) was prepared in water, this was labeled reagent A. To prepare, 5 grams of potassium chromate was dissolved in 50 ml distilled water. Silver nitrate (1N) was added dropwise until a slight permanent red precipitate was formed. The reagent was filtered and the volume was made up to 100 ml with distilled water. To 5 ml of water sample, 4 drops of prepared reagent A was added. This was titrated against 0.05N Silver Nitrate solution until a permanent reddish-brown color appeared. The titration reading with blank (distilled water) samples was zero. The concentration of chlorides in sample was determined using the following formula:

$\mathrm{Cl\ (meq/l)} = [(\mathrm{V} - \mathrm{B}) * \mathrm{N} * 1000]/\mathrm{Ve}$, Where:

V = volume of 0.05N $AgNO_3$ used during titration

B = Blank titration volume (ml) of 0.05N $AgNO_3$

N = Normality of 0.05N $AgNO_3$ solution

Ve = Volume of sample used for titration

### 8.3.3. Calcium and magnesium

Water samples were diluted with distilled water and a few drops of Eriochrome Black T indicator and Ammonium purpurate were added. This sample was titrated against 0.01N Ethylene diamine tetra- acetic acid (EDTA) and calcium + magnesium concentration was calculated in meq/l) [137].

$$\mathrm{Ca} + \mathrm{Mg}\,(\mathrm{meq/l}) = (\mathrm{V} * \mathrm{N} * 1000)/\mathrm{Ve}$$

Sodium Absorption Ratio was calculated using the formula:

$$\mathrm{SAR} = \frac{\mathrm{Na}}{\sqrt{(\mathrm{Ca} + \mathrm{Mg}/2)}}$$

## 8.4.Water solute analysis results

The irrigation water used in all studies was analyzed in the laboratory as described in methods above, and the average concentration of Sodium, Potassium, Calcium and Magnesium and Chlorine are given in Table 51. The Sodium absorption ratio was also calculated.

**Table 51.** Irrigation water analysis data

| Study | Treatment | K (ppm) | Na (ppm) | Ca+Mg (meq/l) | Cl (meq/l) | Sodium Absorption Ratio |
|---|---|---|---|---|---|---|
| *Ricinus communis* Field Trial | Control | 1.49 | 42.25 | 31.00 | 7.00 | 0.47 |
| | 5 dS $m^{-1}$ | 17.51 | 844.98 | 350.00 | 36.50 | 2.78 |
| | 10 dS $m^{-1}$ | 34.14 | 1351.84 | 713.00 | 73.00 | 3.11 |
| | 15 dS $m^{-1}$ | 68.29 | 2672.96 | 1339.00 | 137.00 | 4.49 |
| *Citrullus colocynthis* Field Trial | Control | 0.85 | 39.62 | 33.00 | 6.20 | 0.42 |
| | 5 dS $m^{-1}$ | 16.38 | 893.04 | 368.00 | 38.25 | 2.86 |
| | 10 dS $m^{-1}$ | 33.62 | 1418.00 | 691.00 | 71.00 | 3.32 |
| | 15 dS $m^{-1}$ | 65.99 | 2516.22 | 1245.00 | 142.00 | 4.38 |
| *Citrullus colocynthis* Greenhouse Trial | Control | 1.16 | 50.05 | 29.00 | 6.50 | 0.57 |
| | 2 dS $m^{-1}$ | 2.69 | 69.91 | 217.00 | 12.50 | 0.29 |
| | 4 dS $m^{-1}$ | 12.17 | 851.74 | 299.00 | 26.00 | 3.03 |
| *Brassica juncea* Field Trial | Control | 1.27 | 38.85 | 36.00 | 7.00 | 0.40 |
| | 15 dS $m^{-1}$ | 71.88 | 3006.66 | 1437.00 | 109.00 | 4.88 |
| | TWW | 17.95 | 29.37 | 268.00 | 6.28 | 0.11 |

The treated waste water used in the study was analyzed at the Dubai Municipality Central Laboratory, and the results of that analysis is presented in Table 52.

**Table 52.** Dubai Municipality Central Laboratory Analysis of Treated Waste Water

| 1) Pathogens | Quantity |
|---|---|
| E. Coli (CFU/100ml) | <1 |
| Fecal Streptococci (CFU/100ml) | <1 |
| Total Coliform (CFU/100ml) | 980 |
| **2) Chemical Analysis** | **Quantity** |
| Ammonia Nitrogen (mg/l) | 11.8 |
| Phosphate Phosphorus | 3.36 |
| K | 19.4 |
| BOD (mg/l) | <7 |
| COD (mg/l) | 46 |
| Free Chlorine (mg/l) | <0.2 |

Water salinity is a function of the dissolved salts, whereas soil salinity is due to the presence of dissolved and readily dissolvable salts in a water extract of the soil, and electrical conductivity is a reliable standard estimate of the total salts in water or soil extract [138]. Electrical conductivity is used due to the difficulty of regularly measuring the concentrations of each solute by chemical analysis. The most important salts in water and soil with respect to effect on plant growth are sodium, chlorine, potassium, calcium and magnesium. This is because Na and Cl accumulation and disturbances in the Na, K, Ca homeostasis are some of the main contributors to sensitivity towards salinity [139]. Sodium absorption ratio (SAR) determines the suitability of water for irrigation, with higher SAR values indicating that water is unsuitable due to the high concentration of sodium relative to Ca and Mg, which can result in poor soil structure and water infiltration. Soil salinity was measured one month into salinity treatment and at the end of trial for all studies. This was done in order to understand whether salts accumulate in the root-zone in the field trials, leading to higher effective salinity than the intended treatment. Plant response to salinity is

generally described in terms of relative yield as a continuous function of root-zone salinity, expressed as electrical conductivity of the solution in contact with the roots (ECe) [140]. Soil samples were always collected from dry root zones before the first irrigation in the morning, so as to avoid the salts freshly added from the irrigation water. It was observed that while test salinity is usually achieved within a month after treatment commences, there is no significant increase in the salinity by the end of the treatment. This could be because the soils are sandy with good drainage and more water is applied than required to leach out the salts and prevent salinity build-up in the root zone. A more significant increase in the root-zone salinity was observed in the greenhouse pot trial, because of inadequate drainage of the pots leading to accumulation of salts. The marginal effect of salinity of up to 5dS $m^{-1}$ on *Ricinus communis* seed yield may be due to the fact that sandy soils do not retain salts in the manner that clayey soils do, apart from the leaching due to proper irrigation management. The plant is hence able to take up water from the root zone in spite of the moderate osmotic stress. Our results support this assumption, as it was seen that the soil salinity values were comparable to irrigation water salinity even after six months of irrigation with saline water. In contrast, salts corresponding to 1dS $m^{-1}$ could accumulate within 3-7 days in more loamy soils [141]. The sandy soil in the region with less loam and organic matter is thus of advantage with respect to irrigation with saline water. Soil pH did not change significantly in any of the trials and was in the normal, slightly basic 7.6 to 7.9 range.

Irrigation water salinity, achieved by mixing freshwater and saline groundwater, had to be monitored regularly through all the field trials to check for possible variations form set levels due to changes in mixing ratios or groundwater salinity. Electrical conductivity was mostly maintained within a +/- 1 dS $m^{-1}$ range in every treatment. It was noted that TWW had an average electrical conductivity of 2.25 dS $m^{-1}$, but this is not due to the presence of sodium, as illustrated by the sodium levels less than that in the water used in the control treatment. Water pH was slightly basic, in the range 7.4-8.9. From the results of chemical analysis of water, it is seen that sodium salts are present in the highest concentration in the salinity treatment, followed by calcium and magnesium. Chlorine salts are present in a relatively lower amount. While potassium salts were also present in higher concentrations in the salinity treatments, there was usually almost 50 times as much sodium. Though the sodium levels were high, the sodium absorption ratios were very low due to the high concentration of calcium and magnesium, thus enabling us to disregard any role of SAR in the plant responses [142]. The Dubai Municipality Laboratory analysis of TWW showed a high level of Coliforms, which is within specifications for wastewater use [143], but highlights the concerns with regard to the use of TWW for agricultural irrigation of food crops [144]. The nitrogen in TWW in the form of nitrates could not be estimated, but it can be assumed

that high levels of nitrogen and organic matter are expected, which could be one of the reasons for the improved growth and yield of *Brassica juncea*.

# CHAPTER 9

# ION STRESS

Accumulation of sodium in leaf tissue is a commonly used indicator of ion stress as a result of salinity in irrigation water or soil. The ratio of potassium to sodium in leaf tissue is an important parameter in terms of how a plant responds to salinity. These values were determined and the effect of ion stress on crop response to salinity was studied. The details of this study are elaborated in this section.

### 9.1. Analysis

Plant response to salinity was studied by estimating the accumulation of sodium and potassium ions in leaf tissue. Leaves were dried at 80˚C in a JSR® convection oven till a static weight was observed (to remove all moisture). Dried leaves were ground using mortar and pestle and weighed samples were wet digested using concentrated $HNO_3$ for 48 hours [145]. Leaf extract was filtered using ashless filter paper and analyzed using Inductively coupled plasma/optical emission spectrometry by Perkin Elmer® optical emission spectrometer (Optima 7000 DV) with S10 autosampler. $K^+$ and $Na^+$ concentrations in leaf extracts were determined by ICP OES and $K^+/Na^+$ ratios were calculated [146].

### 9.2. Ion stress in *Ricinus communis*

Concentration of K and Na ions in grams/ gram of leaf tissue is given in Figures 52 and 53. The K/Na ratio across treatments is presented in Table 55.

**Table 53.** *Ricinus communis* leaf potassium levels: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accession | 0.000883 | 10 | 8.83E-05 | 1.01125934 | 0.45693 | 2.16458 |
| Treatment | 0.002051 | 3 | 0.000684 | 7.82885659 | 0.000527 | 2.922277 |
| Error | 0.00262 | 30 | 8.73E-05 | | | |
| Total | 0.005554 | 43 | | | | |

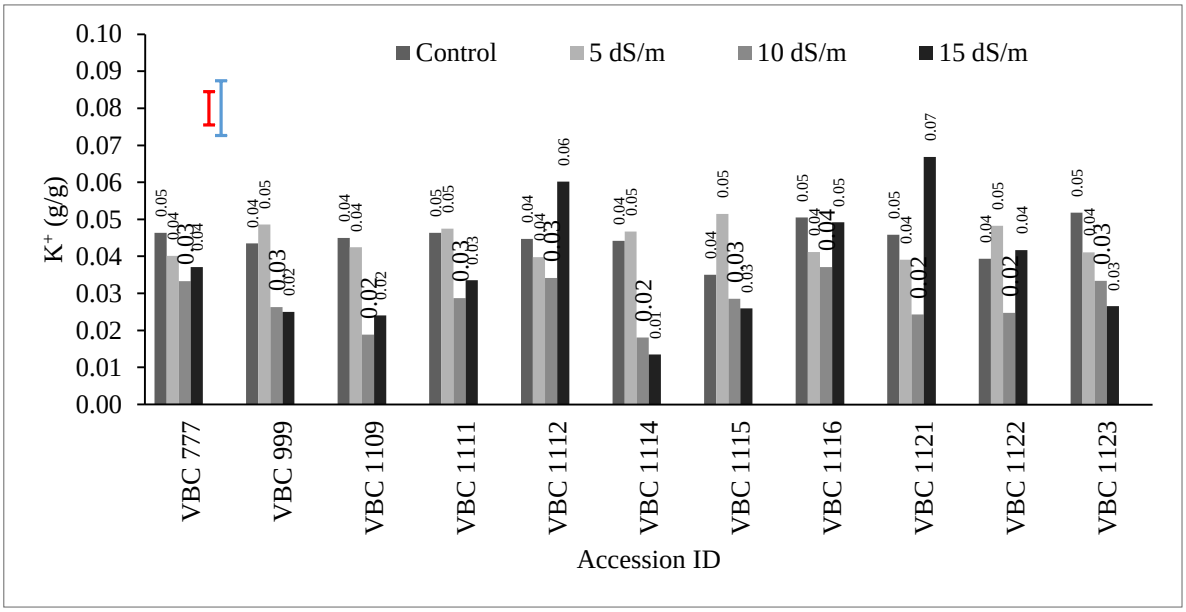


**Figure 52.** Concentration of Potassium ions in *Ricinus communis* leaf tissue (w/w) across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

Concentration of potassium ions in leaf tissue does not vary significantly between the control and 5 dS $m^{-1}$ treatments for all accessions (Figure 52). Then there is a drastic decrease in the 10 and 15 dS $m^{-1}$ treatments except for accessions VBC 1112 and VBC 1121, in which potassium seems to accumulate to a great degree in leaf tissue from the 15 dS $m^{-1}$ treatment.

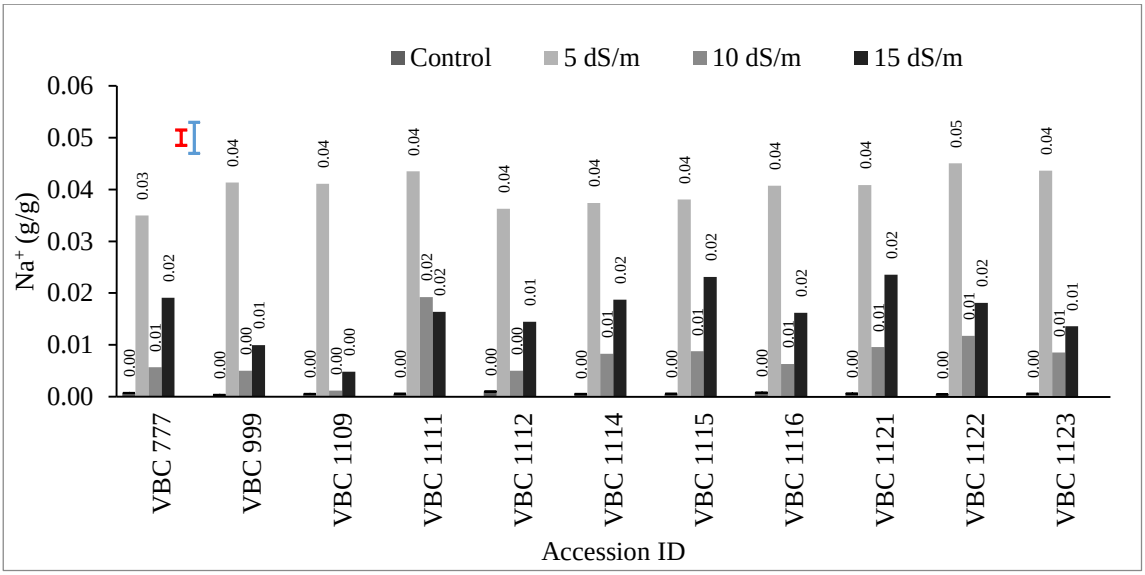


**Figure 53**. Concentration of Sodium ions in *Ricinus communis* leaf tissue (w/w) across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

**Table 54.** *Ricinus communis* leaf sodium levels: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accession | 0.000226 | 10 | 2.26E-05 | 1.731699 | 0.119143 | 2.16458 |
| Treatment | 0.00978 | 3 | 0.00326 | 249.5428 | 2.69E-21 | 2.922277 |
| Error | 0.000392 | 30 | 1.31E-05 | | | |
| Total | 0.010398 | 43 | | | | |

$Na^+$ ion concentration in leaf tissue increases significantly, between 2 and 3 fold in the 5 dS $m^{-1}$ treatment for all accessions, and the increase is significant (Table 54).

**Table 55.** $K^+/Na^+$ ratio in *Ricinus communis* leaf tissue across treatments

| | $K^+/Na^+$ ratio | | | |
|---|---|---|---|---|
| Accession ID | Control | 5 dS/m | 10 dS/m | 15 dS/m |
| VBC 777 | 66.9265 | 1.147798 | 5.886732 | 1.941416 |
| VBC 999 | 137.6584 | 1.176404 | 5.285812 | 2.510818 |
| VBC 1109 | 89.36511 | 1.031638 | 16.76683 | 4.982642 |
| VBC 1111 | 86.83738 | 1.091873 | 1.497415 | 2.049287 |
| VBC 1112 | 47.06015 | 1.096436 | 6.858531 | 4.172635 |
| VBC 1114 | 97.63576 | 1.248665 | 2.18793 | 0.718139 |
| VBC 1115 | 67.03015 | 1.352079 | 3.266076 | 1.125343 |
| VBC 1116 | 66.90482 | 1.011581 | 5.893728 | 3.037011 |
| VBC 1121 | 81.04015 | 0.956879 | 2.545624 | 2.844845 |
| VBC 1122 | 92.21443 | 1.070498 | 2.109404 | 2.304656 |
| VBC 1123 | 95.66195 | 0.942075 | 3.918247 | 1.95113 |

$K^+/Na^+$ ratio, as expected from the previous data, dropped significantly in the salinity treatments (Table 55), but does not vary significantly among accessions (Table 56).

**Table 56.** *Ricinus communis* $K^+/Na^+$ ratio in leaf tissue: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accession | 1392.821 | 10 | 139.2821 | 0.959774 | 0.496544 | 2.16458 |
| Treatment | 54870.12 | 3 | 18290.04 | 126.0341 | 4.27E-17 | 2.922277 |
| Error | 4353.593 | 30 | 145.1198 | | | |
| Total | 60616.53 | 43 | | | | |

### 9.3. Ion stress in *Citrullus colocynthis*

The plants in the *Citrullus colocynthis* field trial did not last long enough for analysis of the leaf tissue. Leaf tissue analysis was conducted using samples from the greenhouse trial. In accessions RMS 215, 253 and 244 there is a steady decrease in Potassium concentration in leaf tissue with

increasing salinity (Figure 54). In RMS 237, there is a steep decrease in the 2 dS $m^{-1}$ treatment but no further decrease in the 4 dS $m^{-1}$ treatment. In RMS 227 there is an increase in the 4 dS $m^{-1}$ treatment in comparison to the 2 dS $m^{-1}$ treatment. The difference between treatments was significant (Table 57).

**Table 57.** *Citrullus colocynthis* potassium levels in leaf tissue: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *Df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accession | 4341.507 | 4 | 1085.377 | 1.108838 | 0.415664 | 3.837853 |
| Treatment | 38829.58 | 2 | 19414.79 | 19.83445 | 0.000793 | 4.45897 |
| Error | 7830.735 | 8 | 978.8419 | | | |
| Total | 51001.82 | 14 | | | | |

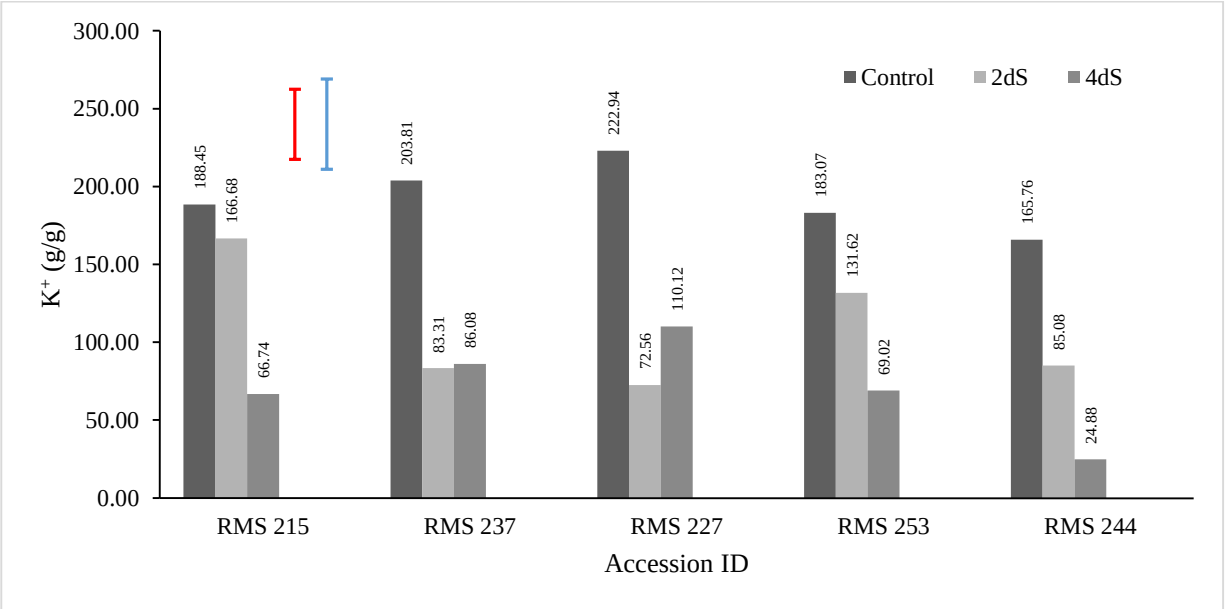


**Figure 54.** Potassium concentration in *Citrullus colocynthis* leaf tissue across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated.

Accession RMS 227 showed a decrease and RMS 253 showed an increase in sodium ion concentration in the 2 dS $m^{-1}$ treatment (Figure 55). All accessions show an increased sodium accumulation in the 4 dS $m^{-1}$ treatment, the increase with salinity was significant (Table 58).

**Table 58.** *Citrullus colocynthis* sodium levels in leaf tissue: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 389.559 | 4 | 97.38975 | 2.728582 | 0.105872 | 3.837853 |
| Treatments | 1355.246 | 2 | 677.6228 | 18.98505 | 0.000917 | 4.45897 |
| Error | 285.5395 | 8 | 35.69244 | | | |
| Total | 2030.344 | 14 | | | | |

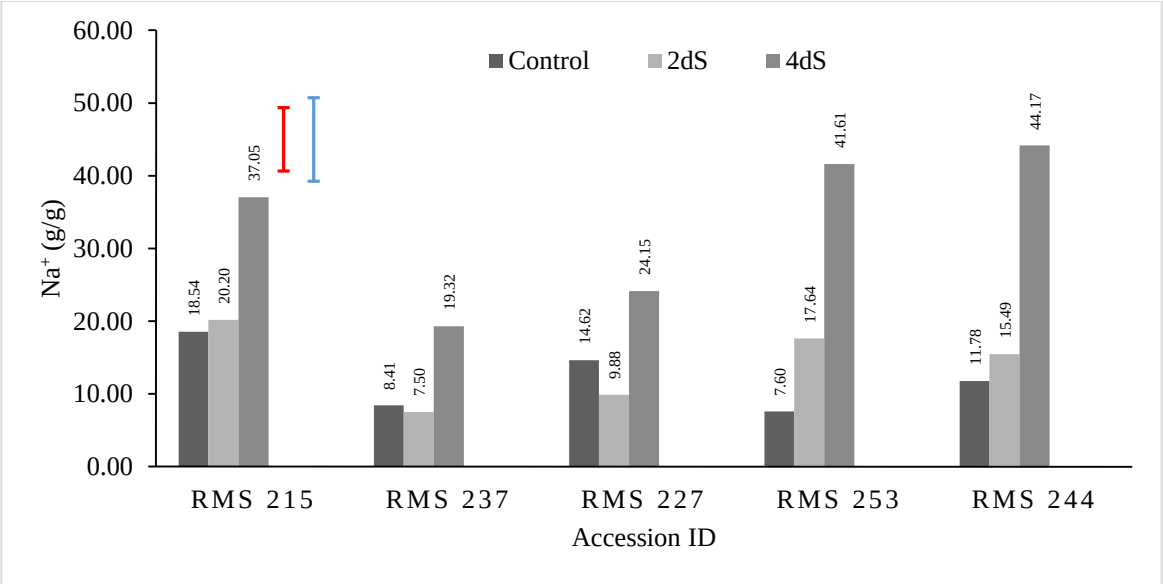


**Figure 55.** Sodium concentration in *Citrullus colocynthis* leaf tissue across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

**Table 59.** *Citrullus colocynthis* $K^+/Na^+$ ratio in leaf tissue: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 96.51139 | 4 | 24.12785 | 2.085969 | 0.174627 | 3.837853 |
| Treatments | 574.3019 | 2 | 287.151 | 24.82559 | 0.000371 | 4.45897 |
| Error | 92.53386 | 8 | 11.56673 | | | |
| Total | 763.3472 | 14 | | | | |

The $K^+/Na^+$ ratio declines steadily with increase in salinity (Table 56), as observed (Figure 56).

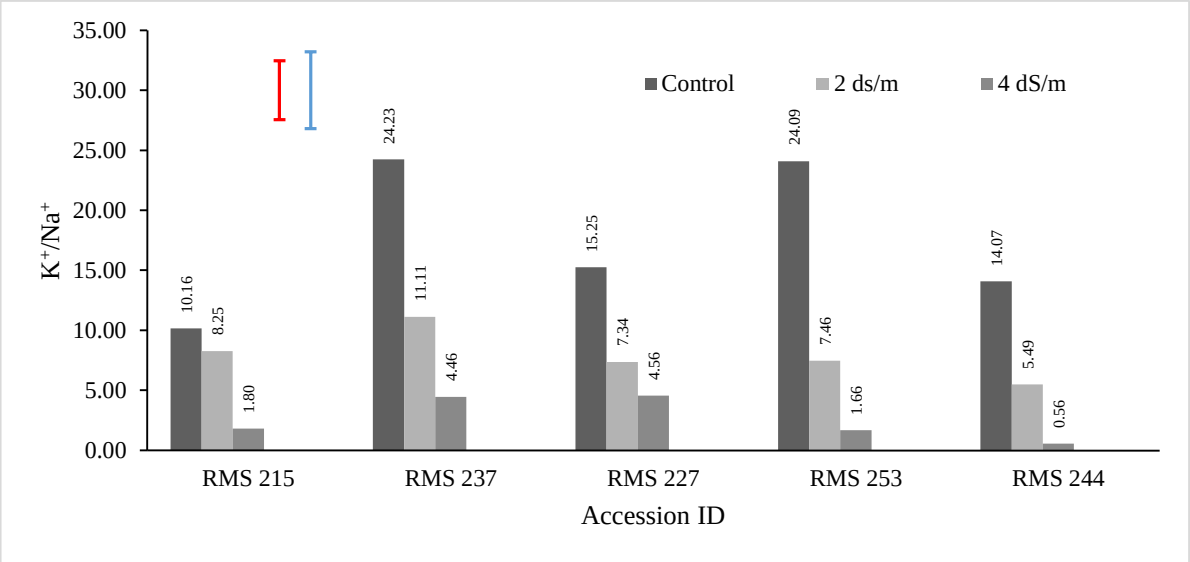


**Figure 56.** $K^+/Na^+$ ratio in *Citrullus colocynthis* leaf tissue across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated.

### 9.4. Ion stress in *Brassica juncea*

The *Brassica juncea* study had a treated waste water treatment and a single salinity treatment at 15 dS $m^{-1}$ in addition to the control. Accession ATC- 90783 showed an increase in potassium concentration in the TWW treatment, no major changes are seen in the other accessions. Accessions ATC- 93161, ATC- 93358 and ATC- 90783 showed an increase in potassium accumulation in the salinity treatment. ATC 93402 remained most stable across treatments with respect to potassium concentration in leaf tissue (Figure 57). The difference among treatments was significant (Table 60).

**Table 60.** *Brassica juncea* potassium levels in leaf tissue: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accession | 24.57561 | 4 | 6.143903 | 1.005023 | 0.458706 | 3.837853 |
| Treatment | 60.5403 | 2 | 30.27015 | 4.951609 | 0.039869 | 4.45897 |
| Error | 48.90557 | 8 | 6.113196 | | | |
| Total | 134.0215 | 14 | | | | |

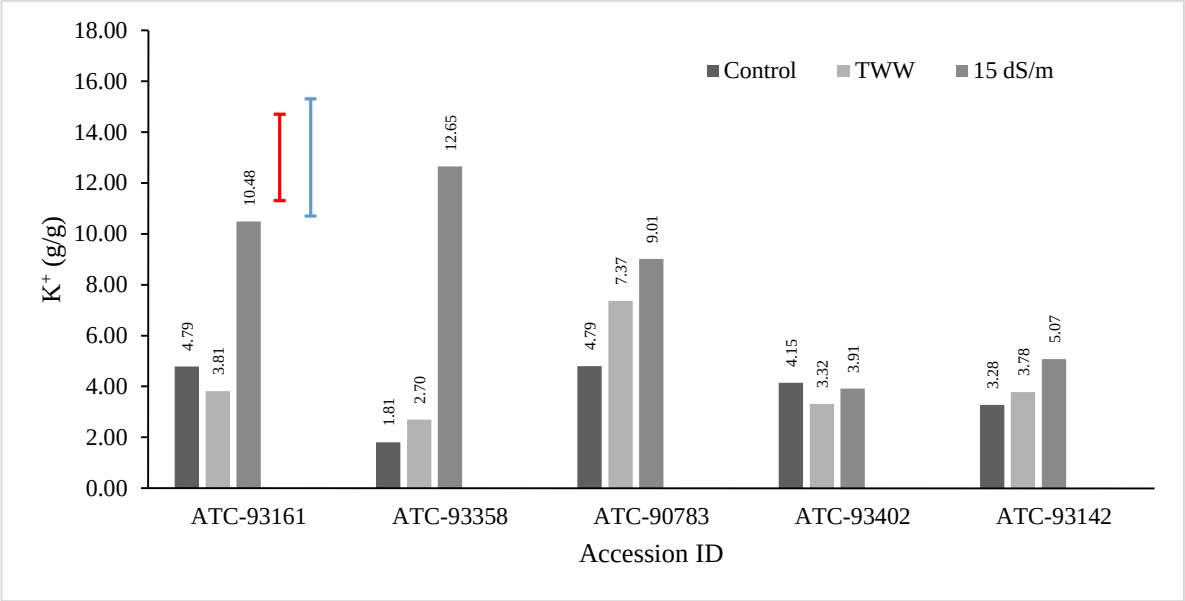


**Figure 57.** Potassium concentration in *Brassica juncea* leaf tissue across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

Sodium does not accumulate significantly in the TWW treatment for accessions ATC- 93358, ATC- 90783 and ATC- 93142 (Figure 58). There is an increase in the other two accessions. There is a significant increase in sodium accumulation in the salinity treatment for all accessions (Table 61).

**Table 61.** *Brassica juncea* sodium levels in leaf tissue: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accession | 2.477024 | 4 | 0.619256 | 1.736654 | 0.234644 | 3.837853 |
| Treatment | 14.5578 | 2 | 7.2789 | 20.41309 | 0.000721 | 4.45897 |
| Error | 2.85264 | 8 | 0.35658 | | | |
| | | | | | | |
| Total | 19.88746 | 14 | | | | |

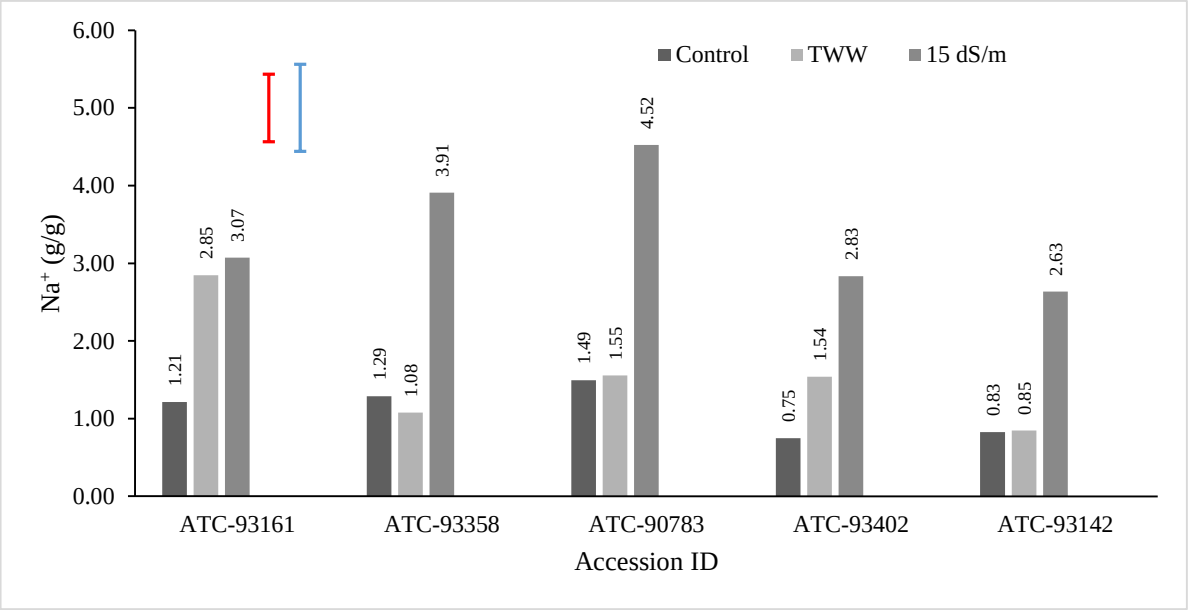


**Figure 58.** Sodium ion accumulation in *Brassica juncea* leaf tissue across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

The differences in $K^+/Na^+$ ratio among treatments and among accessions within each treatment were both statistically not significant (Table 62), suggesting that these accessions have some mechanism to overcome sodium accumulation in leaf tissue and are tolerant to salinity to a certain extent. ATC-93402 appeared most sensitive to salinity and sodium accumulation compared to other accessions in the study (Figure 59).

**Table 62.** *Brassica juncea* $K^+/Na^+$ ratio in leaf tissue: Two-way ANOVA results (SS- Sum of squares, df- degrees of freedom, MS- Mean Square)

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Accessions | 2.102078 | 4 | 0.525519 | 0.221683 | 0.918858 | 3.837853 |
| Treatments | 3.759671 | 2 | 1.879836 | 0.792981 | 0.485084 | 4.45897 |
| Error | 18.96474 | 8 | 2.370593 | | | |
| Total | 24.82649 | 14 | | | | |

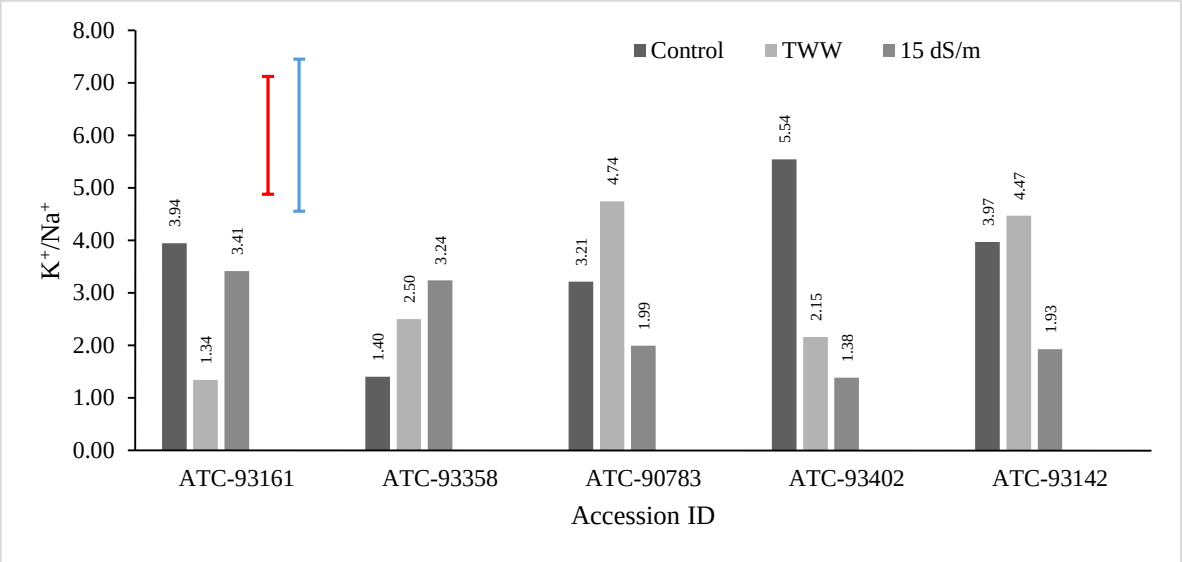


**Figure 59.** $K^+/Na^+$ ratio in *Brassica juncea* leaf tissue across treatments. Least significant difference among treatments (red error bar), and among accessions (blue error bar) are indicated

### 9.5. Discussion of ion stress data

The pattern of $Na^+$ and $Cl^-$ accumulation in higher plants at increasing salinity is remarkable. Na+ accumulates more in older leaves until a certain salt concentration, after which there is no difference between leaves of different ages. It has been established that once the $Na^+$ enters into the root cells it is extruded from the cytoplasm into the apoplastic space and/or compartmentalized into the vacuole [160-161]. At low salinity, $Na^+$ will be mostly removed from the cytoplasm through the above-mentioned mechanisms. The remaining $Na^+$ (the amount that is not stored into the vacuole) will follow the transpiration water flux. Consequently, a higher $Na^+$ accumulation will be observed in leaves that are transpiring since longer time (mature leaves). At advanced salinization, mature leaves will reach a $Na^+$ concentration threshold that will trigger stomatal closure and eventually reduce the transpiration flux, compared to younger leaves. This will contribute to flattening of the differences between the two leaf types at higher salinization. In our study we chose mature leaves as standards in each species so that none of the variation due to differences in leaf age would be encountered. At advanced salinization, activation of structural changes may be required for the plant to adapt. These could include a leaf area reduction to control plant water homeostasis among others, which has also been observed in the salinity treatments.

The $K^+$ deficiency of salinized plants has been inversely correlated to the increased accumulation of $Na^+$, indicating the existence of competition effects between $Na^+$ and $K^+$ ions which most likely share the same transport system at the root surface [147].

As seen in the ICP-OES data for *Ricinus communis*, sodium ions appear to accumulate to an extent enough to reduce plant growth, but not to a toxic level in the 5 dS $m^{-1}$ treatment. The Leaf $K^+/Na^+$

ratio is an important indicator of salinity response [163-164]. The accumulation of excess $Na^+$ from irrigation water in leaf tissue and the resulting competition of $Na^+$ for $K^+$ binding sites in metabolic processes could be the reason for the decreased growth of the plants [20]. This is because $K^+$ is more essential for physiological activity and required in greater amounts by the plant, but is replaced by the also monovalent $Na^+$, thus hindering essential metabolic processes. The lower sodium levels in the 10 and 15 dS $m^{-1}$ treatments in comparison with the 5 dS $m^{-1}$ treatment could be because the leaves from the plants in these treatments were senescent as a result of salinity when sampling was done, because of which there was less salt accumulation and water content in the leaves, and less transpirational flux. The leaves in the 5 dS $m^{-1}$ treatment were still alive and thus accumulating sodium. The decrease in all biomass growth parameters and no difference in reproductive function in the plants from this treatment in spite of the ion stress could be a mechanism adopted by the plant to ensure survival under stress.

In the *Citrullus colocynthis* greenhouse salinity trial a steady decrease is observed in $K^+/Na^+$ ratio from control to 2 dS $m^{-1}$ and the 4 dS $m^{-1}$ treatment. Increased accumulation of sodium in leaf tissue even at such low salinity suggests that there is no compartmentalization of sodium in roots even at low salinities and the plant has no mechanism to deal with salts. This could be because the natural habitat of the plant is sandy desert soils which are extremely low in loam or clayey particles because of which salts do not accumulate. Pertinent to this, the material used in current studies was all collected from sand dunes. If germplasm from coastal regions could be obtained, some mechanism of adaptation to salinity could perhaps be detected.

In *Brassica juncea*, accession ATC- 93161 showed accumulation of sodium even in the TWW treatment, with low levels of sodium in the irrigation water, suggesting a relatively high sensitivity to salinity in comparison with the other accessions. But it is interesting to note that $K^+/Na^+$ ratio in this accession is not altered significantly in the salinity treatment when compared to the control. From the $K^+/Na^+$ ratios, ATC-93358 and to a less extent ATC- 90783 could possibly be less stressed than the other accessions, but when considered in conjunction with the decreased growth and yield it has to be concluded that all accessions used in this study were sensitive to high levels of salinity (15 dS $m^{-1}$).

# CHAPTER 10

# OIL ANALYSIS

Oil extracted from all the various accessions of each crop studied was analyzed in triplicates (or more) for important parameters such as free fatty acid content and viscosity in order to assess the suitability of the oil for bio-diesel production. The methods of analysis, results and implications of the same are detailed in this section.

### 10.1. Analysis

Saponification value of oil samples was determined using ASTM D5558: Standard Test Method for Determination of the Saponification Value of Fats and Oils. Acid number was estimated by titrating a known volume of oil in fat solvent against 0.1N KOH using phenolphthalein as an indicator (EN 14 104). Free fatty acid content (FFA) was calculated from the Acid number [148]. Kinematic viscosity was determined at room temperature using a microprocessor Digital Viscometer model LT-730 (Labtronics).

### 10.2. *Ricinus communis* oil

FFA content of *Ricinus communis* oil is presented (Figure 60). Oil from all accessions except VBC 1109 and VBC 1111 had a FFA content less than 0.5%. Accessions VBC1112 and VBC 1116 from the 10 dS $m^{-1}$ treatment had an average FFA greater the 0.5%.

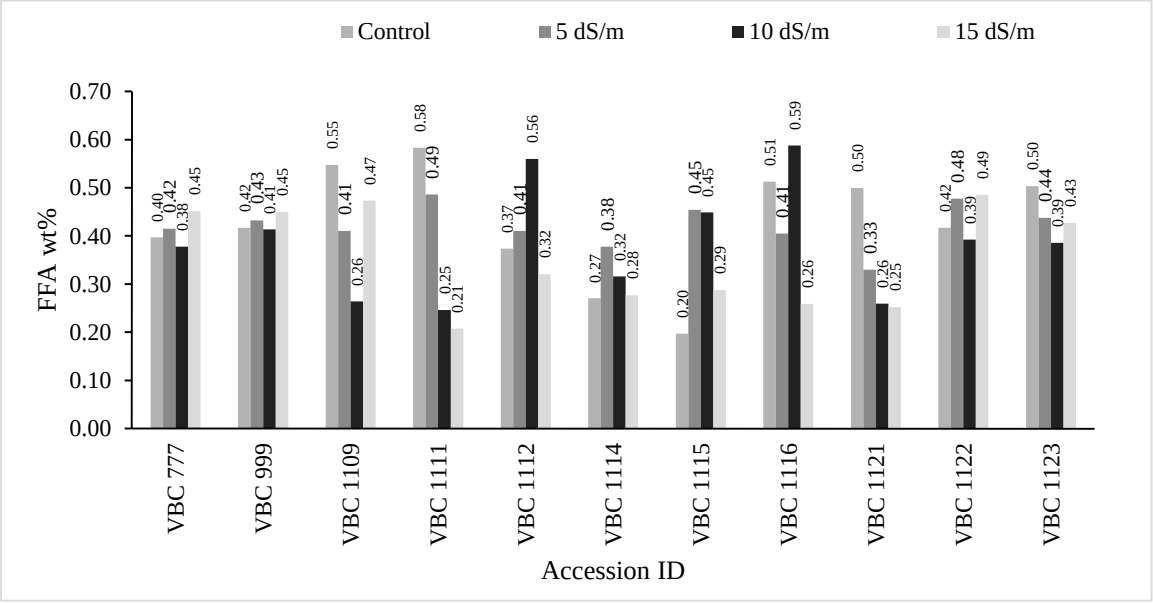


**Figure 60.** Average free fatty acid content of *Ricinus communis* oil from different accessions across treatments

Saponification value (Figure 61) does not appear to vary significantly among accessions or treatments ($p>0.05$).

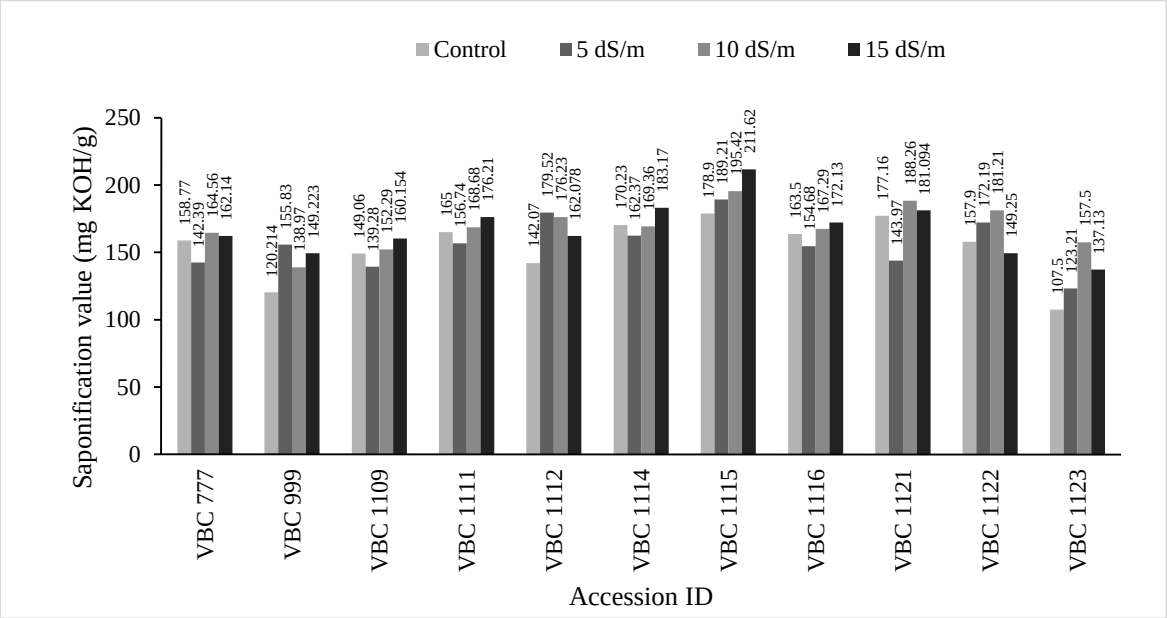


**Figure 61.** Saponification value of *Ricinus communis* oil from different accessions across treatments

Density of *Ricinus communis* oil was found to be 0.942 g/ $cm^3$. Average specific gravity was 0.96. There wasn't sufficient oil yield from the 10 and 15 dS $m^{-1}$ treatments to estimate viscosity. Dynamic and Kinematic viscosity of *Ricinus communis* oil at 29 °C is listed here (Table 63).

**Table 63.** Dynamic and Kinematic Viscosity of *Ricinus communis* oil at 29 °C including mean and standard deviation (SD)

| Accession ID | Treatment | Kinematic viscosity ($mm^2/s$) | Dynamic viscosity (mPa.s) |
|---|---|---|---|
| VBC 777 | Control | 290.53 | 276 |
| VBC 999 | Control | 296.22 | 274 |
| VBC 1109 | Control | 278.95 | 265 |
| VBC 1111 | Control | 298.95 | 284 |
| VBC 1112 | Control | 323.24 | 299 |
| VBC 1114 | Control | 288.42 | 274 |
| VBC 1115 | Control | 309.47 | 294 |
| VBC 1116 | Control | 297.89 | 283 |
| VBC 1121 | Control | 303.78 | 281 |
| VBC 1122 | Control | 289.47 | 275 |
| VBC 1123 | Control | 310.53 | 295 |
| Mean | | 298.86 | 281.82 |
| SD +/- | | 11.82 | 10.04 |
| VBC 777 | 5 dS $m^{-1}$ | 300.54 | 278 |
| VBC 999 | 5 dS $m^{-1}$ | 283.16 | 269 |
| VBC 1109 | 5 dS $m^{-1}$ | 309.47 | 294 |
| VBC 1111 | 5 dS $m^{-1}$ | 312.43 | 289 |
| VBC 1112 | 5 dS $m^{-1}$ | 277.89 | 264 |
| VBC 1114 | 5 dS $m^{-1}$ | 295.79 | 281 |

| | | | |
|---|---|---|---|
| VBC 1115 | 5 dS $m^{-1}$ | 315.68 | 292 |
| VBC 1116 | 5 dS $m^{-1}$ | 309.47 | 294 |
| VBC 1121 | 5 dS $m^{-1}$ | 305.95 | 283 |
| VBC 1122 | 5 dS $m^{-1}$ | 311.35 | 288 |
| VBC 1123 | 5 dS $m^{-1}$ | 298.38 | 276 |
| Mean | | 301.82 | 282.54 |
| SD +/- | | 11.68 | 9.61 |

### 10.3. *Citrullus colocynthis* oil

*Citrullus colocynthis* oil properties seem to vary significantly among accessions (p=8.73E-32). The FFA content of 27 accessions from the field trial are presented (Figure 62). Oil from 10 of the 27 accessions have an FFA content greater than 0.5%.

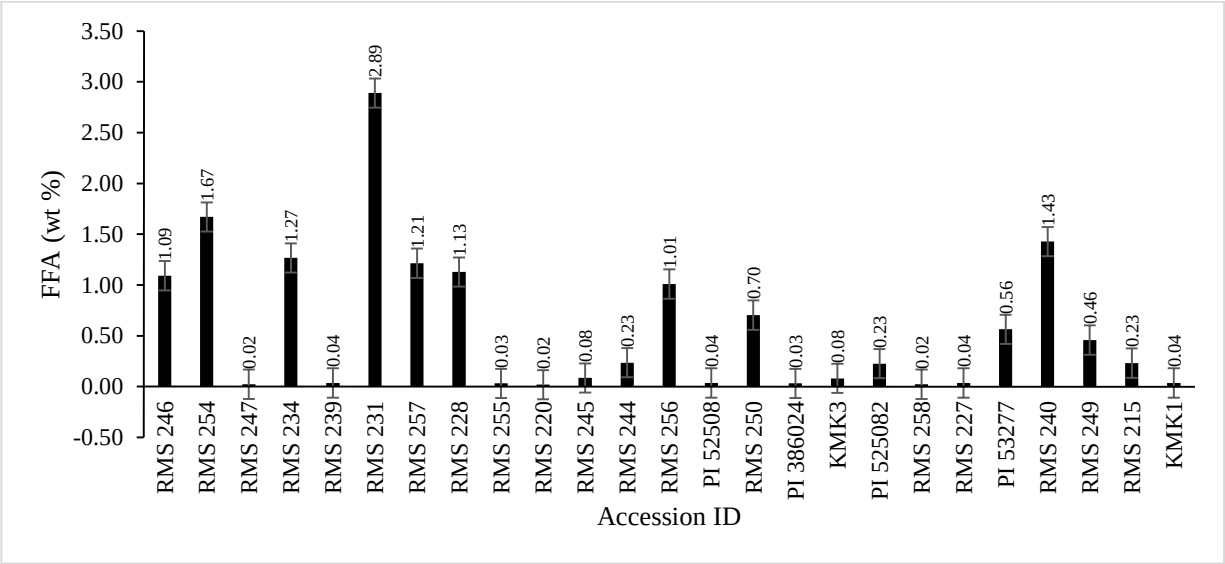


**Figure 62.** Free fatty acid content of *Citrullus colocynthis* oil from different accessions

Average Saponification value also varied greatly among accessions (p=4.44E-18), suggesting differences in oil composition (Figure 63).

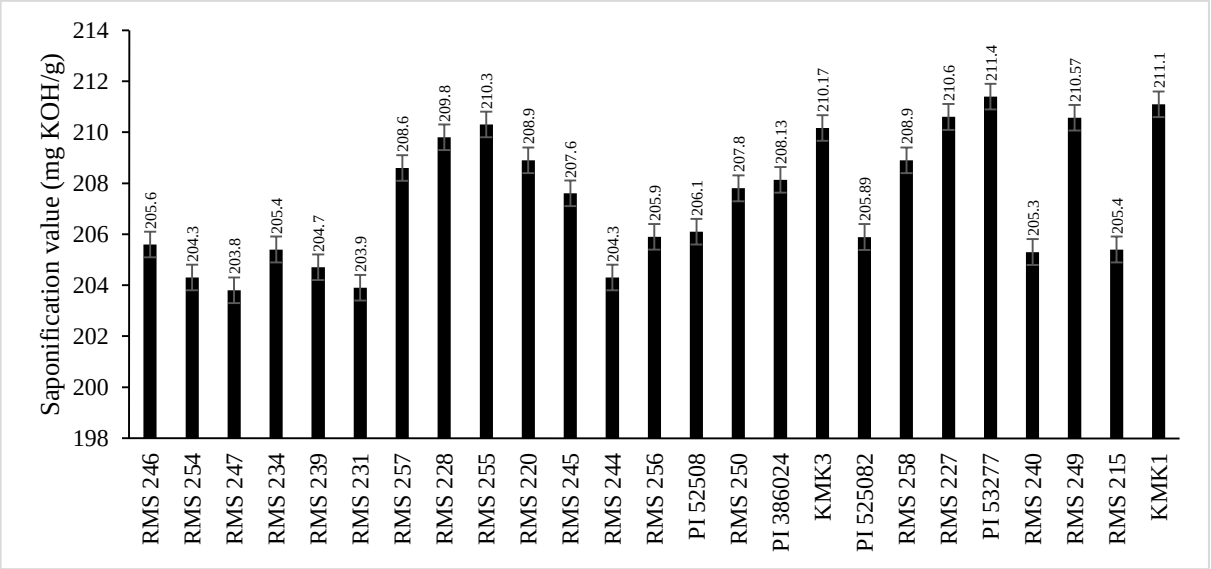


**Figure 63.** Saponification value of *Citrullus colocynthis* oil from different accessions

*Citrullus colocynthis* oil has an average density of 0.92 g/cm$^3$ and a specific gravity of 0.93 at room temperature. Viscosity of *Citrullus colocynthis* oil at 29 °C is given below (Table 64). Kinematic viscosity ranges from 23 to 35 mm$^2$/s depending on accession.

**Table 64.** Dynamic and Kinematic viscosity of *Citrullus colocynthis* Oil at 29 °C including mean and standard deviation (SD) across accessions

| Accession ID | Kinematic viscosity (mm$^2$/s) | Dynamic viscosity (mPa.s) |
|---|---|---|
| RMS 246 | 25.14 | 22.00 |
| RMS 254 | 24.63 | 22.00 |
| RMS 247 | 35.71 | 35.00 |
| RMS 234 | 28.57 | 25.00 |
| RMS 239 | 26.87 | 24.00 |
| RMS 231 | 23.47 | 23.00 |
| RMS 257 | 25.14 | 22.00 |
| RMS 228 | 25.75 | 23.00 |
| RMS 255 | 23.47 | 23.00 |
| RMS 220 | 27.43 | 24.00 |
| RMS 245 | 27.99 | 25.00 |
| RMS 244 | 32.65 | 32.00 |
| RMS 256 | 35.43 | 31.00 |
| RMS 250 | 31.34 | 28.00 |
| KMK3 | 28.57 | 28.00 |
| RMS 258 | 25.14 | 22.00 |
| RMS 227 | 25.75 | 23.00 |
| RMS 237 | 24.49 | 24.00 |
| RMS 240 | 33.14 | 29.00 |
| RMS 249 | 26.87 | 24.00 |
| RMS 215 | 25.51 | 25.00 |
| KMK1 | 26.29 | 23.00 |

| | | |
|---|---|---|
| PI 525080 | 24.63 | 22.00 |
| PI 386024 | 26.53 | 26.00 |
| PI 525082 | 30.86 | 27.00 |
| PI 537277 | 23.51 | 21.00 |
| PI 388770 | 28.57 | 28.00 |
| Mean | 27.53 | 25.22 |
| SD +/- | 3.45 | 3.42 |

### 10.4. *Brassica juncea* oil

Upon analysis of *Brassica juncea* oil, it was observed that FFA (Figure 64) content is below 0.5% for all accessions in all treatments and that there is no significant variability between accessions or treatments ($p>0.05$).

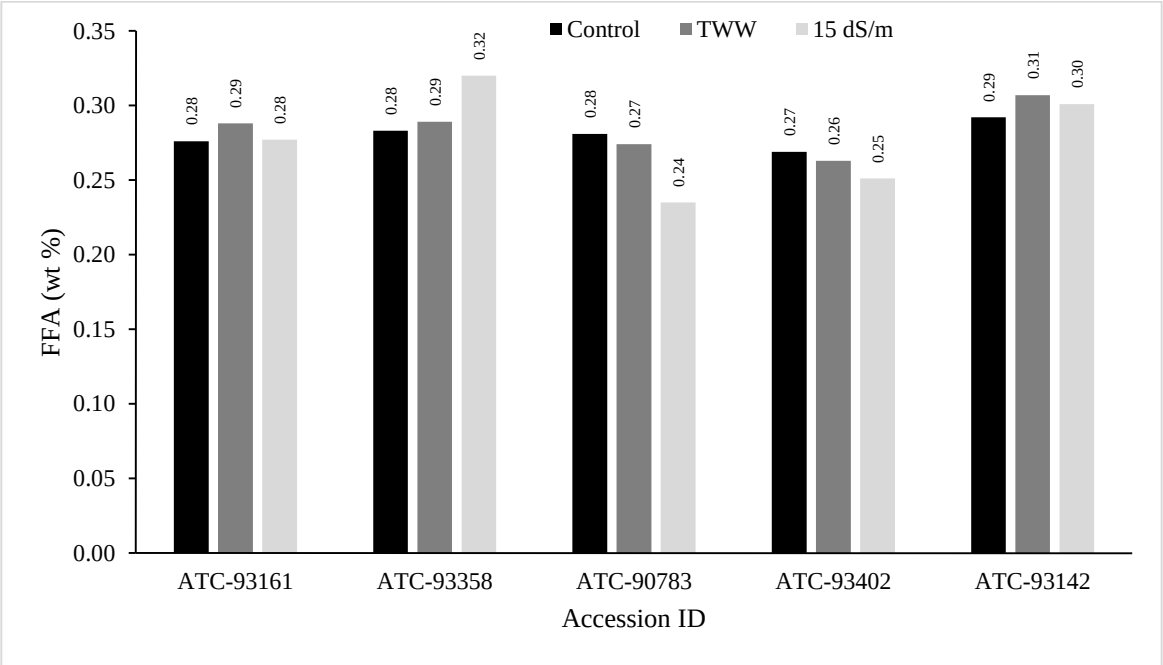


**Figure 64.** FFA content in *Brassica juncea* oil from different accessions across treatments

Saponification value was in the range of 167 to 180, and did not vary significantly between treatments (Figure 65) but did between accessions ($p=0.01$).

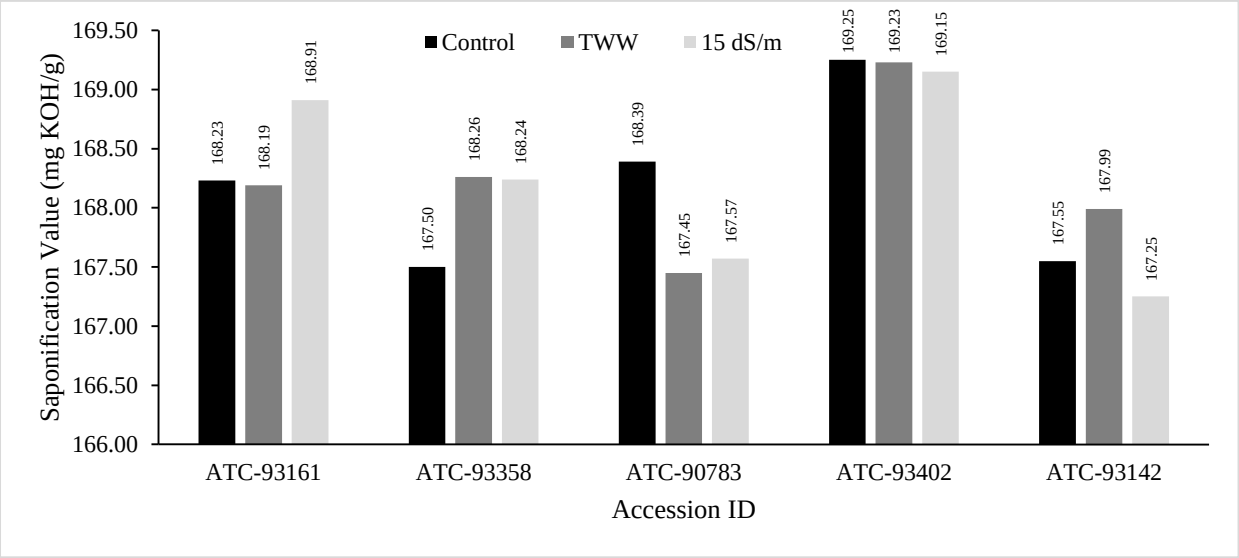


**Figure 65.** Saponification value of *Brassica juncea* oil from different accessions across treatments

Average density of *Brassica juncea* oil was found to be 0.85 g/ $cm^3$ and average specific gravity was 0.86. Viscosity of *Brassica juncea* oil at 29 °C is given below (Table 65). Oil viscosity of ATC-93402 is higher than that of other accessions. Viscosity does not change significantly for a particular accession between treatments ($p>0.05$).

**Table 65.** Dynamic and kinematic viscosity of *Brassica juncea* oil at 29 °C including means and standard deviations (SD) for each treatment

| Accession ID | Treatment | Kinematic viscosity ($mm^2/s$) | Dynamic viscosity (mPa.s) |
|---|---|---|---|
| ATC-93161 | Control | 22.62 | 19.00 |
| ATC-93358 | Control | 26.85 | 23.00 |
| ATC-90783 | Control | 22.56 | 19.00 |
| ATC-93402 | Control | 36.63 | 31.00 |
| ATC-93142 | Control | 25.00 | 21.00 |
| Mean | | 26.73 | 22.60 |
| SD +/- | | 5.20 | 4.45 |
| ATC-93161 | TWW | 22.18 | 19.00 |
| ATC-93358 | TWW | 28.50 | 24.00 |
| ATC-90783 | TWW | 23.63 | 20.00 |
| ATC-93402 | TWW | 36.90 | 31.00 |
| ATC-93142 | TWW | 23.35 | 20.00 |
| Mean | | 26.91 | 22.80 |
| SD +/- | | 5.44 | 4.44 |
| ATC-93161 | 15 dS $m^{-1}$ | 23.75 | 20.00 |
| ATC-93358 | 15 dS $m^{-1}$ | 27.18 | 23.00 |
| ATC-90783 | 15 dS $m^{-1}$ | 22.62 | 19.00 |
| ATC-93402 | 15 dS $m^{-1}$ | 33.85 | 29.00 |
| ATC-93142 | 15 dS $m^{-1}$ | 28.50 | 24.00 |
| Mean | | 27.18 | 23.00 |
| SD +/- | | 3.97 | 3.52 |

### 10.5. Suitability of oil for bio-diesel production

Saponification value is defined as the amount of potassium hydroxide (KOH) in milligrams required to saponify one gram of fat or oil. Based on the length of the fatty acids present in the triacylglycerol molecule, the weight of the triacylglycerol molecule changes which in turn affects the amount of KOH required to saponify the molecule. Hence, saponification value is a measure of the average molecular weight or the chain length of the fatty acids present. As most of the mass of a triglyceride is in the three fatty acids, it allows for comparison of the average fatty acid chain length. Saponification value range of 185-210 corresponds with $C_{16}$-$C_{18}$ fatty acid chain length while $C_{12}$-$C_{14}$ corresponds to a range of 250-280 [149]. Since the oil from these crops is not being analysed from an edibility point of view, the exact fatty acid composition is not of great importance for this study. Fatty acid composition does have an influence on the oxidative stability and kinematic viscosity, and estimation of these physical parameters is sufficient to determine suitability of feedstock for bio-diesel production. Saponification value for the majority of the feedstocks are in the range of 185 to 210 mg KOH/g. This range is typical for feedstocks having predominately fatty acids with a chain length between $C_{16}$ and $C_{18}$. Babassu palm (*Attalea speciosa*) and coconut (*Cocos nucifera*) oil have a relatively higher saponification value of 258.5 and 267.6 mg KOH/g, respectively. Higher saponification values may indicate the presence of shorter chain lengths and babassu and coconut oil have a higher fraction of $C_{12}$ and $C_{14}$ fatty acids. Jojoba (*Simmondsia chinenis*) and *Lesquerella fendleri* oil have lower than average saponification values of 106 and 173.9 mg KOH/g, respectively.

Ricinoleic acid is $C_{18}$ reportedly makes up 90% of the fatty acid composition of *Ricinus communis* oil [150], but the estimated saponification values are slightly lower than the expected range of 185-210. This could mean that there is a lower percentage of Ricinoleic acid, or that the remaining fatty acid composition is made up of fatty acids with higher chain lengths. The slight differences in saponification value between accessions are not of statistical significance and does not suggest any differences in fatty acid composition between treatments or among the hybrids.

In *Citrullus colocynthis* like other traits, oil composition also appeared to vary among accessions. Saponification value ranged from 203.8 to 211.4, which corresponds with an average $C_{16}$- $C_{18}$ fatty acid chain length [151]. According to literature, *Citrullus colocynthis* oil is 60% linoleic acid, a polyunsaturated omega-6 $C_{18}$ fatty acid [152]. The edibility and dietary benefits of the oil may be worth studying in spite of current reports that the oil is unfit for human consumption.

*Brassica juncea* oil saponification value ranged from 167 to 169 and showed no significant variability between treatments or among accessions. According to literature, approximately 45% of the *Brassica juncea* oil is composed of Erucic Acid, which is a mono-unsaturated $C_{22}$ fatty acid [153]. The saponification value range of oil corresponded with this information. As mentioned earlier, it is the reported ill effects of erucic acid on health which has resulted in *Brassica juncea* oils being considered inedible in some parts of the world.

The interaction of FFA in the feedstock and sodium methoxide catalyst may form emulsions which make separation of the bio-diesel more difficult; possibly leading to yield loss. Emulsions can also increase cost by introducing extra cleaning steps and replacement of filters [154]. To minimize the generation of soaps during the reaction, the target reduction for FFA in the feedstock should be 0.5 wt% or less (ASTM D664). Oil from almost all *Ricinus communis* hybrids included in the study have a free fatty acid content below 0.5% and could be transesterified without pre-treatment. Only accessions VBC 1109 and VBC 1111 have a slightly greater FFA content. Oil from some accessions of *Citrullus colocynthis* in our study have high free fatty acid content. This could be due to hydrolysis by enzymes or oxidation upon storage, indicating that the oil is prone to rancidity and oxidation because of its high unsaturated fatty acid content. *Brassica juncea* oil on the other hand had a very low FFA content across treatments and accessions. From our results, it appears that only *Citrullus colcoynthis* oil may need to be pre-treated before bio-diesel production, but since only one of the accessions with high FFA oil is also high yielding and sufficient diversity exists in the collection, it would be simpler to select high-yielding accession with low FFA content for commercial cultivation.

Density is the weight per unit volume. Oils that are denser contain more energy. Density of *Ricinus communis* oil was highest (0.94 g/cm$^3$), followed by *Citrullus colocynthis* and *Brassica juncea* with a density of 0.92 and at 0.85 g/cm$^3$, respectively. It could thus be assumed that *Ricinus communis* contains more energy. Relative density or specific gravity is the density of the component compared to the density of water. The specific gravity of bio-diesel needs to be determined to make mass to volume conversions, calculate flow and viscosity properties, to judge the homogeneity of bio-diesel tanks [155]. The average specific gravity of all three feedstock oils was determined in this study to be 0.96, 0.93 and 0.86 for *Ricinus communis, Citrullus colocynthis* and *Brassica juncea,* respectively.

Viscosity is defined as the resistance to shear or flow; it is highly dependent on temperature and it describes the behavior of a liquid in motion near a solid boundary like the walls of a pipe. The presence of strong or weak interactions at the molecular level can greatly affect the way the molecules of an oil or fat slide pass each other, therefore, affecting their resistance to flow. The kinematic viscosity of bio-diesel is 10–15 times greater than that of diesel fossil fuels. This is because of its large molecular mass and large chemical structure. In some cases, at low temperatures bio-diesel can becomes very viscous or even solidified. Higher viscosity of bio-diesel can affect the volume flow and injection spray characteristics in the engine [2]. At low temperature, it may even compromise the mechanical integrity of the injection pump drive systems. Average kinematic viscosity of *Ricinus communis* oil was found to be very high, ranging from 278 to 323 $mm^2/s$. This makes the oil difficult to use as it can easily clog machine parts. Upon transesterification, the kinematic viscosity reduces approximately by an order of magnitude. But this would still be too high as bio-diesel specifications are 1.9–6.0 $mm^2/s$ in ASTM D6751 and 3.5–5.0 $mm^2/s$ in EN 14214 [156]. This result suggests that 100% bio-diesel (B100) from *Ricinus communis* oil cannot be used in an engine, despite reports to the contrary. Blending with petro-diesel will reduce viscosity and make the fuel usable. One possible reason for this observation is these two oils contain high concentrations of hydroxy containing fatty acids (ricinoleic acid) that are capable of forming hydrogen bonding. Kinematic viscosity of *Citrullus colcoynthis* oil was very low, between 23 and 36 $mm^2/s$, which makes it ideal for bio-diesel synthesis. Kinematic viscosity of edible Indian *Brassica juncea* oil was approximately 50 $mm^2/s$. In comparison, the kinematic viscosity of oil from our trial was much lower, ranging from 22 to 36 $mm^2/s$. This may be due to differences in fatty acid composition.

When considering seed oil content, oil quality and oil yield, *Ricinus communis* is the best feedstock among the three candidates in our study, the only drawback of it being its high viscosity. *Citrullus colocynthis* oil is also a good candidate in terms of viscosity, but the species needs to be domesticated and agricultural production needs to be optimized before any large-scale oil production can be realized. *Brassica juncea* oil is suitable in terms of oil quality, but the low yields make its use as feedstock relatively unviable under the UAE conditions, although more detailed studies are required including identification of high yielding genotypes and optimization of production and management to maximize yields.

# CHAPTER 11

# STATISTICAL ANALYSIS OF DATA

A large volume of data (in replicates) was obtained as a result of the field trials of each crop. In order to analyze the data and reach coherent and logical interpretations from it, the data was analyzed using statistical analysis tools. The tools and techniques used and the results obtained are detailed in this section.

### 11.1. Analysis

For all crops, analysis of variance was carried out on collected agronomic and salinity response parameters using Genstat® Discovery Edition 3 [174-175] in order to identify statistically significant differences in data with respect to accessions and salinity treatments.

Additionally, for *Citrullus colocynthis*, a Correlation matrix was generated and Pearson Principal components analysis was conducted using Microsoft © Excel 2013 add- in XLSTAT 2015.4 (© Addinsoft). A Principal Components Analysis based on correlation was carried out using two significant components identified from the scree plot. Agglomerative Hierarchical cluster analysis was performed based on unweighted pair- group averages.

### 11.2. Analysis of *Ricinus communis* data

Correlation matrix of all observed descriptive, agronomic and salinity response characteristics of eleven *Ricinus communis* accessions is given below (Table 66). Plant height is strongly correlated with other indicators of plant growth and biomass such as stem diameter, leaf size, leaf dry weight, size of inflorescence, etc. a significant positive correlation (0.358) is also observed between plant height and yield per plant. Yield per plant does not correlate very strongly with any of the other agronomic or descriptive characteristics. Seed oil content correlates significantly with number of fruits/spike, spike length, leaf dry weight and plant height. $K^+/Na^+$ ratio in leaf tissue correlates in an obvious manner with improved growth characteristics. The Scree plot for the PCA showed a drop in eigenvalue after the first two components (Figure 66). The first two components account for a cumulative variability of 47.5%, so these were chosen for the PCA biplot (Figure 67) generation. The contribution of each characteristic to each component is given in Table 67. The biplot displays the spread of characteristics as well as accessions along the two components. Leaf dry weight and leaf size, which are indicators of vegetative growth and biomass, lie on the opposite vector to oilseed yield per plant. But surprisingly, so does 1000 seed weight and $K^+/Na^+$ ratio. Seed oil content associates more strongly with inflorescence characteristics, such as spike type, length and number of fruits/spike. Accessions VBC 999 and VBC 1116 associate most strongly along the seed oil content vector, while accessions VBC 1123 and VBC 1112 associate along the yield vector.

**Table 66.** Correlation matrix of *Ricinus communis* data (Pearson)

| Variables | Plant height | Stem diameter | Leaf size | Leaf Dry weight | YPP | Leaf Moisture | Inflorescence Length | No. of Fruits/spike | Seed oil% | Stem Color | Leaf color | Spike Type | 1000 seed wt. | Leaf K/Na ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant height | 1 | 0.327 | 0.172 | -0.408 | 0.358 | -0.009 | 0.148 | -0.065 | 0.226 | -0.236 | 0.052 | 0.346 | -0.114 | -0.049 |
| Stem diameter | 0.327 | 1 | 0.513 | 0.250 | -0.058 | 0.167 | -0.228 | -0.107 | -0.105 | 0.124 | 0.309 | 0.458 | 0.246 | 0.517 |
| Leaf size | 0.172 | 0.513 | 1 | 0.484 | -0.239 | 0.061 | 0.251 | 0.105 | 0.142 | 0.350 | -0.340 | 0.075 | 0.659 | 0.092 |
| Leaf Dry weight | -0.408 | 0.250 | 0.484 | 1 | -0.733 | 0.495 | 0.428 | 0.541 | 0.271 | 0.370 | -0.470 | 0.064 | 0.411 | 0.281 |
| YPP | 0.358 | -0.058 | 0.239 | -0.733 | 1 | -0.271 | -0.174 | -0.436 | -0.440 | -0.223 | 0.375 | 0.046 | -0.188 | -0.179 |
| Leaf Moisture | -0.009 | 0.167 | 0.061 | 0.495 | -0.271 | 1 | 0.303 | 0.216 | 0.009 | -0.184 | -0.363 | 0.137 | -0.368 | -0.087 |
| Inflorescence Length | 0.148 | -0.228 | 0.251 | 0.428 | -0.174 | 0.303 | 1 | 0.757 | 0.423 | -0.196 | -0.594 | 0.385 | 0.226 | -0.152 |
| No. of Fruits/spike | -0.065 | -0.107 | 0.105 | 0.541 | -0.436 | 0.216 | 0.757 | 1 | 0.578 | -0.343 | -0.349 | 0.347 | 0.370 | 0.260 |
| Seed oil% | 0.226 | -0.105 | 0.142 | 0.271 | -0.440 | 0.009 | 0.423 | 0.578 | 1 | -0.048 | -0.613 | 0.152 | 0.309 | 0.218 |
| Stem Color | -0.236 | 0.124 | 0.350 | 0.370 | -0.223 | -0.184 | -0.196 | -0.343 | -0.048 | 1 | -0.149 | 0.418 | 0.362 | -0.009 |
| Leaf color | 0.052 | 0.309 | 0.340 | -0.470 | 0.375 | -0.363 | -0.594 | -0.349 | -0.613 | -0.149 | 1 | 0.356 | -0.085 | 0.083 |
| Spike Type | 0.346 | 0.458 | 0.075 | 0.064 | 0.046 | 0.137 | 0.385 | 0.347 | -0.152 | -0.418 | 0.356 | 1 | 0.015 | 0.116 |
| 1000 seed wt. | -0.114 | 0.246 | 0.659 | 0.411 | -0.188 | -0.368 | 0.226 | 0.370 | 0.309 | 0.362 | -0.085 | 0.015 | 1 | 0.166 |
| Leaf K/Na ratio | -0.049 | 0.517 | 0.092 | 0.281 | -0.179 | -0.087 | -0.152 | 0.260 | 0.218 | -0.009 | 0.083 | 0.116 | 0.166 | 1 |

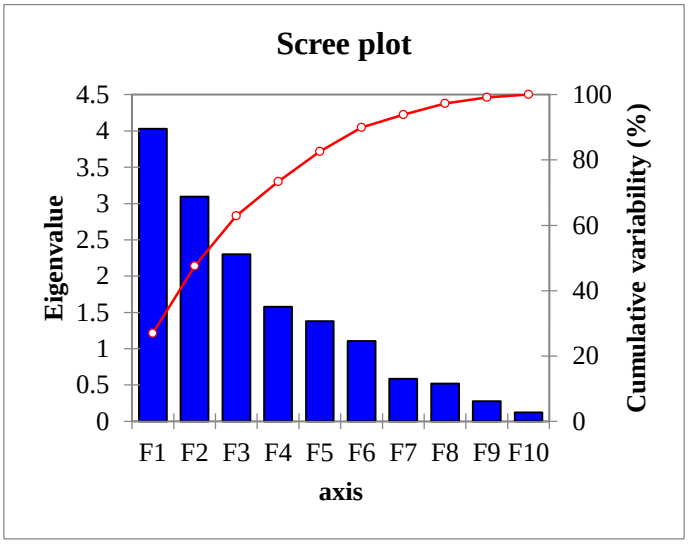


**Figure 66.** Scree Plot of *Ricinus communis* principle components analysis factors F1 and F2

**Table 67.** Contribution of each characteristic to components F1 and F2 (%) of *Ricinus communis* principle components analysis

| | F1 | F2 |
|---|---|---|
| Plant height | 0.802 | 3.792 |
| Stem diameter | 0.497 | 0.650 |
| Leaf size | 8.909 | 2.255 |
| Leaf Dry weight | 19.816 | 0.366 |
| YPP | 12.614 | 0.129 |
| Leaf Moisture | 2.349 | 4.112 |
| Inflorescence Length | 9.051 | 10.610 |
| No. of Fruits/spike | 11.120 | 11.872 |
| Seed oil% | 9.179 | 2.341 |
| Growth Habit | 0.000 | 0.000 |
| Stem Color | 2.369 | 25.309 |
| Leaf color | 11.357 | 0.366 |
| Spike Type | 0.001 | 10.000 |
| Spike compactness | 0.000 | 0.000 |
| Waxy coating | 2.369 | 25.309 |
| 1000 seed wt. | 8.096 | 2.878 |
| Fruit surface | 0.000 | 0.000 |
| Fruit dehiscence | 0.000 | 0.000 |
| Leaf $K^+/Na^+$ ratio | 1.470 | 0.009 |

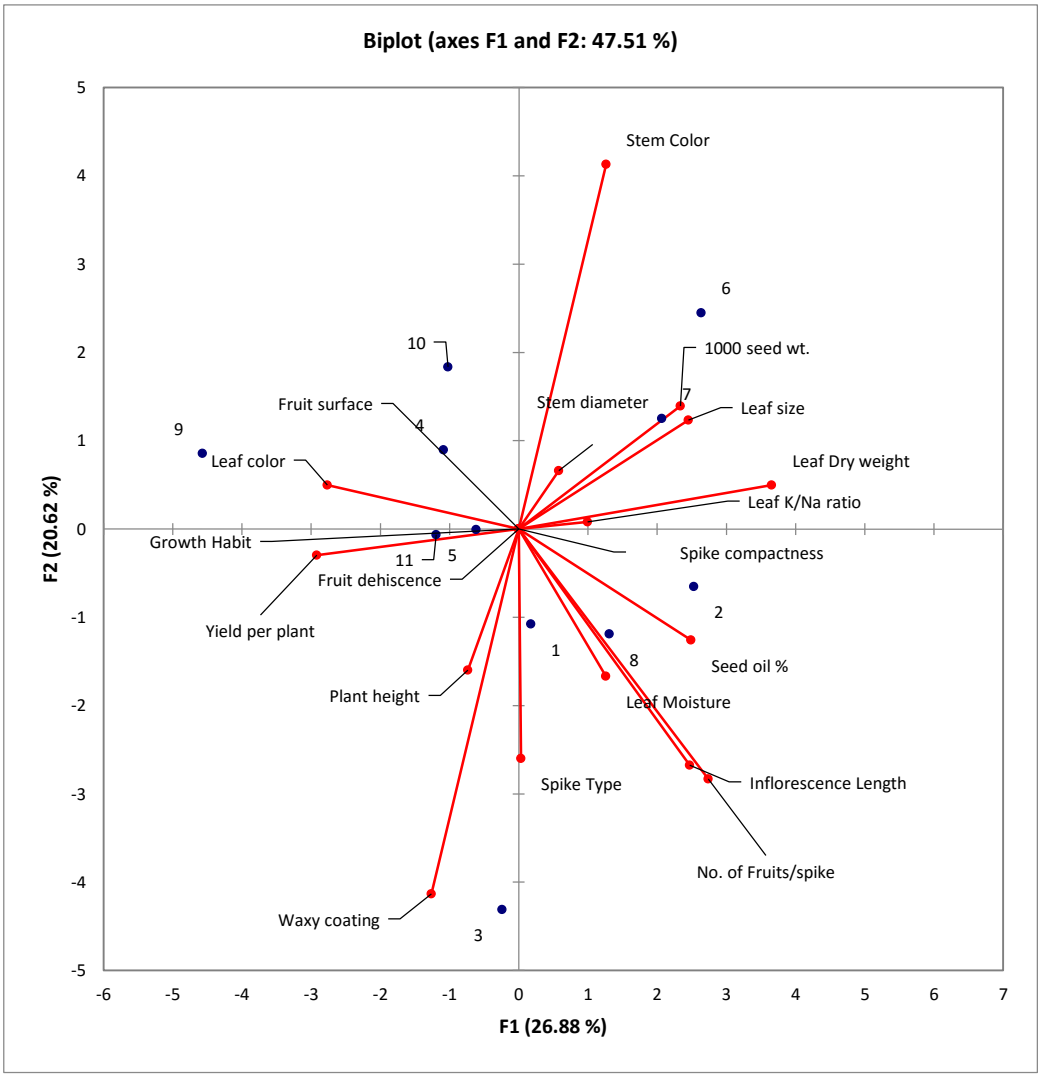


**Figure 67.** Principle components analysis biplot of factors F1 and F2 for *Ricinus communis* data

## 11.3. Analysis of *Citrullus colocynthis* data

The correlation matrix (Table 68) for observed agronomic traits of 27 accessions shows the expected positive correlation between growth related characteristics such as number of branches, branch length and leaf size. Interestingly, the plants with greater primary branches and longer branches correlated with lower number of fruits and lower yield per plant ($r<0$). Yield per plant did not positively correlate with any parameter other than the number of fruits ($r=0.481$), which is expected. The number of fruits also correlated positively with seed oil content. Based on the scree plot (Figure 68) the first two components contributing to 61.78% of the cumulative variability, were chosen for the principle components analysis (PCA).

**Table 68.** Correlation matrix of observed agronomic characteristics of 27 *Citrullus colocynthis* accessions (Pearson)

| Variables | No. of branches | Vine length | Leaf Length | Leaf width | No. of fruits/plant | Fruit diameter | Fruit color | Oil% | Yield /plant | 1000 seed weight | Seed surface area | Seed color |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of branches | 1 | 0.797 | 0.751 | 0.668 | -0.367 | 0.458 | 0.059 | 0.013 | -0.350 | 0.683 | 0.668 | -0.099 |
| Vine length | 0.797 | 1 | 0.869 | 0.835 | -0.415 | 0.510 | 0.054 | 0.223 | -0.387 | 0.754 | 0.856 | 0.090 |
| Leaf length | 0.751 | 0.869 | 1 | 0.911 | -0.335 | 0.323 | -0.002 | 0.177 | -0.406 | 0.621 | 0.685 | 0.092 |
| Leaf width | 0.668 | 0.835 | 0.911 | 1 | -0.266 | 0.325 | 0.110 | 0.304 | -0.383 | 0.574 | 0.646 | 0.105 |
| No. of fruits/Plant | -0.367 | -0.415 | -0.335 | -0.266 | 1 | -0.234 | 0.522 | 0.134 | 0.481 | -0.469 | -0.436 | 0.065 |
| Fruit diameter | 0.458 | 0.510 | 0.323 | 0.325 | -0.234 | 1 | 0.349 | 0.307 | -0.108 | 0.592 | 0.695 | -0.015 |
| Fruit color | 0.059 | 0.054 | -0.002 | 0.110 | 0.522 | 0.349 | 1 | 0.196 | 0.253 | -0.004 | 0.114 | 0.036 |
| Oil% | 0.013 | 0.223 | 0.177 | 0.304 | 0.134 | 0.307 | 0.196 | 1 | -0.127 | 0.340 | 0.170 | 0.005 |
| Yield/plant | -0.350 | -0.387 | -0.406 | -0.383 | 0.481 | -0.108 | 0.253 | -0.127 | 1 | -0.430 | -0.354 | -0.095 |
| 1000 seed weight | 0.683 | 0.754 | 0.621 | 0.574 | -0.469 | 0.592 | -0.004 | 0.340 | -0.430 | 1 | 0.795 | 0.040 |
| Seed surface area | 0.668 | 0.856 | 0.685 | 0.646 | -0.436 | 0.695 | 0.114 | 0.170 | -0.354 | 0.795 | 1 | 0.243 |
| Seed color | -0.099 | 0.090 | 0.092 | 0.105 | 0.065 | -0.015 | 0.036 | 0.005 | -0.095 | 0.040 | 0.243 | 1 |

The percentage contribution of each characteristic to each component is represented in Table 69. As can be observed from the table, the characteristics of interest from the agricultural point of view such as yield per plant, number of fruits, and oil content, all load more significantly on the second component to which fruit size, color, seed size and color also contributes. The first components loads characteristics such as leaf size, number of branches, length of branches and 1000 seed weight, which are of less importance from the agricultural point of view. This means these characteristics associate more strongly with each other than with other characteristics associated with vegetative growth. The biplot (Figure 69) helps visualize this information.

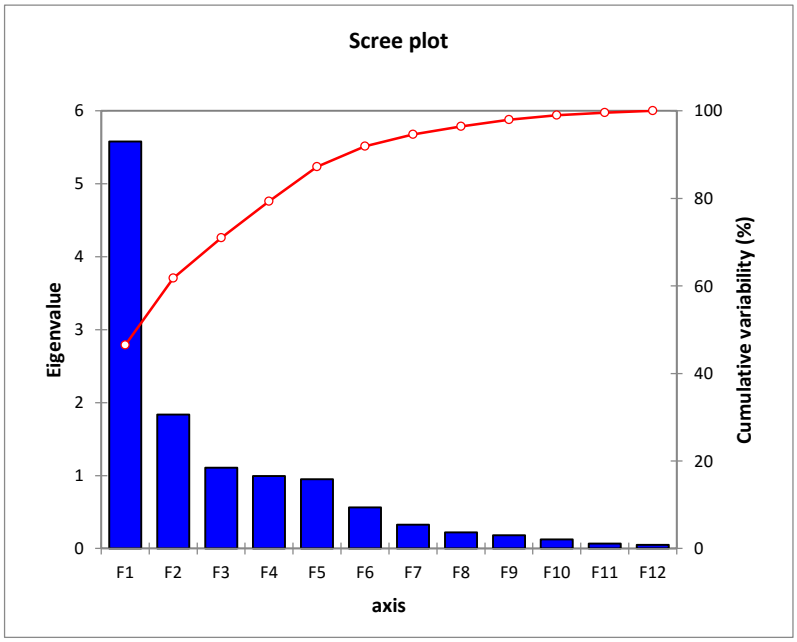


**Figure 68.** Scree plot of *Citrullus colocynthis* characteristics Principle Components Analysis

**Table 69.** Contribution of each characteristic to components F1 and F2 (%) of *Citrullus colocynthis* principle components analysis

| | F1 | F2 |
|---|---|---|
| No. of branches | 12.343 | 0.053 |
| Vine length | 16.038 | 0.077 |
| Leaf length | 13.626 | 0.039 |
| Leaf width | 12.615 | 0.493 |
| No. of fruits/plant | 4.667 | 25.676 |
| Fruit diameter | 6.788 | 8.958 |
| Fruit color | 0.023 | 41.438 |
| Oil% | 1.376 | 11.506 |
| Yield/Plant | 4.780 | 10.832 |
| 1000 seed weight | 13.252 | 0.005 |
| Seed surface area | 14.331 | 0.558 |
| Seed color | 0.160 | 0.365 |

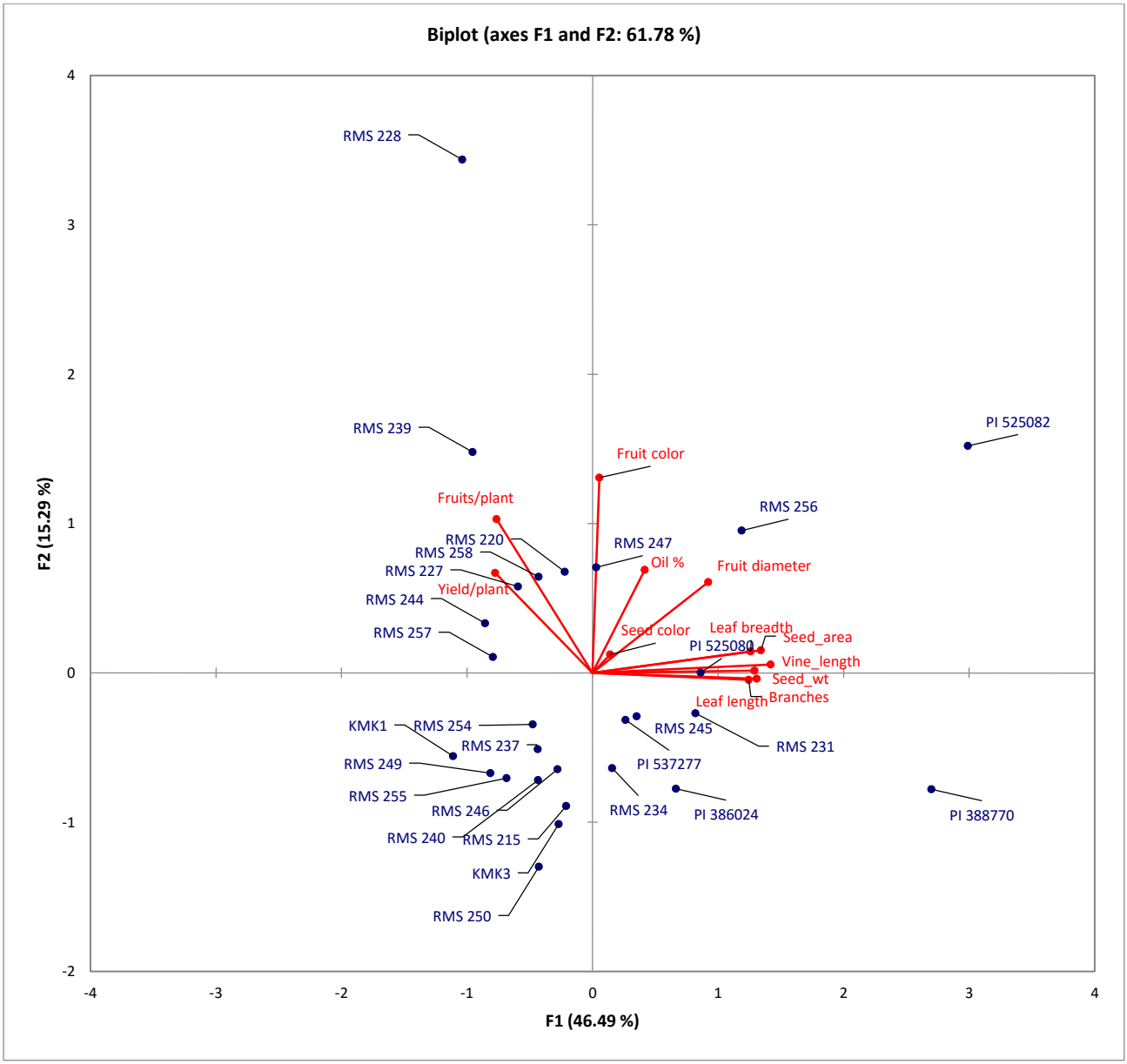


**Figure 69.** Biplot of *Citrullus colocynthis* principle components analysis for components F1 and F2

The dendrogram from the Agglomerative Hierarchical clustering of all accessions based on the collected agronomic data is presented below (Figure 70).

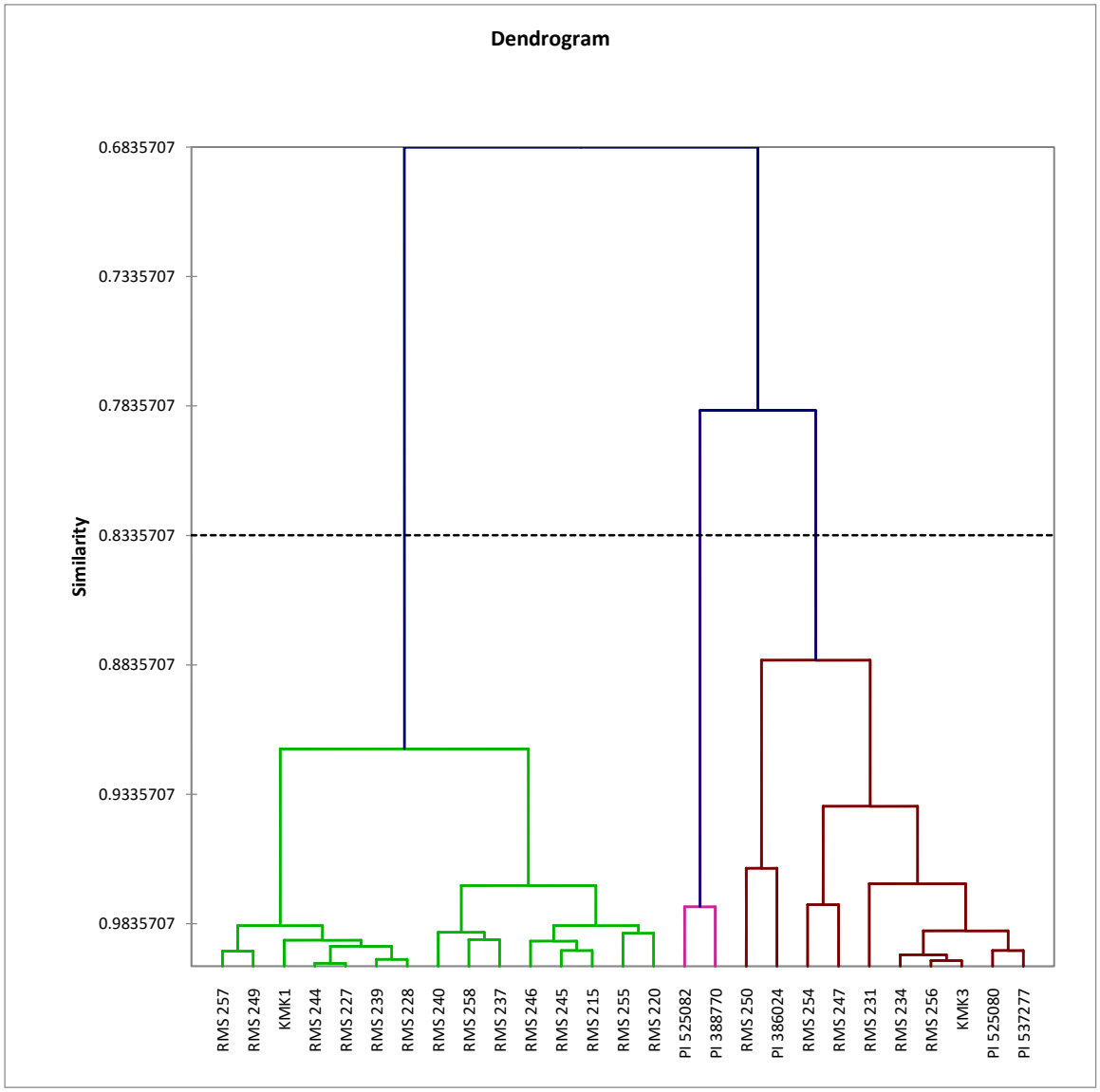


**Figure 70.** Agglomerative Heirarchical clustering of *Citrullus colocynthis* accessions using Ward's linkage algorithm

## 11.4. Analysis of *Brassica juncea* data

The correlation matrix of *Brassica juncea* characteristics is presented in Table 70. Yield per plant correlates most significantly with leaf moisture, 1000 seed weight, pod length and plant height. Seed oil content and 1000 seed weight are observed to correlate strongly with pod size. The scree plot (Figure 71) shows two components that contribute to 77.45% of cumulative variability. Contribution of each characteristic to the components is represented in Table 71. Yield per plant, plant height, leaf size and leaf dry weight all load more significantly on the first component and leaf moisture, 1000 seed weight and $K^+/Na^+$ ratio on the second component. These associations and the spread of accessions along these vectors is represented in the biplot (Figure 72).

**Table 70.** Correlation matrix of *Brassica juncea* agronomic characteristics (Pearson)

| Variables | Plant height | Leaf Length | Leaf breadth | Leaf Dry Weight | Leaf Moisture content | Pod Length | YPP (g) | 1000 seed weight | Seed Oil content (%) | K/Na |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant height | 1 | -0.548 | -0.802 | -0.643 | -0.073 | 0.601 | 0.267 | -0.114 | 0.692 | 0.180 |
| Leaf Length | -0.548 | 1 | 0.918 | 0.992 | -0.257 | -0.043 | -0.659 | 0.145 | 0.100 | -0.357 |
| Leaf breadth | -0.802 | 0.918 | 1 | 0.951 | 0.012 | -0.168 | -0.448 | 0.307 | -0.277 | -0.446 |
| Leaf Dry Weight | -0.643 | 0.992 | 0.951 | 1 | -0.229 | -0.125 | -0.661 | 0.151 | -0.016 | -0.366 |
| Leaf Moisture content | -0.073 | -0.257 | 0.012 | -0.229 | 1 | 0.504 | 0.769 | 0.885 | -0.579 | -0.680 |
| Pod Length | 0.601 | -0.043 | -0.168 | -0.125 | 0.504 | 1 | 0.363 | 0.679 | 0.344 | -0.624 |
| YPP (g) | 0.267 | -0.659 | -0.448 | -0.661 | 0.769 | 0.363 | 1 | 0.449 | -0.267 | -0.095 |
| 1000 seed weight | -0.114 | 0.145 | 0.307 | 0.151 | 0.885 | 0.679 | 0.449 | 1 | -0.400 | -0.925 |
| Seed Oil content (%) | 0.692 | 0.100 | -0.277 | -0.016 | -0.579 | 0.344 | -0.267 | -0.400 | 1 | 0.351 |
| K/Na | 0.180 | -0.357 | -0.446 | -0.366 | -0.680 | -0.624 | -0.095 | -0.925 | 0.351 | 1 |

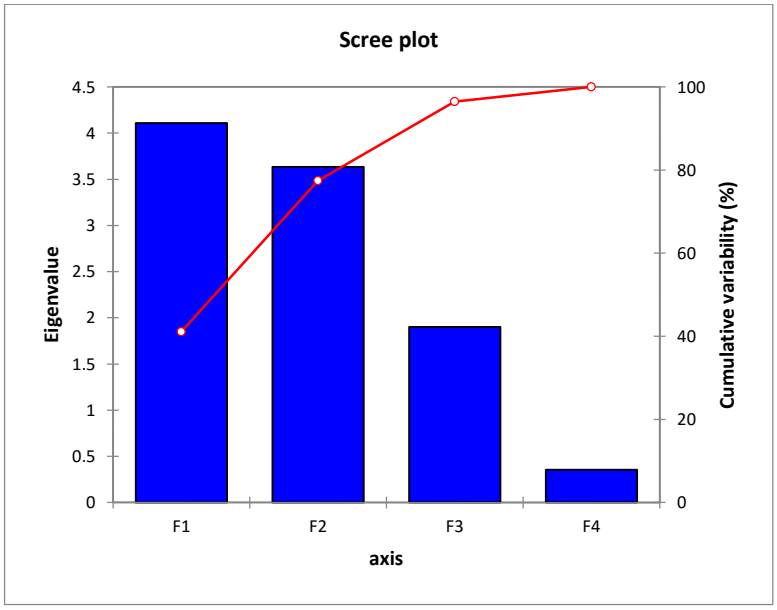


**Figure 71.** Scree plot of *Brassica juncea* principle components analysis

**Table 71.** Contribution of individual characteristics to PCA components F1 and F2 (%) for *Brassica juncea* data

| | F1 | F2 |
|---|---|---|
| Plant height | 15.660 | 0.100 |
| Leaf Length | 20.965 | 0.131 |
| Leaf breadth | 23.131 | 0.540 |
| Leaf Dry Weight | 22.774 | 0.081 |
| Leaf Moisture content | 0.477 | 25.634 |
| Pod Length | 1.743 | 11.168 |
| YPP (g) | 9.297 | 10.445 |
| 1000 seed weight | 0.751 | 26.077 |
| Seed Oil content (%) | 1.606 | 6.432 |
| K/Na | 3.595 | 19.392 |

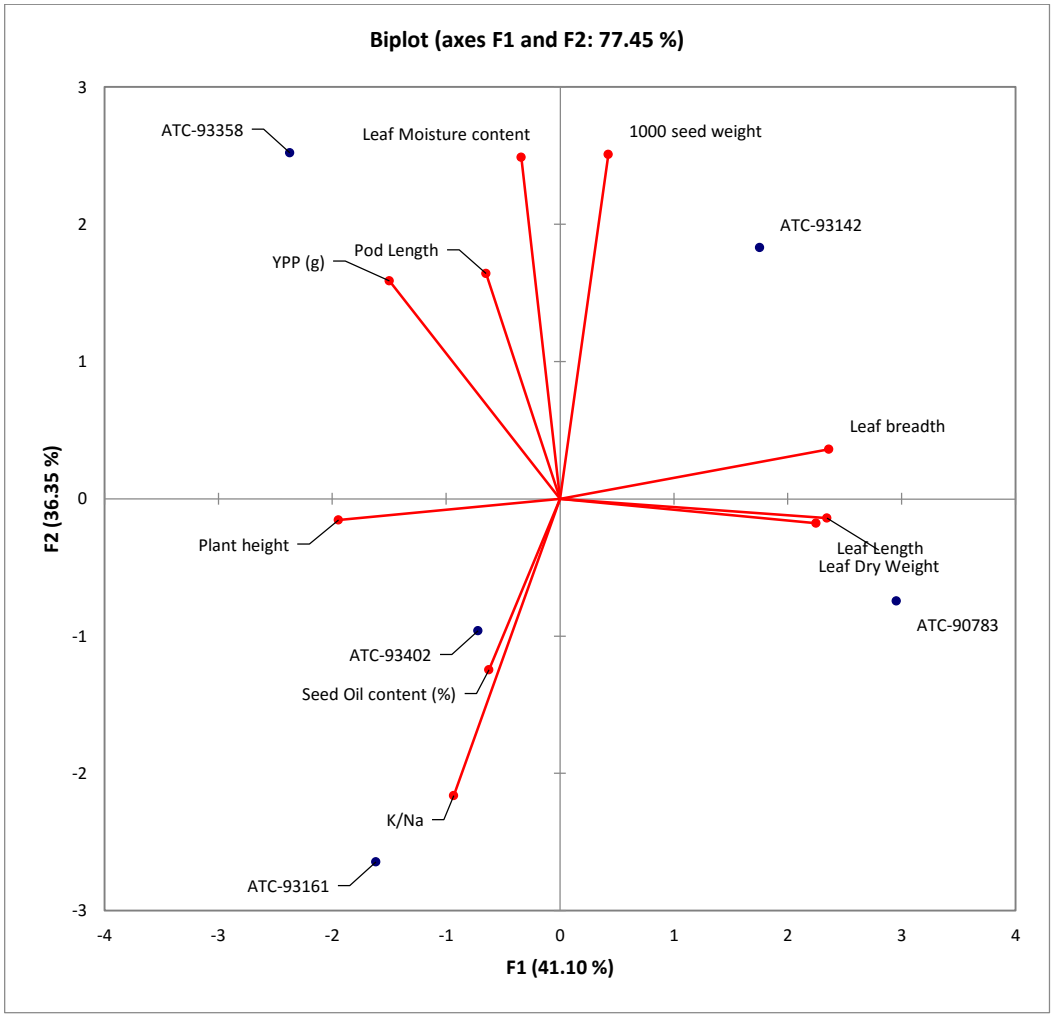


**Figure 72.** Biplot of *Brassica juncea* principle components F1 and F2

## 11.5. Discussion of statistical analysis of data

Multivariate statistical analysis of *Ricinus communis* and *Brassica juncea* data was performed to help in the selection of accessions for cultivation on the basis of desired traits. The correlation matrices emphasized the inverse correlation between salinity treatment and growth parameters. In both the crops, the correlation matrices emphasized that seed oil content is mostly independent of all other plant characteristics.

The statistical analysis was most useful in the case of *Citrullus colocynthis*, which has a high yield per plant in comparison with many other oilseed crops including *Ricinus communis* (current study*)* and *Jatropha curcas.* From the correlation matrix, it is seen that higher yielding varieties generally have lower values for branch length and other growth factors, indicating more compact growth habit. This is of advantage from the point of view of large scale cultivation because a compact growth habit not only seems to correlate with higher yield per plant, but also means a higher density of plants can be achieved per hectare, increasing per hectare yield. The principle components analysis also showed that characteristics of agricultural and economic interest such

as yield per plant, oil content and number of fruits group together and load more significantly on the second component. 1000-seed weight is mostly characteristic to a particular accession and does not correlate with seed yield. If the accessions are ranked on the basis of important characteristics such as germination efficiency, oilseed yield and oil yield RMS 244, 227, 228 and KMK1 are the best performing of the 27 accessions studied. If oil quality, mainly FFA< 0.5% is also taken into consideration, RMS 227 appears to be the best performing accession in this study. From the hierarchical clustering and the principle components biplot of accessions, it can be seen that the accessions grouped into two large clusters, with the exceptions of accessions PI 388770 and PI 525082 grouping together and forming a smaller class. Accessions PI 388770 and PI 525082 are low yielding and different from other accessions and similar to each other because of their highly branched growth habit and greater vegetative growth in terms of vine length and leaf size compared to other accessions. Considering the degree of variation in characteristics even among the accessions of the larger group, it would seem that these accessions (PI 388770 and PI 525082) are simply the most diverse in an already highly diverse group. Accessions RMS 228 and 239, while separate in the PCA biplot along the second component because of their exceptionally high yield, grouped together with accessions RMS 246, RMS 257, RMS 255, RMS 220, RMS 245, RMS 244, RMS 258, RMS 247, RMS 237, RMS 240, RMS 249, RMS 215 and KMK1 as per the cluster analysis dendrogram, to form one large group composed of the higher yielding accessions. The remaining ten accessions formed the third group.

# CHAPTER 11

# CONCLUSIONS

***Conclusions***

The high rates of GHG emissions and increasingly visible and alarming effects of global warming has highlighted the need for green energy over this past decade. The year 2015 was the hottest year in history, or at least on record. Obvious climate change indicators such as wildfires in Indonesia, drought in California, the ocean's worst-ever coral bleaching event, the strongest tropical cyclones ever to hit Mexico and Yemen and satellite assessments finding that massive sections of the great Antarctic and Greenland ice sheets have begun to slip into the sea have made even climate change sceptics note the urgency for change in order to protect the environment. As a result of the UN climate conference (COP21) held in Paris in December 2015, the UAE has pledged a 24% dependency on clean energy by 2021. In order to achieve this target, the Dubai Executive Council has recently approved the Dubai Carbon Abatement Strategy 2021 which aims to stem greenhouse gas emissions by 16% by the year 2021. Bio-diesel is the only green fuel that can be used in existing transportation vehicles without modification, making it a very important area of research for UAE and for other countries in this region. This study was a comparison of the bio-diesel production potential of three different crops under marginal growing conditions, carried out over a period of 3-4 years. Each crop was studied for a single season to provide a general overview of crop performance in the described conditions, and has helped identify potential candidate accessions. The study found that *Ricinus communis* (castor) and some accessions of *Citrullus colocynthis* (desert gourd) have good potential to pursue further in-depth studies including development of optimal production and management practices to maximize yields, and so on.

Oil yields of some bio-diesel feedstocks obtained from literature are given in Table 72. Yield obtained from *Ricinus communis* and *Citrullus colocynthis* with freshwater irrigation in our study are comparable to/higher than the yield from most of these feedstock crops with the exception of palm oil. What is also important to note is that most of these popular feedstocks are also edible cooking oils, while castor and desert gourd are not.

While a highly salinity tolerant accession could not be identified in this study, tolerance of castor to low levels of salinity was discovered. From the survey of literature, it is obvious that the over-extraction and improper irrigation management of groundwater are leading causes of increased salinity in the UAE and other countries in arid regions such as the Middle East and North Africa (MENA). Under these circumstances, it seems counter-intuitive to use saline groundwater for growing bioenergy crops Thus, the more productive and sustainable solution could be to find alternative irrigation sources rather than identifying plant varieties that can tolerate saline irrigation water. In this respect, the results of the Treated Waste Water (TWW) treatment in the

*Brassica juncea* field trial, where an up to two-fold increase in yield was observed, are promising. Currently, 40% of treated waste water in the UAE is dumped into the ocean while 60% is utilized in irrigation of public landscaped spaces. The water used is tertiary treated, and not hazardous to health or the environment. TWW irrigation may thus well be the most sustainable and productive source of irrigation water in the region. It also opens the way to study the potential for more heat-tolerant oilseed crops for cultivation in the region that need not exhibit potential for salinity tolerance. This is a nutrient rich alternative irrigation source that needs to be tapped and could be ideal for the irrigation of bioenergy crops and further improve the yields of crops such as *Ricinus communis* and *Citrullus colocynthis*.

**Table 72.** Oil yields of various bio-diesel feedstocks (Reproduced partially from Atabani *et al*., 2012)

| Feedstock | Oil content (%) | Oil yield (L/ha/year) |
|---|---|---|
| Castor | 53 | 1413 |
| Jatropha | Seed: 35-40<br>Kernel: 50-60 | 1892 |
| Linseed | 40-44 | - |
| Neem | 20-30 | - |
| *Pongamia Pinnata* (Karanja) | 27-39 | 2250 (kg/ha) |
| Soybean | 15-20 | 446 |
| Sunflower | 25-35 | 952 |
| *Calophyllum inophyllum* | 65 | 4680 |
| *Moringa olifera* | 40 | - |
| *Euphorbia lathyris* L. | 48 | 1500- 2500 (kg/ha.) |
| *Sapium sebiferum* L. Kernel | 12-29 | - |
| Rapeseed | 38-46 | 1190 |
| Tung | 16-18 | 940 |
| *Pachira glabra* | 40-50 | - |
| Palm oil | 30-60 | 5950 |
| Peanut oil | 45-55 | 1059 |
| Olive oil | 45-70 | 1212 |
| Corn (Germ) | 48 | 172 |
| Coconut | 63-65 | 2689 |
| Cottonseed | 18-25 | 325 |

| | | |
|---|---|---|
| Rice bran | 15-23 | 828 |
| Sesame | - | 696 |
| Jojoba | 45-50 | 1818 |
| Rubber seed | 40-50 | 80-120 (kg/ha.) |
| Sea mango | 54 | - |

With respect to commercial-scale cultivation, yield is of paramount importance. From the comparison carried out in this study, *Ricinus communis* and *Citrullus colocynthis* seem to have most potential for cultivation in the UAE and other countries in the region. The disadvantage of *Ricinus communis* however, is the high viscosity of oil. While this can pose difficulties in the processing of the oil and conversion to bio-diesel, blending can prevent any potential problems in the end-use of the bio-diesel. *Citrullus colocynthis* also has immense potential due to the high yield of individual plants under irrigated conditions. Among the species studied *Citrullus colocynthis* is likely to have the least water requirements, as evidenced from its adaptation to arid natural habitat and growth under non-irrigated, rain-fed conditions in the region. *Citrullus colocynthis* could prove to be a high value crop considering the possible medicinal applications of extracts from the crop, some of which have been studied as part of this project (Appendix II). The possibility of multiple products from the crop makes its study and use more attractive in terms of economic feasibility. The disadvantage of desert gourd is that it is yet a wild species. Selection and improvement for favorable characteristics will be required before the species can be cultivated on a commercial scale. The principal components analysis grouped yield bearing traits together, which could facilitate yield improvement and selection of accessions for breeding.

While the results of previous pilot study of *Brassica juncea* accessions conducted by ICBA were promising, the yields in our field trial were not on par with those results. The small seed size and lower yield per hectare in comparison with *Ricinus communis*, are the main disadvantages of this crop. The observations of increased yield with TWW irrigation was exciting and has provided a platform for further studies into the subject.

In terms of potential as bio-diesel feedstock, oil characteristics determined appear to be in keeping with existing literature and much work has already been done by researchers across the globe with regards to the suitability of *Ricinus communis* oil, establishing it as a feedstock for bio-diesel production. There is less published information about *Citrullus colocynthis* and *Brassica juncea* oil for bio-diesel production. In the present study, the most important oil characteristics for bio-diesel feedstock oil such as viscosity, density, free fatty acid content and saponification value were

analyzed and suitability for such use was determined on the basis of these results. As is, the results of this study justify further studies of *Ricinus communis* and *Citrullus colocynthis* to further validate the suitability of these crops for cultivation as bio-diesel feedstock.

***Specific contributions***

*Ricinus communis* cultivation and yield in this region has not been reported previously. In this study the performance of eleven hybrid accessions of the crop was studied in field trials and yield was found to be on par with the global average. *Ricinus communis* has not been previously studied for salinity tolerance under field conditions. While accessions tolerant to high levels of salinity could not be identified, tolerance to low levels of salinity (5 dS $m^{-1}$) in terms of yield was observed in spite of sodium accumulation in plant parts. Suitability of *Ricinus communis* oil for bio-diesel production has been reported before, and those findings were corroborated and accessions yielding suitable oil were identified.

*Citrullus colocynthis* is native to the region and a relatively unknown crop. There were no previous reports of cultivation of this crop and yields under irrigated conditions. Pre-treatments to break seed dormancy was studied in addition to the field trials for salinity tolerance and germplasm diversity. While the accessions studied were sensitive to salinity, they proved to be very high yielding under irrigated conditions. Seed oil was also analyzed and accessions more suitable than others for use as bio-diesel feedstock were identified. The results of this study warrants further investigation of this crop and its cultivation in the region.

*Brassica juncea* cultivation and yields in the region have been reported before, but performance under irrigation with treated wastewater had not been previously studied. While the yields from our field trials were low in comparison to previously reported data, a significant improvement in yield under TWW irrigation was observed. The results of this study emphasizes the need to dedicate the use of more TWW towards irrigated agriculture in the region.

***Future prospects***

Studies on a wider range of genotypes of *Ricinus communis*, and collections of *Citrullus colocynthis* from coastal areas may possibly identify salinity tolerant accessions. The use of treated wastewater for irrigation of oilseed crops, *Ricinus communis* and *Citrullus colocynthis* included, merits further investigation to assess whether it can improve yields in these crops as well. The specific nutrients and components in TWW, apart from nitrates, which contributes to increased plant growth and yield will also be worth identifying. Irrigation management studies could be conducted to determine rate and volume of irrigation required and avoid over-irrigating of crops, especially *Citrullus colocynthis*, and optimize cultivation economics. Additionally, a study of

*Brassica juncea* accessions over multiple seasons in multiple locations may help elucidate their lower than average performance in our study.

While the suitability of oil as bio-diesel feedstock based on important physical characteristics has been established in this study, larger scale studies of identified accessions and conversion of oil to bio-diesel may be carried out in order to determine transformation efficiency and fuel performance of the bio-diesel generated from these crops. The cost of bio-diesel production could be analyzed in such a study.

Bio-diesel feedstock cultivation results in carbon fixation but also uses energy, and the use of the fuel leads to further emissions. It may be of merit to determine the net energy and carbon balance of the cultivation and production of bio-diesel from these crops in comparison to emission from the fuel to ensure that production and use is at the very least, carbon neutral.

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# APPENDIX 1

**Table A1. 1.** Randomized complete block design for *Ricinus communis*

| Replicate | Plot | Accession ID | Replicate | Plot | Accession ID | Replicate | Plot | Accession ID |
|---|---|---|---|---|---|---|---|---|
| 1 | 101 | VBC 1123 | 2 | 201 | VBC 1109 | 3 | 301 | VBC 1114 |
| 1 | 102 | VBC 1116 | 2 | 202 | VBC 1111 | 3 | 302 | VBC 999 |
| 1 | 103 | VBC 1112 | 2 | 203 | VBC 777 | 3 | 303 | VBC 1112 |
| 1 | 104 | VBC 1114 | 2 | 204 | VBC 1123 | 3 | 304 | VBC 777 |
| 1 | 105 | VBC 1121 | 2 | 205 | VBC 1114 | 3 | 305 | VBC 1109 |
| 1 | 106 | VBC 777 | 2 | 206 | VBC 1112 | 3 | 306 | VBC 1122 |
| 1 | 107 | VBC 1122 | 2 | 207 | VBC 1121 | 3 | 307 | VBC 1121 |
| 1 | 108 | VBC 1115 | 2 | 208 | VBC 1122 | 3 | 308 | VBC 1123 |
| 1 | 109 | VBC 999 | 2 | 209 | VBC 1116 | 3 | 309 | VBC 1111 |
| 1 | 110 | VBC 1111 | 2 | 210 | VBC 999 | 3 | 310 | VBC 1116 |
| 1 | 111 | VBC 1109 | 2 | 211 | VBC 1115 | 3 | 311 | VBC 1115 |

| | | | | | |
|---|---|---|---|---|---|
| 101 | 102 | 103 | 104 | 105 | 106 |
| 201 | 111 | 110 | 109 | 108 | 107 |
| 202 | 203 | 204 | 205 | 206 | 207 |
| 302 | 301 | 211 | 210 | 209 | 208 |
| 303 | 304 | 305 | 306 | 307 | 308 |
| | | | 311 | 310 | 309 |

**Figure A1.1.** Field layout for *Ricinus communis* salinity treatments based on the randomization in Table A1.1.

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 204 | 203 | 202 | 201 | 111 | 110 | 109 | 108 | 107 | 106 | 105 | 104 | 103 | 102 | 101 |
| 205 | 206 | 207 | 208 | 209 | 210 | 211 | 301 | 302 | 303 | 304 | 305 | 306 | 307 | 308 |
| | | | | | | | | | | | | 311 | 310 | 309 |

**Figure A1.2.** Field layout for *Ricinus communis* control treatment study based on the randomization scheme in Table A1.1.

**Table A1. 2.** Randomized complete block design for *Citrullus colocynthis* diversity study

| Plot | Accession ID | Plot | Accession ID |
|---|---|---|---|
| 101 | RMS 246 | 119 | RMS 250 |
| 102 | RMS 254 | 120 | PI 386024 01 SD |
| 103 | RMS 247 | 121 | KMK3 |
| 104 | RMS 238 | 122 | PI 525082 01 SD |
| 105 | RMS 234 | 123 | RMS 258 |
| 106 | RMS 248 | 124 | RMS 227 |
| 107 | RMS 239 | 125 | PI 53277 01 SD |
| 108 | RMS 231 | 126 | RMS 241 |
| 109 | RMS 257 | 127 | RMS 237 |
| 110 | RMS 228 | 128 | PI 652554 02 SD |
| 111 | RMS 255 | 129 | RMS 240 |
| 112 | RMS 220 | 130 | KMK2 |
| 113 | RMS 245 | 131 | RMS 253 |
| 114 | RMS 244 | 132 | RMS 232 |
| 115 | RMS 236 | 133 | PI 388770 01 SD |
| 116 | RMS 259 | 134 | RMS 249 |
| 117 | RMS 256 | 135 | RMS 215 |
| 118 | PI 52508 02 SD | 136 | PI 386014 01 SD |
| | | 137 | KMK1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 110 | 109 | 108 | 107 | 106 | 105 | 104 | 103 | 102 | 101 |
| 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 |
| 130 | 129 | 128 | 127 | 126 | 125 | 124 | 123 | 122 | 121 |
| 131 | 132 | 133 | 134 | 135 | 136 | 137 | | | |

**Figure A1.3.** RCBD layout for *Citrullus colocynthis* (L.) Schrad. germplasm diversity study based on randomization scheme from Table A1.2.

**Table A1.3.** Randomized complete block design for *Citrullus colocynthis* (L.) salinity tolerance study

| Replicate | Plot | Accession ID | Replicate | Plot | Accession ID |
|---|---|---|---|---|---|
| 1 | 101 | RMS 244 | 2 | 204 | RMS 244 |
| 1 | 102 | RMS-253 | 2 | 205 | RMS-237 |
| 1 | 103 | RMS-227 | 3 | 301 | RMS-253 |
| 1 | 104 | RMS-215 | 3 | 302 | RMS-227 |
| 1 | 105 | RMS-237 | 3 | 303 | RMS-215 |
| 2 | 201 | RMS-227 | 3 | 304 | RMS 244 |
| 2 | 202 | RMS-215 | 3 | 305 | RMS-237 |
| 2 | 203 | RMS-253 | | | |

| | | | | |
|---|---|---|---|---|
| 101 | 102 | 103 | 104 | 105 |
| 205 | 204 | 203 | 202 | 201 |
| 301 | 302 | 303 | 304 | 305 |

**Figure A1.4.** Field layout for *Citrullus colocynthis* (L.) Schrad. salinity tolerance study based on randomization scheme in Table A1.3.

**Table A1.4.** Randomized complete block design for *Brassica juncea* (L.) Czern field trial

| Replicate | Plot | Accession ID | Replicate | Plot | Accession ID |
|---|---|---|---|---|---|
| 1 | 101 | ATC-93402 | 2 | 204 | ATC-93402 |
| 1 | 102 | ATC-93142 | 2 | 205 | ATC-90783 |
| 1 | 103 | ATC-93358 | 3 | 301 | ATC-93142 |
| 1 | 104 | ATC-93161 | 3 | 302 | ATC-93358 |
| 1 | 105 | ATC-90783 | 3 | 303 | ATC-93161 |
| 2 | 201 | ATC-93358 | 3 | 304 | ATC-93402 |
| 2 | 202 | ATC-93161 | 3 | 305 | ATC-90783 |
| 2 | 203 | ATC-93142 | | | |

| | | | | |
|---|---|---|---|---|
| 101 | 102 | 103 | 104 | 105 |
| 205 | 204 | 203 | 202 | 201 |
| 301 | 302 | 303 | 304 | 305 |

**Figure A1.5.** Field layout for *Brassica juncea* (L.) Czern. based on randomization scheme in Table A1.4.

# APPENDIX II

## A2.1.1. Anti-microbial activity and phytochemicals:

As *Citrullus colocynthis (L.)* Schrad. varieties native to the UAE were being studied for the first time and the plant has been used in traditional Arab medicine, we decided that this aspect merited further investigation. The ability to extract multiple products from a single crop can also make its' cultivation more economically feasible. Antibiotic resistance in common microbial pathogens is an increasingly worrying phenomenon, underlining the need for new drugs to combat such pathogens. Plants have long been a source for novel drug leads, and traditional folk medicines give us a starting point in the search for these new drugs. Therapeutic plants possess bioactive compounds or secondary metabolites such as flavonoids, phenols, tannins, alkaloids, glycosides, terpenoids, steroids, carbohydrates etc. [157]. Phytochemicals find their use in scientific research, veterinary, therapeutics, agriculture and many more fields [158]. A variety of bioactive compounds originate from different chemical classes and prohibit the growth of wide ranging microbes [159]. Bioactive compounds may be isolated from different plant tissues such as leaves, flowers, roots, fruits and seeds [160]. The various mixtures that are prepared from these therapeutic plants would comprise of decoctions, apozems, decoctions, electroactives, liniments and powders. Medicinal plants are also extensively utilized in the food, cosmetic and perfumery industries [161].

Phytochemicals are plant constituents which are non-nutritive but possess defensive or disease deterrent assets. The word phytochemicals denotes a vast diversity of components synthesized by plants, but is mainly used to define those constituents that have the potential to alter human health. These phytochemicals are obtained in edible plant based foods like fruits, vegetables, beans and grains. Researchers have discovered thousands of such phytochemicals. Only a small portion have been analyzed carefully. Some the widely known phytochemicals would comprise of beta carotene, other carotenoids, vitamin C (ascorbic acid), vitamin E and folic acid. Phytochemicals can also be classified into antioxidants, flavonols, flavonoids, flavanones, isoflavones, catechins, epicatechins, anthocyanins, anthraquinones, anthocyanidins, proanthocyanidins, isothiocyanates, carotenoids, allyl sulfides, polyphenols, phenolic acids, sterols, glycosides, terpenoids etc. certain phytochemicals possess antioxidant or hormones like effects in the human body.

## A2.2. Antimicrobial assay methods

Crude extracts of *Citrullus colocynthis* leaves, fruit pulp, seed and rind were prepared using a reflux condenser set-up and different solvents (Methanol, Ethanol, Water and n-Hexane) in order to extract compounds with different polarities. These crude extracts were tested on 6 laboratory bacterial strains Escherichia coli, Bacillus cereus, Klebsiella spp., Lactobacillus spp., Staphylococcus aureus and Salmonella spp. Three different methods were used to study anti-microbial activity of crude extracts.

### A2.2.1. Agar well diffusion assay:

1% agar plates were prepared and wells were bored using a sterilized cork-borer of 12 mm diameter. Plates were inoculated using spread-plate technique with a standard inoculum volume from 16 hour cultures of the respective bacterial strain. 250 µl crude leaf extract was added to the wells and zone of inhibition was noted at 24 hours. This experiment was conducted in triplicates for each extract and bacterial strain.

### A2.2.2. Disk diffusion assay:

Disks were prepared by punching out 5 layers of blotting paper, irrigating and drying multiple times and sterilized before use. Disks were 3.73 mm in diameter. They were saturated with crude extract (75 µl) and placed on spread-plated 1% agar. 3 disks per plate, in duplicates for extracts and triplicates for each strain. Zone of inhibition was recorded after 24 hours.

### A2.2.3. Broth dilution assay:

200 ml nutrient broth was inoculated with a standard inoculum volume from 16 hour cultures of the respective bacterial strain. 500 µl crude extract was added to the culture at time of inoculation and OD600 was noted at 24 hours. 500 µl of sterilized distilled water was added to control. This experiment was conducted in triplicates for each extract and bacterial strain.

## A2.3. Phytochemical screening methods

Crude ethanol and aqueous extracts of *Citrullus colocynthis* were screened for the presence of various phytochemicals. Triplicates were maintained in all tests. The spectrophotometer used in all tests was Lambda 25 UV-Vis spectrometer (Perkin Elmer ®).

### Total phenols:

300µl extract was reacted with 100µl 5% ferric chloride solution and observed for color change to deep blue or black [162].

**Tannins:**

Braymer's test- 300µl extract was reacted with 100µl 10% alcoholic ferric chloride and observed for color change to blue or green [163].

**Sterols and Terpenoids:**

Liebermann- Buchard test – 500µl of extract was reacted with 100µl chloroform, 100µl acetic anhydride and a few drops of concentrated sulphuric acid. The solution was observed for color change to dark pinkish red color for sterols and dark green for terpenoids, respectively [164].

**Coumarin**:

500µl of extract was reacted with 200µl of 10% of sodium hydroxide solution. The solution was observed for color change to yellow [165].

**Anthroquinones**:

Borntrager's test: 500µl of extract was heated with 200µl of 10% ferric chloride solution and 500µl of concentrated hydrochloric acid. The solution was allowed to cool and was reacted with 200µl of diethylether and shaken well. After this 200µl of strong ammonia solution was added to all the tubes and observed for pink or deep red coloration of aqueous layer [166].

**Quinones**:

500µl of extract was reacted with 200µl of concentrated hydrochloric acid and observed for formation of a yellow color precipitate [167].

**Alkaloids**:

Wagner's test - 500µl of extract was reacted with 200µl of Lugol's reagent (iodine and potassium iodide reagent) and observed for formation of reddish brown precipitate [168].

**Flavonoids**:

Sulphuric acid test - 500µl of extract was reacted with 200µl of concentrated sulphuric acid and observed for color change to orange [169].

**Anthocyanins**:

500µl of extract was reacted with 200µl of 2M Sodium hydroxide and observed for color change to blue- green [165].

**Starch**:

500µl of extract was reacted with 200µl of Lugol's reagent (iodine and potassium iodide reagent) and observed for color change to blue- black.

**Carbohydrates**:

300µl of extract was allowed to react with 1ml of Fehling's A solution followed by 1ml of Fehling's B solution. The test tubes were heated to 70˚C in a water bath for 10-15 minutes and the formation of reddish brown precipitate was observed.

Quantitative tests were also carried out for tannins, flavonoids, coumarins and terpenoids [176, 189-190].

### A2.4. HPLC methods

Centrifuged and filtered aqueous and ethanol extracts were analyzed for flavonoids (quercetin standard) and coumarin (hydroxycoumarin standard) with the detector at 370 nm and 443 nm. A $C_{18}$ column and a flow rate of 0.1 ml/min was used. Two different solvent ratios were tested, 60:40 Methanol:Water and 20:60:20 Acetonitrile:Methanol:Water.

### A2.5. Spectrometric quantitation methods

Tannins- Dried extracts of were re-suspended in 80% methanol, to obtain a final concentration of 1mg/ml. 500µl of extract was transferred to respective test tube, to which 1ml of sodium carbonate was added followed by 8ml of distilled water. The samples were incubated at room temperature for 30 minutes and absorbance readings noted at 760nm.

Flavonoids- Dried extracts were re-suspended in 80% methanol, to obtain a final concentration of 1mg/ml. 500µl of extract was taken in test tubes and 500µl of acetic acid solution was added, followed by 2ml of pyridine solution, 1ml of Aluminum chloride reagent and 6ml of 80% methanol. Samples were incubated at room temperature for 30 minutes and absorbance readings taken at 420nm.

Coumarins- Dried extracts were re-suspended in 80% methanol, to obtain a final concentration of 1mg/ml. 500µl of extract was taken in test tubes and 1ml of distilled water was added, followed by 500µl of lead acetate reagent. The tubes were shaken well and 3ml of 0.1M HCl was added. Tubes were incubated for 30 minutes at room temperature and absorbance noted at 320nm.

Total phenols- 500µl of extract was taken and 100µl of Folin-Ciocalteau reagent was added and the mixture incubated at room temperature for 15mins. 2.5ml of saturated sodium carbonate was added and further incubated at room temperature for 30min. Absorbance was noted at 760nm.

Terpenoids- Dried extracts were re-suspended in 80% methanol to obtain a final concentration of 1mg/ml. 1.5ml of chloroform was added to 200µl of the extract. Samples were vortexed and allowed to rest for 3 minutes. 100µl of sulphuric acid was added and incubated for 1.5-2hrs in the dark. After incubation period a reddish brown precipitate formed at the bottom of each tube. Supernatant was discarded and precipitate was dissolved in 1.5ml of 80% methanol. Absorbance was noted at 538nm.

## A2.6. Antimicrobial assay results

### A2.6.1. Agar well diffusion assay

In the preliminary anti-microbial assays using agar well diffusion, most activity was observed with the ethanol and aqueous extracts against *S.aureus* and *Salmonella spp*. N-hexane extracts showed no inhibitory activity. Methanol and Ethanol extracts also had activity against *Klebsiella spp.* Methanol and Ethanol have inhibitory activity of their own and comparison with solvent control was difficult using this assay.

### A2.6.2. Broth dilution assay

A decrease in O.D. 600 in comparison with pure solvent control was observed for the following species and eight extracts (Table.A2.1.). Aqueous extracts of leaf and rind and ethanol extract of leaf were most effective.

**Table A2.1.** Anti-microbial activity of *Citrullus colocynthis* extracts

| S.NO | TISSUE | SOLVENT | MICROORGANISM |
|---|---|---|---|
| 1 | Leaf | Aqueous | *S. aureus, E. coli, B. cereus, Lactobacillus spp., Salmonella spp.* |
| 2 | Rind | Aqueous | *S. aureus, E.coli, B. cereus, Lactobacillus spp., Klebsiella spp.* |
| 3 | Seed | Aqueous | *B. cereus, Lactobacillus spp.* |
| 4 | Pulp | Aqueous | *B. cereus, Lactobacillus spp.* |
| 5 | Pulp | n-Hexane | *S. aureus* |
| 6 | Leaf | Ethanol | *B. cereus* |
| 7 | Seed | Ethanol | *B. cereus* |
| 8 | Pulp | Ethanol | *B. cereus* |

### A2.6.3. Disk diffusion assay:

In order to discount any solvent effects and to confirm the results of the broth assay a disk diffusion assay was performed with only aqueous extracts (Table A2.2.). None of the pure solvent controls

(Autoclaved Millipore water) showed zones of inhibition. The data corresponds with the results of the broth assay and confirms those results for the most part, except that leaf extract did not show activity against *Salmonella spp.* Leaf extracts showed most activity against *B. cereus* and *E.coli*. Seed extracts showed most activity against *E.coli* and *Lactobacillus spp*. and pulp extract showed significant activity against *B. cereus*.

**Table A2.2.** Antimicrobial activity of *Citrullus colocynthis* aqueous extracts

| Plant Tissue | Micro-organism | Mean Zone of inhibition (mm) |
|---|---|---|
| Leaf | *S.aureus* | 4.1 |
| | *E.coli* | 5.2 |
| | *B.cereus* | 7.6 |
| | *Lactobacillus spp.* | 4.6 |
| | *Salmonella spp.* | - |
| | *Klebsiella spp.* | 4.7 |
| Seed | *S.aureus* | - |
| | *E.coli* | 6.9 |
| | *B.cereus* | 5.1 |
| | *Lactobacillus spp.* | 7.2 |
| | *Salmonella spp.* | - |
| | *Klebsiella spp.* | 5.4 |
| Rind | *S.aureus* | 4.2 |
| | *E.coli* | 4.4 |
| | *B.cereus* | - |
| | *Lactobacillus spp.* | 4.2 |
| | *Salmonella spp.* | - |
| | *Klebsiella spp.* | 4.8 |
| Pulp | *S.aureus* | 4.2 |
| | *E.coli* | - |
| | *B.cereus* | 6.3 |
| | *Lactobacillus spp.* | 4.3 |
| | *Salmonella spp.* | - |
| | *Klebsiella spp.* | - |

## A2.7. Phytochemical screening results

Crude ethanol and aqueous extracts of *Citrullus colocynthis* were screened for the presence of various phytochemicals. The results are presented in Table A2.3. Quinones appear to be present only in leaf extracts, while sterols could be detected only in the aqueous rind extract. Tannins could not be detected in Aqueous pulp extract and terpenoids could not be detected in Aqueous rind extract. Starch and anthocyanins were absent in all extracts.

**Table 73.** Phytochemical screening of *Citrullus colocynthis* aqueous and ethanol extracts

| EXTRACT | ALKALOIDS | FLAVONOIDS | QUINONE | ANTHOCYANIN | STARCH | TANNIN | PHENOL | CARBOHYDRATES | TERPENOIDS | STEROL | COUMARINS | ANTHROQUINONES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aq. Leaf | + | + | + | - | - | + | + | + | + | - | + | + |
| Aq. Seed | + | + | - | - | - | + | + | - | + | - | + | + |
| Aq. Rind | + | + | - | - | - | + | + | + | - | + | + | + |
| Aq. Pulp | + | + | - | - | - | - | + | - | + | - | + | + |
| EtOH Leaf | + | + | + | - | - | + | + | + | + | - | + | + |
| EtOH Seed | + | + | - | - | - | + | + | - | + | - | + | + |
| EtOH Rind | + | + | - | - | - | + | + | + | + | - | + | + |
| EtOH Pulp | + | + | - | - | - | + | + | - | + | - | + | + |

## A2.8. HPLC results

Two different solvent ratios were tested, 60:40 Methanol:Water and 20:60:20 Acetonitrile:Methanol:Water. The second solvent profile gave a better resolution, but since crude extract was analyzed, peak resolution was too poor to use for quantification of phytochemicals.

## A2.9. Spectrometric quantitation results

Quantitative assays were carried out for some phytochemicals and the results are presented in this section. The extracts are represented by acronyms: AEL is aqueous extract of leaf, EEL is ethanol extract of leaf, and so on.

Tannins:

The highest quantity of tannins was present in aqueous and leaf extracts, in addition to the ethanol rind extract (Fig.A2.1.).

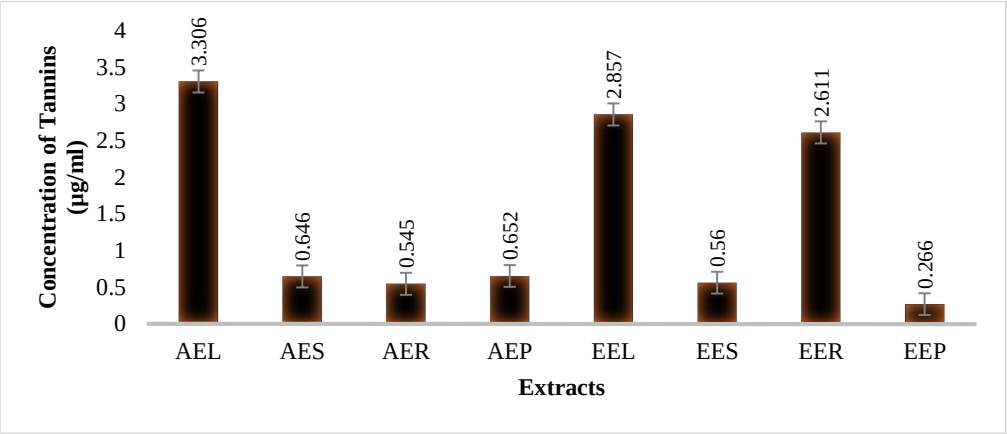


**Figure 73.1.** Quantification of Tannins in Aqueous and Ethanol extracts of *Citrullus colocynthis*

Flavonoids:

Flavonoids could not be quantified in the aqueous pulp and Ethanol rind extracts even though they were detected in preliminary screening (Fig.A2.2.). Highest quantities were detected in leaf and seed extracts, and they appear to be better extracted by water when compared to ethanol. Considering absolute values, this is the most abundant class of compound detected in the plant extracts.

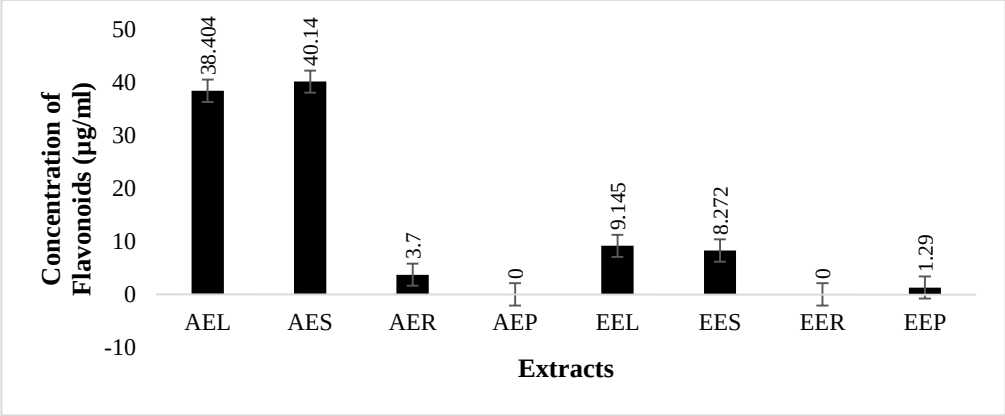


**Figure 74** Quantification of Flavonoids in Aqueous and Ethanol extracts of *Citrullus colocynthis*

Coumarins:

Coumarins are also present in the highest quantity in leaf extracts (Fig.A2.3.).

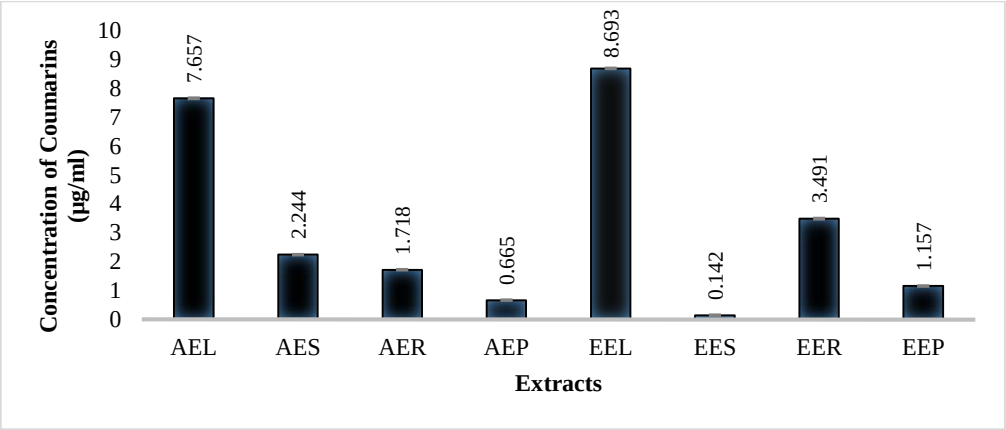


**Figure 75** Quantification of Coumarins in Aqueous and Ethanol extracts of *Citrullus colocynthis*

Terpenoids:

Terpenoids are present in all extracts to more or less the same degree (between 8.1 and 11.7 mg/ml) as seen in Fig.A2.4.

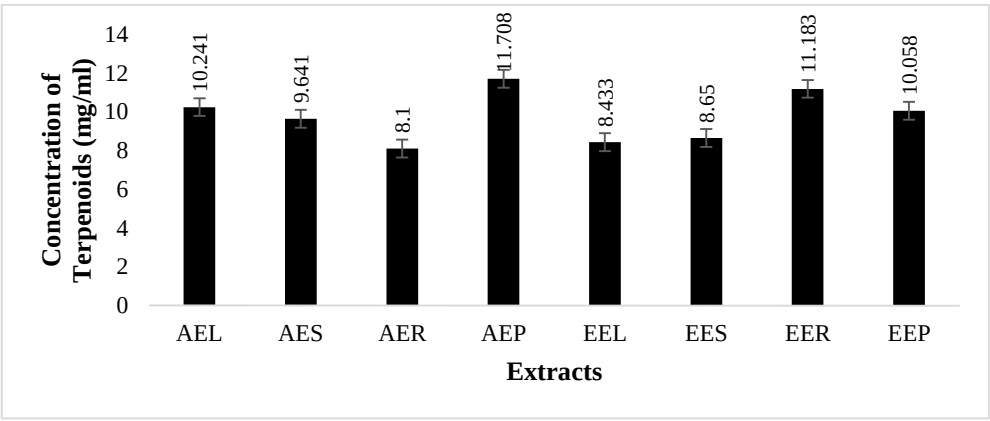


**Figure A2.4.** Quantification of Terpenoids in Aqueous and Ethanol extracts of *Citrullus colocynthis*

## A2.10. Antimicrobial assays discussion

Crude extracts of *Citrullus colocynthis* was tested for anti-microbial activity in order to study reported medicinal properties and uses of the plant. Usually plant extracts are lyophilized to obtain a powder, different concentrations of which are then prepared and tested for minimum inhibitory concentration required for anti-microbial activity. Since the facilities for lyophilization were unavailable and heat drying can alter properties of the bioactive compound in the extracts and deactivate them, crude extract was used in the following preliminary tests. Solvents of different polarities were used to extract fractions of phytochemicals of a range of polarities. From test results, it appears that the aqueous and ethanol solvents were most suitable for extracting

compounds with anti-microbial properties as these extracts had maximum inhibitory activity. Because of the large extract volumes required and difficulty quantifying results and comparing with pure solvent controls in the agar well diffusion assay, crude extracts were added to liquid broth cultures and OD values were used as an estimate of bacterial growth. Aqueous extracts of leaf and rind showed maximum antibacterial activity in these tests and the inhibitory activity was not restricted to gram positive or gram negative species. Because ethanol has its own anti-microbial activity, aqueous extracts were further tested using disk diffusion tests and inhibitory activity was recorded. The results corresponded with those of the broth test. Leaf extract had inhibitory activity against most bacterial species and maximum against *B.cereus*. Seed extracts showed most inhibitory activity against *E.coli, Klebsiella spp.* and *Lactobacillus spp*. Surprisingly, pulp extract was active only against Gram-positive species. Fresh extracts had to be prepared for each assay because it was noted that growth inhibitory function decreased when the same extracts were used over a period of time. This suggests that the active phytochemicals are prone to oxidation.

Preliminary tests confirm that Citrullus plant extracts have antimicrobial activity. It would be of great interest to purify these extracts and test them against drug-resistant pathogens. Since the exact use of these plants in traditional medicine is not clear, it would also be of interest to carry out cell cytotoxicity studies and test the purified extracts on multicellular laboratory species.

### A2.11. Phytochemical screening discussion

Phytochemical screening revealed that most classes of bioactive phytochemicals are present in all extracts. A number of these phytochemical classes overlap, for example flavonoids and tannins are sub categories of phenolic compounds. Coumarins were found to be present in a significantly higher concentration in leaves than in other plant tissue and could be responsible for the higher anti-microbial activity of these extracts in comparison with other extracts [170]. Flavonoids and Tannins were also present in maximum concentration in leaf extracts. Flavonoids were also detected in high concentration in aqueous seed extract while tannins were also present in high concentration in ethanol rind extract. Anti-bacterial function of flavonoids is well documented and can be attributed to various mechanisms such as inhibition of nucleic acid synthesis, cytoplasmic membrane function or disruption of energy metabolism [171]. There are several mechanisms of anti-bacterial activity of tannins as well. The astringent property of the tannin may induce complexation with enzymes or substrates. Many microbial enzymes in raw culture filtrates or in purified forms are inhibited when mixed with tannins. A tannin's toxicity may also be related to its action on the membranes of the microorganisms. Alternatively, complexation of metal ions by

tannins may account for tannin toxicity [172]. Further fractionation and purification the extracts can help elucidate the exact bioactive compounds present.

# APPENDIX III

## A3.1. Salinity stress and ROS:

Abiotic stress such as drought and salinity induce similar metabolic changes in plants, especially under high light intensity or in combination with other stresses. They disrupt photosynthesis and increase photorespiration, altering the normal homeostasis of cells and cause an increased production of reactive oxygen species (ROS). ROS play a dual role in the response of plants to abiotic stresses functioning as toxic by-products of stress metabolism, as well as important signal transduction molecules [173]. ROS such as hydrogen peroxide and oxygen radicals are toxic molecules capable of causing oxidative damage to intracellular proteins, DNA and lipids [174]. Under optimal growth conditions, ROS are mainly produced at a low level in organelles such as chloroplasts, mitochondria and peroxisomes. However, during stress, their rate of production is dramatically elevated. The level of toxicity resulting from ROS accumulation depends on the plants' ROS scavenging ability. ROS scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX) have a major role in combating oxidative stress. The differential expression of these enzymes in salt –tolerant vs salt-sensitive varieties of the same species emphasizes the role of these enzymes in combating salinity stress [196-197]. It is also interesting to note that the increased or decreased expression of these enzymes in response to salinity stress is not uniform across species. If CAT expression increases in one species subjected to salinity, it may decrease in another [198-200].

Catalase activity:

The catalase enzyme is found extensively in life forms exposed to oxygen (plants and animals). The gene that codes for this enzyme is the CAT gene. It catalyzes the decomposition of hydrogen peroxide ($H_2O_2$) to water ($H_2O$) and oxygen ($O_2$) [175]. The reaction takes place in a living tissue as follows:

$2\ H_2O_2 \rightarrow 2\ H_2O + O$

The optimum pH for its activity depends on the plant species. Catalase is a tetramer of four polypeptide chains, each being around 500 amino acids long. The enzyme consists of four iron groups (porphyrin heme) which makes it possible to react with hydrogen peroxide.

For a plant cell under oxidative stress superoxide dismutase is the first line of defence against reactive oxygen species [176]. It converts superoxide ($O_2^-$) into $H_2O_2$, which plays an important role in inducing salt tolerance by activating the in-built plant antioxidant system.

A decrease in catalase activity has been observed under NaCl stress in leaves of *Halimione portulacoides* [177] and in both tolerant and sensitive varieties of wheat [178] possibly due to conformational changes caused to the enzyme upon prolonged exposure to salt stress.

Glutathione Reductase:

Glutathione reductase also known as GR/GSR is a flavo-protein oxidoreductase which catalyzes the reduction of glutathione disulphide (GSSG) to the sulphydryl form glutathione (GSH) using the coenzyme NADPH [179]. Mainly found in the chloroplast, the isoforms of GR can also be found in the cytosol, mitochondria and peroxisomes in small amounts. Glutathione reductase along with SOD is a major component of ascorbate-glutathione (ASH-GSH) pathway which plays an important function in shielding the cells against ROS and the potential anomalies accumulated by its' reaction products [180]. GR is present in both prokaryotic as well as eukaryotic lifeforms. GSH, which is a product of GR activity, holds the enzyme in its dimeric form under cellular conditions. Glutathione reductase is responsible for keeping a high GSH/GSSG ratio in cells. GR catalyzes the reduction of GSSG (consisting of two GSH linked by a disulfide bridge). GSH plays an important role in the ASH-GSH pathway and maintenance of the sulfhydryl group. Both GR and GSH play a significant role in establishing the salinity tolerance in plants. GR maintains the GSH pool which in turn maintains protein function. GR activity increases under conditions of NaCl stress in salt tolerant plants [197, 207].

Ascorbate peroxidase:

Ascorbate peroxidases (or APX) are enzymes that detoxify peroxides such as hydrogen peroxide using ascorbate as a substrate. APX is an integral component of the glutathione-ascorbate cycle. $H_2O_2$ is reduced to water by APX using ascorbate as the electron donor. The oxidized ascorbate (monodehydroascorbate/ MDA) is regenerated by monodehydroascorbate reductase (MDAR). MDA is a radical and if not rapidly reduced it gets converted into ascorbate and dehydroascorbate. Dehydroascorbate is reduced to ascorbate by dehydroascorbate reductase at the expense of GSH, yielding oxidized glutathione (GSSG) which is reduced by GR using NADPH as electron donor.

APX activity increases in radish upon salt stress, even though no increase is observed in mRNA levels [181]. An increase in activity was also observed in tolerant varieties of wheat [182] and in leaves of sea purslane [177].

Superoxide dismutase:

Superoxide dismutases are enzymes that alternately catalyse the dismutation (or partitioning) of the toxic superoxide ($O_2^-$) radical into either ordinary molecular oxygen ($O_2$) or hydrogen peroxide ($H_2O_2$).

- $Cu^{2+}$-SOD + $O_2^-$ → $Cu^+$-SOD + $O_2$
- $Cu^+$-SOD + $O_2^-$ + $2H^+$ → $Cu^{2+}$-SOD + $H_2O_2$

SOD activity is seen to increase with increase in NaCl stress in many higher plants [207, 210].

## A3.2. Oxidative stress enzymes methods

A small pilot scale study was conducted in order to understand the role of oxidative stress enzymes in response to salinity. A single accession each of castor, *Citrullus colocynthis*, mustard and *Salicornia bigelovii* was germinated in pots containing regular potting soil. Three pots with 5 seeds each were maintained for each treatment (Control, 50 mM NaCl and 100 mM NaCl. Germinated seedlings were treated with respective irrigation water daily for five days after germination and enzyme extraction was carried out using leaf tissue of 21 day old seedlings. Enzyme extraction and oxidative stress enzyme protocols were replicated or adapted from the protocols described by Hakeem *et al*. [183].

### A3.2.1. Enzyme extraction

Leaf tissue was crushed in Eppendorf tubes on ice, homogenized with ice cold enzyme extraction buffer (50 mM sodium phosphate buffer (pH 6.8) containing 1 mM EDTA and 2% w/v PVPP) at 4°C. Homogenate centrifuged at max rpm for 40 minutes 4°C. Supernatant was used for assays.

### A3.2.2. Superoxide dismutase assay

1 ml reaction buffer, 100 µl enzyme extract, shaken and placed near fluorescent light lamps in an Aluminum foil lined box for 10 minutes. Absorbance was read at 600 nm to follow reduction of Nitroblue Tetrazolium (NBT). Blanks were run without enzyme, controls without illumination. One unit of SOD is defined as amount of enzyme producing 50% inhibition of NBT reduction under assay conditions. The maximum reduction was observed in the absence of the enzyme. Activity expressed as EU/mg protein per hour. Gallic acid [53], ascorbic acid, a-tocopherol, curcumin [56] can be used as a positive control. SOD reaction buffer used was 1M sodium bicarbonate, 200 mM methionine, 3 mM EDTA, 60 µM riboflavin, 2 mM NBT.

### A3.2.3. Glutathione Reductase assay

1 ml reaction buffer, 100 µl enzyme extract in triplicates. Absorbance noted at 340 nm. Activity calculated using extinction coefficient 6.2 $mM^{-1}$ $cm^{-1}$ and expressed as EU/mg of protein.

$$Enzyme\ Activity = \frac{A * 1000}{\varepsilon * \Delta t * c}$$

Glutathione reductase Reaction buffer used was 0.2 mM NADPH, 0.5mM oxidized glutathione (GSSG).

### A3.2.4. Catalase

3 ml of 2 mM $H_2O_2$, 40 µl enzyme extract. The reaction mixture (1ml) contains potassium phosphate buffer (pH 7.0), 2 mM $H_2O_2$ to initiate the reaction. The reaction was measured at 240 nm for 3 min and $H_2O_2$ consumption was calculated using extinction coefficient, 39.4 $mM^{-1}cm^{-1}$.

### A3.2.5. Ascorbate Peroxidase

290 nm, oxidation of ascorbic acid (decrease of absorbance at 290 nm). Reaction Mixture: 10 µl leaf extract + 1 ml reaction mix. The decrease in absorbance at 290 nm was measured and monitored for 100 s. The reaction was calculated using extinction coefficient, 2.8 mM-1cm-1. Ascorbate Peroxidase reaction buffer used was 0.2 M Tris/HCL buffer (pH 7.8), 0.25 mM ascorbic acid, 0.5 mM $H_2O_2$.

### A3.2.6. Bradford's assay for protein content in extract

Total protein in extracts was quantified using Bradford's method [184] with 50 µl of enzyme extract and 2.5 ml of Bradford reagent, in triplicates. 1mg/ml BSA was used to prepare standards. Samples were incubated at room temperature for at least 5 min. Absorbance increased over time; samples were not allowed to incubate for more than 1 hour. Absorbance was measured at 595 nm. Equation from calibration curve was used to calculate total protein concentration in enzyme extracts.

## A3.3. Oxidative stress enzymes results

There wasn't sufficient germination of *Citrullus colocynthis* for an adequate sample size, so enzyme extraction and assays could not be carried out.

### A3.3.1. Superoxide Dismutase

There was no significant change in SOD activity in the halophyte S*alicornia bigelovii*. *Ricinus communis* and *Brassica juncea* responded in opposite manners, with a decrease in enzyme activity in the case of *Ricinus communis* in response to increasing salinity, while an increase in activity was observed in *Brassica juncea* (Figure A3.1.).

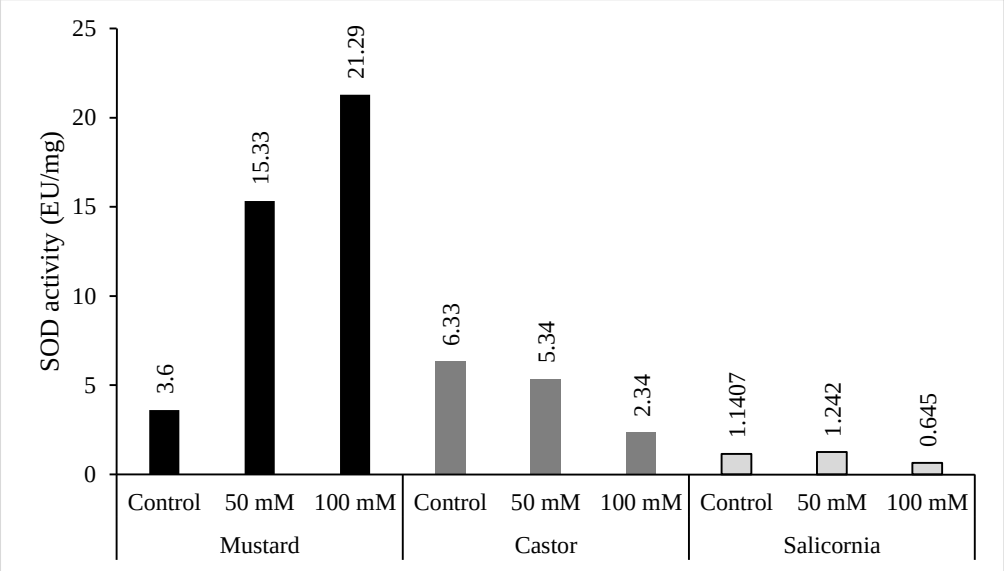


**Figure A3.1.** Superoxide dismutase enzyme activity in response to salinity in different species

### A3.3.2. Glutathione Reductase

Glutathione reductase activity decreased by half in the salinity treatments for *Brassica juncea*, a greater decrease was observed in *Salicornia bigelovii*, while no clear pattern was obtained with *Ricinus communis*, except for a two-fold increase in enzyme activity in the 100 mM treatment (Figure A3.2.).

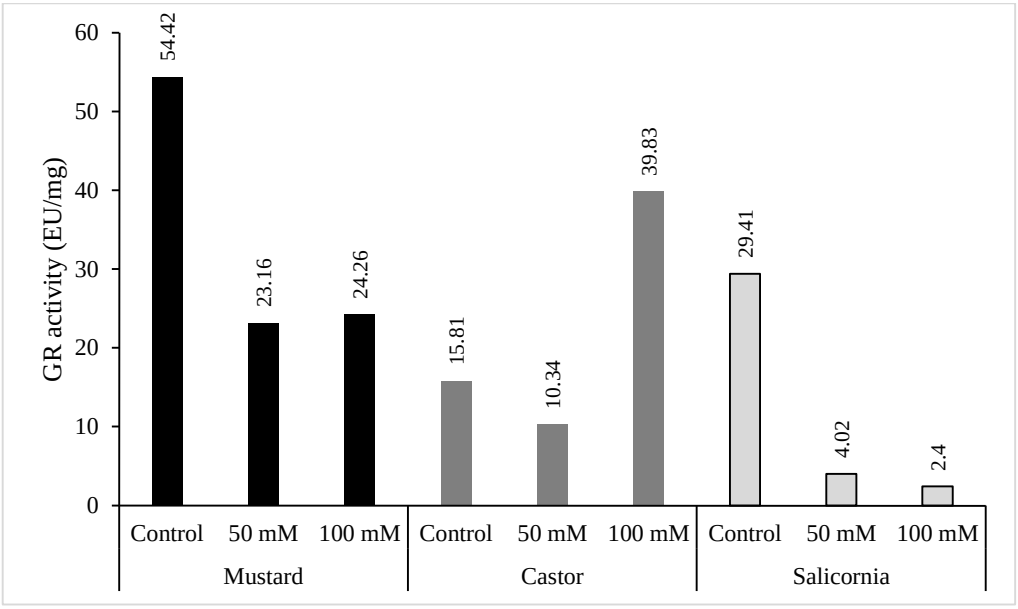


**Figure A3.2.** Glutathione reductase enzyme activity in response to salinity in different species

### A3.3.3. Catalase

No significant change in catalase activity was observed in *Brassica juncea* up on salinity treatment, as with glutathione reductase, a decrease in activity in the 50 mM treatment and then an increase was observed in the 100 mM treatment for *Ricinus communis* (A3.3.). In *Salicornia bigelovii*, there was a drastic decrease (almost by 75%) in the salinity treatments.

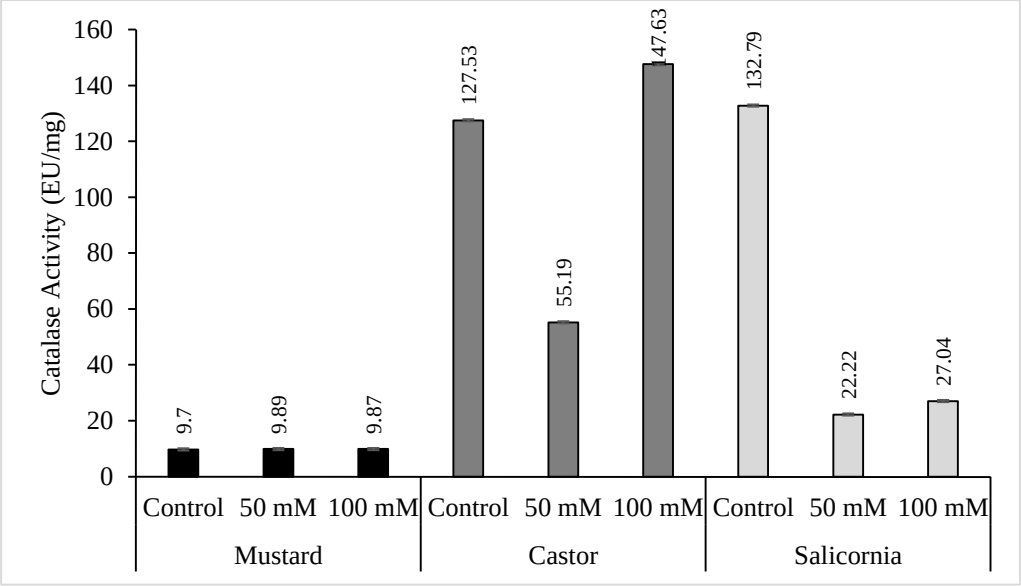


**Figure A3.3.** Catalase enzyme activity in response to salinity in different species

### A3.3.4. Ascorbate Peroxidase

Ascorbate peroxidase activity did not change significantly in *Brassica juncea* in the salinity treatments (Figure 76). This enzyme's activity is much higher in *Salicornia bigelovii* when compared to the other two species, and there is an increase in enzyme activity in both *Ricinus communis* and *Salicornia bigelovii* with increasing salinity.

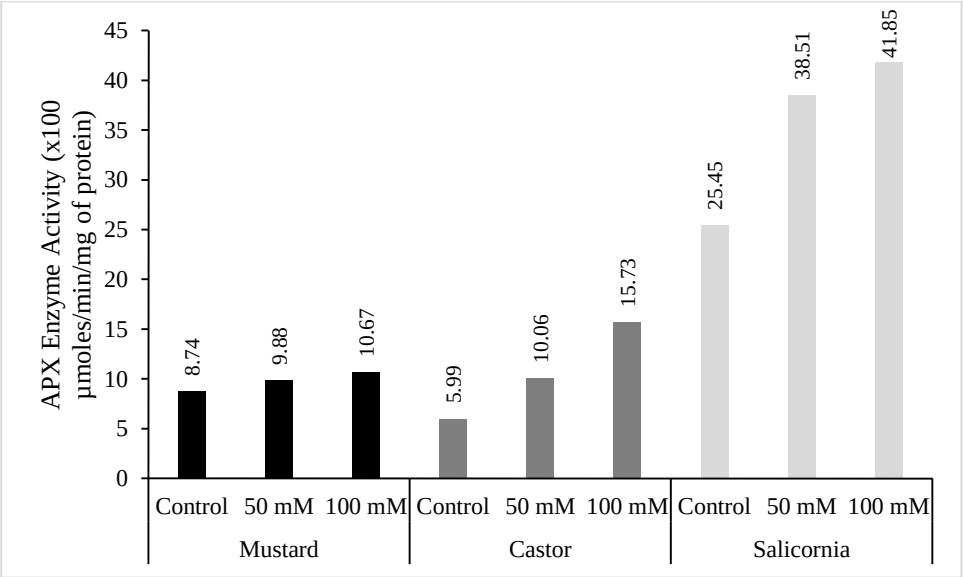


**Figure 76.** Ascorbate Peroxidase activity in response to salinity in different species

## A3.4. Oxidative stress enzymes discussion

As described previously, there is a strong link between tolerance to salinity stress and the function of oxidative stress enzymes and anti-oxidants [185]. The enzymes assays were planned with the idea of comparing the response of a tolerant accession with a sensitive accession of the same species. This however, was not possible because no significant differences were found among the accessions studied by us. We therefore used a halophyte in the form of *Salicornia bigelovii* in the study to view the enzyme activities in parallel even though any inter-species comparisons would not be valid and will not be attempted in this section. In general, stress-induced downregulation of anti-oxidative isozymes can be correlated with increased oxidative damage. In contrast, a stress-induced upregulation of the anti-oxidative isozymes can be correlated with alleviation of oxidative stress [186].

The increase in SOD activity in *Brassica juncea* suggests that the plant may be attempting to combat the effects of oxidative stress. Ideally, in tolerant varieties we would see and even greater level of expression. In *Ricinus communis,* SOD activity decreased with increasing salinity, indicating oxidative damage to plant tissue.

According to literature, GR and APX activity are expected to increase under salinity stress, more so in the tolerant varieties [187]. While the increase in APX activity was observed in all three species, there was a steady decrease in GR activity. There was also a decrease in catalase activity in the 50 mM treatment for *Ricinus communis* before increasing in the 100 mM treatment, similar to GR. This in addition to the unaltered yield and other characteristics at 5 dS $m^{-1}$ suggests that *Ricinus communis* responds very differently to low and moderate salinity. The over or under expression of these antioxidant enzymes in response to salinity also varies in different species and not just different accessions or between tolerant and sensitive cultivars [188] . It is thus necessary to identify contrastingly sensitive and tolerant accessions of the same species before these results can be discusses in a coherent manner.

## *List of Publications*

The following is a list of research articles and conference proceeding published or communicated from parts of this thesis:

| **Title** | **Journal/Conference** | **Details** | **Status** |
|---|---|---|---|
| The Potential of Castor as a Biodiesel Feedstock Crop for the Arabian Peninsula | ICREGA'14 - Renewable Energy: Generation and Applications, Springer Proceedings in Energy 2014, Pages 1-9, Springer International Publishing. | Chapter in book published by Springer International Publishing | Published |
| Seed Dormancy and Effect of Salinity on Germination of *Citrullus Colocynthis* | International Journal of Environmental Science and Development, Volume 5, Issue 6, Pages 566-569, 2014. | Open Access Journal indexed in: Chemical Abstracts Services (CAS), CABI, DOAJ, Ulrich Periodicals Directory, Engineering & Technology Digital Library, Electronic Journals Library, Crossref, ProQuest | Published |
| Study of morpho-agronomic diversity and oil content in desert gourd (*Citrullus colocynthis* (L.) Schrad.) | Australian journal of crop science | Southern cross Publishing, Impact Factor: 1.17 Indexed in: Scopus, CABI, Chemical Abstracts, Agricola, Bioline international, EBSCO, E-Journals, DOAJ, Scirus, National library of Australia, Science Alert, ProQuest, ERA, Thomson Reuters Open Access Journal | Communicated, (22 November 2015), Accepted with major revision |
| *In vitro* Anti-bacterial activity and phytochemical screening of crude *Citrullus colocynthis* (Schrad.) extracts | Medicinal Plants - International Journal of Phytomedicines and Related Industries | Society for conservation and resource development of medicinal plants, Impact Factor: 0.15, NAAS Rating for 2013:4.23. Indexed in: Scopus, Indian Science Abstract, MAPA, Indian | Communicated, (25 October 2015), Under review |

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|---|---|---|---|
| | | Citation Index, Google Scholar, Index Copernicus (2011, ICV- 5.40), CABI, Proquest | |
| Phytochemical Screening of Anti-microbial *Citrullus colocynthis* Schrad. Extracts. | The 2nd Middle East Molecular Biology Congress and Exhibition, Istanbul (2015). | Frontiers Publications | In press |
| Activity of Oxidative Stress Enzymes in Response to Salinity Stress in *Salicornia bigelovii* and *Ricinus communis*. | The 2nd Middle East Molecular Biology Congress and Exhibition, Istanbul (2015). | Frontiers Publications | In press |

***Brief biography of the supervisor:***

| | |
|---|---|
| **Name of the Supervisor** | Dr. Neeru Sood |
| **Present designation and organization** | Professor, Department of Biotechnology<br>Associate Dean, Academic Research Division<br>BITS Pilani, Dubai Campus<br>DIAC, Dubai, UAE<br>Phone : 009714 4200700<br>Mobile : 0097150-3752805<br>Email : sood@dubai.bits-pilani.ac.in |
| **Qualification** | Ph. D. (1998) from P.A.U., Ludhiana<br><br>Thesis Title: Studies on Hardening of Micro-propagated Clones |
| **Area of research** | 1. Botany<br>2. Agricultural Sciences<br>3. Plant biotechnology<br>4. Phytochemical analysis of medicinal plants<br>5. Effect of natural products for treatment of diseases<br>6. Development of Biosensors-BIOMEMS technology,<br>7. Development of Biofertilizers using stress tolerant rhizobial isolates,<br>8. Biodegradation of textile dyes<br>9. Antimicrobial activities of medicinal plants<br>10. Biodegradation/ bioremediation of environmental pollutants |
| **Work experience (years)** | 19 |
| **Number of publications** | 62 |
| **Number of Ph.D students supervised** | 3 (ongoing) |

***Brief biography of the co- supervisor:***

| | |
|---|---|
| **Name of the Supervisor** | Dr. Nanduri Kameswara Rao |
| **Present Designation and organization** | Plant Genetic Resources Scientist<br>International Center for Biosaline Agriculture<br>Academic City/Al Ruwayyah<br>Dubai, UAE<br>Phone: 971 4 3361100 ext 202<br>Fax: 971 4 3361155<br>Email: n.rao@biosaline.org.ae |
| **Qualification** | Ph.D. (1986) from University of Reading, UK<br><br>Thesis Title: Chromosomal aberrations and gene mutations induced in lettuce (*Lactuca sativa* L.) seeds during storage |
| **Areas of Research** | Genetic resources conservation and use<br>Biosaline agriculture<br>Crop diversification and improvement<br>Seed production and quality control<br>Plant taxonomy<br>Genetics and Cytogenetics |
| **Work experience (Years)** | 38 |
| **Number of publications** | 130 |
| **Number of Ph.D. students supervised** | 4 |

***Brief biography of the candidate:***

| | |
|---|---|
| Name of the Candidate | Kiran Menon |
| Contact information | Mob: +971551545670<br><br>Ph: +97143709515<br><br>Email: menonkiran86@gmail.com |
| Qualification | M.Sc. Biotechnology from Hochschule Mannheim, Germany (2010)<br><br>B.Tech. Biotechnology from Vellore Institute of Technology University, Vellore, India (2008) |
| Areas of research | Agricultural sciences, bio-diesel, molecular biology, animal disease models, gene expression, bio- MEMS |
| Work experience (years) | 5 |
| Number of publications | 12<br><br>- 3 peer-reviewed journals<br>- 1 book chapter<br>- 8 conference proceedings |