Eco-physiological Assessment of Water Stress in Selected Native Plant species for Sustainable Landscaping

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2013-2020

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FINAL APPROVAL

It is certified that we have read the thesis submitted by Mr. Hasnain Alam and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biotechnology.

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DEDICATION

This work is dedicated to my sweet and beloved parents and my family whose constant support and guidance enabled me to achieve this milestone.

DECLARATION

It is certified that work present in the following Doctoral Thesis "Eco-physiological Assessment of Water Stress in Selected Native Plant Species for Sustainable Landscaping" is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of this thesis has been previously presented for any other degree.

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Hasnain Alam

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ABBREVIATIONS AND ACRONYMS

%	=	Percent
^{0}C	=	Degree Centigrade
APX	=	Ascorbate Peroxidase
CAT	=	Catalase
cm	=	Centimeter (s)
CVG	=	Coefficient of velocity of germination
dSm^{-1}	=	deciSiemens per meter
GI	=	Germination Index
GP	=	Germination Percentage
GR	=	Germination Rate
GSI	=	Germination Stress tolerance Index
LCMS	=	Liquid Chromatography–Mass Spectrometry
MDG	=	Mean Daily Germination
MGT	=	Mean Germination Time
Mha	=	Million hectares
MPa	=	Megapascals
MS	=	Mass Spectrometry
PEG	=	Polyethylene Glycol
PI	=	Promptness Index
POD	=	Peroxidase
Pr	=	Photosynthetic Rate
RDW	=	Root dry weight

RL	=	Root Length
SDW	=	Shoot Dry Weight
SL	=	Shoot Length
spp.	=	Species
WUE	=	Water Use Efficiency
ΨLeaf	=	Leaf Water Potential

ABSTRACT

In the perspective of climate change and increasing water insecurity worldwide, afforestation and horticultural trends have largely shifted towards using indigenous species for sustainable landscaping. Domesticating wild species for landscaping can combat current global salinity issues, particularly in the Arabian Gulf region. Study was conducted in two phases. First phase was identification of native plants suitable for landscaping and collection of seeds. This phase was completed within one year (Oct 2015 to Sept 2016.). Second phase involved germination and field studies. For germination experiment nine native plants species were evaluated against salt and water stress. Two osmotic agents (OA) i.e. NaCl and PEG 6000 were used for germination test and four osmotic levels (OL) of 0, -0.2, -0.4 and -0.6 MPa were prepared for each OA. Osmotic stress significantly decreased most of the germination traits, while mean germination time was increased with decreasing OL. Booth salt and water stress created by NaCl and PEG had negative and variable effect on germination traits of each native species. However, germination was not inhibited completely even under highest osmotic stress in many selected species. This minimal effect of osmotic stress can be associated to genetic potential of native plant species to resist the osmotic stress instead of ionic or osmotic effect of NaCl or PEG. In this study *Salsola imbricata, Tetraena mandavillei, Tephrosia apollinea, A. leucoclada* and *Sinna italica* stood out as the best plant species to survive under induced salt and water stress at the germination stage. These five species were selected for further studies for their field performance. Established plants were subjected to four salt water treatments i.e. 5, 10, 15 and 20 dSm^{-1} (S1, S2, S3 and S4) and four different water regimes that were 100 % (control), 80 %, 60 % and 40 % (WL1, WL2, WL3 and WL4) of field capacity. It was observed that a species resistant to salt or water stress during germination may or may not give the same result in the field. Out of five selected species in germination experiment only three species i.e. *S. imbricata, T. mandavillei* and *A. leucoclada* survived in the field experiment. Although, *T. apollinea* and *S. italica* performed well in germination experiment but could not survive under salt stress in the field trial. All three species showed morphological and physiological adaptation to salt and water stress and had no significant effect on survival percentage. *S. imbricata* a succulent species from the family

Amaranthaceae can be classified as obligatory halophyte. *S. imbricata* showed highest growth under lowest water stress and had no effect of salt stress. NaCl in salt stress had shown a protective effect on shoot weight and shoot length in water stress. We found that salt stress had no significant effect on *S. imbricata* and *A. leucoclada.* On the other hand, increasing water stress had shown major decrease in growth. Water stress treatment decreased the RL of *S. imbricata* under low salt stress. However, as supposed, water stress increased the RL under higher salt stress. Plant total nitrogen, phosphorus and potassium content were decreased with increasing water stress only at low salt stress level. As the salt stress increased negative effect of water stress became minimal. Both salt and water stress had increased Na^+ and Cl^- uptake. Catalase (CAT) activity of *S. imbricata* had non-significant effect of salt and water stress levels. Interactive effects of salinity and water stress levels were significant ($P \leq 0.05$) on the ascorbate peroxidase (APX) and peroxidase (POD) activity of *S. imbricata*. *T. mandavillei,* which belongs to family *Zygophyllaceae,* can be classified as a facultative halophyte. *T. mandavillei* grew well without salt and water stresses and survived under higher salt stress although reduced the growth. *T. mandavillei* had the maximum shoot weight, root weight, shoot length and root length under S1WL1. Salt and water stress had significant interaction for plant total nitrogen, phosphorus and potassium content. As the salt stress increased negative effects of water stress became minimal on nutrient accumulation. Sodium and chloride content was increased not only with increasing salt stress but also with increasing water stress. However, Na^+ uptake decreased with increasing water stress at highest salinity level of S4. At low salinity levels ABA and proline content in leaves decreased with increasing water stress. However, at higher salinity proline increased with increasing water stress in *T. mandavillei*. CAT activity increased with increasing water stress level in leaves of *T. mandavillei*. APX and POD activity of *T. mandavillei* significantly affected by salt x water stress levels. *A. leucoclada* might be classified as obligatory halophyte. Shoot growth increased with increasing salt stress. However, root growth decreased with increasing salt stress while increased with increasing water stress. Plant total nitrogen content was decreased with increasing water stress only at low salt stress level. As the salt stress increased the negative effect of water stress become minimal. Na^+ content increased not only with increasing salt stress but also with increasing water stress. Cl^- uptake in *A. leucoclada* increased with

increasing water stress at lowest salinity level S1 only. Both salt and water stress had an additive role in increasing ABA and proline content. Higher salt stress levels of S2, S3 and S4 increased proline content with increasing water stress level. CAT activity in leaves of *A. leucoclada* increased with increasing water stress level under S1 and decreased with increasing water stress under S2, S3 and S4. With increasing water stress level, POD activity of *A. leucoclada* in leaves also increased. Increasing salinity stress level from S1 to S3 also increased peroxidase. However, at higher salinity stress level of S4 POD activity decreased. APX activity in *A. leucoclada* increased with increasing salt stress. Similarly increasing water stress also increased the APX activity in *A. leucoclada.* In conclusion *S. imbricata, T. mandavillei* and *A. leucoclada* used salt resistance mechanism to accumulate higher concentrations of salts in its cells. Studied species used physiological adaptation to cope with higher salt stress and ROS (reactive oxygen species) produced. In addition, salt stress had a protective role on plant growth of these species under water stress condition. These results are more important in determining the irrigation requirements of salt tolerant species in established landscapes.

1. INTRODUCTION

1. INTRODUCTION

Climate change had increased the global mean temperature and water scarcity throughout the world (Zamin *et al*., 2019) and is expected to become even more in the future, which poses a serious threat specially to the desert ecology (Haddeland *et al.*. 2014). Pakistan is positioned 28th among the nations that might be most seriously influenced by environmental changes. Mean temperature increase up to 3 degrees is expected by 2040 and by the end of the century raise in temperature up to 5-6 degrees is predicted. Monsoon rains are predicted to be reduced drastically but will have much higher intensity (Amir, 2011). This rising temperature may enhance the overall de-glaciation process endangering the sustained sources of fresh water (Hussain *et al.*, 2005). Middle Eastern region is also most susceptible to climate change impacts. This region has highest water scarcity in the world. Mean temperature in the next 15–20 years in this region is expected to increase up to 2 °C (Elasha, 2010).

Total land area of Pakistan is 79.6 million ha (Mha) out of which 22 Mha is cultivated land and only 17 Mha is canal irrigated (Kijne, 1999; Anjum *et al.*, 2010; Khoso *et al.,* 2015). Pakistan is among the water deficit countries (Rasul *et al*., 2012). The rapid rate of urbanization, industrialization and agricultural expansion has resulted in the overexploitation and contamination of groundwater resources in several parts of the country (Khattak *et al.*, 2014). Pakistan had salt affected area estimated up to 6.3 Mha (Zaman and Ahmad, 2009; Qureshi *et al*., 2008). Salt water intrusion also emerged in various areas of the Indus basin due to the unregulated and uncontrolled use of groundwater and difficulty of the overdraft of aquifers (Kijne, 1999) which is threatening ecosystem of wetlands (Qureshi *et al.,* 2010). Water demand increased due to changes in rainfall and rising temperature (Alam *et al*., 2017).

United Arab Emirates (UAE) had adopted a unique approach to cope with desertification. UAE had the motto "greening the desert" and transformed the huge desert areas into agriculture land (Abdelfattah *et al*., 2009). Huge plantation was done to promote forestation which have annual water requirement of 709 million m^3 (Shahin and Salem, 2014). UAE is located within arid zone with very limited annual precipitation and

underground water resources. Out of total water consumed 70 % is obtained from underground aquifers, 95 % of which is utilized for greening the desert (Shahin and Salem, 2014). As declared by EAD (Environmental Agency Abu Dhabi), by the year 2030 all water from underground aquifers will be vanished (Pitman *et al*., 2009; Shahin and Salem, 2014).

Demand for water supply is continuously increasing with the increasing urbanization (Wu and Tan, 2012). Increasing water requirements due to rapid urbanization and decreasing groundwater supply will be the main future problem for green sector. Thus, landscaping sector will face serious challenges, to meet up the irrigation requirements (Shahin and Salem, 2014). Increasing water shortage will also have negative impact on tourism and revenue source of UAE. Currently UAE government is determined to take necessary measures for sustainable development (Almheiri, 2015).

Mostly imported exotic species are utilized in landscaping. These plants are imported from temperate and semi-temperate countries, therefore it is difficult for these species to adopt in arid environment as their water requirements are high (Frenken *et al*., 2009). The irrigation requirements of these species are hard to meet. Increasing water shortage and salinity are main abiotic stresses for the plants. These stresses disturb plant physiology and growth by disrupting their gene expression (Wu and Tan, 2012).

Halophytes had been recommended as a solution for production with salt/brackish water (Khan and Weber, 2006). Native halophyte plants can grow in harsh environmental conditions and can be introduced in urban landscaping under drought and saline conditions (Franco *et al.*, 2006).

Plants have adopted certain physiological and biochemical mechanisms to resist abiotic stresses and sustain the protoplasmic viability. Under stressful conditions production of reactive oxygen species (ROS) also increases and plants face oxidative stress. In order to adopt such conditions, plants have a built-in complex antioxidant defense system (Lee *et al*., 2001; Sabovljevic and Sabovljevic, 2007). Most of our information about water stress responses at the molecular level is mostly of cultivated crops under

laboratory conditions (Umezawa *et al*., 2004). The broader studies on physiological and molecular level for the water stress responses of native landscape plants have not been done yet (Vásquez-Robinet *et al*., 2010).

Although many plant species are studied for salt tolerance, plant selection should be site specific. Different responses can be expected after screening of plant species under localized conditions. Examples are saltbushes (*Atriplex* sp.) and band blue bushes (*Maireana* sp.) which performed well in Australia but not when introduced in Pakistan (Ismail, 1998). The effective method so far is to choose the native/wild plant species having landscape and economic potential and are genetically tolerant to salt and water stress.

Many native plants species are vanishing because of urbanization. They can be preserved by utilizing them through xeriscaping for landscapes of desert cities (Al-Mashhanadi, 2015). Landscape architects have found native species suitable for difficult or unique site conditions, rather than using strictly for conservation purposes (Brzuszek *et al*., 2007). Using native plants cultured landscapes can be transformed into natural areas (Potts *et al.*, 2002). Native plants can be used for landscaping, fodder production, afforestation, wind break, sand stabilization and alternative crops in salt and water stress environments. All the associated ecological benefits of native plants contribute to maintain sustainable greenery, providing shelter to local fauna, conserving local flora and maintaining specific, traditional unique landscape of any country.

1.1 STUDY OBJECTIVES

- To screen native plants for their potential to be used in landscaping of arid environments.
- To determine seed quality and effect of salt and water stress on seed germination.
- To evaluate the combined effects of salt and water stress on selected species at morphological, physiological and biochemical levels.
- To assess the water use efficiency of selected native plants species.

2. LITERATURE REVIEW

2. LITERATURE REVIEW

Global demand for water had increased three fold since the 1950s as the fresh water supply had been on the way out (Gleick, 2003). Water shortage is a serious issue as 1.1 billion people are lacking access to drinking water (Gleick, 1998). According to an estimate an average of 90 % of global fresh water is being used by agriculture and crop production (Shiklomanov, 2000). UAE had neglectable precipitation and limited underground fresh water resources while ranked top for per capita water consumption in the world. Approaches employed for remediating water resources issues poses adverse effect on water's quality and quantity (Scanlon *et al.*, 2007).

2.1 Water scarcity and artificial greenery

UAE has average annual rainfall of 80-140 mm (Sherif *et al*., 2014). Abdelfattah *et al*. (2009) estimated 757.6 km^3 potential for groundwater aquifers but less than 7.5 % of that was fresh water. Annual groundwater recharge of UAE was about 350 million m^3, while the annual groundwater extraction was about 2668 million m^3 (Abdelfattah *et al*., 2009). This huge difference between the incoming and the consuming water had resulted in seawater intrusion and dryness of wells (MOEW, 2015). Excess pumping had diminished the groundwater by one tenth during the last three decades (Mohamed *et al.*, 2016). Ground water is the major source of irrigation in UAE for green sector which comprises of agriculture, forestry and landscaping (Frenken *et al.*, 2009). Agriculture sector consumes 95 % of groundwater. Total irrigation requirement for the amenity maintenance was 547 Mm^3/year in 2007. In the communities also major portion of water is devoted for irrigating home gardens as compared to actual consumption of the occupants (Pitman *et al.*, 2009).

To solve the problems of water shortage and ground water salinity many irrigation systems and equipment are being introduced (Shahin and Salem, 2014). Two types of approaches had been taken on so far to overcome salinity problem. First one is modifying the environment by managing the irrigation and drainage and second approach is genetically modifying the plants to enhance their stress tolerance. But still huge areas

cannot be managed in this way and majority of the possible solutions are much expensive (Läuchli and Lüttge, 2002; Mahmood *et al.*, 2003). There are many abiotic factors which make genetic alteration in plants for tolerance (Wang *et al.,* 2003). According to Saghir (1999) the utilization of biotic approach can be most feasible and an economic solution. However, stress tolerance responses of plants are complex and functions of many genes controlling these mechanisms are unknown (Manuela *et al.*, 2003).

Excess concentration of sodium/magnesium salts is regarded as salinity (Chapman, 1975). A saline soil is that one when electrical conductivity (EC) of the saturation extract (ECe) exceed 4 dSm^{-1} at 25 °C and had exchangeable sodium of 15 %. This EC decreases growth of most of crop species (Jamil *et al*., 2011; Munns, 2005; Richards, 1969). According to (FAO, 2005) report salinity had affected 800 Mha of land globally. It is expected that salinity will vanish annually about 10 Mha agricultural land, of which 1.5 Mha is irrigated land (Khan *et al*., 2006). According to Jamil *et al*. (2011) it is estimated that by the year 2050, 50 % of the arable land would be salinized. In UAE seawater intrusion had become the leading factor of groundwater salinity especially near the coast and the Gulf of Oman coast extended 8 km (Sherif *et al*., 2011). Mohamed *et al*. (2016) found that in the last three decades the average groundwater flow is decreased by one tenth due to excess pumping groundwater for irrigation. In UAE water has strategic nation importance. For the sustainable development and mitigate the ground water resources it is necessary to reduce the current extraction rates at least by 25 % (Mohamed *et al*., 2016).

2.1.1 Native plants and landscaping

Native plants generally refer to plants that are found to occur and grow naturally in particular area without the human aid or introduction (Al-Mashhanadi, 2015). Most of the introduced plants species are difficult to acclimatize in local environment, whereas native plants are most suitable for local environment (Bhat *et al.,* 2009).

Bodle (2001), Hostetler *et al*. (2003) and Haehle and Brookwell (2004) argued that native species should perform better than exotic species in their indigenous habitat.

Native plants also exhibit advantage in rebuilding the suitable environment for wild life by providing food and shelter (Anna *et al*., 2007; Fiedler, 2006 : Anella, 2000).

In ancient plazas "miadien" or "sahaat" masses of palm trees were used as a shaded pavilion and to direct the pedestrian movement or add to the sense of space inside the courtyards. The palm canopy was used again to create microclimate conditions. In conjunction with the *Arabian mashrabia* the species like *Phoenix dactylifera, Ziziphus spina Christi, Nerium oleander* and *Vinca rosea* were used as a natural air-conditioning and cooling method (Salama, 1990).

Native plants prefer different soil types. Most of Arabian native plant species grow well in coarsely textured, freely draining, well aerated soils, but some including *Olea, Aerva*, *Argemone, Carissa* and *Dodonaea* also thrive under rocky mountainous conditions where the stony soil is shallow. Others such as the *Acacias, Palms*, *Azadirachta, Ficus salicifolia*, *Prosopis, Abutilon* and *Calotropis* are well suited to the poorer draining silty soils found in alluvial areas. A few species, such as *Suaeda* can tolerate soil conditions with a very high water table, whilst *Tamarix, Arundo, Phragmites* and *Typha* will survive in salt marshes and sabkha regions. *Atriplex* and *Limonium* are particularly impressive as they can withstand inundation by sea-water whilst *Cornulaca* and *Zygophyllum* are not adversely affected by salt spray. Soil pH affects the availability of certain plant micronutrients. However, many of the native species, for example *Acacia tortilis, Azadirachta, Tamarix*, *Atriplex*, *Capparis*, *Dodonaea*, *Retama* and *Asphodelus* are not affected in this way and are especially useful under alkaline conditions (Ricks, 1992).

Architects and contractors have promoted demand for natives due to their ecofriendly nature in several states of North America with water restrictions for landscape use (Potts et al., 2002). Native plants getting more importance in landscaping due to their associated environmental benefits. They are always well adapted to local environment and need less maintenance. They also exhibit the ability to adjust or grow in stresses conditions more than their cultivated relatives (Morales, 2001; Fiedler, 2006; Stephens *et al.,* 2006; and Ochoa *et al*., 2009). Native plants can also be used in biological control as habitat for natural enemies (Fiedler and Landis, 2007).

Many reports had validated the importance of native plants in different countries including Saudi Arabia (Ricks, 1992), China (Zheng and Chen, 2008), Oman (Hopkins and Al-Yahyai, 2015) and different states of USA (McPherson and Haip, 1989; Mee *et al*., 2003; Brzuszek *et al*., 2007; Love *et al*., 2009; Hilaire *et al*., 2010; Anonymous, 2011; Ricordi *et al*., 2014; Reid et al., 2008).

Native plants not only contribute in vernacular landscapes but also help in water conservation and solution for rising temperatures (Crewe, 2013). Arizona, city of Tucson was entirely dependent on groundwater. Groundwater supplies start declining in 1980s. The city government made it mandatory and to use native plants with low water requirements and use of turf was restricted (McPherson and Haip, 1989).

2.1.2 Public interest for native plants in landscaping

Landscaping with native plants is becoming more popular due to their ecological and cultural functions (McMahan, 2006). Native plants are well thought-out as a rising slot in green industry (Hamill, 2005). Consumer demand for native plants has increased rapidly because of their ecofriendly nature especially in drought-affected areas (Yue, *et al*., 2010 and Anderson, 2011). In addition native plants and desert landscaping (designed or natural landscape with desert plants) are preferred by public (Hilaire *et al*., 2010; Yabiku *et al*., 2008; Yue *et al*., 2011).

As compared to invasive or nonnative plants, customers show more interest in plants labeled as native and agreeable to pay extra (Yue *et al*., 2011; 2012). There were different programs to promote native plants like “Nevada Grown’’ and “Utah’s Choice (marketed by the Intermountain Native Plant Growers Association)’’. One third of people were ready to give 20 % more for plants labeled as native (Meyer, 2005; Curtis *et al*., 2009; Yue *et al*., 2011).

Amenity plantations in urban areas are generally inspired by water-rich European-style (Alam *et al*., 2017). According to EAD adopting native plants and increasing hardscape could save significant amount of water and energy. This arid landscape policy

can reduce half of maintenance cost and energy requirements (Pitman *et al*., 2009).

In horticulture industry, introduction and promotion of native plants had been slow but consumers are preferring native plants (Gagliardi and Brand, 2007; Yue *et al*., 2011). Customer's perceptions about native plant's aesthetics are a limiting factor for the use of native plants. Other limiting factor is customer's lack of information about specific native plant species uses and care (Hooper, 2003). For increasing the native plant market and implementation need to educate consumer and industry about native plants. In addition various reports (Peppin *et al*., 2010; Woosaree, 2000) recommended focusing on consumer awareness and education about native plants.

2.1.3 Promotion of native plants market

Several studies had been carried out to examine the customer's choice difficulties faced by producers and trends in native plant markets. Major issues regarding these are difficulty in seed treatments, seed viability and dormancy testing of seeds, threats posed to wild populations by collection of wild native plants/seeds and above all, the most limiting factor is lack of sufficient scientific research on propagation techniques (Potts *et al*., 2002; Kauth and Pérez, 2011; Neufeld, 2010). According to Potts *et al*. (2002) specific propagation guidelines are very valuable for green industry as many nurserymen are confronting issues in poor and slow germination rates. Many researchers mentioned some other issues like lack of commercial seeds availability, unawareness and lack of knowledge of customer about native plants, unavailable plant material, un-established maintenance requirements, selection of plants, unavailability of required size and species are some issues that often limit the acceptance of landscape projects incorporating native plants (Hooper, 2003; Potts *et al*., 2002; Ricordi *et al*., 2014; Tamimi, 1996). To sustain the native plant industry there is a dire need to educate the consumer and grower about native plants (Meyer, 2005; Peppin *et al.*, 2010; Woosaree, 2000).

2.1.4 Native Plant selection

Choosing appropriate selection parameters of plants for urban landscapes is of vital importance. Aims and strategies of the selection program may change with environmental conditions and urban needs, but the selection method and the species evaluation process can be applicable elsewhere (Agarzadeh *et al.,* 2014). Different species can be developed that go well with the particular area (Paine *et al*., 1992). Wang and Huang (2011) offered the suitable system with two important components for the selection of the key street tree species including expert knowledge approach and Analytic Hierarchy Process (AHP) method to develop an inventory of plant species. Sadeghian and Vardanyan (2013) also developed selection criteria for urban parks of Isfahan (Iran) based on three categories including climate adaptation, tolerance of diseases and pests and phenotypic plasticity. Phondani *et al*. (2016) prioritized and categorized 50 potentially native plant species of Qatar based on 12 criteria and 49 indicators (including weather conditions tolerance, multiple use value, standard crown size and water requirement). Asgarzadeh *et al.* (2014) also employed Analytical Hierarchy Process (AHP) to categorize plant species and selection criteria for the landscape of Tehran. By employing this system. They identified different new species for the development of more attractive and economic landscape.

2.2 Germination responses to salt and water stress

Seed germination studies are more suitable prior to field testing (Almaghrabi, 2012). Both salt and water stress by PEG 6000 had shown variable effects on different plant species. In case of triticale, PEG 6000 adversely influenced the germination percentage more than NaCl and increased root-to-shoot ratios at equivalent osmotic potential (Kaydan and Yagmur, 2008; Yagmur and Kaydan, 2008). In case of NaCl and drought stress, germination is delayed in both cases. Abnormal germination percentage and mean germination time are higher in PEG than NaCl. It was concluded that depressive effects on germination by iso-osmotic solutions of PEG and NaCl resulted from osmotic effect of PEG/NaCl rather than specific ion toxicity during salt stress (Kaya *et al*., 2005).

Z. *qatarense* reduced germination by increasing salinity from -0.1 to -0.8 MPa

However, *Z. simplex* tolerated moderate salinity (Ismail, 1990). For *Atriplex halimus* low osmotic stress (NaCl and mannitol) only delayed the germination, while higher osmotic stress can reduce the final germination percentage. However, both osmotic stress of salinity and drought created by NaCl and mannitol had similar effect on germination. In conclusion, salinity effects on the germination were also related to its osmotic component. These effects on germination may include the impact on minerals mobilization (Bajji *et al*., 2002).

2.3 Plants Eco-physiological responses to salinity

2.3.1 Salt stress resistance mechanism

Plants have evolved numerous mechanisms to adapt to salt stress conditions. It is possible to distinguish three types of plant response or tolerance (Munns and Tester, 2008).

2.3.1.1 Salt avoidance

Salt avoidance is the mechanism adopted by plants to keep salt ions away from plant parts where they can cause damage (Allen, *et al*., 1994). During salt avoidance, salt concentration in cells is minimized by physiological exclusion or physiological adaptations (Koyro *et al*., 2011). It may be achieved by dilution through the growth of succulent tissues and vigorous extrusion. Mainly four methods are involved for salt avoidance in halophytes such as: succulence i:e reduction of growth and surface area, through specialized glands salts are excreted from plants (Weber, 2009), from roots salt exclusion (Waisel *et al*., 1986) and older leaves shedding (Chapman, 1968).

2.3.1.2 Salt tolerance

Tissue tolerance is the compartmentalization of salt ion in vacuoles for maintaining protoplasmic viability through physiological and biochemical adaptations (Greenway and Munns, 1980).

2.3.2 Morphological response and salinity

To avoid salt stress, plants employ many resistance mechanisms. Considering effect on visual quality is of critical importance in selecting herbaceous perennials for saline landscapes (Cassaniti, *et al*., 2012). Different species have different mechanisms of the salt tolerance (García-Caparrós *et al*., 2016). Different cultivars of ornamental plants also show different responses to increasing salinities. High salinity alters many metabolic functions of landscape plants including photosynthesis, respiration, enzymatic activity, nutrient absorption and protein and nucleic metabolism (Munns and Tester, 2008). The impact of salt stress on these physiological functions depends on level of salt stress and plant exposure period (Niu *et al*., 2012).

Chrysanthemums (*Chrysanthemum* × *morifolium*) didn't show any negative effect of water salinity with 1 $g \cdot L^{-1}$ NaCl. Higher salinity of 3 $g \cdot L^{-1}$ NaCl or more produced poor-quality plants, reduce SDW stomatal conductance (g_S) and a 4-days delay in flowering or severely stunted plants (Lee *et al*., 2001a). *Gazania rigens* and *Delosperma cooperi* were found suitable for landscaping with saline water irrigating as they did not show any injury symptoms in spite of decreased growth rate (Cassaniti *et al*., 2012). *Teucrium fruticans* and especially *Eugenia myrtifolia*, can maintain their visual quality under saline conditions (Cassaniti *et al*., 2012). Generally, *Trifolium* species are sensitive to salinity stress but comparing native and commercial *Trifolium. T. pratense* (native) and *T. repens* were found more tolerant to salinity stress (Vahdati *et al*., 2012).

2.3.3 Physiological response

Salinity affects the plants including both halophytes and non-halophytes and mostly cause adverse effects on plant growth. This reduced growth is result of several physiological responses to counter the negative effects of salt stress (Flowers *et al*., 1977; Munns and Termaat, 1986).

2.3.3.1 Cation uptake

Increased NaCl levels accumulate more Na^+ and Cl^- content of the safflower (*Carthamus tinctorius* L.) seedlings, with no change in K content (Kaya *et al.*, 2011). The Na^+ and Cl^- accumulation in leaf increases up to four times with increasing salt stress (Niu *et al*., 2012). *Hyacinthus orientalis* showed sudden increase in Na^+ concentration by increasing salinity (koksal *et al*., 2014). *Aloe vera* plants accumulate Na^+ at the root level. *Kalanchoe blossfeldiana* release Na^+ by shedding older leaves. *Gazania splendens* plants accumulated Na^+ and Cl^- at the root level and secreted salt from leaves (García-Caparrós *et al*., 2016).

2.3.3.2 Photosynthetic rate (Pr)

Salinity reduces net Pr in plants (Gibberd *et al*., 2002; Kumar *et al*., 2000; Tezara *et al*., 2002). However, salt tolerance is associated with the preservation of net photosynthesis (Kumar *et al*.. 2000). The Pr and stomatic conductance was decreased by saline stress in *Argyranthemum coronopifolium* (De Herralde *et al*., 1998).

It is well established that plants decrease the photosynthesis under salt stress but had no or little relationship with growth e.g. *Triticum aestivum* (Hawkins and Lewis, 1993) and *Olea europea* (Loreto *et al*., 2003). In contrast, for many crops yield may decrease also with decreasing photosynthesis in saline conditions e.g. *Asparagus officinalis* (Faville *et al*., 1999), *Brassica* species (Ashraf, 2001) and in grass species (Hester *et al*., 2001). Mild salinity levels even increased the rate of photosynthesis in some other species (Muhammad Ashraf, 2004).

2.3.3.3 Stomatal conductance

Liu and Yu (2017) studied salinity tolerance in alfalfa. Increasing salinity decreased average value of all studied morphological traits. Compared to the control plants, average plant height and dry weight of stressed plants decreased by 17.43 % and 43.84 % respectively. At high salinity plants have lower stomatal conductance to prevent dehydration. Increasing Na^+ concentration decrease of the transpiration flux by increasing

stomatal closure (Maggio *et al*., 2007). Tomato plants can maintain its turgor potential and stomatal conductance by adjusting its osmotic potential under saline environment (Katerji *et al.*, 1998).

2.3.3.4 Chlorophyll content

Salt stress reduces the chlorophyll content in plants. Chlorosis start to develop in oldest leaves and fall down if salt stress continue for longer period (Agastian *et al.*, 2000). Salt stress can result in smaller and thicker leaves by changing the leaf anatomy. This decrease in leaf area decrease the photosynthesis per plant (Munns and Tester, 2008). Munns (2005) stated that this mechanism compensates the stomatal conductance to have high leaf transpiration efficiency and to maintain the Pr. Montesano and Iersel (2007) also had similar findings. Similar effects of salinity had been reported previously on the photosynthesis of non-halophytes (Downton *et al*., 1985; Farquhar *et al.*, 1987; Seemann and Critchley, 1985) and halophytes (Ball and Farquhar, 1984; Flanagan and Jefferies, 1989).

Drought and salinity can affect the photosynthesis primarily or secondarily. Direct effect is limitation of diffusion through stomata and alterations in photosynthetic metabolism and secondary effect is due to oxidative stress (Manuela *et al*., 2003a).

2.3.4 Biochemical responses

Salt tolerance mechanisms have two important components that are oxidative stress signaling and ROS detoxification (Bose *et al*., 2014). Lee *et al*. (2001) found an increased APX and decreased CAT activity in rice leaves. Salt tolerance in root tissues also achieved by ROS-scavenging system. In roots, under salinity CAT and APOX activities increased in salt-tolerant cultivar. SOD activity remain same under increasing salinity in both cultivars (Demiral and Türkan, 2005).

Halophytes show increase activity of catalase after salt treatment which decreased thereafter, whereas in glycophytes the activity of these enzymes remains higher (Ellouzi *et al*., 2011). Under collective salt and waterlogging stress halophytes showed higher activity

of antioxidants as compared to drained conditions (Alhdad *et al*., 2013). The APX and CAT activities increased significantly within 2 hours after exposure of salt stress to cotton calli (Vital *et al.,* 2008). True salt tolerant plants can efficiently exclude Na^+ from the cytosol therefore they don't have increased production of ROS and hence may not require higher level of antioxidant activity. These halophytes have higher levels of indigenous SOD which activate adaptive responses (both genetic and physiological) when exposed to salinity (Bose *et al*., 2014). It can be concluded that under slat stress halophytes protect themselves from deleterious ROS by antioxidant enzymes (Jithesh *et al*. 2006).

Seckin *et al.* (2010) compared *Hordeum vulgare* and *Hordeum marinum* under salt stress. He reported that *Hordeum marinum* (sea barley grass) had increased activity of all antioxidant enzymes giving it a better protective mechanism against salt-induced oxidative damages when compared with *H. vulgare* (cultivated barley) (Seckin *et al*., 2010).

Salt tolerance of halophytic species had been found correlated with the antioxidant capacity in case of *Plantago maritima* (Hediye *et al*., 2007) and *Centaurea tuzgoluensis* (Yıldıztugay *et al.,* 2011). Therefore, salt-tolerant plant species possess efficient ROS-scavenging mechanism, along with ability to regulate water and ionic relations (Rout and Shaw, 2001). However, in previous investigations ROS scavenging capacity of plant species had focused mainly on different stresses applied separately (Sekmen *et al.,* 2014).

2.4 Plants Eco-physiological responses to water stress

2.4.1 Drought resistance mechanisms

Plants had developed many various resistance mechanisms to counter water stress. Water stress-avoiding refer to range of morphological and physiological adaptations of plants to sustain suitable water status, either by preserving water during water stress periods or by ensuring an efficient water supply to above ground organs (Clarke and Durley, 1981). These adaptations can be of three types: (i) enhanced water uptake (ii) reduced loss of water through transpiration and (iii) storing water in plant tissues. Other approach to withstand water stress is water stress tolerance that includes physiological and biochemical mechanisms (Clarke and Durley, 1981). Combined mechanisms of avoidance and

tolerance had been found in most of grasses for their survival in drought conditions (Arraudeau, 1989).

2.4.2 Morphological response and drought

To get high quality plants of different species, understanding of their morphological and physiological response and optimization of irrigation regimes are of critical importance (Franco *et al*., 2006; Sánchez-Blanco *et al*., 2009). Compared to herbaceous plant, woody plants are found more water stress tolerant (Augé *et al*., 2003). During severe drought conditions shoot growth and leaf area decreases, root growth increases, plants may over hardened and even die (Franco *et al*., 2006). Cameron *et al.* (2006) narrated that severe stress can reduce leaves area, internode sections size and flower number and size. However, Arreola *et al*. (2006) concluded that moderate-stress can improve the seedling quality by increasing shoot length and root weight. The highly-stressed seedling will be over-hardened and too small (Sánchez-Blanco *et al*., 2009). Deficit irrigation also maintains plant shape and quality can also be substitute for labor-intensive pruning techniques by reducing the plant height e.g. in case of *Rosmarinus officinalis*, plants under deficit irrigation showed a conservative strategy in the water consumption and reducing stomatal conductance (Cameron *et al*., 1999 and Sánchez-Blanco *et al*., 2009).

Flowering stage is most susceptible to salt and drought stress (Álvarez *et al.*, 2013). Araújo-Alves *et al*. (1999) estimated the minimum water requirement in two native *Santolina chamaecyparissus* L. and *Arbutus unedo* L. without affecting their ornamental value. *P. barbatus* tolerate drought by decreasing stomatal conductance and increasing root: shoot ratio. *Lavandula angustifolia* P. Mill. and *Penstemon* × *mexicali* Mitch. are tolerant to moderate drought but dies if exposed to severe drought (Zollinger *et al*., 2006).

2.4.3 Physiological mechanism

Understanding plants responsive mechanism to drought is vital to get better plant quality under water stress conditions. This is also important to understand water requirement of landscape plants and for water conservations in arid zone landscapes.

2.4.3.1 Photosynthesis

Photosynthesis is the primary process influenced by water stress because of stomatal closure and decreased CO_2 diffusion to the chloroplast. The capacity of plants species to deal with environmental stresses is related with their ability to acclimate the photosynthesis level. Soil drying stimulate stomatal closure by drought-induced root-to-leaf signaling, through the transpiration stream (Anjum *et al*., 2011). As a plant is exposed to drought, net CO_2 uptake decreased because of stomatal closure. As a result, CO_2 concentration in the chloroplast decreased in plants exposed to drought (Cornic and Massacci, 1996). Patane *et al*. (2013) revealed that photosynthesis correlates with stomatal conductance in all species generally for C3 plants.

Cornic and Massacci (1996) studied that plants can maintain the water status by regulating water loss and water uptake, in which abscisic acid act as signaling agent. Siddique *et al*. (2000) studied that water stress lead to significant decrease in leaf water potential. This decrease in LWP is associated with a decreased Pr.

2.4.3.2 Stomatal conductance

Stomatal control of water stress is an avoidance measure and forms part of the plant's first line of defense to water stress (Chaves, 1991; Cornic and Massacci, 1996). Stomatal closure is the important limiting factor to photosynthesis and is the primary reaction to water stress (Flexas and Medrano, 2002b).

There is a high correlation of Crop Water Stress Index (CWSI) with Stomatal Conductance (SC), from potato tuber initiation to maturity based on ground and aerial data (Rud *et al*., 2014). Plants were able to conserve water by closing their stomata, reducing plant size, leaf number and LAI when exposed to water stress (Mabhaudhi *et al*., 2011).

Stikić *et al.* (2015) mentioned in his paper that due to drought avoidance mechanisms, Quinoa (*Chenopodium quinoa*) sustained water uptake by reducing transpiration rate. Transpiration rate is reduced by decrease of stomatal conductance and leaf area development. Caplan and Yeakley (2010) compared water relations of *Rubus*

armeniacus with native species i.e. *R. spectabilis* and *R. parviflorus. R. armeniacus* maintained higher stomatal conductance (gs) compared to the native species. Hanson *et al*. (2015) studied Alfalfa populations under drought treatment. Two drought-tolerant germplasm exhibited the lowest stomatal conductance under severe drought among the 11 populations.

2.4.4 Biochemical responses

High salt concentration and drought, damage the cellular electron transport which in turn affects the chloroplast and mitochondria. As a result electrons leak out due to damaged electron transport system which increases Reactive Oxygen Species (ROS) and other toxic compounds (Ali and Alqurainy, 2006; Foyer and Noctor, 2012; Bhattacharjee, 2005 and Suzuki and Mittler, 2006).

Beside the destructive nature, ROS act as signaling molecules in many biological processes such as growth, development and stomatal closure (Demiral *et al*., 2011). Hydrogen peroxide (H_2O_2), the main signaling molecule studied so far, is the most likely ROS to act as messenger because of its relative stability and it can cross membranes through aquaporins (Furlan, *et al*., 2016). To deal with the destructive effects of ROS, plants had established a complex antioxidant defense mechanism (Lee *et al*., 2001; Suzuki *et al*., 2012). Antioxidant defense systems protect plants from osmotic stresses and ROS-associated injury (Miller *et al*., 2008).

APX and CAT activity increased significantly under drought in barley (Harb *et al*., 2015) and *Continues coggygria var. cinerea* (Zhao *et al*., 2011). SOD, POX and CAT activities increased in *Sesamum indicum* L. (Fazeli *et al*., 2007). In *Brassica napus* L. POD, CAT and APX activities increased when exposed to water stress (Mirzaee *et al*., 2013). Türkan *et al.* (2005) found increased antioxidant enzymes activities in *P. acutifolius* than in the *P. vulgaris* (Türkan *et al*., 2005).

2.5 Cross tolerance in Plants

Cross-tolerance is characterized as a natural phenomenon by which, a plant resistant to one stress can develop tolerance to another form of stress. Plants had improved special mechanisms that help plants to sense and respond to individual or multiple environmental stresses. This is still an important challenge in research and is known as cross tolerance. Scientists focus on to get stable multiple stress tolerant traits in agronomical crops to improve yields (Bahmani and Maali-Amiri, 2017).

The simultaneous incidence of different stresses can have positive or negative impacts on plants depending on stresses nature and exposure period (Niinemets, 2010). Water stress can have negative effects on plant pathogen resistance (Ijaz *et al*., 2013). So, biotic and abiotic stress combinations can interact negatively and cause damage to plants. Many stress-induced genes during combined salt and water stresses had been reported to overlap in *Arabidopsis* (Chinnusamy *et al*., 2004).

2.5.1 Cross tolerance to salt and water stress

Plants have similar physiological mechanisms to handle salt and water stress. Water potential during saline conditions decrease similarly to drought conditions. As the salinity in soil is increased water available to plant is decreased leading to the shortage of water (Hasegaw *et al*., 2000). Physiological and morphological changes during stress conditions can be avoidance or tolerance. Avoidance mechanisms refer to morphological and physiological responses. By contrast, tolerance mechanisms are based on molecular biochemical and cellular modifications that can be practically manipulated (Vinocur and Altman, 2005). However, effects of abiotic stresses which include salt, water and oxidative stress are often indistinguishable and interconnected. For example, salt and water stress disrupt homeostasis and ion distribution resulting from osmotic stress in the cell (Wang *et al*., 2003). Water stress studies for signaling are also focused on salt stress mostly because of overlapping mechanism and similar responses to drought and saline conditions (Zhu, 2002). Therefore, studies on plant tolerance to salt and water stress are of fundamental importance.

2.6 Benefits of stress studies for using native plants in landscape industry

Most of molecular investigation about salt and water stress response done on cultivated crops (Umezawa *et al*., 2004). ROS scavenging capacity of cultivated plants mostly investigated applying different stresses separately (Sekmen *et al*., 2014). The broad studies of physiological and molecular response of native landscape plants to combine salt and water stress have not yet been done (Harb *et al*., 2010). Studying native plants under stress conditions will help us to better understand the salt and water stress resistance mechanisms of arid zone plants native to UAE and Pakistan. Moreover, introducing the identified species in landscaping will not only save huge amount of water but also preserve the biodiversity, wildlife habitats, horticulture heritage and national unique landscape of the country.

3. RESEARCH METHODOLOGY

3. RESEARCH METHODOLOGY

Study on eco-physiological responses of plants native to Pakistan and UAE under different salt and water stresses were conducted at United Arab Emirates University, Abu-Dhabi during 2015-18. Study was conducted in two phases:

Phase 1: Identification of native plants suitable for landscaping and collection of seeds. This phase was completed within one year (Oct 2015 to Sept 2016).

Phase 2: During this phase, native plants were evaluated for germination and field performance under salt and water stress conditions. This phase consisted two studies and below.

1. Germination study: In this phase nine selected native plant species. were tested against salt and water stress to evaluate their performance during seed germination stage whereby the best performing spp. were forwarded to the next study of these plant species.
2. Field study: Field evaluation of three selected native plant species in germination experiment under salinity and water stress conditions. Selection of plants

In the first phase, perceptions of community peoples and industry experts were obtained for selection of potential native plant species for sustainable landscaping. Native plants in landscaping is new trend specifically in UAE and very little information is available about the species native to arid zones and their uses in landscaping. Plant species from both Pakistan and UAE were assessed for their government and municipalities recommendations, survival rate, habitat, growth rate, life forms, inflorescence and customer demand. Plant species most suitable for landscape use and supposed to have salt and water stress tolerance were selected for further studies.

Even though there is no clear list of recommended native plants for landscaping. However some help was taken from the published studies and municipalities guidelines including Al-Mashhadani and Alameri (2014), Al-Mashhanadi (2015), Phondani *et al*. (2013), EAD (2015), Trakhees (2018), Bhatt (2015), Salama (1990), Hopkins and Al-Yahyai (2015), Al Mashhadani (2014), Ricks (1992).

All dominant native plant species used in landscapes, commercially grown for different purposes and the species recommended by municipalities were investigated and

a list of potential native plant species was developed.

3.1 Native plants identification and seed collection

Plants samples of selected native plants species were collected from wild populations and were preserved for further use in this trial. Collected plant samples were classified by United Arab Emirates University, Al Ain, Abu Dhabi, UAE and International Islamic University, Islamabad, Pakistan. For germination experiment fresh seeds of selected native plants were collected during 2016-2017.

3.2 Native plant's adaptation to salt and water stress during seed-germination stage

Species used in germination experiment were *Rhazya stricta* Decne**,** *Leptadenia pyrotechnica* (Forssk.) Decne**,** *Convolvulus virgatus Boiss***,** *Atriplex leucoclada* Boiss**,** *Senna italica* Mill**,** *Taverniera glabra* Boiss**,** *Tephrosia apollinea* (Delile)**,** *Tetraena mandavillei (Hadidi) Beier & Thulin* and *Salsola imbricata* Forssk. Fresh mature seeds of selected shrubs were collected from wild population of UAE during 2016-2017. Experiment was conducted at Plant Physiology Lab, Department of Biology, UAE University, Al Ain, Abu Dhabi, UAE.

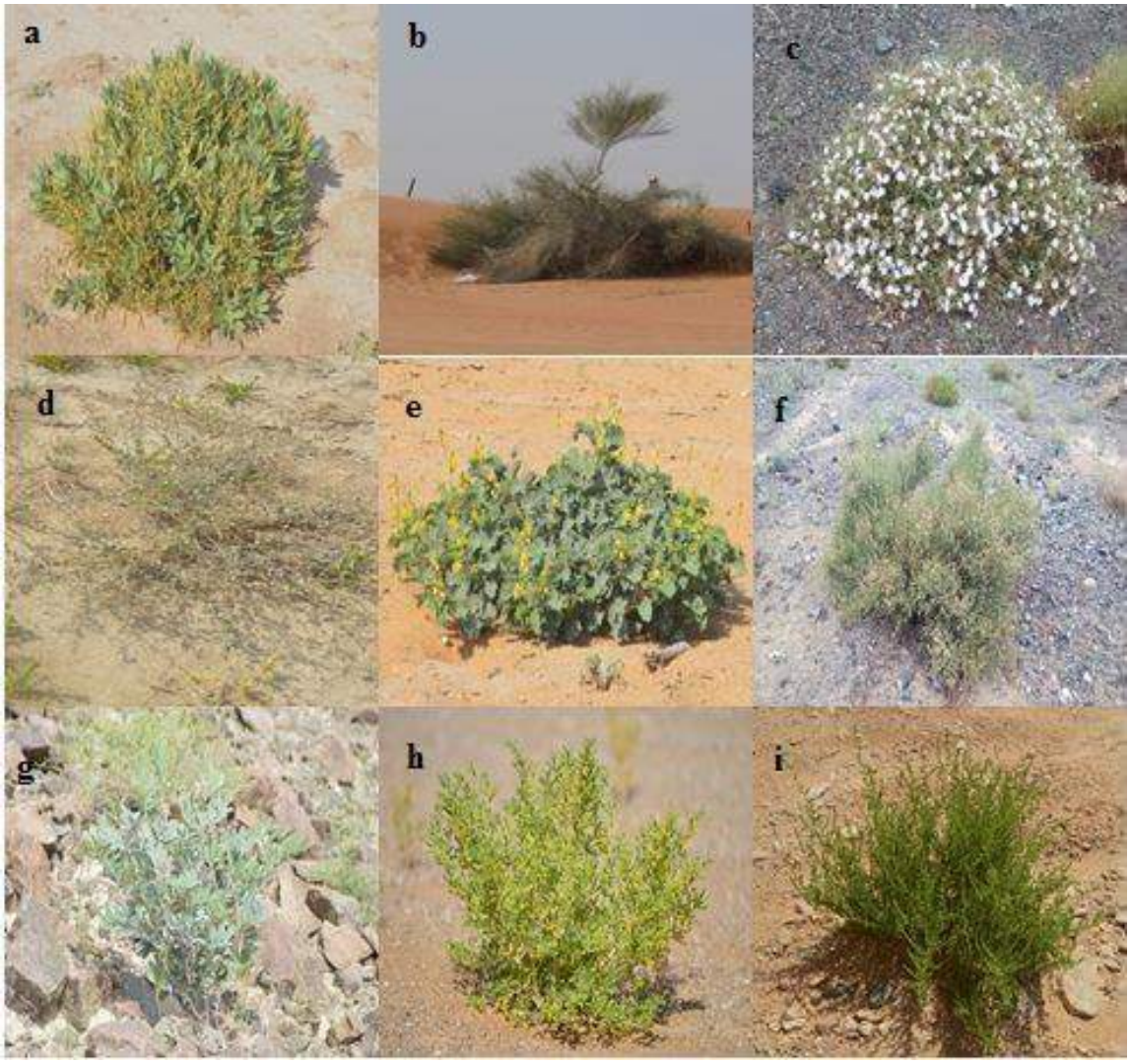

Fig. 3.2. Native plant species selected for germination and field experiment (a) *Rhazya stricta* (b) *Leptadenia pyrotechnica* (c) *Convolvulus virgatus* (d) *Atriplex leucoclada* Boiss (e) *Senna italica* Mill (f) *Taverniera glabra* Boiss (g) *Tephrosia apollinea* (Delile) (h) *Tetraena mandavillei (Hadidi) Beier & Thulin* (i) *Salsola imbricata*.

3.2.1 Preparation of Osmotic solutions

NaCl and Polyethylene glycol (PEG 6000) solutions were used as two osmotic agents (OA) for germination experiment. Four osmotic levels (OL) i.e. S0, S1, S2 and S3 of osmotic level 0 (control), -0.2, -0.4 and -0.6 MPa water potential; respectively were prepared of both NaCl and PEG (6000). NaCl concentrations of 0, 50, 100 and 150 mM were prepared to get solutions of desired osmotic potential which were confirmed in an automatic cryoscopic osmometer (Osmomat 030 model; Gonotec, Berlin, Germany). PEG (6000) solution was prepared to get desired osmotic potential level. The quantity of PEG 6000 to be added to obtain each osmotic level was calculated according to Michel and Kaufmann (1973) equation.

3.2.2 Imbibition

Imbibition rate for each species was studied in petri dishes. Each treatment was triplicated. In each petri dish a twofold layer of blotting paper was used and thereafter 25 mature seeds of equal size of each species were placed on blotting paper. Each petri-dish was added with 10 ml solution of respective treatment. These petri-dishes were then kept in an incubator at 25 ^{o}C. The experiment design was two factors split plot (2 x 4) design and randomized complete block design (RCBD) arrangement with three replications. Seeds were reweighed after 24 hours (Heather *et al*., 2010). Imbibition Rate (IR) was calculated using Song *et al*. (2005) formula:

$$\text{Imbibition rate (IR)} = \frac{Wf - Wi}{Wi} \times 100$$

Where:

IR = Relative increase in fresh weight

Wi = Initial weight of the seeds

Wf = Final weight of the seeds

3.3 Seed germination studies

Germination experiment was conducted on another set of 25 seeds placed on filter paper in petri-dishes by repeating the above-mentioned procedure. Germinated seeds were counted every day. Seed was considered as germinated when the radicle length was 2 mm (Jajarmi, 2009).

3.3.1 Experimental design:

The experiment was conducted in two factors split plot (2x4) design with randomized complete block design (RCBD) arrangement. The experiment was replicated thrice and 25 seeds per replicate. First factor was osmotic agents (OA; i.e. NaCl and PEG), the second was osmotic level (OL; 0, -0.2, -0.4 and -0.6 MPa). For statistical analysis, the data was transformed to square roots as $x= \sqrt{X}/100$ to meet variance assumptions. Data was subjected to analysis of variance (ANOVA) procedures (SAS Institute Inc., 1988) and LSD test was applied at 5 % probability level to compare the differences among treatment means.

3.3.2 Parameters to be studied

Table 3. 1 Parameters studied during experiment

S. No	Parameter	Formula	Description	Reference
1	**Germination Percentage (GP)**	GP= Ng / Nt x 100	Ng=Total number of seeds germinated Nt=Total number of seeds	(Kader, 2005)
2	**Germination Index (GI)**	GI = ∑(Gt/Tt)	Gt is number of the germinated seeds in the t day, Tt is time corresponding to Gt in days	(Hu *et al*., 2005)
3	**Mean Daily Germination (MDG)**	MDG=FGP/d	GP = germination percentage, d = maximum days to final germination	(Almaghrabi, 2012)
4	**Mean Germination Time (MGT)**	MGT= ∑n. D /∑n	n= number of seeds newly germinated on day D; D= days counted from start of trial, ∑n= final germination	(Ellis and Roberts, 1978)
5	**Promptness Index (PI)**	PI = nd2 (1.0) + nd4 (0.8) + nd6 (0.6) + nd8 (0.4) + nd10 (0.2)	Where nd2, nd4, nd6, nd8 and nd10 shows percentage of seeds germinated after 2, 4, 6, 8, and 10 days.	(Sapra *et al.*, 1991)
6	**Germination Stress Tolerance Index (GSI)**	GSI (%) = [P.I of stressed seeds / P.I of control seeds] x 100	P.I. = Promptness index	(Bouslama and Schapaugh, 1984)
7	**Coefficient of Velocity of Germination (CVG)**	CVG=ΣNi / ΣNiTi x 100	Ni is the number of seeds germinated on each day, Ti is number of days from beginning of experiment	(Scott *et al.*, 1984)
8	**Germination Rate (GR)**	GR=Timson germination index = ∑G/t,	"G" is seed germination percentage at two days' intervals and "t" is total germination time (days)	(Khan and Ungar, 1997)

3.4 Assessment of salt and water stress response in native plants

3.4.1 Study area and plant material

A field experiment was conducted to study the eco-physiological response of selected native plants to different salt and water stresses at AL-Foa Research Farm, United Arab Emirates University, Al Ain, Abu Dhabi, UAE, during the year 2015-16. Seeds for three selected plant species i.e. *Salsola imbricata, T. mandavillei* and *A. leucoclada* were sown in germinating trays with a growing media of potting soil and sand 1:1 v/v. After three weeks of germination, seedlings were transplanted to 20 cm pots that were filled with desert sand. All cultural practices, i.e. fertilization, weeding etc. were the same for all plants during the experiment.

3.4.2 Plant stress treatment

Four salt water treatments i.e. 5, 10, 15 and 20 dSm^{-1} (S1, S2, S3 and S4 respectively) were designed according to irrigation water salinities. S1 represents the lowest salinity in irrigation water, S2 is the current salinity level in farmer's fields, S3 is the maximum salinity level suggested by extension services and S4 is highly saline water. The target irrigation water salinities (5, 10, 15, 20 dSm^{-1}) were obtained by dissolving NaCl in irrigation water (Al-Dakheel *et al.*, 2015; Zamin *et al.* 2019). All the plants were well supplied with ground water for 60 days after germination. After establishment, the plants were subjected to four different water regimes i.e. 100 % (control), 80 %, 60 % and 40 % of field capacity. The experiment was conducted in a randomized complete block design with a split-plot arrangement replicated three times. The main plot had four salinity levels and the four irrigation regimes were in the subplot.

3.4.3 Harvesting and sampling

Three plants from each treatment were harvested at the end of each month. Data regarding morphological parameters was recorded monthly. For the quantitative chemical analysis, representative specimens of each plant were instantly ground in liquid nitrogen and stored at -80°C.

3.4.4 Morphological traits

After harvest, the plant samples were carefully cleaned from sands, washed with distilled water and soaked with the help of tissue paper. Each plant was divided into shoots and roots and oven dried (60 ^{o}C) and weighed (±0.0001g). For morphological traits, all the samples were put in Ziploc bags, placed in an ice bag at 4 ^{o}C and transferred to the laboratory. Shoot length (SL) was measured from the base of stem till the apical bud while Root Length (RL) was measured from the root base up to the end of primary root. Root and shoot parts were separated, oven-dried at 60 ^{o}C to calculate shoot dry weight (SDW) and root dry weight (RDW).

3.4.5 Physiological traits

Water use efficiency (WUE) was figured out by dividing the mineral-free dry mass of shoots by the amount of water transpired over the experiment (Hsiao, 1993). Chlorophyll index was measured using Hanstech Cl-01. Photosynthetic rate (Pr) (μ mole/m^2/sec) of upper, lower and basal leaves was measured weekly using a plant photosynthesis meter (EARS, Netherlands) (Samarah, 2005). The midday leaf water potential (ΨLeaf) was measured using WP4C Dewpoint psychrometer (Decagon Devices, Inc., USA) after one month and five months of treatment application (Xiong *et al.*, 2015). The youngest fully developed leaves on the main tiller were detached and cut into small sections, immediately followed by leaf water potential (Ψleaf) measurement. Nitrogen was estimated in plants leaves by kjeldahl method (Bremner, 1960). Phosphorus concentration was estimated in plants leaves at the end of experiment by Olsen (1954) methodology. K was estimated through Wright and Stuczynski (1996) methodology. Na and Cl^- were determined through Helmke and Sparks (1996) method.

3.4.6 ABA and Proline-LCMS/MS analysis (μg.g^{-1} FW)

ABA and proline extraction was performed on 10 mg of freeze-dried tissue as described by Forcat *et al*. (2008). The samples were analyzed for ABA and proline using LCMS/MS. The Samples were filtered through a 0.45 μm cellulose acetate syringe to

remove any large particles. Phytohormones separation was performed using a C18 column (ZORBAX Eclipse Plus). An injection of 2 µl was loaded onto the C18 column (1.8 µm particle size, 2.1 mm inner diameter and 50 mm long) at a flow rate of 0.2 mL/min and the column temperature was kept at 35°C. The liquid chromatography was connected to an Agilent Technologies mass spectrometer (6420 Triple Quad detector). The solvents used for elution in this method were, solvent which was water with 0.1 % formic acid and solvent B which was an LCMS- grade acetonitrile. The analytical procedure was as follows:

The first 5 min were only solvent A, then a gradient of 0 to 100 % for solvent B continued from 5 to 20 min, after which solvent B was kept constant for 5 min. At 25.1 min, solvent A was at 100 % until the end of the 30-min method. During the LCMS/MS analysis, only the negative polarity mode was used for ABA and proline analysis. For fragmentation, nitrogen gas was used. The capillary voltage was 4000 V, the gas flow was 8 L/min, the gas temperature was 3000°C and the nebulizer pressure was 45 psi.

3.4.7 Enzyme extractions and assays

For the isolation, 0.5 g plant material was rubbed with quartz sand in a deep-frozen mortar with the addition of 2.5 ml ice-cold 0.5 M tris HCl buffer (pH 7.4) containing 3 mM $MgCl_2$ and 1 mM EDTA. The homogenate was centrifuged (40°C, 20 min, 15,000 g), and the supernatant was divided between Eppendorf tubes. During the analysis, enzyme activity was recorded after freezing at -20°C. Enzyme activity was measured photometrically (UUV-VIS 160 A, Shimadzu, Japan). The samples were kept on ice prior to measurement, but the measurement was performed at room temperature. Enzyme activity was expressed as the change in absorbance caused by 1g enzyme protein for 1 min ($\Delta\Delta$ min^{-1} g^{-1} protein).

CAT (Catalase) (EC 1.11.1.6) activity was quantified as proposed by (Ádám *et al*., 1995).

POD (Peroxidase) (EC1.11.1.7) activity was quantified using the procedure of Kumar and Khan (1982).

APX (Ascorbate Peroxidase) (EC 1.11.1.11) activity was quantified as proposed by (Nakano and Asada, 1987).

3.4.8 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) procedures using Statistix Software 8.1, the standard errors of the means were calculated, and the means were separated by LSD test at the 0.05 significance level.

4. RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1 Study 1: Native plant's adaptation to salt and water stress during seed-germination stage

In this section, we presented species vise results, significant parameters are discussed in detail. However, non-significant parameters are present in graphs only.

4.1.1 *Rhazya stricta* Decne

Local/ Arabic name: Harmal, حرمل

Germination was completely inhibited by all NaCl levels and PEG -0.6 MPa (data not shown for NaCl). Germination percentage (GP), germination index (GI), mean daily germination (MDG), mean germination time (MGT), promptness index (PI), germination stress tolerance index (GSI) and germination rate (GR) of *R. stricta* were significantly affected by osmotic levels (OL) of PEG. GP, GI, MDG, PI, GSI and GR decreased with decreasing water potential of PEG from control to -0.6 MPa. However, MGT increased with decreasing water potential of PGE from control to -0.6 MPa (Fig. 4.1.1).

As OL for PEG decreased so did GP. Highest GP (47.78 %) was recorded in control treatment followed by 45.5 % for PEG -0.2 MPa whereas; lowest GP (0.00 %) was recorded in PEG-0.6 MPa (Fig. 4.1.1b). GI for different levels of PEG was 2.96 %, 2.23 %, 0.16 % and 0 % for control, PEG -0.2 MPa, PEG -0.4 MPa and PEG -0.6 MPa respectively (Fig. 4.1.1c). Control had the highest MDG (5.53 %) followed by PEG -0.2 MPa (5.10 %). PEG -0.4 MPa treatment had lowest MDG of 0.54 % (Fig. 4.1.1d).

As water potential decreased, MGT increased. The highest recorded MGT was 8.3 days for PEG -0.4 MPa PEG, while the average MGT was 5.2 days and 6.5 days for control and PEG -0.2 MPa respectively (Fig. 4.1.1e). PI was highest (16.6) for control while minimum PI was at PEG -0.4 MPa and PEG -0.6 MPa i.e. 0.13 and 0.00 respectively (Fig. 4.1.1f). GTI decreased with decreasing water potential and was 65.74 %, 0.82 % and 0.0 % for PEG -0.2 MPa, PEG -0.4 MPa and PEG -0.6 MPa. GR was 5.53 day^{-1}, 5.10 day^{-1} and 0.54 day^{-1} for control PEG -0.2 MPa and PEG -0.4 MPa respectively (Fig. 4.1.1i).

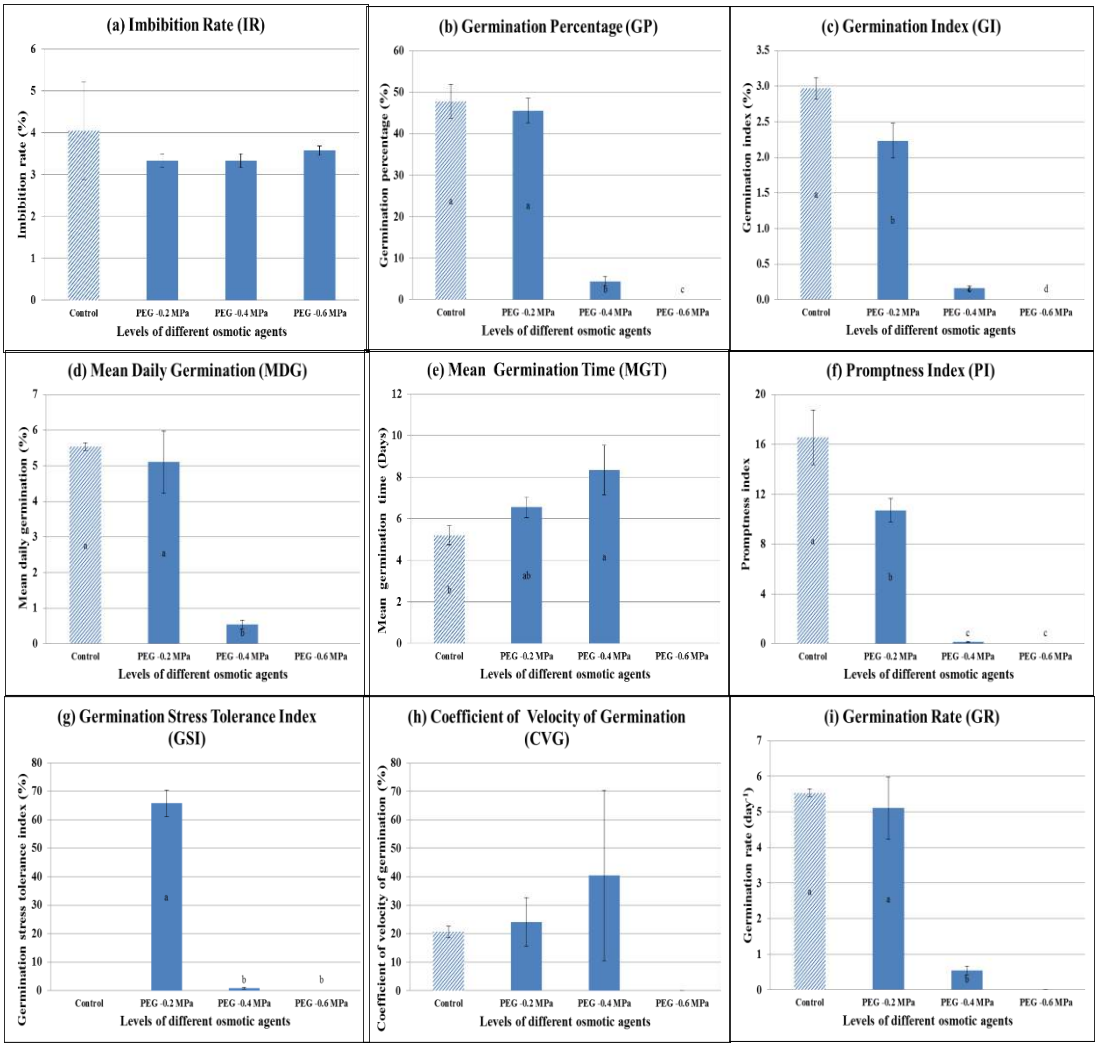

Fig. 4.1.1. Means of the different germination parameters for *R. stricta* under three osmotic levels of PEG. Means with different letters are significantly different at P≤0.05

4.1.2 *Leptadenia pyrotechnica* (Forssk.) Decne

Local/ Arabic name: Markh, مرخ

GP, GI, MDG, MGT, PI, CVG and GR of *L. pyrotechnica* showed significant interaction for OA* OL. GP, GI, MDG, GSI, PI and GR decreased with decreasing OL. However, decrease with PEG was more than the decrease due to NaCl. MGT increased with decreasing the OL while increase more in PEG as compared to NaCl. GSI was affected by the OA and OLs. GSI was higher for NaCl compared to PEG and decreased with decreasing OL.

Highest GP was recorded for PEG -0.2 MPa (96.67 %) followed by control (94.44 %) while lowest GP was observed at PEG -0.6 MPa (0.00 %) (Fig. 4.1.2b). Maximum GI was observed for control treatment (9.62 %) followed by PEG -0.2 MPa (7.41 %) while minimum was observed at PEG -0.6 MPa (0.00 %) (Fig. 4.1.2c). Control treatment had highest MDG of 20.39 % while NaCl -0.6 MPa and PEG -4 MPa had lowest MDG of 3.70 % and 3.82 % respectively (Fig. 4.1.2d).

Lowest MGT was observed for control i.e. 3.25 days. Highest MGT was 5.29 days followed by 4.45 days for PEG -4 MPa and NaCl -0.6 MPa respectively (Fig. 4.1.2e). Maximum PI (69.98) was observed for control while minimum PI (0.00) was observed for PEG -0.6 MPa (Fig. 4.1.2f). GSI was 45.5 % for NaCl and 26.55 for PEG. Maximum GSI was 67.92 % for -0.2 MPa while minimum (6.31 %) for -0.6 MPa (Fig. 4.1.2g).

Control treatment had maximum GR (20.39 day^{-1}) while PEG -0.2 MPa, PEG -0.4 MPa, NaCl -0.2 MPa, NaCl -0.4 MPa and NaCl -0.6 MPa had 18.26 day^{-1}, 3.82 day^{-1}, 9.62 day^{-1}, 6.72 $day^{-1}$and 3.70 day^{-1} respectively (Fig. 4.1.2i).

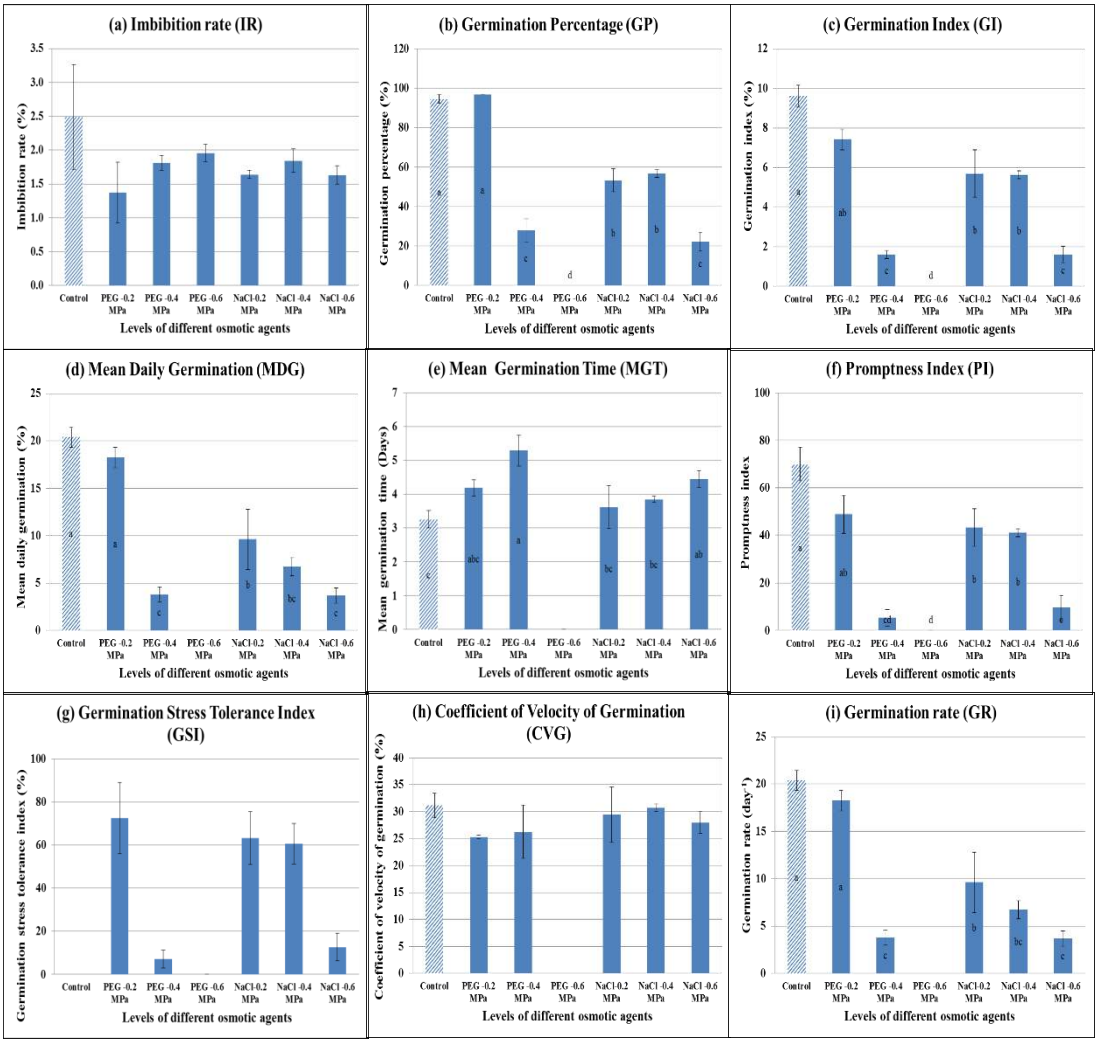

Fig. 4.1.2 Means of the different germination parameters for *L. pyrotechnica* under three osmotic levels of PEG and NaCl. Means with different letters are significantly different at $P \leq 0.05$.

4.1.3 *Convolvulus virgatus* Boiss

Local/ Arabic name: Hub-e-Reesha, حب الريشه

Germination was completely inhibited by all salt levels under investigation therefore data is not shown here. MGT increased significantly with decreasing the OL while GI and PI decreased significantly with decreasing OL of PEG (Fig. 4.1.3).

GI was 4.18 %, 3.39 %, 1.15 % and 0.71 % for control, PEG -0.2 MPa, PEG -0.4 MPa and PEG -0.6 MPa respectively (Fig. 4.1.3c). MGT was 2.98 days for control treatment and was 3.69 days, 5.28 days and 5.42 days for PEG -0.2 MPa, PEG -0.4 MPa and PEG -0.6 MPa respectively (Fig. 4.1.3e). PI was 29.0, 24.3, 8.8 and 2.4 for control, PEG -0.2 MPa, PEG -0.4 MPa and PEG -0.6 MPa respectively (Fig. 4.1.3f).

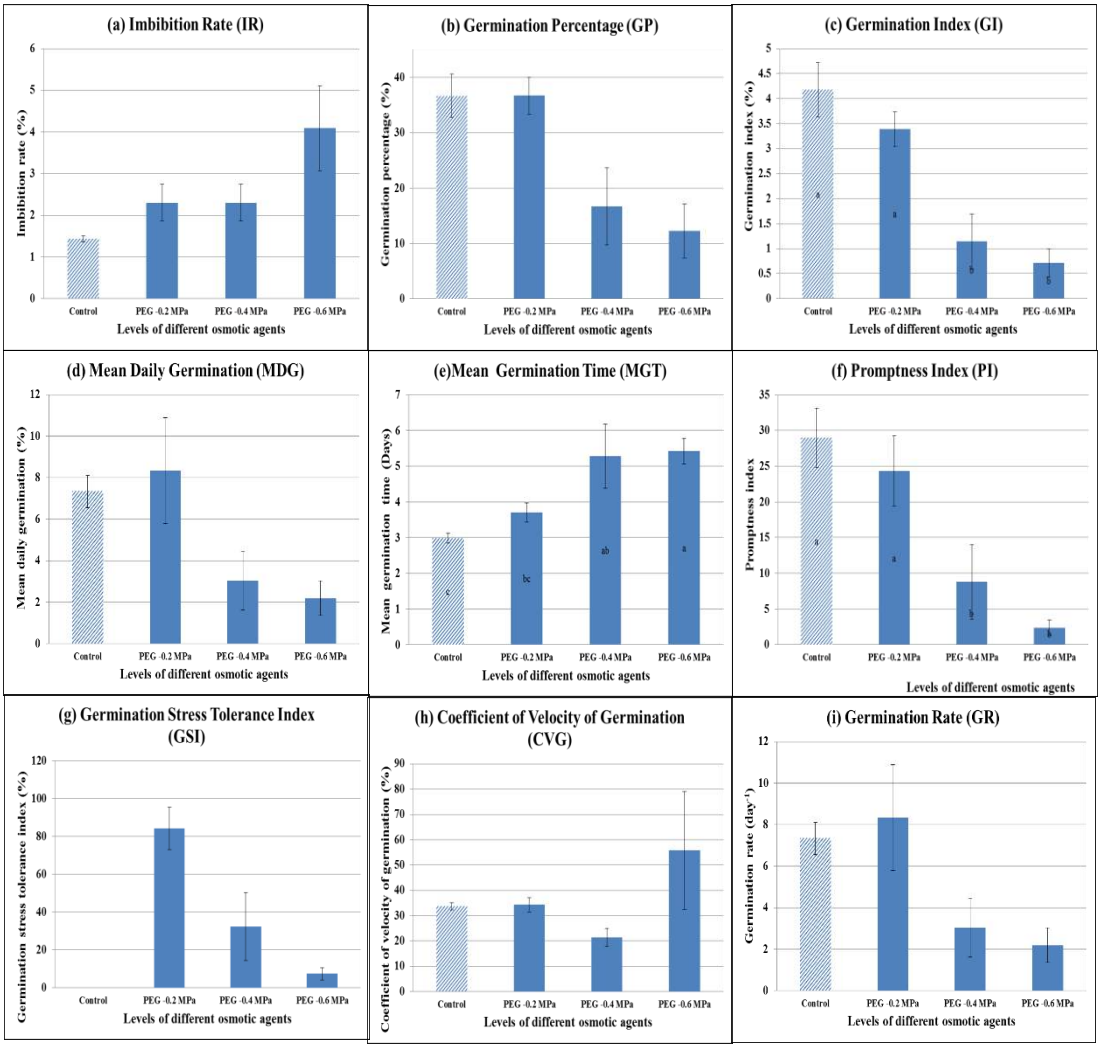

Fig. 4.1.3 Means of the different germination parameters for *C. virgatus* under three osmotic levels of PEG. Means with different letters are significantly different at P≤0.05.

4.1.4 *Atriplex leucoclada* Boiss

Local/ Arabic name: Ragal, رغل

GP and PI of *Atriplex leucoclada* were significantly affected by the OA and OL interaction. MDG, MGT, CVG and GR were significantly affected only by OL. GI and GSI were significantly affected both by OA and OL. GP and PI was higher for PEG treatment as compared to NaCl. However, decreased in above parameters due to decreasing OLs was more in PEG as compared to decrease in NaCl. MDG, CVG and GR decreased with decreasing OLs while MGT increased with decreasing OLs. GI and GSI were more for PEG as compared to NaCl and decreased with decreasing water potential level.

The GP showed significant interaction for OA*OL. Highest GP was recorded for control (98.9 %) followed by control 95.6 % and 87.8 % for PEG -0.2 MPa and PEG -0.4 MPa, while the lowest GP was 22.22 % recorded at NaCl -0.6 MPa (Fig. 4.1.4b). The Germination Index was significantly affected by OA and OL. Overall, GI was higher for PEG (11.14 %) and lower for NaCl (6.71 %). GI decreased with decreasing OL. GI was 15.83 %, 9.16 %, 7.79 % and 2.93 % for control, -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4.1.4c). ANOVA for OL showed statistically significant results for MDG and MGT. For different OL, MDG was 32.96 %, 19.91 %, 13.35 % and 5.98 % for control, -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4.1.4d). MGT was 2.02 days, 2.30 days, 2.64 days and 4.3 days for control, -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4.1.4e). Interaction of OA and OL also affected the promptness index. Highest PI (96.22) was observed for control while lowest (5.44) was observed for NaCl at -0.6 MPa water potential (Fig. 4.1.4f). OA and OL had significant effect on GSI. PEG treatment had GSI of 50.22 % and NaCl had GSI of 13.26 %. GSI was 59.96 %, 50.39 % and 17.02 % for -0.2, -0.4 and -0.6 MPa; respectively (Fig. 4.1.4g). CVG significantly affected by OL. Maximum CVG (50 %) was observed for the control while the minimum (25 %) was recorded for -0.6 MPa (Fig. 4.1.4h). GR was also significant for OA and OL. Control treatment had maximum GR of 32.96 day^{-1} while minimum GR was 5.98 day^{-1} recorded by -0.6 MPa (Fig. 4.1.4i).

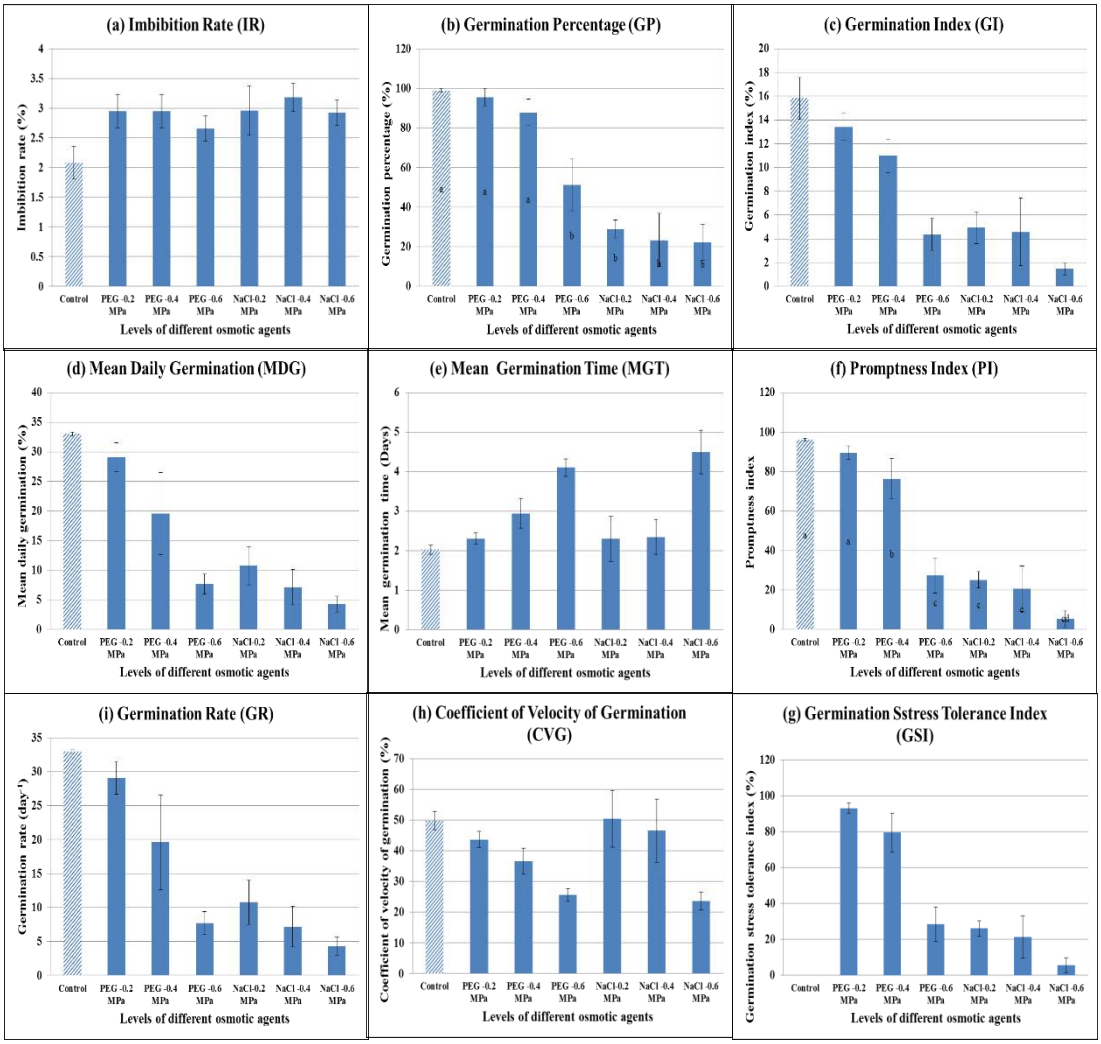

Fig. 4.1.4 Means of the different germination parameters for *A. leucoclada* under three osmotic levels of PEG and NaCl. Means with different letters are significantly different at P≤0.05.

4.1.5 *Senna italica* Mill.

Local/Arabic name: Ishraj, عشرج

GP and GI were significantly affected by the OA and OL. MGT, PI and CVG were significantly affected by the OA and OL interaction. While MDG, GSI and GR was affected by OL only.

GP and GI were lower for NaCl as compared to PEG and decreased with decreasing OL. MGT increased with decreasing OL. However, MGT increased more in PEG than the NaCl. PI and CVG decreased more by decreasing OL of PEG as compared to decreasing OL of NaCl. MDG and GSI decreased with decreasing OLs. GR increased with decreasing OL.

GP was significantly affected both by OA and OL. PEG and NaCl had 24.44 % and 31.39 % GP respectively, while different OL had GP of 43.33 %, 33.89 %, 20.00 % and 14.44 % for control -0.2 MPa, -0.4 MPa and -0.6 MPa water potential respectively (Fig. 4.1.5b). For the GI, ANOVA revealed a significant effect of OA and OL. GI was 3.25 % and 4.92 % for PEG and NaCl; respectively. GI was 6.32, 6.38, 2.42 and 1.22 % for Control, -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4.1.5c). MDG was significantly affected by OL. Control had the maximum MDG of 14.44 day^{-1} while -0.6 MPa had the minimum MDG of 2.73 day^{-1} (Fig. 4.1.5d).

MGT was significantly affected by OL and OA interaction. Highest MGT was 6.17 days at PEG -0.6 MPa and lowest MGT was 2.2 days for NaCl -0.2 MPa (Fig. 4.1.5e).

PI also showed a significant impact of OL and OA interaction. Highest PI (42.44) was recorded for control while lowest (0.64) was observed for PEG -0.6 MPa solution (Fig. 4.1.5f). Different OL affected the GSI. Maximum GSI of 78.2 % was recorded for -0.2 MPa while -0.6 MPa had minimum GSI of 23.01 % (Fig. 4.1.5g). ANOVA revealed a significant interactive effect of OA and OL on CVG. CVG was observed as 75 % for PEG -0.6 MPa and minimum (31.2 %) was recorded for NaCl -0.6 MPa (Fig. 4.1.5h). GR was affected significantly by different OL. Maximum GR was recorded for -0.6 MPa i.e. 2.56 day^{-1} while minimum was recorded for control i.e. 1.45 day^{-1} (Fig. 4.1.5i).

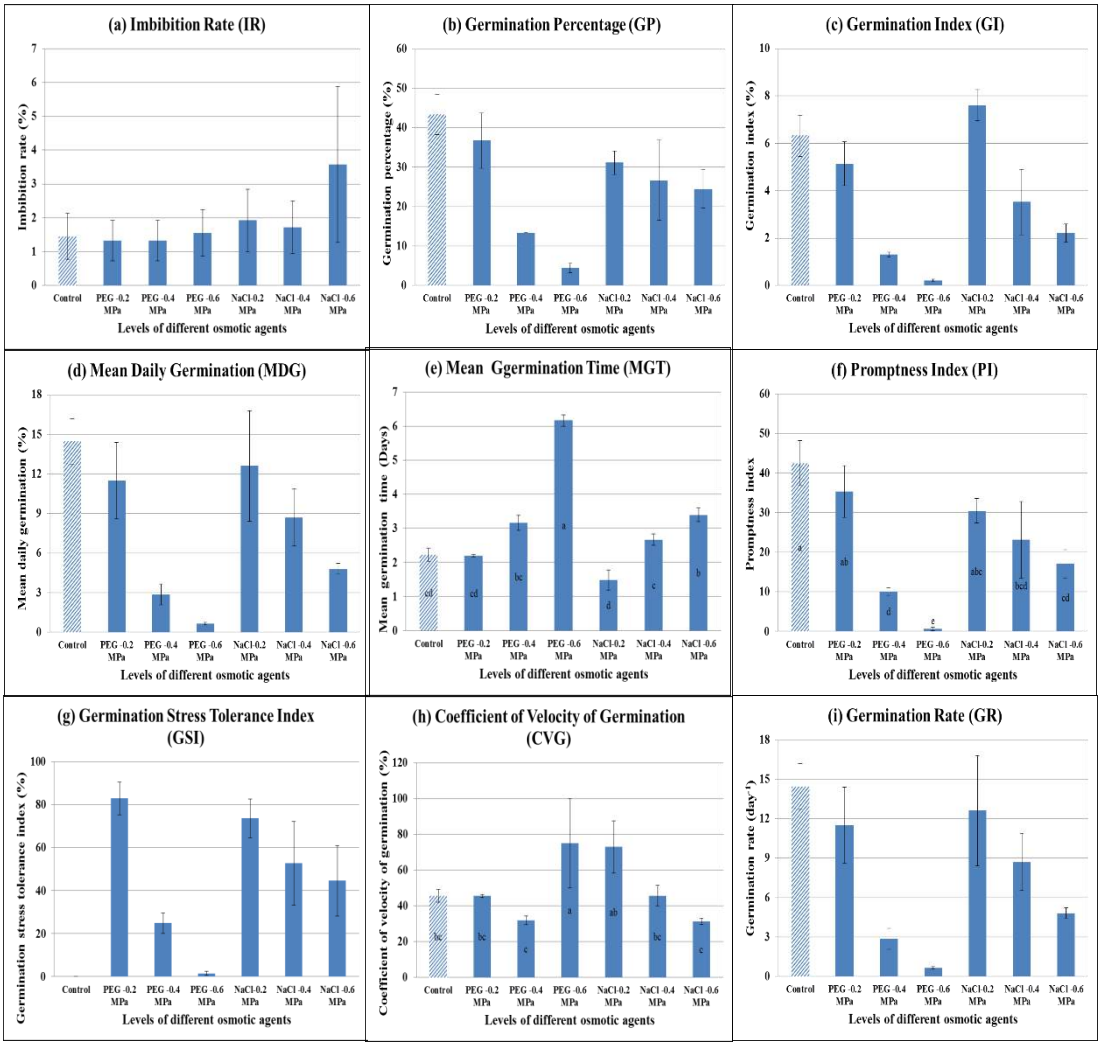

Fig. 4.1.5 Means of the different germination parameters for *S. italica* under three osmotic levels of PEG and NaCl. Means with different letters are significantly different at P≤0.05.

4.1.6 *Taverniera glabra* Boiss.

Local/ Arabic name: Ward-e-Jabal, ورد الجبل

GP, GI, MDG, MGT, PI, GSI and GR of *T. glabra* was significantly affected by OA*OL. CVG was significantly affected by OL only. GP, GI, MDG, PI, GSI, CVG and GR of *T. glabra* were higher for PEG as compared to NaCl. MGT was higher for NaCl as compared to PEG. All the studied parameters decreased with decreasing OL more in PEG as compared to NaCl (Fig. 4.1.6). However, MGT was increased more for NaCl as compared to PEG. In conclusion *T. glabra* was more resistant to low level of PEG. Higher level of PEG and all levels of NaCl adversely affected all germination parameters.

Control treatment had the maximum GP of 54.44 % while NaCl solution at -0.6 MPa had minimum GP of 0.00 % (Fig. 4.1.6b). Maximum GI was 7.73 % recorded for control treatment while minimum GI was 0.00 % recorded for NaCl -0.6 MPa (Fig. 4.1.6c).

MDG was more affected by PEG than by NaCl. Maximum MDG was 14.11 % for control treatment while MDG was 0.75 % for NaCl -0.4 MPa while no seed germinated at NaCl -0.6 MPa during studied period (Fig. 4.1.6d). MGT increased with decreasing water potential. Maximum MGT was 7.33 days for NaCl -0.4 MPa while minimum MGT was 2.27 days for PEG -0.2 MPa followed by 2.32 days for control (Fig. 4.1.6e).

Maximum PI was 51.4 recorded for control treatment whereas minimum PI was 0.00 recorded for NaCl -0.6 MPa (Fig. 4.1.6f). *T. glabra* had maximum GSI (74.5 %) under control treatment while minimum GSI (0.00 %) under NaCl -0.6 MPa (Fig. 4.1.6g).

Maximum CVG was observed for control (44.09 %) while minimum CVG was recorded for -0.6 MPa (27.74 %) (Fig. 4.1.6h). Control treatment had the highest average GR of 14.11 day^{-1} followed by PEG -0.2 MPa (11.48 day^{-1}) while NaCl -0.4 MPa had the minimum average germination rate of 0.75 day^{-1} (Fig. 4.1.6i).

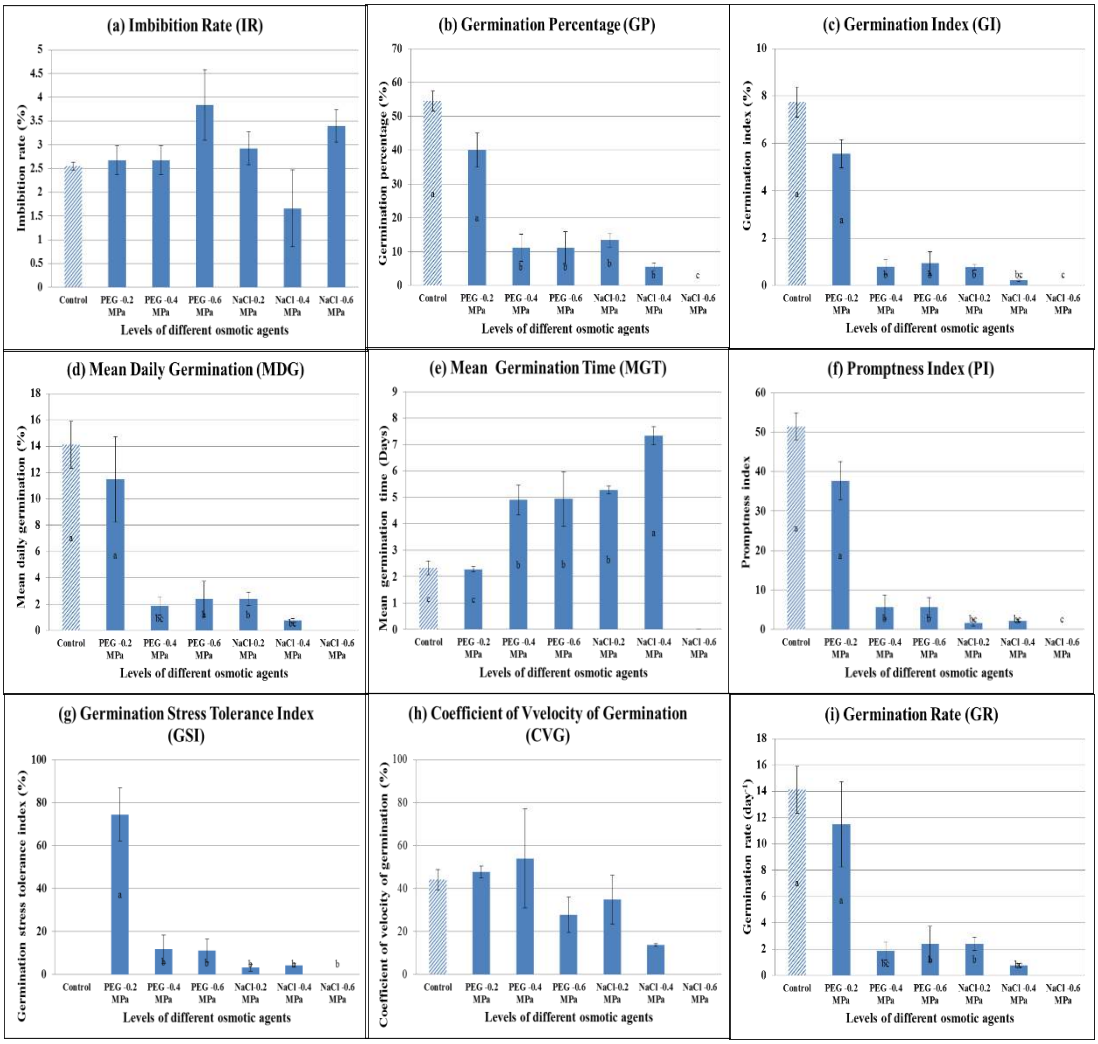

Fig. 4.1.6 Means of the different germination parameters for *T. glabra* under three osmotic levels of PEG and NaCl. Means with different letters are significantly different at P≤0.05.

4.1.7 *Tephrosia apollinea* (Delile)

Local/ Arabic name: Zafra, ظفره

GP, GI, MDG, PI, GSI and GR were significantly affected by the OL and have no significant effect of OA. MGT had a significant interactive effect of OA and OL. IR and CVG had non-significant effect of OA and OL. GP, GI, MDG, PI, GSI and GR reduced with decreasing water potential. However, MGT increased with decreasing OL. This increase in MGT was more prominent in NaCl as compared to PEG (Fig. 4.1.7).

T. apollinea revealed a significant effect of OL on GP. The average GP was 38.89 %, 30.56 %, 23.33 % and 12.78 % for control, -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4.1.7b). GI was also affected significantly by different OL. GI decreased with decreasing water potential of PEG and NaCl and was 3.92, 3.92, 2.23 and 0.89 % for control, -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4.1.7c). MDG was significantly affected by the different OL. As OL decreased, so did MDG. The average of MDG was 8.50 %, 7.69 %, 4.87 % and 2.19 % for control, -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4.1.7d). For MGT ANOVA showed a significant result for OA and OL. PEG had MGT of 4.04 days and NaCl had MGT of 3.43 days. MGT was 3.36 days, 2.57 days, 3.75 days and 5.26 days for control, -0.2, -0.4 and -0.6 MPa; respectively (Fig. 4.1.7e). Different OL also affected the PI. Highest PI was 31.31 and 26.32 for control and -0.2 MPa; respectively. Minimum PI (5.01) was recorded for -0.6 MPa (Fig. 4.1.7f). GSI was significantly affected by different OL. Average GTI for different OLs was 85.0 %, 53.09 % and 17.85 % for -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4.1.7g). GR of *T. apollinea* was significantly affected by OL. Highest GR (8.5 day^{-1}) was observed for control while lowest GR (2.19 day^{-1}) was recorded for PEG -0.6 MPa (Fig. 4.1.7i).

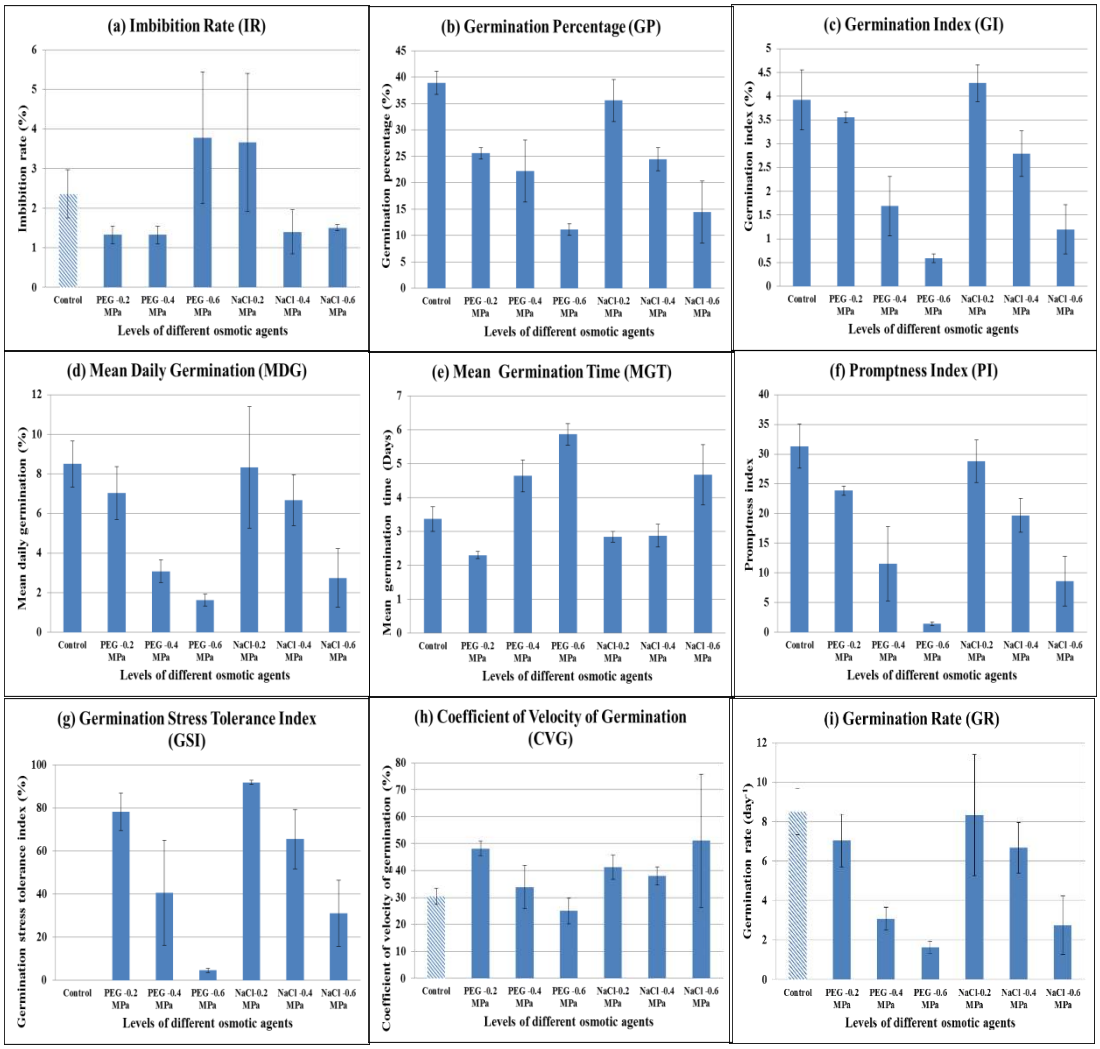

Fig. 4.1.7 Means of the different germination parameters for *T. apollinea* under three osmotic levels of PEG and NaCl. Means with different letters are significantly different at P≤0.05.

4.1.8 *Tetraena mandavillei* (Hadidi) Beier & Thulin

Syn. Zygophyllum mandavillei (Moq.)

Local/ Arabic name: Haram, هرم

For *T. mandavillei* most of the measured parameters i.e. GP, MDG, MGT, PI and GR were significantly affected by OL of different OA. GI had an interactive effect of OA and OL. GSI was significantly affected by OA, while IR and CVG were not significantly affected by OA or OL. GP, MDG, PI and GR were significantly decreased with decreasing OL. MGT increased with decreasing OL. GSI of *T. mandavillei* was higher for PEG as compared to NaCl.

The average GP was 23.3, 16.7, 15.6 and 18.3 % for control, -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4b). GI was significantly affected by interaction of OA and OL. Maximum GI was 4 % for control and minimum GI was 1.77 % for NaCl -0.2 MPa; respectively (Fig. 4.1.8c). MDG was also significantly affected by the different OL. As OL decreased so did the MDG (Fig. 4.1.8d). The average of MDG was 7.78, 5.14, 4.53 and 3.29 % for control, -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4.1.8d). ANOVA analysis of MGT showed a significant effect for different OL. MGT was 1.83, 2.29, 1.7 and 2.63 days for control, -0.2 MPa, -0.4 MPa and -0.6 MPa; respectively (Fig. 4.1.8e). ANOVA results showed that OL affected the PI. PI decreased with decreasing OL. PI was 23.33, 15.74, 15.22 and 16.22 for control, -0.2, -0.4 and -0.6 MPa; respectively (Fig. 4.1.8f). GSI for PEG (53.13 %) was significantly higher than NaCl (49.47). GR of *T. mandavillei* was significantly affected by OL. Average GR was 7.78, 5.51, 4.53 and 3.29 day^{-1} for -0.2, -0.4 and -0.6 MPa; respectively (Fig. 4.1.8i).

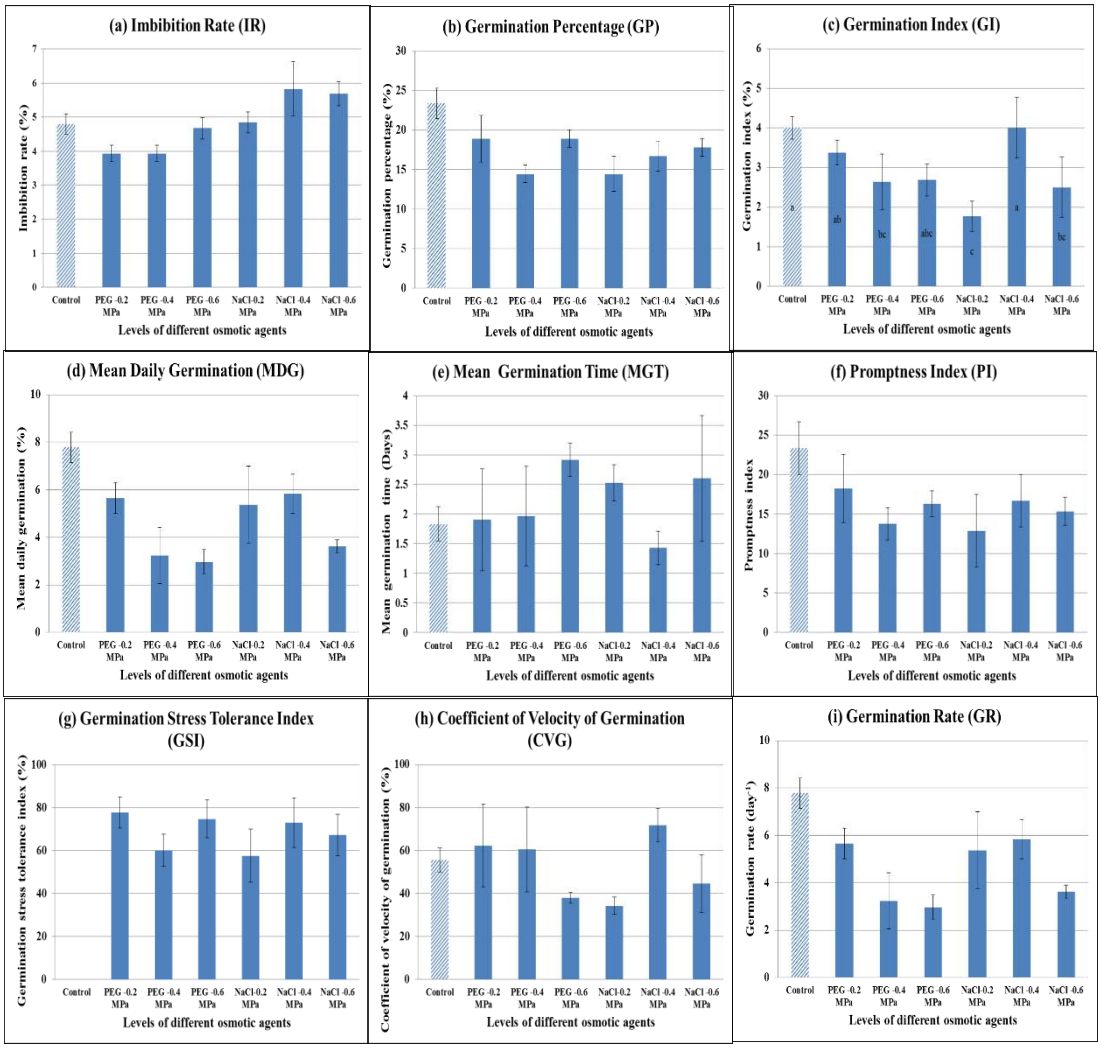

Fig. 4.1.8. Means of the different germination parameters for *T. mandavillei* under three osmotic levels of PEG and NaCl. Means with different letters are significantly different at P≤0.05.

4.1.9 *Salsola imbricata* Forssk

Local/ Arabic name: Ghazraf, غضرف

GP, GI, MDG, PI and GR of *S. imbricata* were significantly affected by osmotic agents (OA) and osmotic levels (OL). MGT was significantly affected by the OA*OL. GSI and CVG were significantly affected by OA only.

GP, GI, MDG, PI, GSI, CVG and GR were lower for PEG as compared with NaCl and decreased with the decreasing OL. GP, GI, MDG, PI and GR decreased with decreasing OL. MGT was higher for NaCl treatment and increases with decreasing OL.

GP for NaCl (83.89 %) was significantly higher than PEG (60.83 %). Control treatment had maximum germination of 90 % while lowest GP was (56.67 %) recorded for -0.6 MPa (Fig. 4.1.9b). GI was higher for NaCl (12.8 %) and lower was recorded for PEG (11.7 %). Maximum GI was observed for control treatment (15.5 %) while minimum was observed at -0.6 MPa (9.1 %) (Fig. 4.1.9c). NaCl had significantly higher MDG (18 %) than MDG of PEG i.e. 15 %. Control treatment had maximum MDG of 21 %, while -0.6 MPa had minimum MDG of 12 % (Fig. 4.1.9d).

Maximum MGT was 2.88 and 2.8 days recorded for NaCl -0.6 and NaCl -0.4 MPa respectively while PEG -0.4 MPa treatment had minimum MGT of 1.86 days (Fig. 4.1.9e). PI for NaCl (69.08) was higher than PEG (56.4). Control treatment had maximum PI of 81.53 and -0.6 MPa had minimum PI of 47.63 (Fig. 4.1.9f).

GSI was significantly affected by OA and was 59 % and 80 % for PEG and NaCl respectively (Fig. 4.1.9g). NaCl had higher CVG of 51.3 % while PEG had lower CVG of 39.9 %. GR was 15.16 day^{-1} for PEG and 17.89 day^{-1} for NaCl while GR was 21.00, 18.3, 14.88 and 11.92 day^{-1} for control, -0.2, -0.4 and -0.6 MPa respectively.

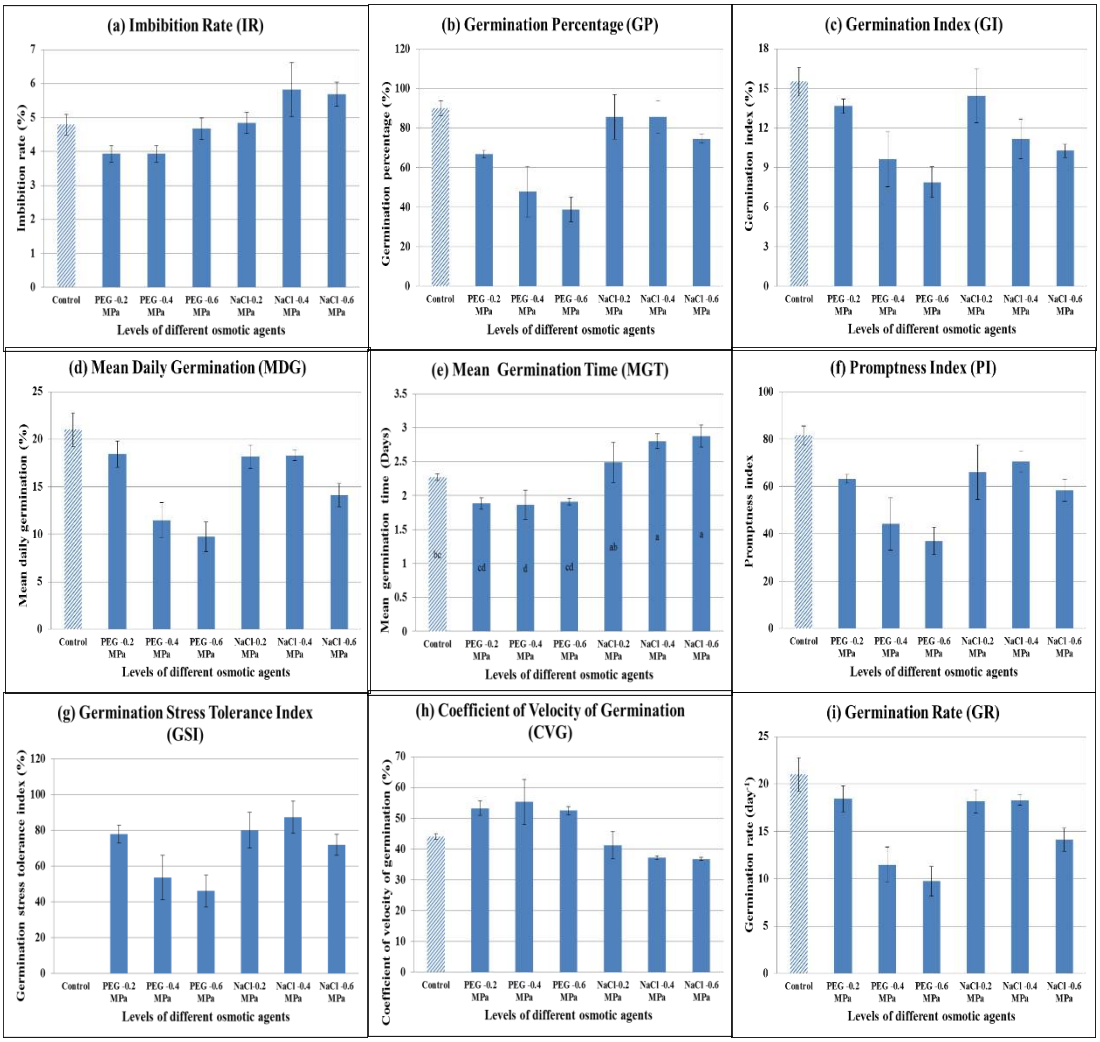

Fig. 4.1.9. Means of the different germination parameters for *S. imbricata* under three osmotic levels of PEG and NaCl. Means with different letters are significantly different at P≤0.05.

4.1.10 Discussion

In our experiment entitled "Native plant's adaptation to salt and water stress during seed-germination stage" nine native plant species were germinated under osmotic stress levels induced by NaCl and PEG.

Different species respond differently to isotonic solutions of NaCl during seed germination (Tobe *et al*., 2001). Germination was significantly inhibited by stress levels of NaCl in *R. stricta* and *C. virgatus* as compared to PEG. It might be because NaCl having adverse effect on seed germination due to ion toxicity (Bal and Chattopadhyay, 1985; Tobe *et al*., 2001; Wu *et al.*, 2011).

PEG stimulate drought and induced water stress (Zhang and Kirkham, 1996). Under water stress conditions germination percentage was significantly reduced by PEG as compared to NaCl (Abudjain, 2003). Mostafavi and Golzardi (2012) reported significant decrease in germination percentage by NaCl due to ion toxicity of NaCl on seeds (Bal and Chattopadhyay, 1985; Tobe *et al*., 2001; Wu *et al.*, 2011).

However, GP in other species including *A. leucoclada* and *T. glabra* was decreased with decreasing OL for PEG. The decrease in GP by PEG was less than that of decrease in GP by NaCl. Here, the maximum GP was shown by *A. leucoclada* i.e. 98.89 % at -0.2 MPa PEG. *A. leucoclada* was less affected by PEG as compared to NaCl, whereas the minimum GP was observed in *T. glabra* i.e. 0.00 % at -0.6 MPa NaCl. The ANOVA revealed a significant interaction of osmotic agents and osmatic levels ($P \leq 0.05$). Afzali *et al*. (2006) had similar results for *Matricaria chamomilla;* Katembe *et al*. (1998) for *Atriplex prostrata* and *A. patula* and Shahriari and Davari (2015) for *Alyssum hamalocarpum.* Seed germination all these species was more affected by NaCl than by iso-osmotic PEG solution.

S. italica and *L. pyrotechnica* were adversely affected more by PEG as compared to NaCl. In case of *L. pyrotechnica* germination was inhibited completely at -0.6 MPa PEG. NaCl causes osmotic stress along with specific ion toxicity. However, the above species are adapted to salinity more efficiently. This may be due to Na^+ ions accumulation in the seed embryo that allow water uptake during germination (Shitole and Dhumal, 2012).

Our results for *S. italica* and *L. pyrotechnica* are in line with many authors who reported more depressing effects of PEG compared to specific ion effect by NaCl e.g. cowpea (Murillo-Amador *et al.*, 2002), sugar beet (Sadeghian and Yavari, 2004) and several halophytes (Ungar, 1978). It can be concluded that at equivalent water potentials osmotic stress by PEG may have more inhibitory effects then NaCl (Okçu, *et al.*, 2005). One explanation of this reduction could be, that plants grown in PEG containing media may had suffered from hypoxia because of a large viscosity possibility that a boundary layer of oxygen depleted solution may form around the roots (Verslues *et al.*, 1998). Similar results are reported for *Solanum melongena* (Demir *et al*., 2003), Sun flower (*Matricaria chamomilla*) (Afzali *et al*., 2006) and pea seeds (Petrović *et al*., 2016).

In the present investigation, *T. apollinea* and *T. mandavillei* were found to be resistant to both iso-osmotic conditions of drought and salt produced by PEG and NaCl and there was no significant difference for both osmotic agents. GP however, slightly decreased by decreasing water potential. GSI was also not significant for both PEG and NaCl. MGT increased with increasing osmotic potential level while MDG decreased with decreasing osmotic potential level. El-Keblawy and Al-Shamsi (2008) reported similar results with no significant differences in germination at lower concentrations of NaCl (0-300 mM), all of them attained significantly greater germination, compared to the higher concentrations (400-800 mM). Khan and Ungar (1997) also indicated that *Zygophyllum simplex* seeds were tolerant to moderate salinity but germination reduced by increasing salinity. Agami (1986) reported for *Zygophyllum dumosum* which reduced germination by increasing salinity but still occur even at 0.5 M NaCl. Ismail (1990) also reported negative effects of increasing salinity of germination.

GP was affected significantly ($P \leq 0.05$) by different levels of osmotic agents for most of the species except *C. virgatus*. GP decreased with decreasing the osmotic potential. *L. pyrotechnica* and *A. leucoclada* had the maximum germination percentages (96.7 % and 98.9 %) at -0.2 MPa PEG. GI also affected significantly ($P \leq 0.05$) by different levels of osmotic agents. Maximum GI was calculated as 15.17 % for PEG (-0.2 MPa) by *A. leucoclada* whereas the minimum GI was 0.0 % by NaCl (-0.6 MPa) in *R. stricta, T. glabra* and *C. virgatus*. GI decreased with decreasing water potential by NaCl.

MDG was more affected by PEG than by NaCl. Maximum MDG were 31.85 % in

control treatment of *A. leucoclada* while minimum MDG was 0 % in relation to *Rhazya stricta* for PEG (-0.6 MPa) level. Here, imbibition rate was not statistically affected by different osmotic agents ($P>0.05$). Different levels of osmotic agents had significant effect on MGT. The effect of PEG levels was more on MGT than NaCl. The maximum MGT was recorded for *Rhazya stricta* as 8.33 days at -0.4 MPa for PEG while minimum MGT of 1.83 days was recorded for *T. mandavillei* in control treatment. Throughout the experiment MGT increased with the increasing water potential. For Promptness index, osmotic agents, osmotic levels and interaction between them had shown a significant effect. In most of the treatments PI decreased with increasing water potential in PEG treatments whereas NaCl had a very limited role. The -0.2 MPa PEG treatment of *A. leucoclada* showed the maximum PI value as 21.40 whereas the minimum was shown by *R. stricta, T. glabra* and *L. pyrotechnica*.

ANOVA revealed a significant interaction of Osmotic Agents and their levels ($P\leq0.05$) for GSI in all the selected plants species except *R. stricta* which showed a non-significant relation between different osmotic agents. By the application of different levels of osmotic agents, the germination was decreased with the decrease of water potential.

Ahmad *et al*. (2009) had similar results in sunflower where GSI values were minimum at highest PEG concentration. In short, in the present investigation, *S. imbricata, T. mandavillei, T. apollinea, A. leucoclada* and *S. italica* stood out as the best plant species to survive induced salt and water stress at the germination stages.

4.2 Study 2: Assessment of salt and water stress response in native plants

Field study was conducted to assess the response of selective native plant species at four different salinity levels (5, 10, 15 and 20 dSm^{-1}) and four irrigation levels i.e. 100 % (control), 80 %, 60 % and 40 % of field capacity. Total five species were tested in field experiment. However, two species *T. apollinea* and *S. italica* didn't survived more than one month under stress conditions. Only three species including *S. imbricata*, *T. mandavillei* and *A. leucoclada* survived till end of experiment for six months. Data regarding different parameters was collected for three successful species. Chlorophyll content and Pr were determined weekly for six months. Data was analyzed for survival percentage, plant water potential, shoot weight, root weight, shoot length and root length every month up to six months. Anti-oxidant enzymes, ionic content, nutrient content, proline and abscisic acid were also determined at the end of experiment.

Here, results are presented for each species investigated individually. For species discussed in this section, only the significant parameters are discussed. Non-significant parameters are represented in respective Figures. Results of experiments carried out for different plant species in respect to their growth, biochemical and ionic constituents are explained below.

4.2.1 *Salsola imbricata* Forssk

Local/ Arabic name: Ghazraf, غضرف

Data regarding different parameters of *S. imbricata* as affected by different levels of salt and water stress is shown in Fig. 4.2.1.1- 4.2.1.19. Analysis of variance (ANOVA) is presented in respective Appendix. 4.2.1.1- Appendix 4.2.1.19.

4.2.1.1 Survival Percentage (%)

Data regarding survival percentage of *S. imbricata* as affected by different levels of salt and water stress is presented in Fig. 4.2.1.1 and their analysis of variance in Appendix 4.2.1.1. Both salt and water stress levels and the interaction between them had the non-significant effect on the survival percentage of *S.* imbricata ($P>0.05$). Survival

percentages of *S. imbricata* ranged between 90-98 % for different salt and water stress levels. In conclusion data showed that *S. imbricata* survived successfully for six months under all salt and water stress levels applied.

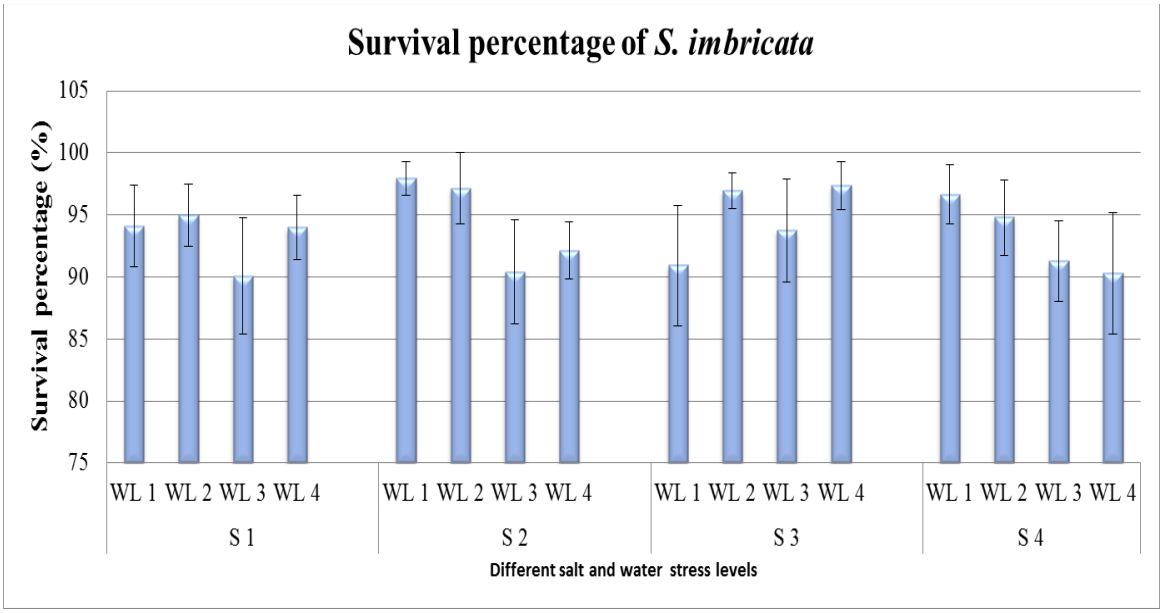

Fig. 4.2.1.1 Survival percentage of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15dS m^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.2 Shoot dry weight (SDW) (g)

Results of the interaction between salt and water stress on SDW of *S. imbricata* are presented in Fig. 4.2.1.2. and the ANOVA in Appendix 4.2.1.2. Analysis of variance revealed that different water levels significantly affected ($P \leq 0.05$) SDW of *S. imbricata* while salt stress and salt x water stress had no significant effect ($P > 0.05$) on SDW of *S. imbricata*. SDW tended to incline during the cores of experiment despite all salinity and water stress. Water deficit declined the SDW at low salinity levels only, while at higher salinity level of S4, water stress had shown no negative effect on the SDW of *S. imbricata*.

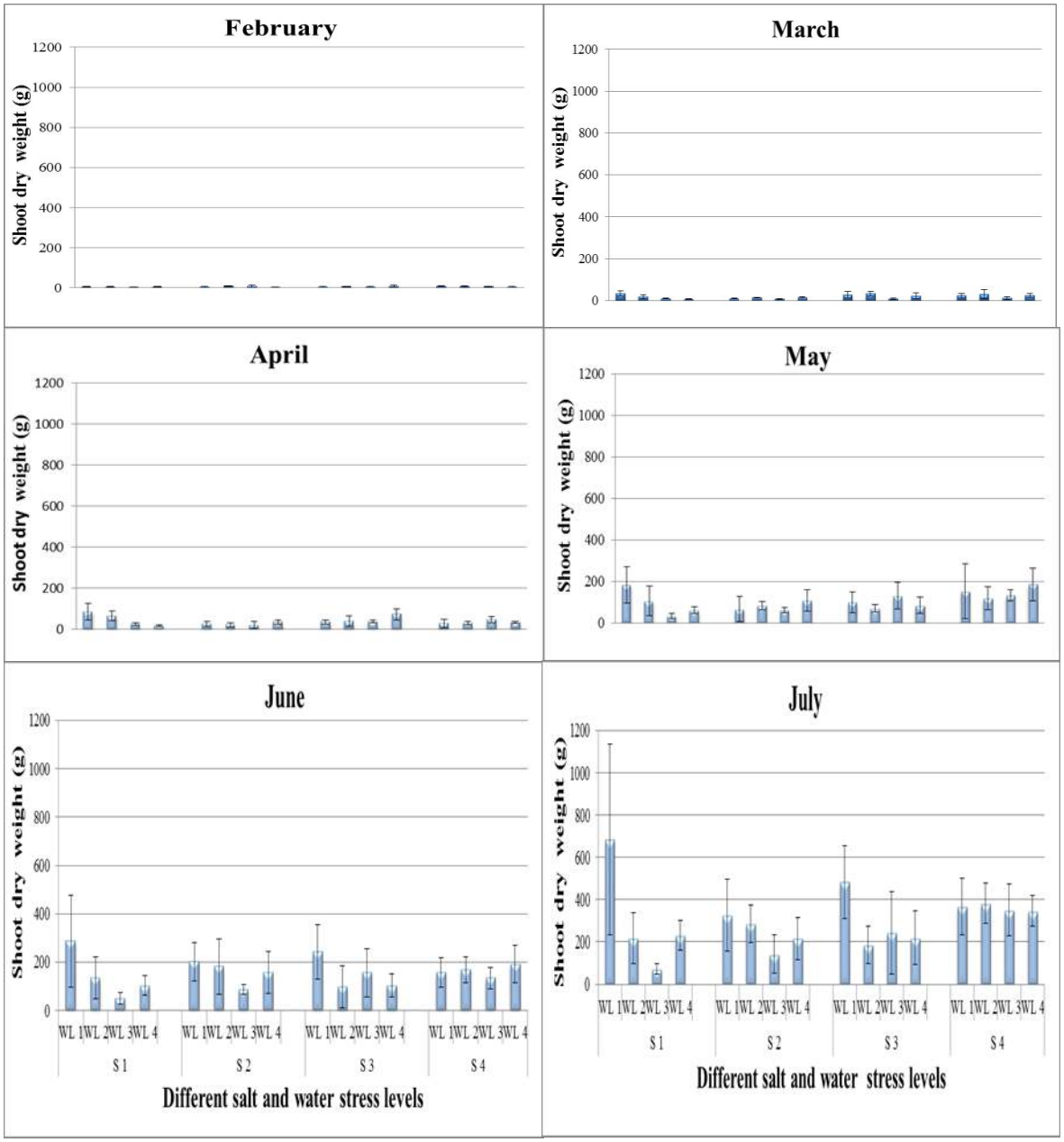

Fig. 4.2.1.2 Shoot dry weight of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.3 Root dry weight (RDW) (g)

Effect of salt and water stress on RDW of *S. imbricata* is presented in Fig. 4.2.1.3. Analysis of variance showed a significant effect ($P \leq 0.05$) of different water stress levels and time interval (month) on RDW of *S. imbricata* (Appendix 4.2.1.3.). However, salt stress levels had non-significant effect ($P > 0.05$) on RDW of *S. imbricata*. Water stress reduced the RDW at low salt stress levels only, while at higher salinity level of S4 water stress had no negative effect on the SDW of *S. imbricata.*

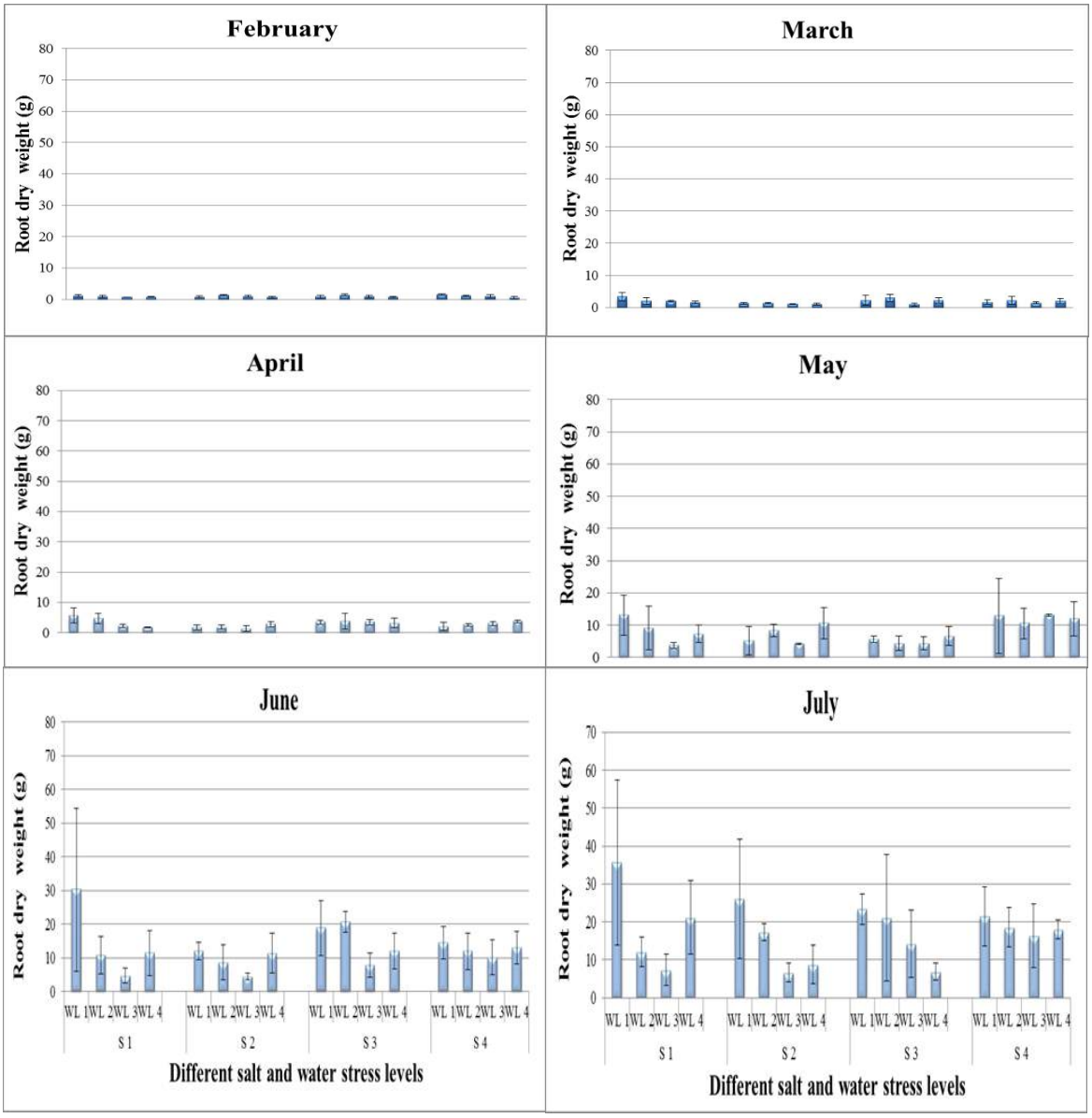

Fig. 4.2.1.3 Root dry weight of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.4 Shoot length (SL) (cm)

SL as affected by different salt and water stress levels is presented in Fig. 4.2.1.4. and the ANOVA in Appendix 4.2.1.4. Different salt and water stress levels showed a significant interactive effect ($P \leq 0.05$) on SL of *S. imbricata*. At the end of experiment SL decreased with increasing water stress under S1. However, at salinities more than S1, SL increased with increasing water stress. SL (83.3 cm) was recorded for S1WL1 during the month of August. Lowest SL (24 cm) was observed for S3WL1 in March.

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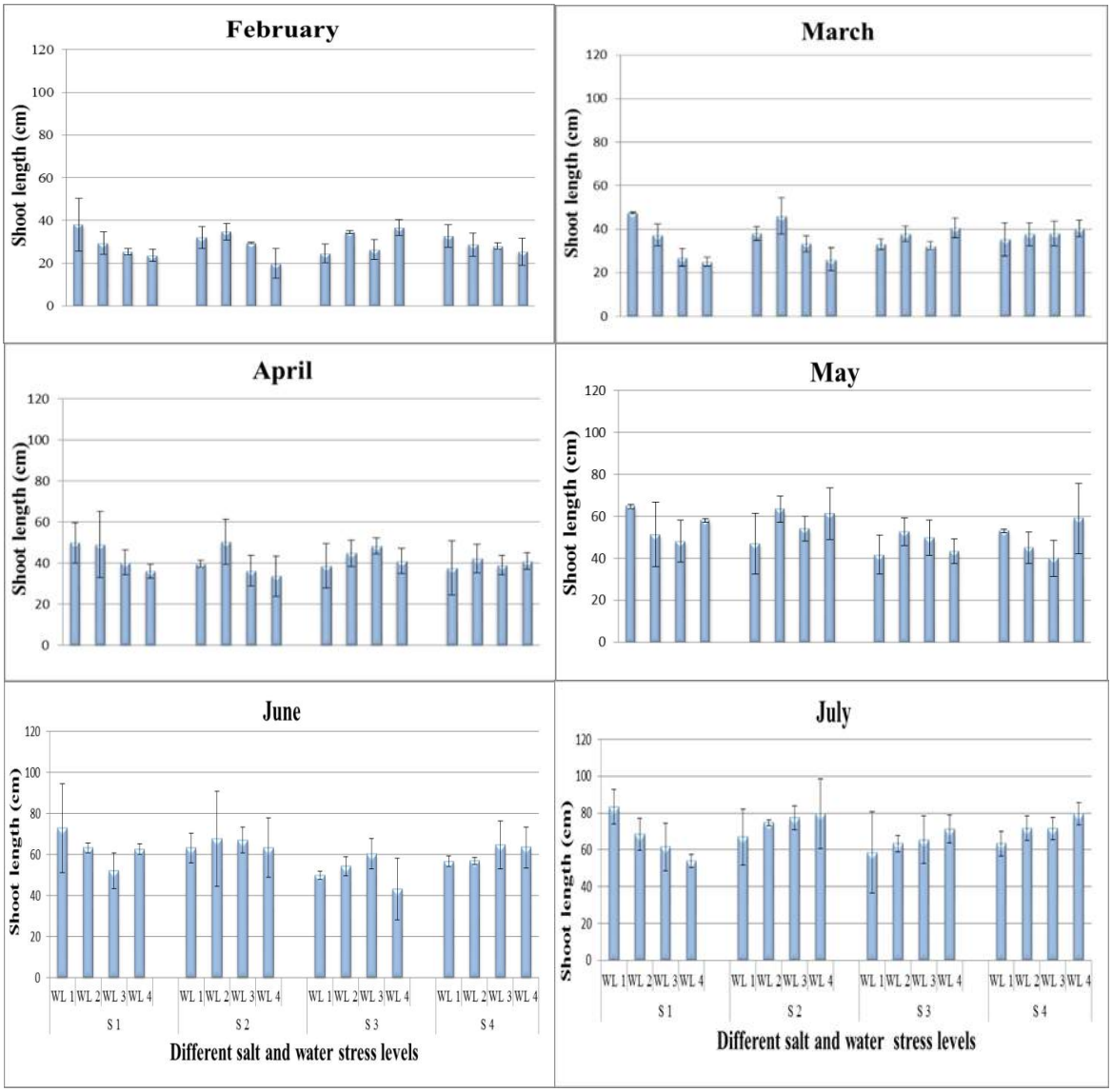

Fig. 4.2.1.4 Shoot length of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.5 Root length (RL) (cm)

Effect of salt and water stress on RL of *S. imbricata* is presented in Fig. 4.2.1.5 and the ANOVA in Appendix 4.2.1.5. Different salt and water stress levels showed a significant interactive effect ($P \leq 0.05$) on RL of *S. imbricata* (Appendix 4.2.1.5). Highest RL (70.7 cm) was recorded for S1WL1. Lowest RL (50.3 cm) was observed for S2WL4. In our experiment RL had interactive result for salt and water stress levels. Under salt stress conditions RL increased with increasing the water stress. Salt stress together with water stress stimulated the RL.

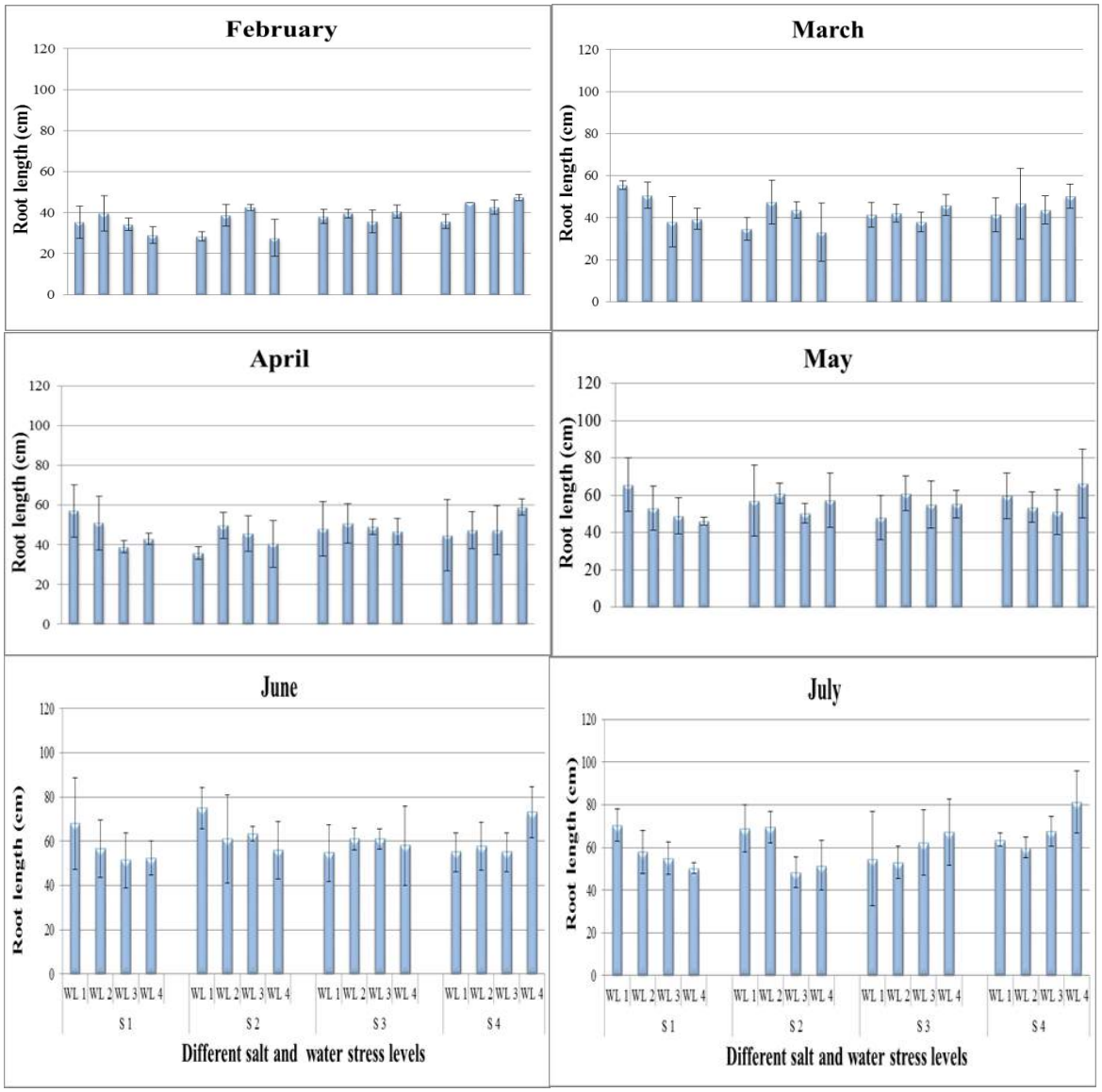

Fig. 4.2.1.5 Root length of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.6 Water use efficiency (WUE) ($g.L^{-1}$)

Results of different salt and water stress levels on WUE of *S. imbricata* are presented in Fig. 4.2.1.6 and the ANOVA in Appendix 4.2.1.6. Water stress had significant effect (P≤0.05) on WUE of *S. imbricata*. ANOVA revealed that salt stress had non-significant (P>0.05) effect on WUE of *S. imbricata*. However, there was a non-significant increase in WUE of *S. imbricata* with increasing salt stress.

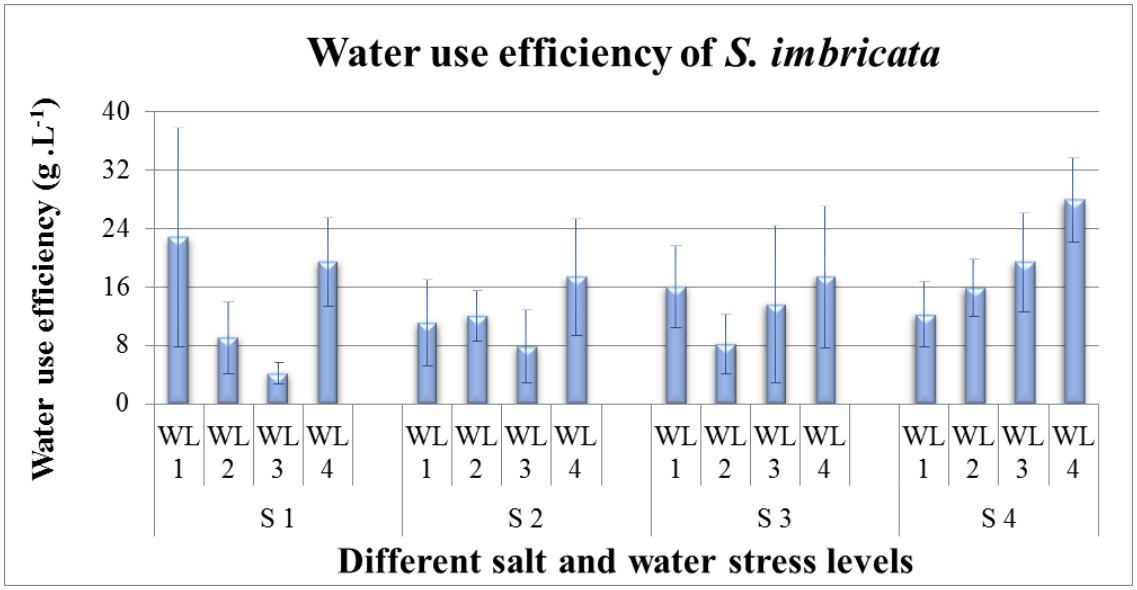

Fig. 4.2.1.6 Water use efficiency of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.1.7 Chlorophyll index

Results of different salt and water stress levels on chlorophyll index are presented in Fig. 4.2.1.7 and the ANOVA in Appendix 4.2.1.7. Analysis of variance revealed significant effect (P≤0.05) of different water stress levels and months (time) on chlorophyll index of *S. imbricata*, while salt stress and salt x water stress had no significant effect (P>0.05) on chlorophyll index of *S. imbricata*.

Chlorophyll index decreased during the experimental period despite all salinity and water stress levels and maximum chlorophyll index (1.82) was recorded for February while

minimum chlorophyll index (0.56) was recorded for month of June. Among the water stress levels WL1 had the higher chlorophyll index of 0.93 while WL4 had the lowest chlorophyll index of 0.75.

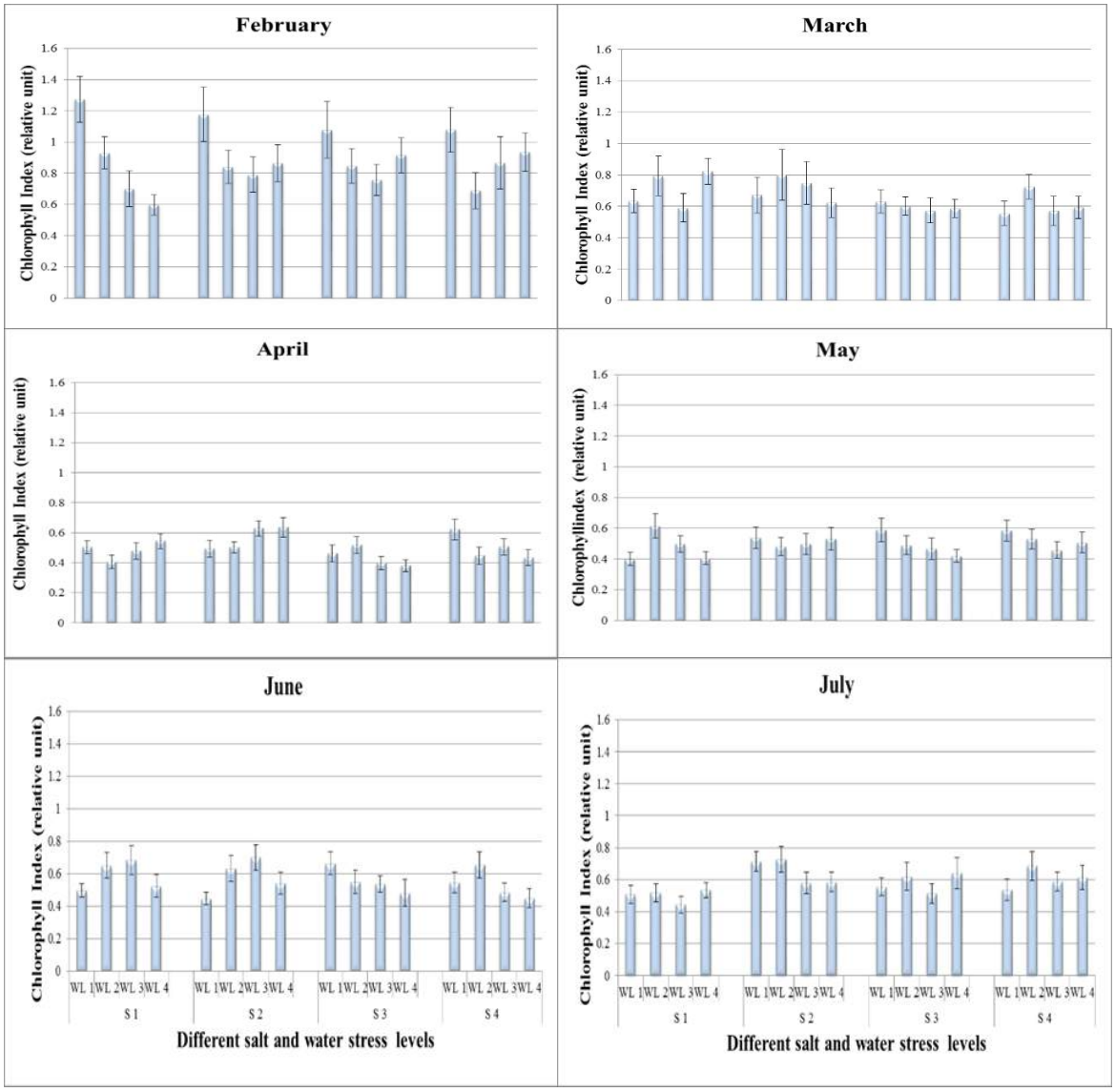

Fig. 4.2.1.7 Chlorophyll index of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.8 Photosynthetic rate (Pr) (µmol/m^2/s)

Data regarding Pr of *S. imbricata* as affected by different levels of salt and water stress is presented in Fig. 4.2.1.8 and their analysis of variance in Appendix 4.2.1.8. Different months, salt and water stress levels and salt x water stress had the significant ($P \leq 0.05$) effect on the Pr of S. *imbricata*. Pr was higher in the month of March and gradually starts decreasing with increasing temperature till the month of June. Highest Pr (84.63 µmol/m^2/s) was recorded during the month of March for SL1WL1 while lowest Pr (2.6 µmol/m^2/s) was recorded during the month of May for SL3WL2 and SL3WL4. Pr showed opposite results of water stress at lowest and highest salt stress level. At lower salt stress, Pr decreased with increasing water stress. On the other hand, Pr at highest salinity of S4 increased with increasing water stress level.

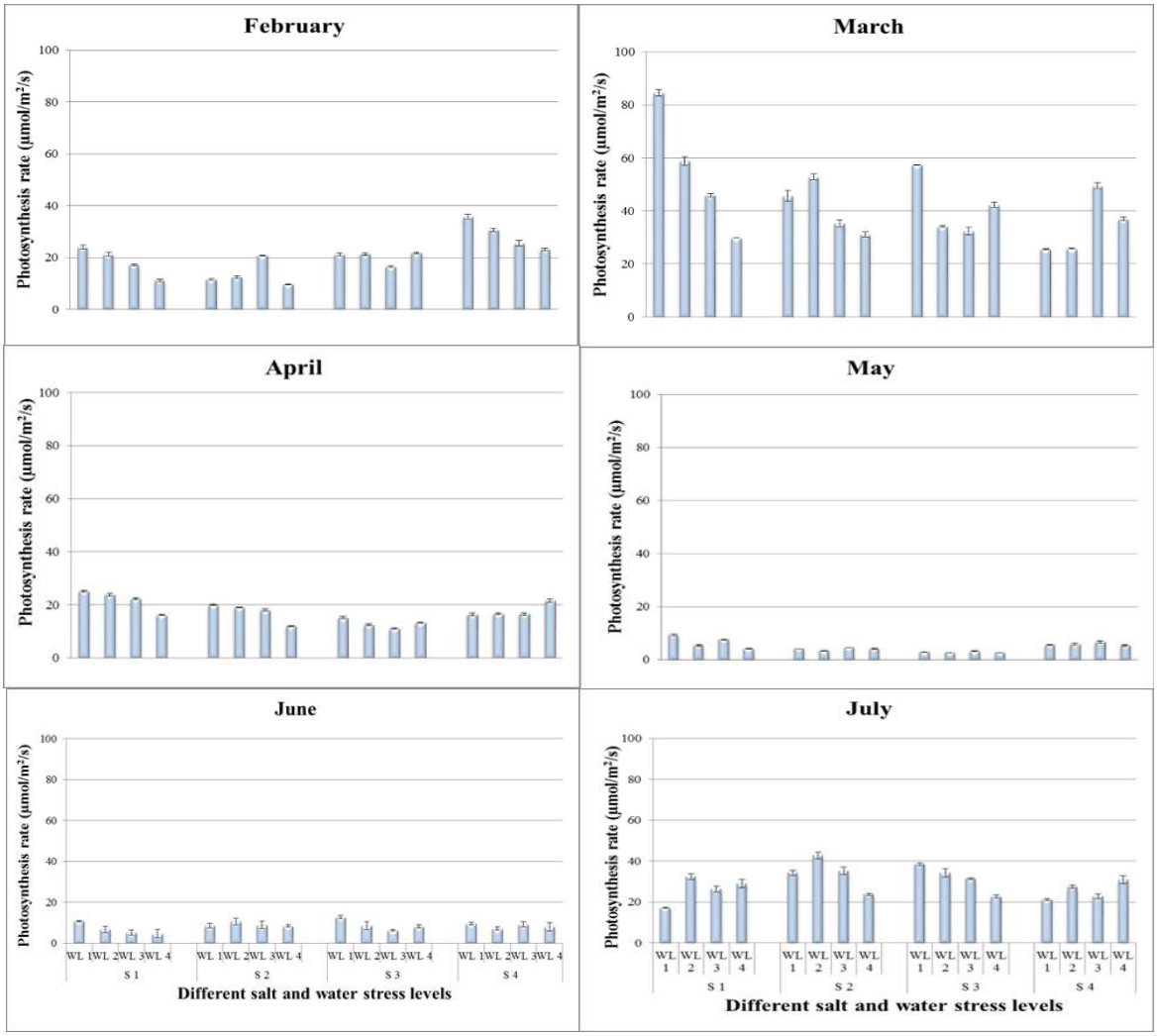

Fig. 4.2.1.8 Photosynthetic rate (Pr) of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.9 Leaf water potential (MPa)

Leaf water potential (LWP) was measured two times: after one month of treatment application and after five months of treatment application. LWP was significantly affected ($P \leq 0.05$) by salt and water stress levels both intervals (Appendix 4.2.1.9a and Appendix 4.2.1.9b). However, LWP after five months was much more negative than after one month. After one month of treatment application, LWP was significantly reduced ($P \leq 0.05$) by increasing salt and water stress. Contrary to this, LWP after five months was less affected by salinity stress. LWP decreased with increasing salt stress level from S1 until S3 and increased again at S4. Water stress had much more negative effect on the LWP which showed continuous decrease with increase in water stress from WL1 to WL3. However, lowest water stress levels i.e. WL3 and WL4 had statistically similar results ($P > 0.05$; Fig. 4.2.1.9).

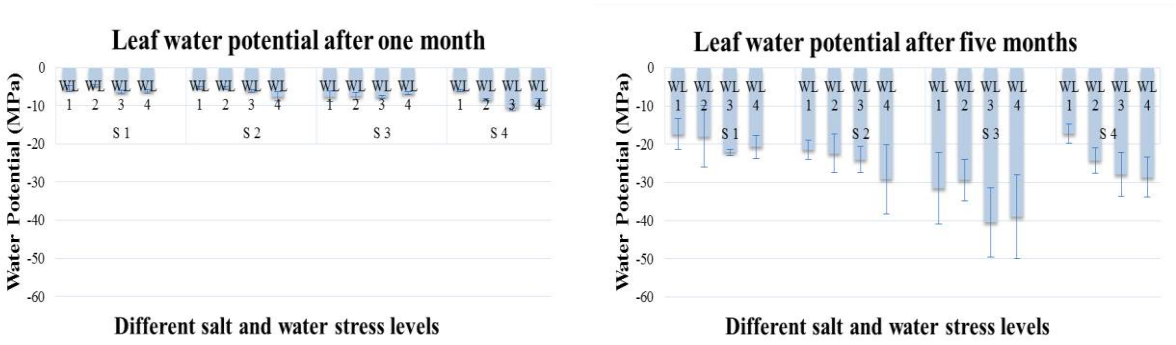

Fig. 4.2.1.9 Leaf water potential of *S. imbricata* at two different time intervals under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.10 Plant total Nitrogen (%)

Different salt and water stress levels and their interaction had the significant effect ($P \leq 0.05$) on the total plant nitrogen content of *S. imbricata* (Appendix 4.2.1.10). Maximum total nitrogen (0.763 %) was recorded at S1WL1 followed by 0.657 % at S2WL1. Lowest total plant nitrogen content was recorded for 0.677, 0.6, 0.343, 0.337 and 0.380 by S1WL4, S2WL4, S3WL3, S4WL3, and S4WL4 respectively. At the lower salinities plants showed decrease in total nitrogen with increasing water stress, while at highest salt stress level of S4 water stress didn't had any significant effect (Fig. 4.2.1. 10).

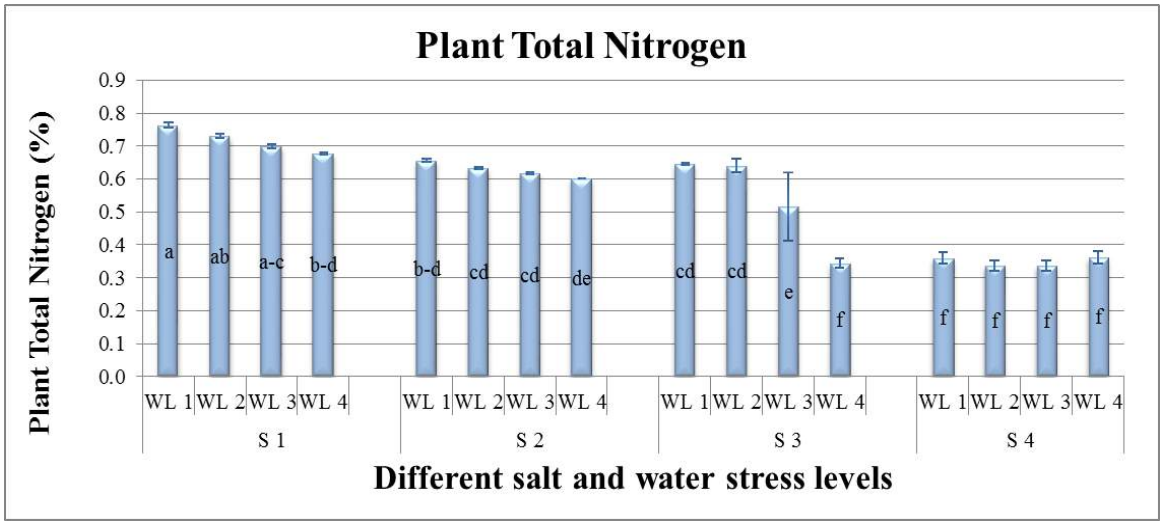

Fig. 4.2.1.10 Plant total nitrogen of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.11 Phosphorus content (%)

ANOVA table revealed that plant total phosphorus of *S. imbricata* was significantly affected ($P \leq 0.05$) by the different salt and water stress levels and their interaction (Appendix 4.2.1.11). Maximum plant total phosphorus (0.052 %) was recorded for S1WL1. Minimum plant total phosphorus (0.013 %) was recorded for S4 WL3. At minimum salt stress level phosphorous content was maximum, while higher salinity showed a drastic decline in phosphorus concentration. Water stress had negative impact on phosphorous at lower salinity while higher salt stress helped to counter the effect of water stress. Therefore, at higher salt stress level effect of water stress was non-significant ($P > 0.05$) (Fig. 4.2.1.11).

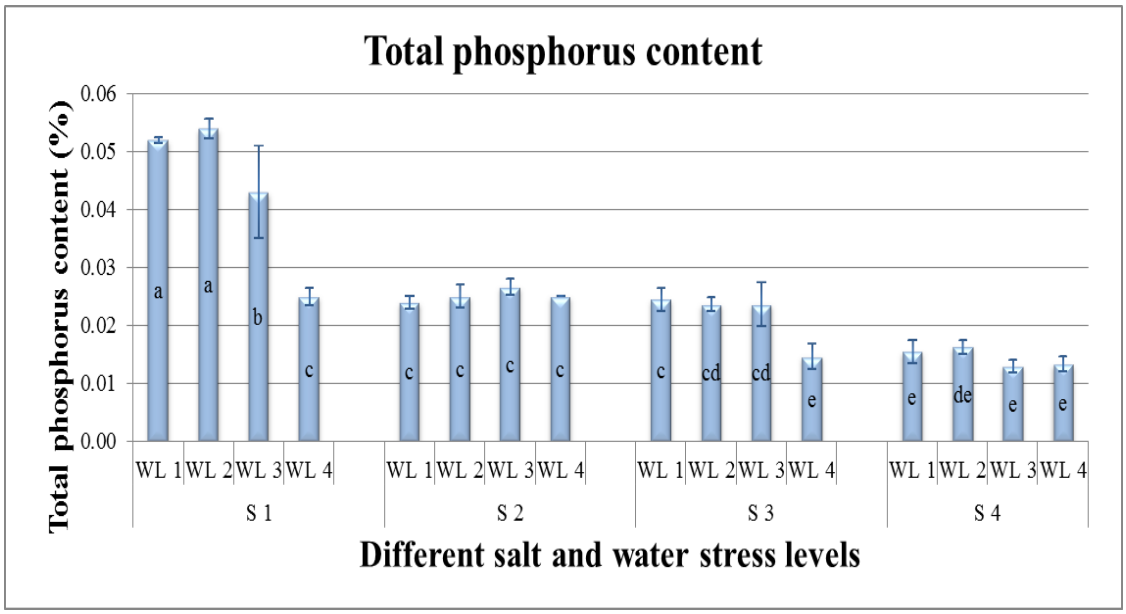

Fig. 4.2.1.11 Plant total phosphorus of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.12 Total potassium content (%)

Total potassium content of *S. imbricata* was significantly affected (P≤0.05) by the interaction of different salt and water stress levels (Appendix 4.2.1.12). Maximum total potassium content (1.180 %) was recorded for S1WL1 followed by 1.127 % at S2WL4. Minimum total potassium content (0.957 %) was found at S3WL4 (Fig. 4.2.1.12).

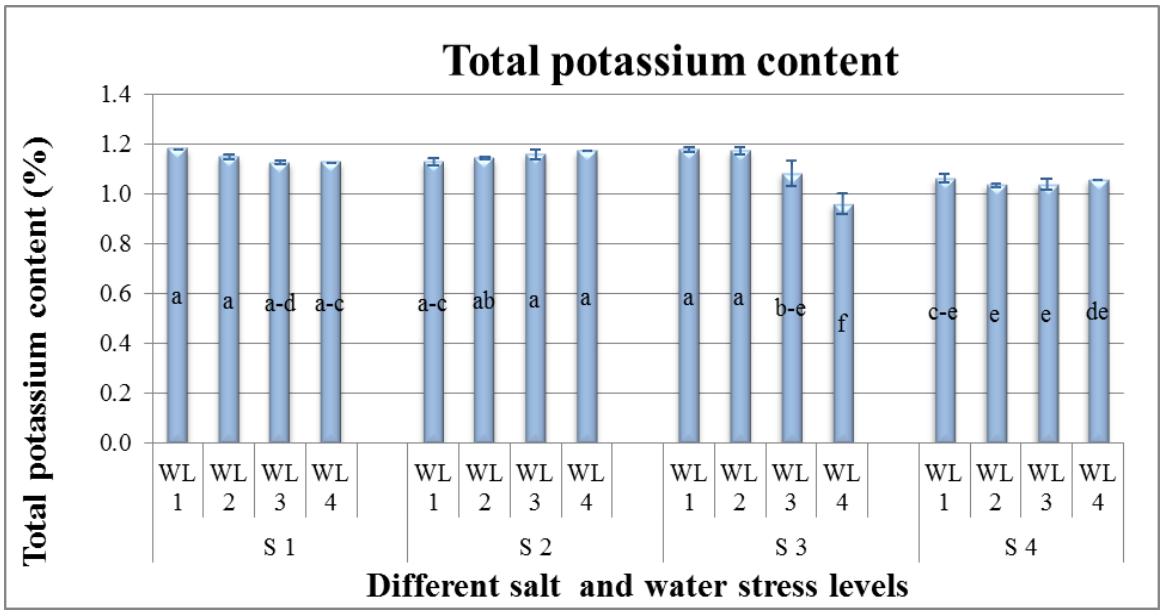

Fig. 4.2.1.12 Plant total potassium content of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.1.13 Sodium content (µ mole/g)

Na^+ concentration had an interactive effect ($P \leq 0.05$) for salt and water stress. Na^+ content increased with increasing salt and water stress (Appendix 4.2.1.13.). Even at the low salt stress level, when external Na^+ was low, Na^+ concentration increased under water stress in shoots (Fig. 4.2.1.13).

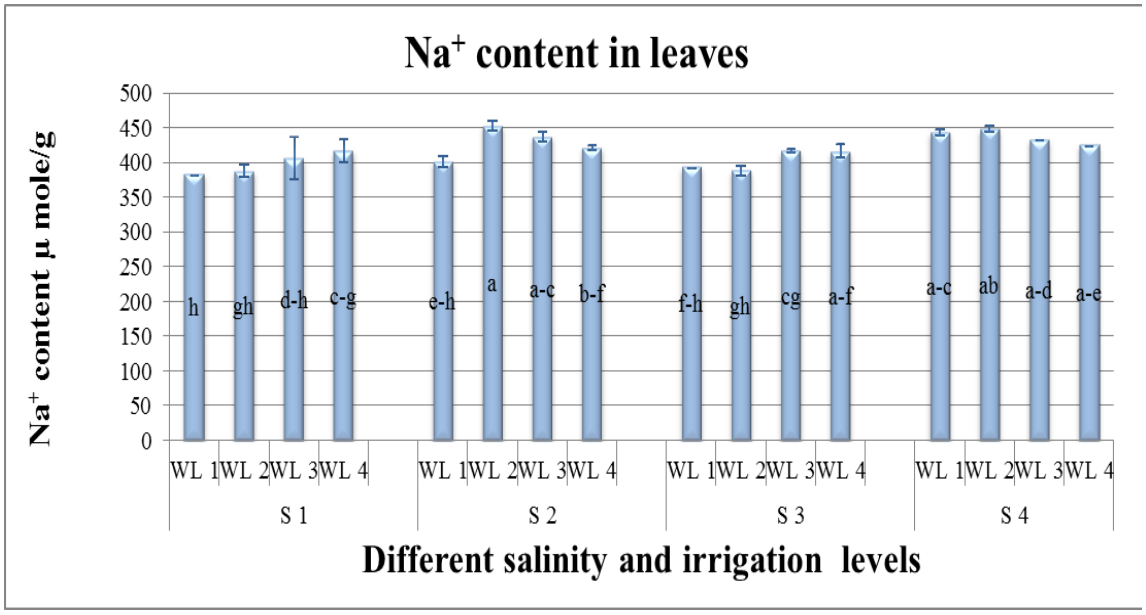

Fig. 4.2.1.13 Na^+ content in leaves of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.14 Chloride content (µ mole/g)

Different salt and water stress levels and their interaction had a significant effect (P≤0.05) on the chloride content of *S. imbricata* (Appendix 4.2.1.14). Maximum Cl^- content (53 µmole/g) was recorded at S4WL3 followed by 50 µmole/g at S4WL2. Lowest Cl^- content was 31 µmole/g recorded at S2WL3. Cl^- content increased with increasing water stress at lower salinity, while at higher salinity level Cl^- content decreased after water stress reached to a certain level (Fig. 4.2.1.14).

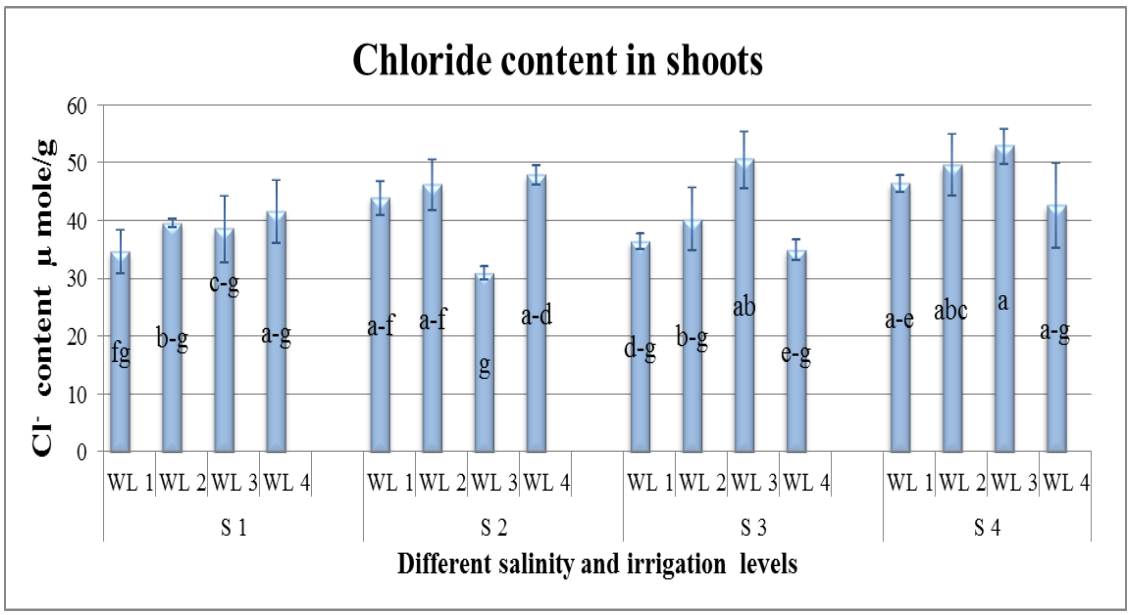

Fig. 4.2.1.14 Cl^- content in leaves of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.15 Abscisic acid (µg.g $^{-1}$ FW)

Salt and water stress levels had a non-significant effect on ABA production (P>0.05), while significant interaction (P≤0.05) of salt and water stress levels was recorded for ABA content of *S. imbricata* (Appendix 4.2.1.15). At lower salt stress of S1, S2 and S3 ABA quantity decreased with increasing water stress. However, at highest salt stress level of S4, ABA quantity increased with increasing the water stress (Fig. 4.2.1.15).

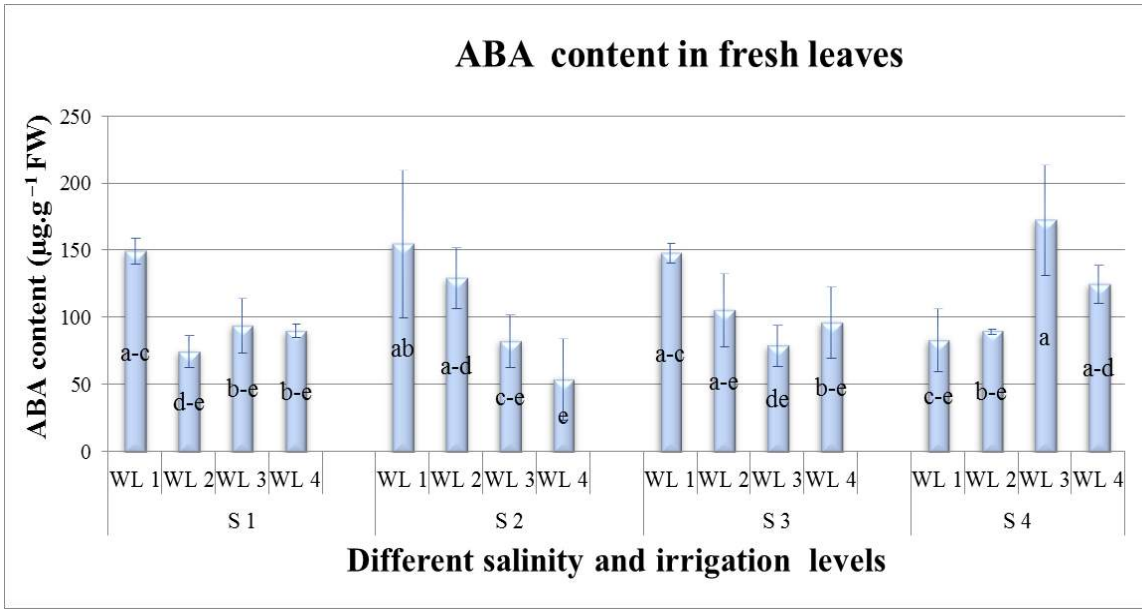

Fig. 4.2.1.15 ABA content (µg.g^{-1}FW) of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.1.16 Proline content (µg.g $^{-1}$ FW)

ANOVA revealed different water stress levels had non-significant effect on the proline content of *S. imbricata* (Appendix 4.2.1.16). Effect of salt stress and salt and water stress interaction was significant (P≤0.05). Proline content in leaves decreased with increasing water stress at lower salt stress. However, at higher salt stress proline had an inverse trend. At higher salinity level of S4 proline quantity increased with increasing water stress level up to WL3 and decreased at WL4 (Fig. 4.2.1.16).

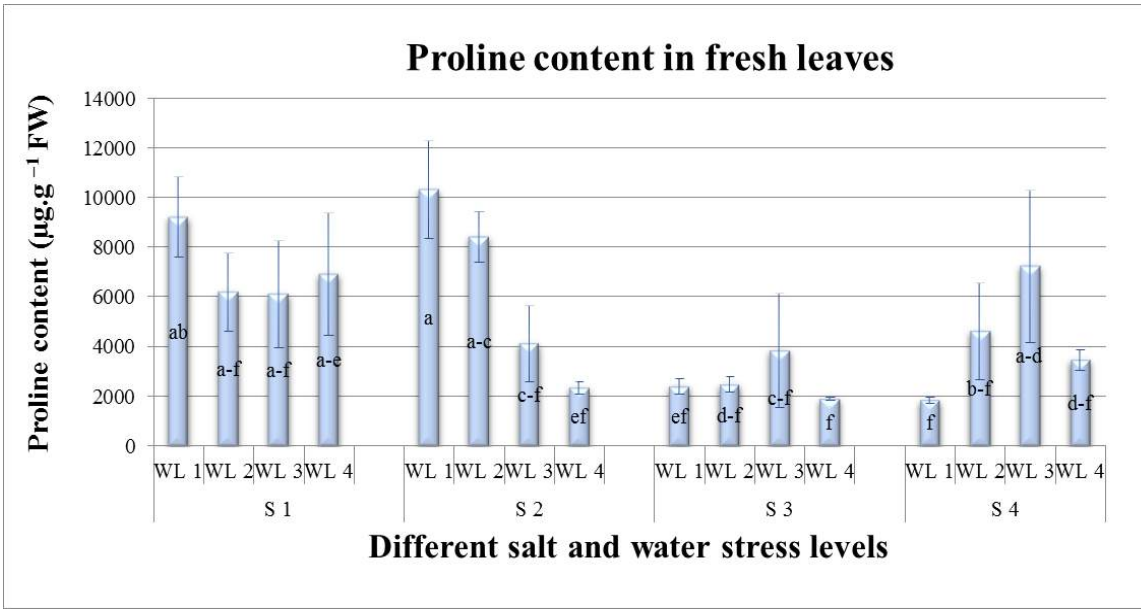

Fig. 4.2.1.16 Proline content (µg.g^{-1}FW) of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.1.17 Catalase activity (units/min/g FW)

ANOVA table revealed a non-significant effect (P>0.05) of different salt and water stress levels on CAT activity of *S. imbricata* (Appendix 4.1.2.17). However, water stress levels showed a non-significant increase in the CAT activity of *S. imbricata* (Fig. 4.2.1.17).

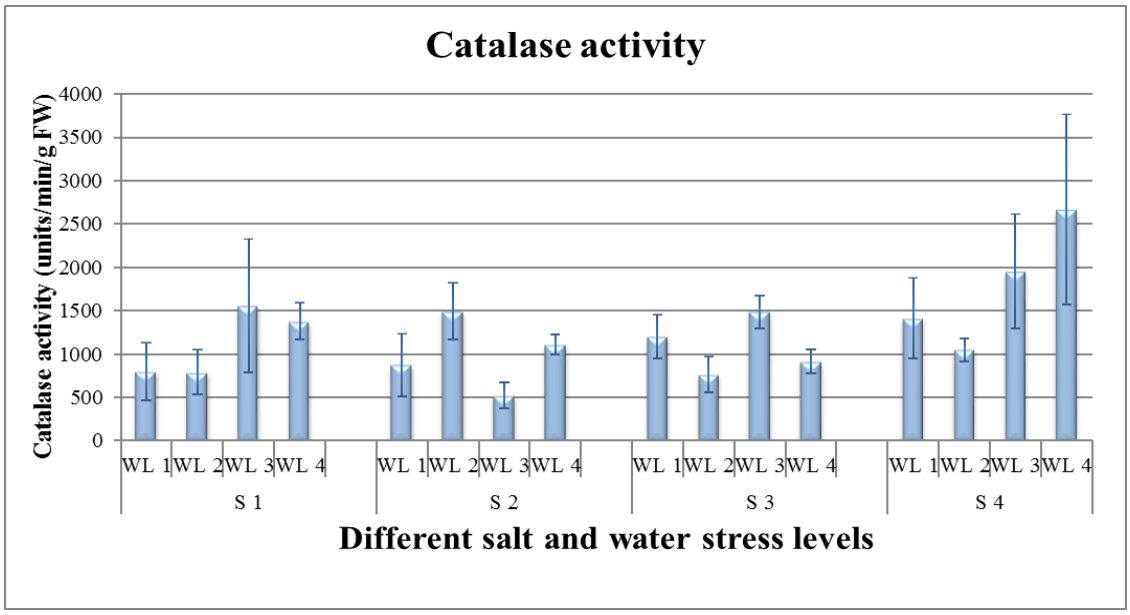

Fig. 4.2.1.17 Catalase activity (units/min/g FW) for *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.1.18 Peroxidase activity (POD) (units/min/g FW)

Interactive effect of salinity and water stress levels was significant (P≤0.05) on the POD activity of *S. imbricata* (Appendix 4.2.1.18). At lower salt stress level, POD activity of *S. imbricata* did not change with increasing water stress level. At salt stress level of S2 and S3 POD activity deceases with increasing water stress level. However, at higher salt stress level of S4 POD activity of *S. imbricata* increased with increasing water stress level.

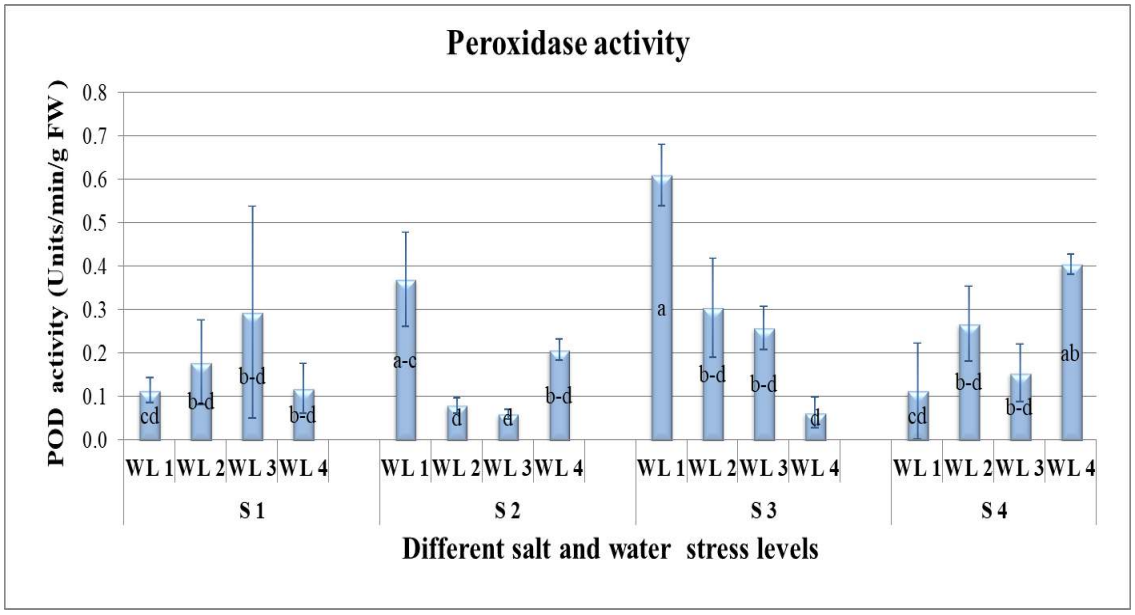

Fig. 4.2.1.18 POD activity (units/min/g FW) for *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.1.19 Ascorbate peroxidase (APX) activity (units/min/g FW)

Data regarding APX activity of *S. imbricata* as affected by different levels of salt and water stress is presented in Fig. 4.2.1.19 and their analysis of variance in Appendix 4.2.1.19. Different salt and water stress levels and the interaction between salt and water stress levels significantly (P≤0.05) affected the APX activity of *S. imbricata*. At lower salt stress levels of S1-S3 APX activity increased with increasing water stress. However, at S4 effect of water stress had no significant effect on APX activity of *S. imbricata* (Figure 4.2.1.19).

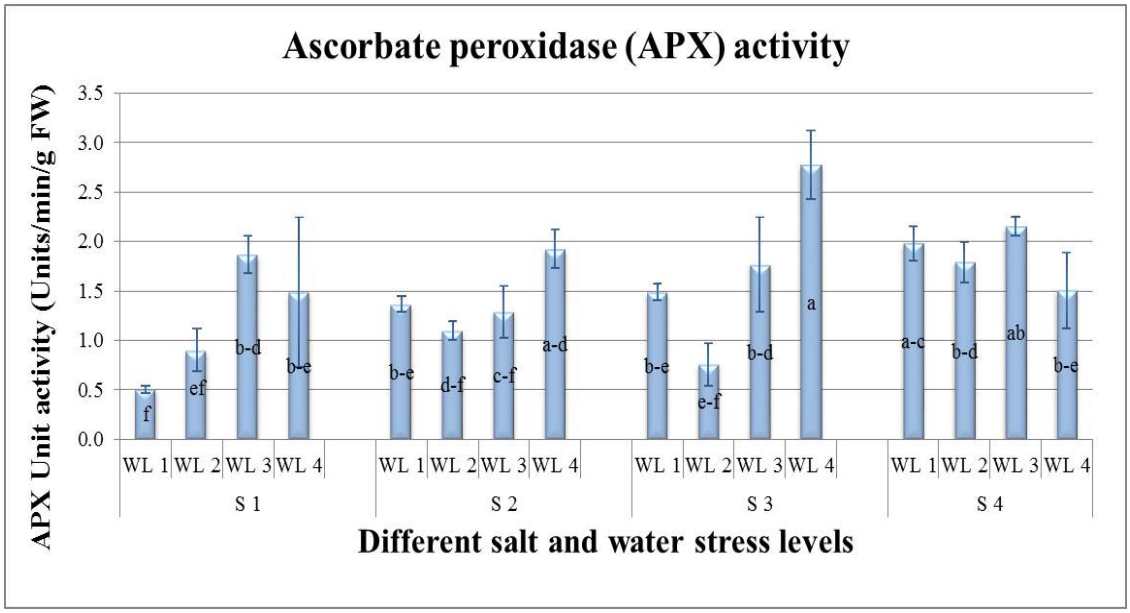

Fig. 4.2.1.19 APX activity of *S. imbricata* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.2 *Tetraena mandavillei* (Hadidi) Beier & Thulin

Syn. Zygophyllum mandavillei (Moq.)

Local/ Arabic name: Haram, هرم

Data regarding different parameters of *T. mandavillei* as affected by different levels of salt and water stress is shown in Fig. 4.2.2.1 - 4.2.2.19. Analysis of variance (ANOVA) is presented in respective Appendix 4.2.2.1 - Appendix 4.2.2.19.

4.2.2.1 Survival percentage (%)

Data regarding survival percentage of *T. mandavillei* as affected by different levels of salt and water stress revealed that both salt and water stress levels and the interaction between them had the non-significant effect ($P>0.05$) on the survival percentage of *T. mandavillei* (Appendix 4.2.2.1). Survival percentage had shown non-significant differences for different salinity and water stress levels. *T. mandavillei* had successfully grown on all levels of salt and water stress levels with survival percentages ≥ 89 %. Lowest survival percentage was 89 % recorded for S3WL3. *T. mandavillei* survived water stress levels of S4 with irrigation water EC even up to 20 dSm^{-2} (Fig. 4.2.2.1).

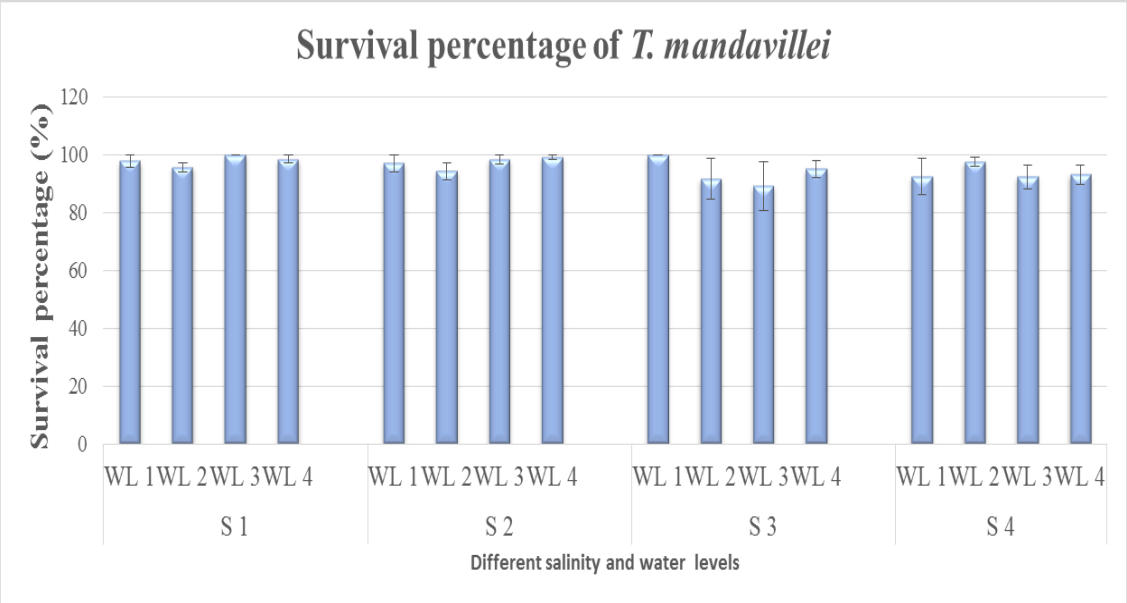

Fig. 4.2.2.1 Survival percentage of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.2 Shoot dry weight (SDW) (g)

Mean effect of salt and water stress on SDW is presented in Fig. 4.2.2.2 and the ANOVA in Appendix 4.2.2.2. Analysis of variance revealed that different salt and water stress levels significantly ($P \leq 0.05$) affected SDW of *T. mandavillei* while salt x water stress had no significant effect ($P > 0.05$) on SDW of *T. mandavillei*.

SDW tends to incline during experiment and maximum SDW recorded was 32.93g for the month of July. Increasing salt stress level decreased the SDW. Lowest salt stress level of S1 had the higher SDW of 11.65g while other three salinities i.e. S2, S3 and S4 had statistically similar ($P > 0.05$) SDW and lower than S1. Increasing the water stress also decreased the SDW significantly ($P \leq 0.05$). SDW was maximum at the lowest water stress level WL1. Increasing water stress decreased the SDW. However, all the other stress levels except WL1 had no significant difference on SDW of *T. mandavillei*.

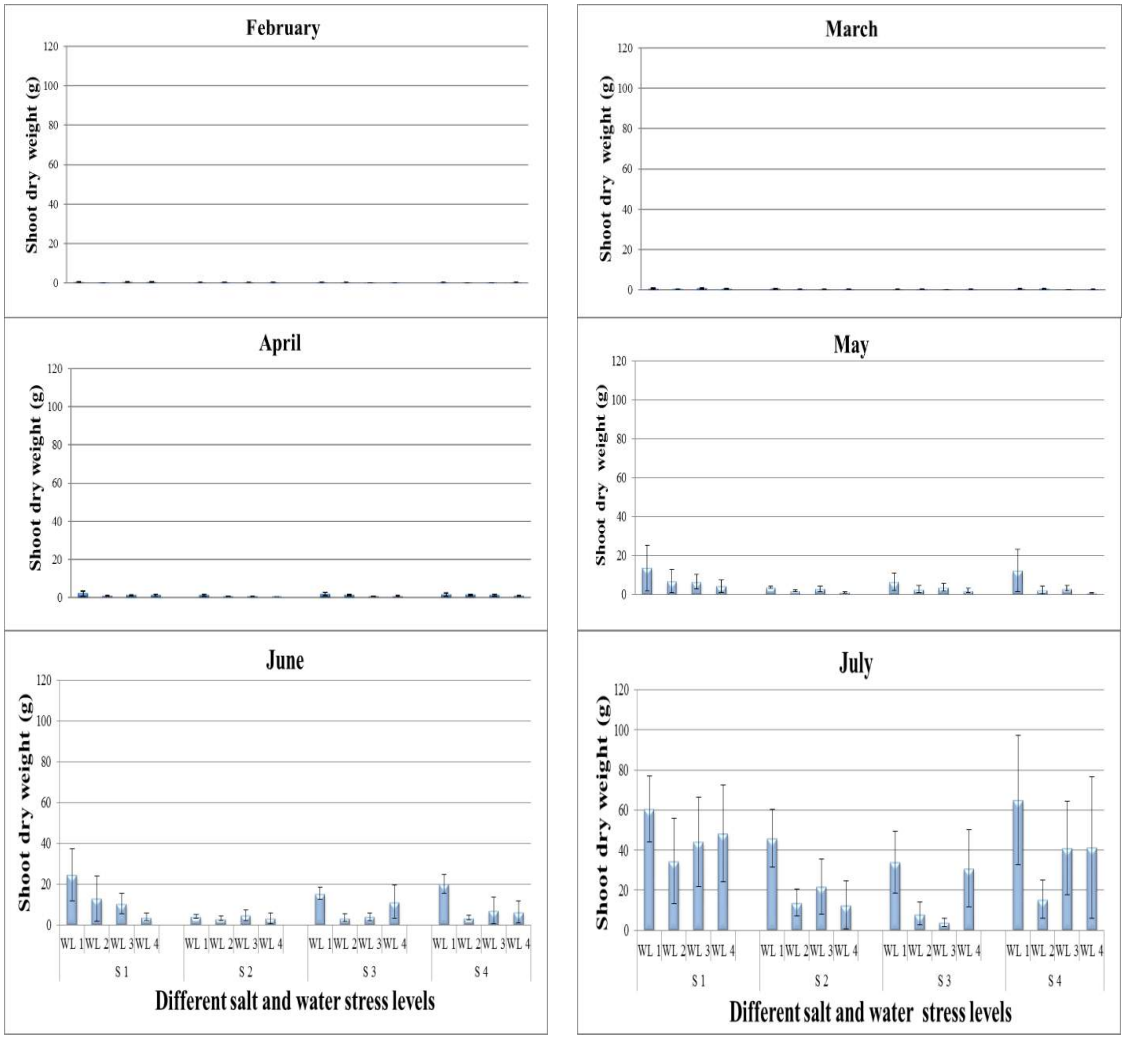

Fig. 4.2.2.2 Shoot dry weight of *T. mandavillei* under four salt stress treatments: 5 dS m^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.3 Root dry weight (RDW) (g)

Mean effect of salt and water stress on RDW is presented in Fig. 4.2.2.3. Analysis of variance showed a significant effect ($P \leq 0.05$) of different salt and water stress levels and time interval (month) on RDW of *T. mandavillei* (Appendix 4.2.2.3). However, interaction between salt and water stress levels had non-significant ($P > 0.05$) effect on RDW of *T. mandavillei*. Increasing salt and water stress reduced the RDW of *T. mandavillei.* RDW was lower in the cooler months of February, March and April and increased in May, June and July (Fig. 4.2.2.3).

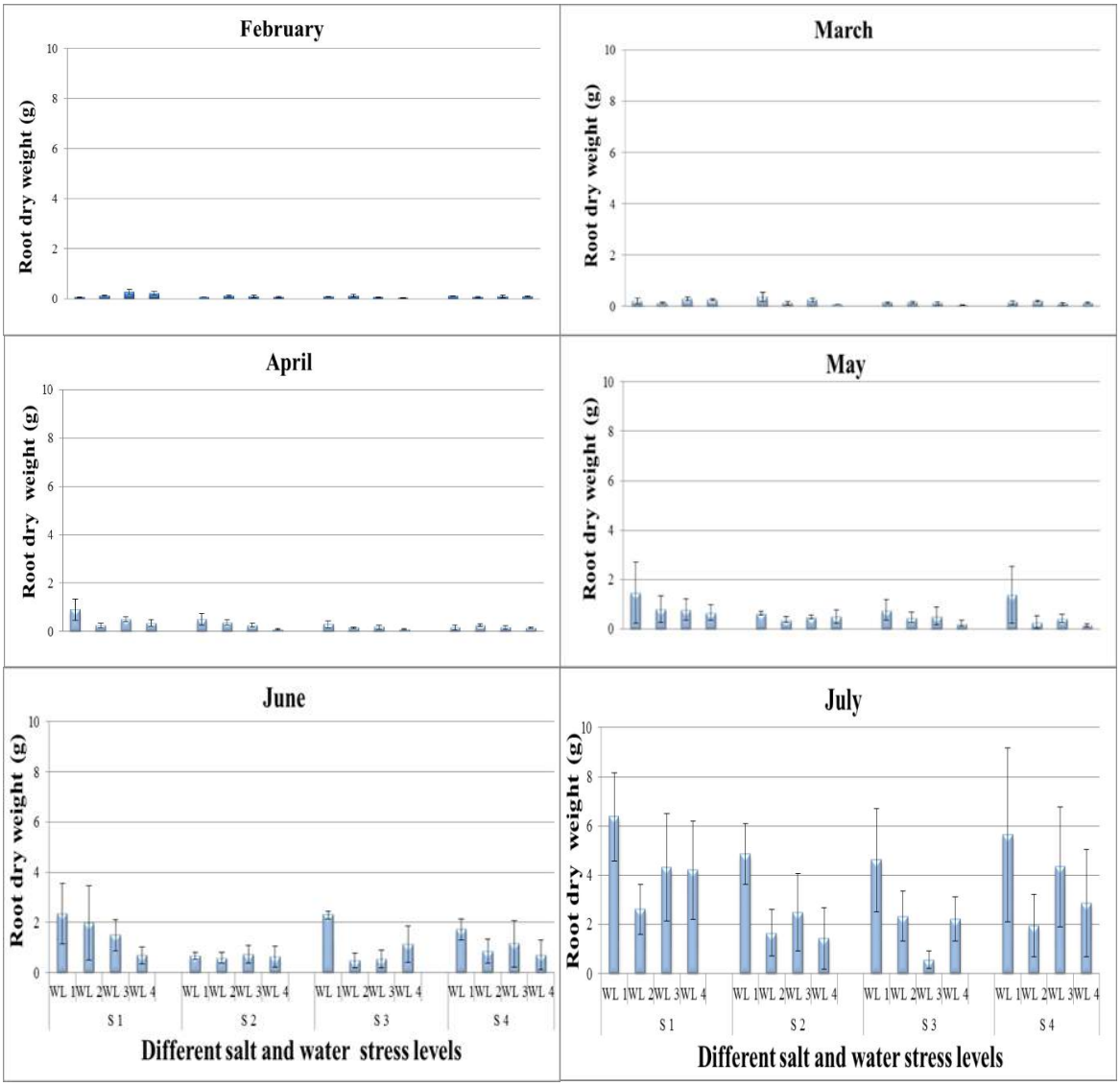

Fig. 4.2.2.3 Root dry weight of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.4 Shoot length (SL) (cm)

Results of the interaction of different salt and water stresses for SL are presented in Fig. 4.2.3.4. The ANOVA in Appendix 4.2.2.4 showed a significant effect ($P \leq 0.05$) on SL of *T. mandavillei*. SL of *T. mandavillei* increased with the time interval. Therefore, time (month) had significant effect on SL of *T. mandavillei*. During the month of February and March, *T. mandavillei* growth was slower and increased thereafter and reached to maximum SL of 27.50 cm in the month of July. SL was maximum (16.78 cm) at lower salt stress level of S1 while reduced at salinity more than that. Maximum irrigation level of WL1 produced highest plants (16.67 cm) while plants at lower irrigation levels of WL2, WL3 and WL4 statistically shorter than WL1 and similar to each other.

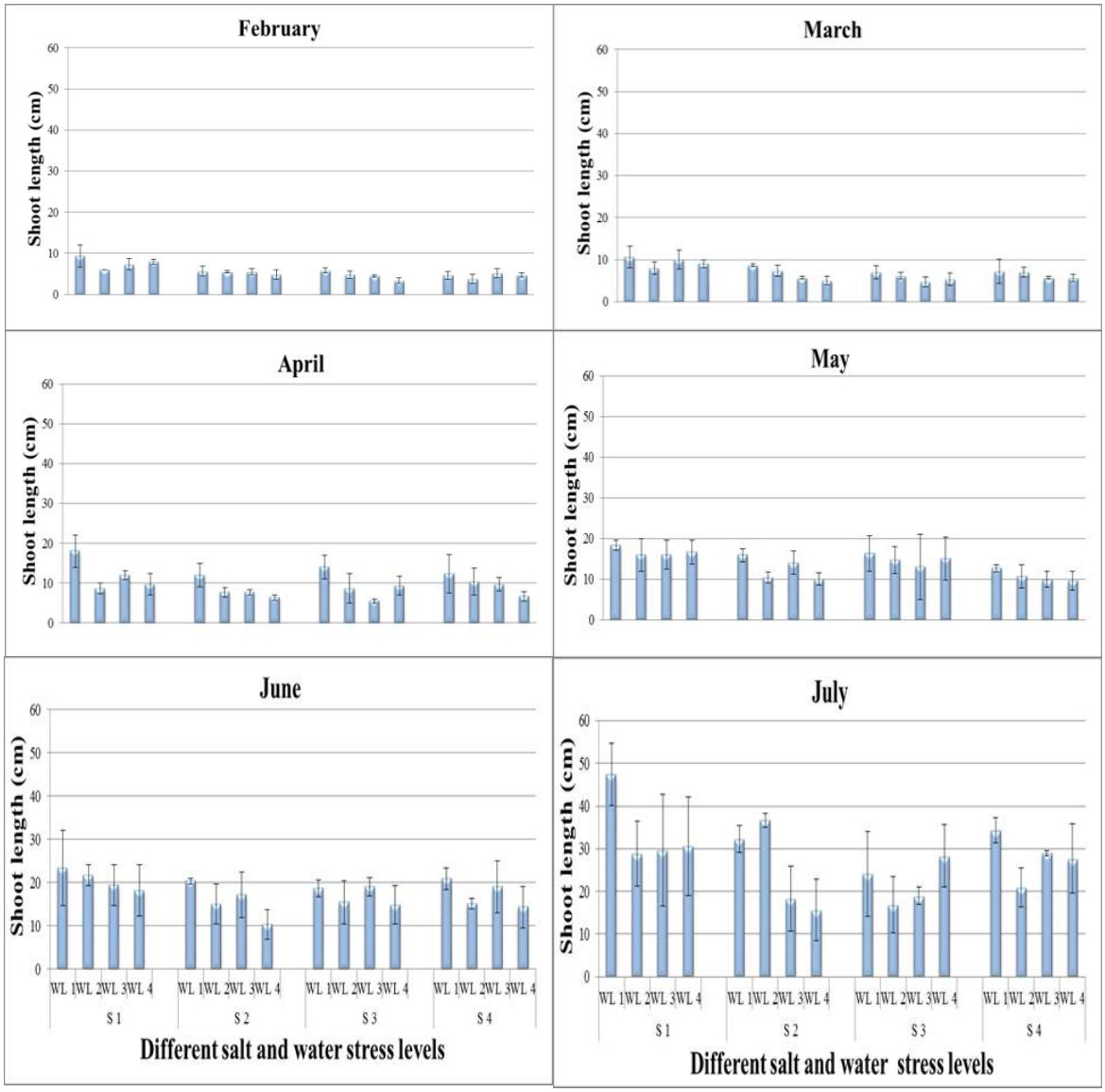

Fig. 4.2.2.4 Shoot length of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.5 Root length (RL) (cm)

Effect of salt and water stress on RL of *T. mandavillei* is presented in Fig. 4.2.2.5. and the ANOVA in Appendix 4.2.2.5. Different salt and water stress levels and time (months) had significant effect ($P \leq 0.05$) on RL of *T. mandavillei*. RL of *T. mandavillei* increased with time and maximum RL (39.32cm) was recorded at the end of experiment for July.

Increasing salt stress decreased the RL for S1, S2 and S3 but increased at S4 with mean values of 27.15, 21.62, 21.45 and 24.18 cm respectively. Increasing water stress decreased the mean RL of *T. mandavillei*. Maximum RL of *T. mandavillei* was (28.82) recorded for WL1 while WL2, WL3 and WL4 had the statistically similar ($P > 0.05$) mean RL of 21.77, 21.21 and 22.61 respectively.

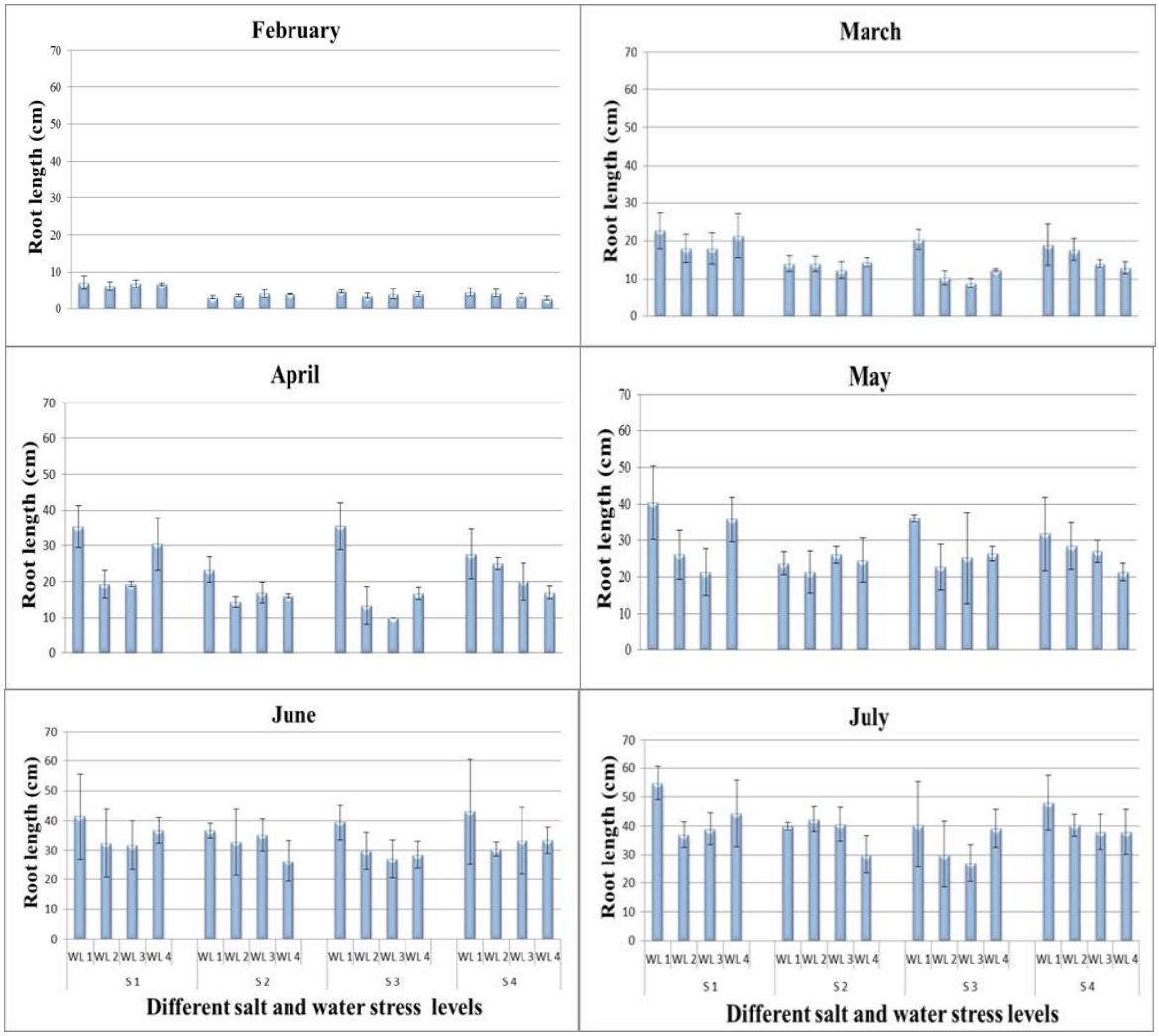

Fig. 4.2.2.5 Root length of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.6 Water use efficiency (WUE) ($g.L^{-1}$)

Results of different salt and water stress levels on WUE are presented in Fig. 4.2.2.6 and the ANOVA in Appendix 4.2.2.6. ANOVA revealed that different salt and water stress levels had non-significant (P>0.05) effect on WUE of *T. mandavillei*. However, there was a non-significant increase in WUE with increasing water stress.

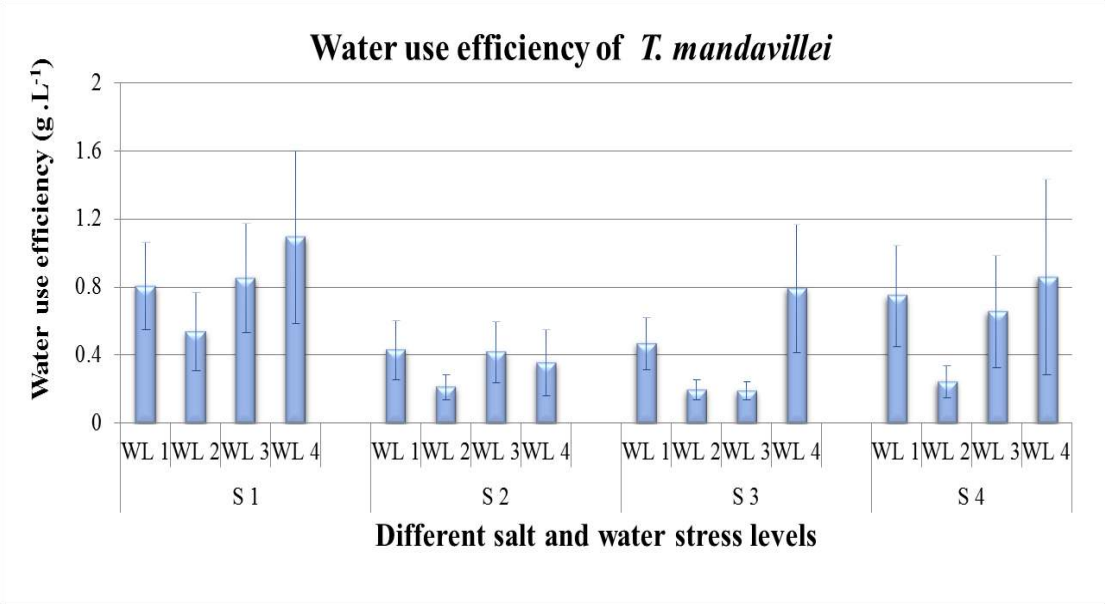

Fig. 4.2.2.6 Water use efficiency of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.7 Chlorophyll index

Results of different salt and water stress levels on chlorophyll index are presented in Fig. 4.2.2.7 and the ANOVA in Appendix 4.2.2.7. Analysis of variance revealed that different salt and water stress levels, and salt x water stress levels significantly ($P \leq 0.05$) affected chlorophyll index of *T. mandavillei*. Highest chlorophyll index was recorded in the month of May which increased with increasing salt and water stress except S3 where water stress increased the chlorophyll index but decreased at WL4. Highest chlorophyll index was 1.79 for S3WL3 and S4WL4 in the month of May. Lowest chlorophyll index was 0.39 recorded for S1WL4 in the month of March.

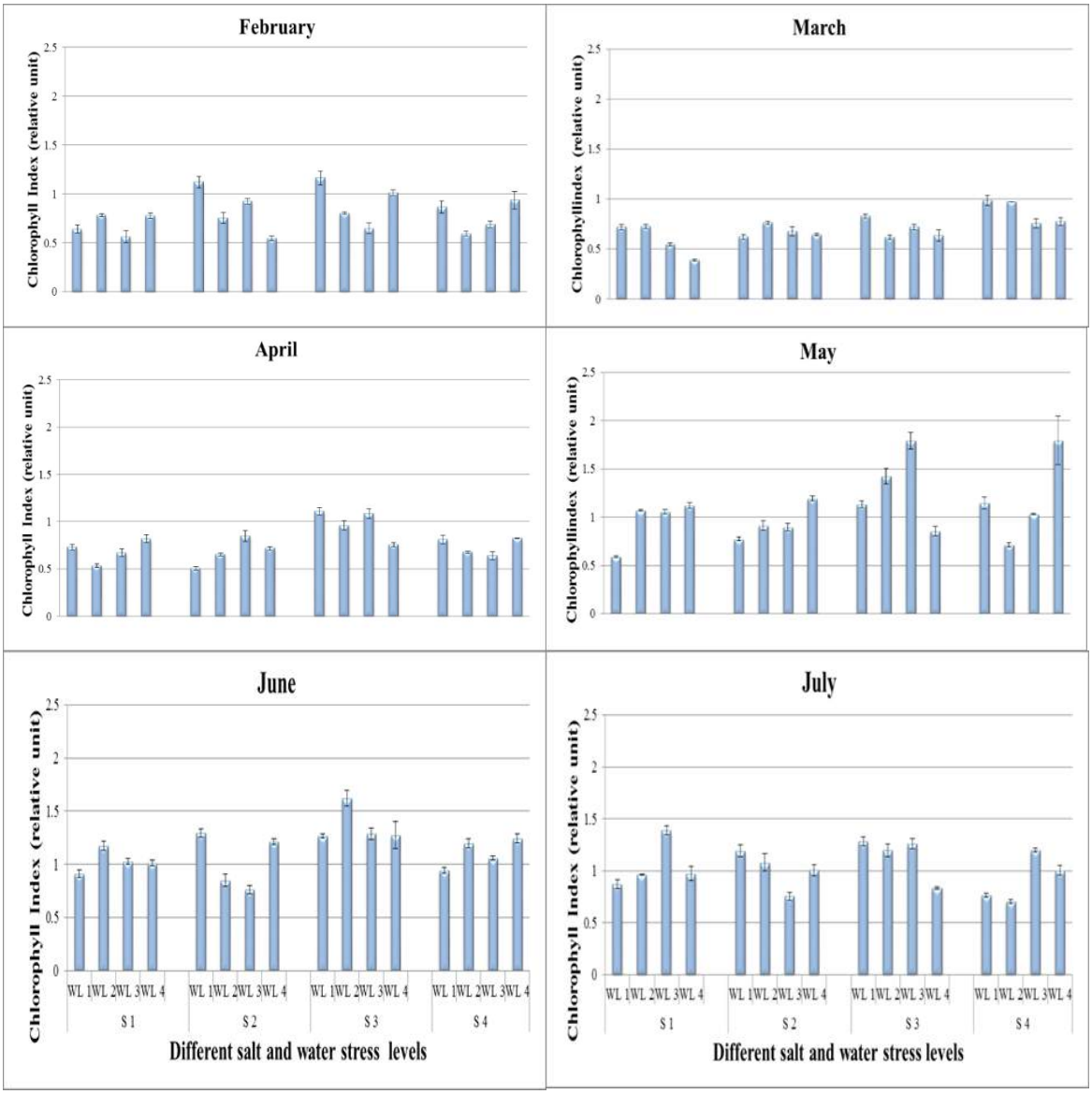

Fig. 4.2.2.7 Chlorophyll index of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.8 Photosynthetic rate (Pr) (µmol/m^2/s)

Pr of *T. mandavillei* was affected by different levels of salt and water stress as presented in Fig. 4.2.2.8 and their analysis of variance in Appendix 4.2.2.8. Different months, salt and water levels and their interaction had the significant ($P \leq 0.05$) effect on the Pr of *T. mandavillei*. Highest Pr (149.2 µmol/m^2/s) was recorded during the month of March for S4WL4 while lowest Pr (5.8 µmol/m^2/s) was recorded during the month of May for S3WL3. Pr was lower at the start of the trial when seedlings were small in the month of February but higher during March. As the temperatures increased the Pr decreased. However, increased again in the month of July. Pr usually decreased with increasing salt and water stress. However, in the month of March when Photosynthetic activity was maximum Pr increased with increasing water stress at higher salt stress level.

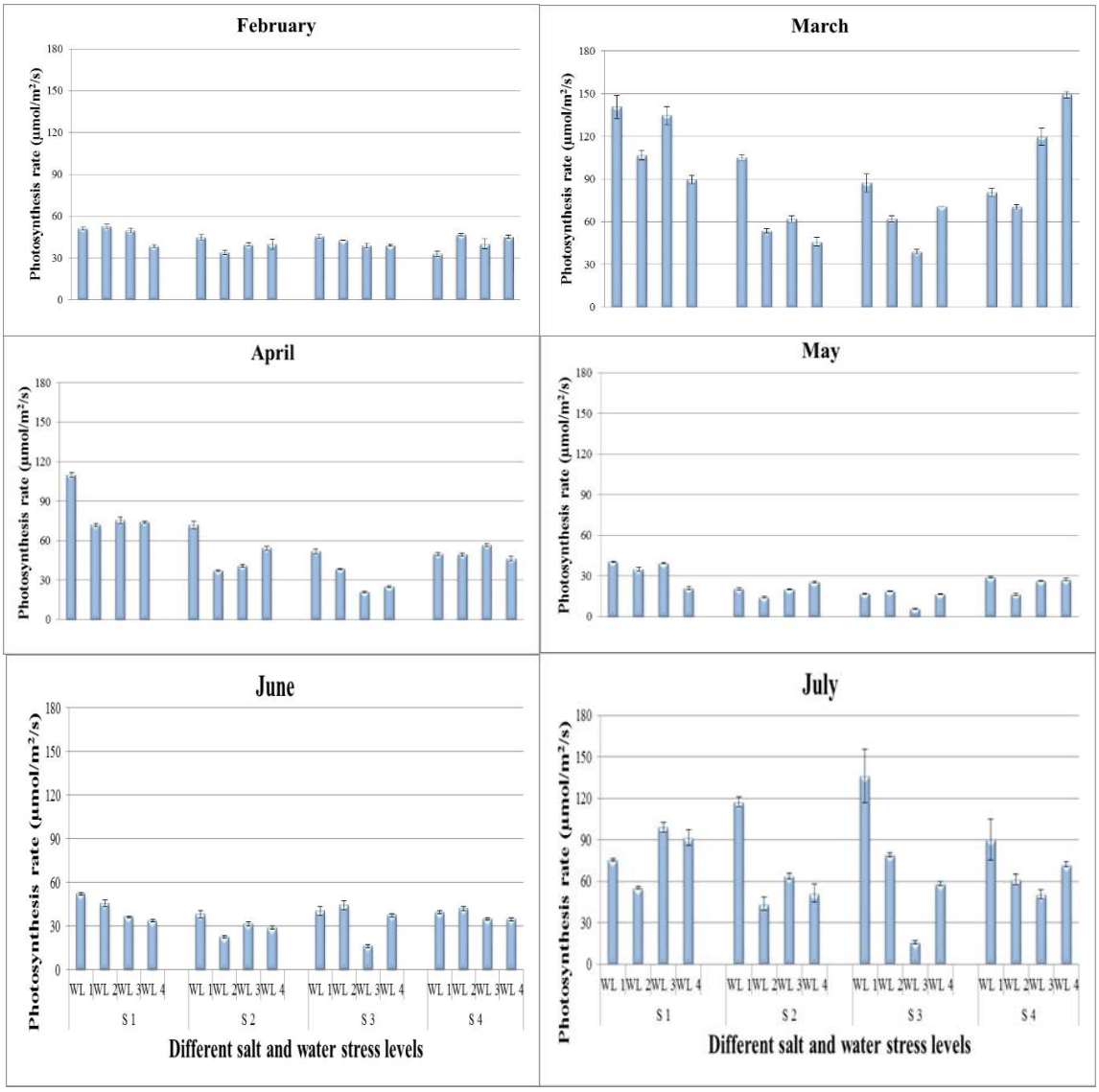

Fig. 4.2.2.8 Photosynthetic rate (Pr) of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.9 Leaf water potential (LWP) (MPa)

T. mandavillei was measured for leaf water potential at two times: one and five month from treatment application. During both readings LWP reduced from fist month to fifth month (Fig. 4.2.2.8). LWP was decreased significantly by both salt and water stress after one month. After five months of treatment application, salinity had significantly reduced the leaf water potential of *T. mandavillei* (Appendix 4.2.2.9a. and Appendix 4.2.1.9b). LWP was decreased significantly ($P \leq 0.05$) by both salt and water stress after one month of treatment application. After one month of stress application, increasing salt stress from S1 to S4 decreased the LWP from -4.3 MPa to -9.8 MPa. Water stress significantly reduced the LWP for WL3 and WL4 compared to WL1 and WL2 (Fig. 4.2.2.8a). After five months of treatment application salinity had significantly reduced the leaf water potential of *T. mandavillei.,* However, water stress had no significant effect on LWP. S1 had the maximum LWP of -8.1 MPa while S3 and S4 had minimum LWP of -15.1 and -14.6 MPa respectively (Fig. 4.2.2.8b).

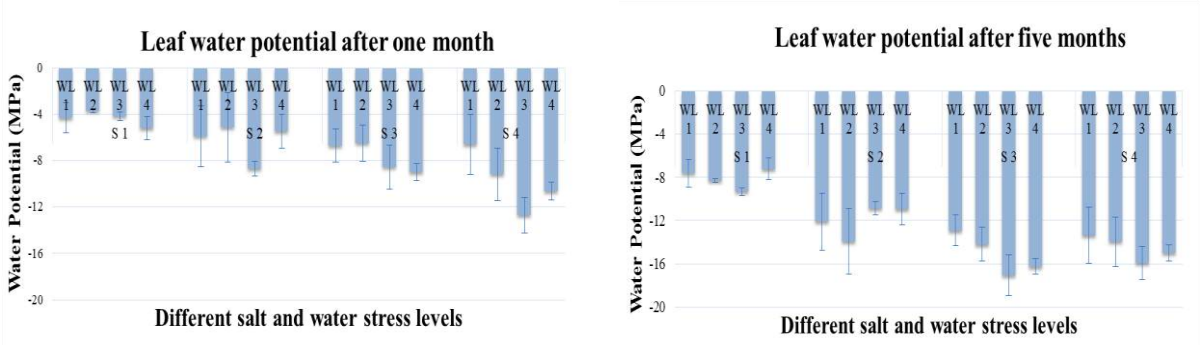

Fig. 4.2.2.9 Leaf water potential of *T. mandavillei* at two different time interval under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.10 Plant total nitrogen (%)

Different salt stress levels and the interaction between salt and water stress levels had a significant effect (P≤0.05) on the plant total nitrogen content of *T. mandavillei* (Appendix 4.2.1.10). Maximum plant total nitrogen was recorded as 0.74 % for each of S1WL2 and S2WL2; respectively. Lowest plant total nitrogen content (0.296 %) was recorded for S3WL3. At the lower salt stress levels of S1 and S2 plants had lower total nitrogen at lowest water stress. However, it increased at WL2 and decreased thereafter. At higher salt stress of S3 and S4 plant total nitrogen decreased with increasing water stress continuously (Fig. 4.2.2.10).

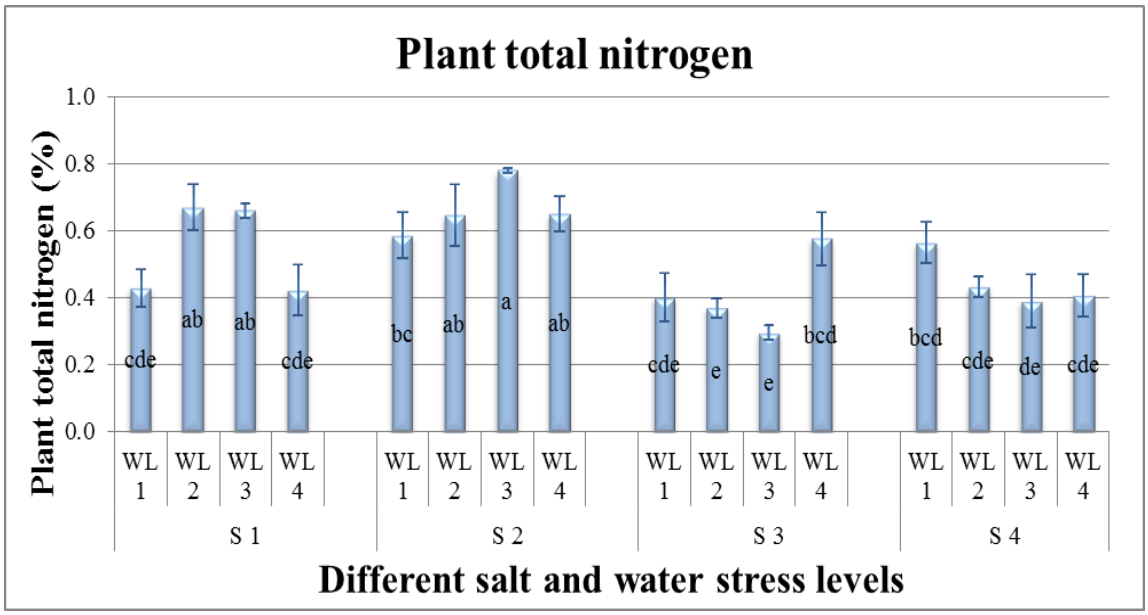

Fig. 4.2.2.10 Plant total nitrogen of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.2.11 Phosphorus content (%)

Plant total phosphorus of *T. mandavillei* was significantly affected (P≤0.05) by the different salt and water stress levels and salt x water stress (Appendix 4.2.2.11). Maximum plant total phosphorus (0.041 %) was recorded for S1WL1 and S1WL2. Minimum plant total phosphorus (0.0193 %) was recorded for S3WL3. At the lower salinity stress levels of S1 and S2, plants total phosphorus was lowest at lowest water stress level. However, it increased at WL2 and decreased thereafter. At higher salinities of S3 and S4 plant total phosphorus decreased with increasing water stress continuously (Fig. 4.2.2.11).

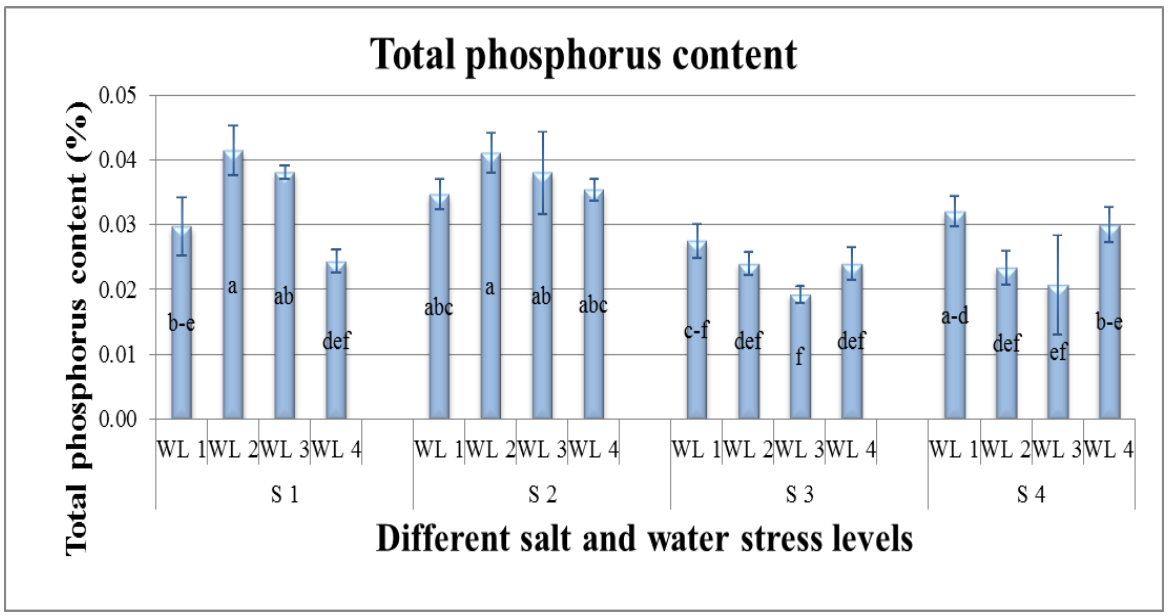

Fig. 4.2.2.11 Plant Total phosphorus *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.2.12 Total potassium content (%)

Total potassium content in shoots of *T. mandavillei* had shown a significant interaction of salt by water stress (P≤0.05) (Appendix 4.2.1.12). Total potassium content of *T. mandavillei* decreased with increasing water stress. S2WL2 had the maximum total potassium content of 1.16 % while S3WL3 had the minimum total potassium content of 0.97 % in shoots of *T. mandavillei* (Fig. 4.2.2.12).

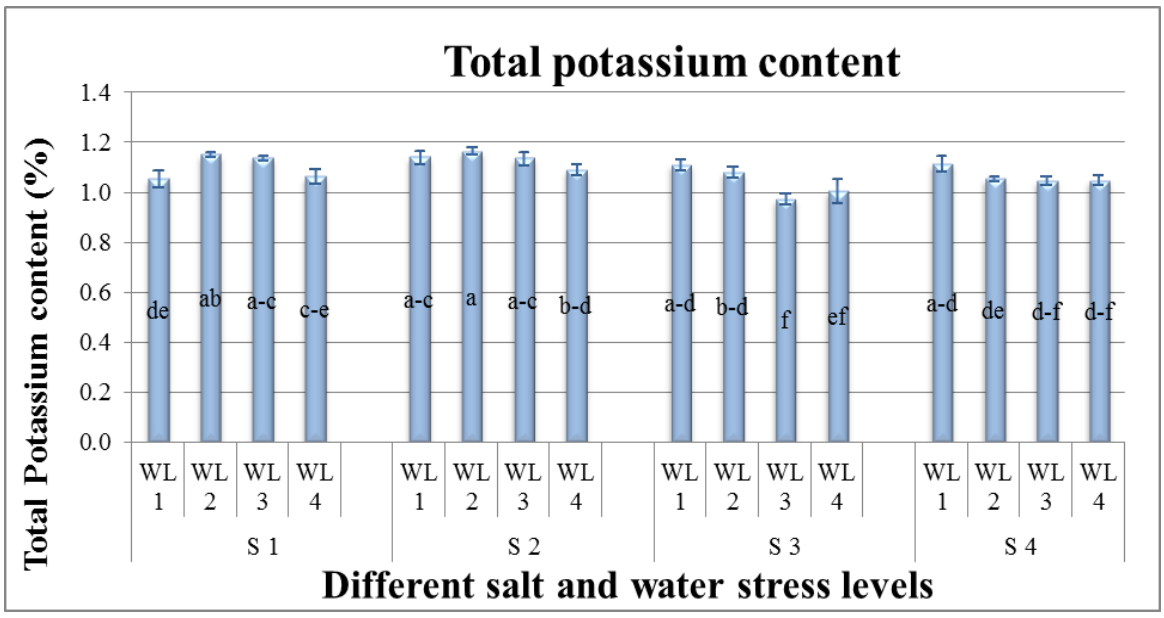

Fig. 4.2.2.12 Plant total potassium content of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.2.13 Sodium content (µ mole/g)

Na^+ concentration had an interactive effect ($P \leq 0.05$) for salt and water stress. Na^+ increased with increasing salinity (Appendix 4.2.2.13). Even at the low salt stress level, Na^+ concentration increased in shoots when combined with water stress (Fig. 4.2.2.13). At higher salinity stress level of S3 and S4 Na^+ content decreased with increasing water stress. Highest Na^+ concentration (665) was found in S4WL1 while lowest was recorded as 503, 514, 519 and 503 for S1WL1, S1WL2, S1WL3 and S3WL3 respectively.

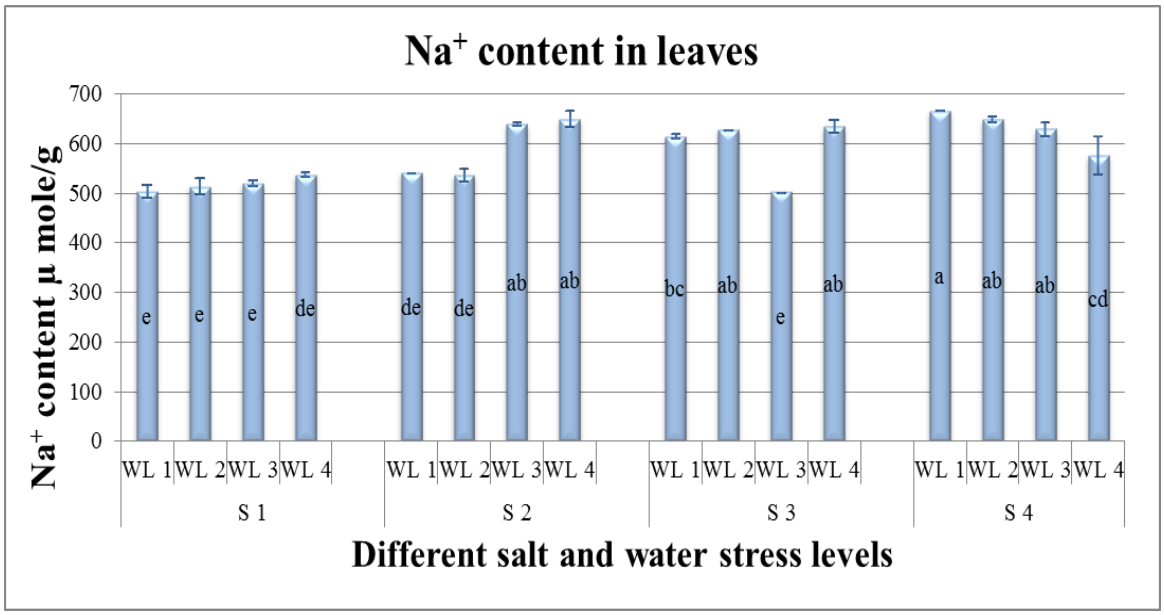

Fig. 4.2.2.13. Na^+ content in leaves of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.14 Chloride content (µ mole/g)

Both salt and water stress had an interactive effect on the Cl^- content of *T. mandavillei* (Appendix 4.2.1.14). Lowest Cl^- content (177 µmole/g) was recorded for S1WL1 while highest Cl^- content (499 µmole/g) was recorded for S4WL3. Cl^- content increased with increasing water stress even on low salinity levels when external NaCl concentration was low. However, at WL4 it decreased again (Fig. 4.2.2.14).

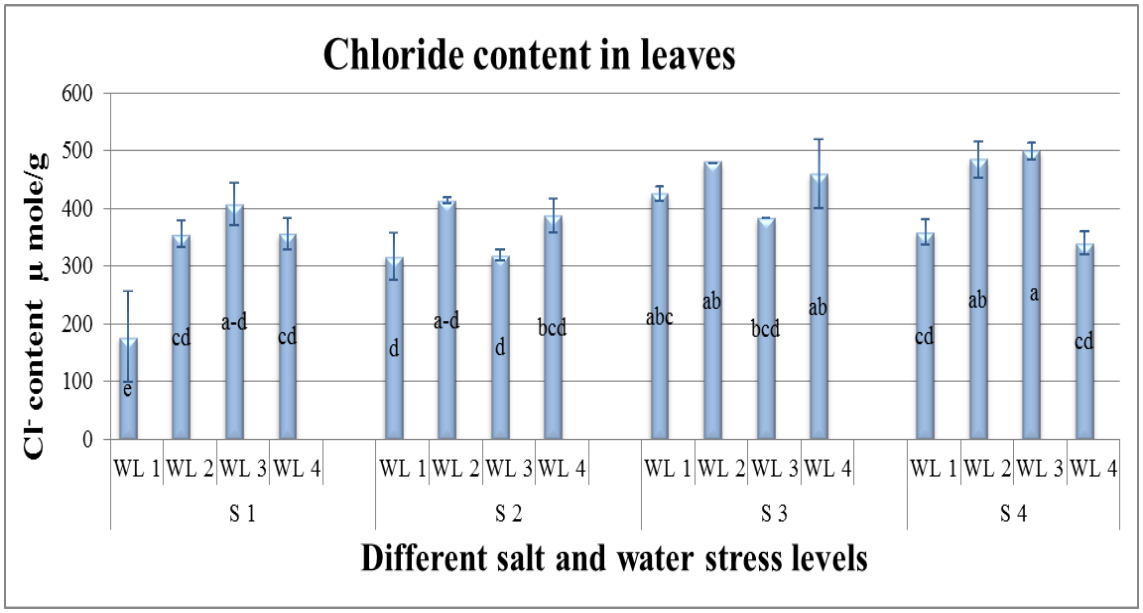

Fig. 4.2.2.14 Cl^- content in leaves of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.15 Abscisic acid (µg. g $^{-1}$ FW)

ABA content was quantified at the end of experiment means of ABA quantified are represented in Fig. 4.2.2.14. Salt and water stress levels had a significant (P≤0.05) interactive effect on ABA production (Appendix 4.2.2.15). ABA production showed an opposite trend at lower and higher salinity stress levels. ABA production decreased with increasing water stress levels at the low salinity stress levels of S1 and S2. However, at higher salinity stress levels of S3 and S4, the ABA content increased with increasing water stress. Maximum ABA content was 33 and 31 µgg^{-1} FW quantified at S4WL4 and S1WL1 respectively.

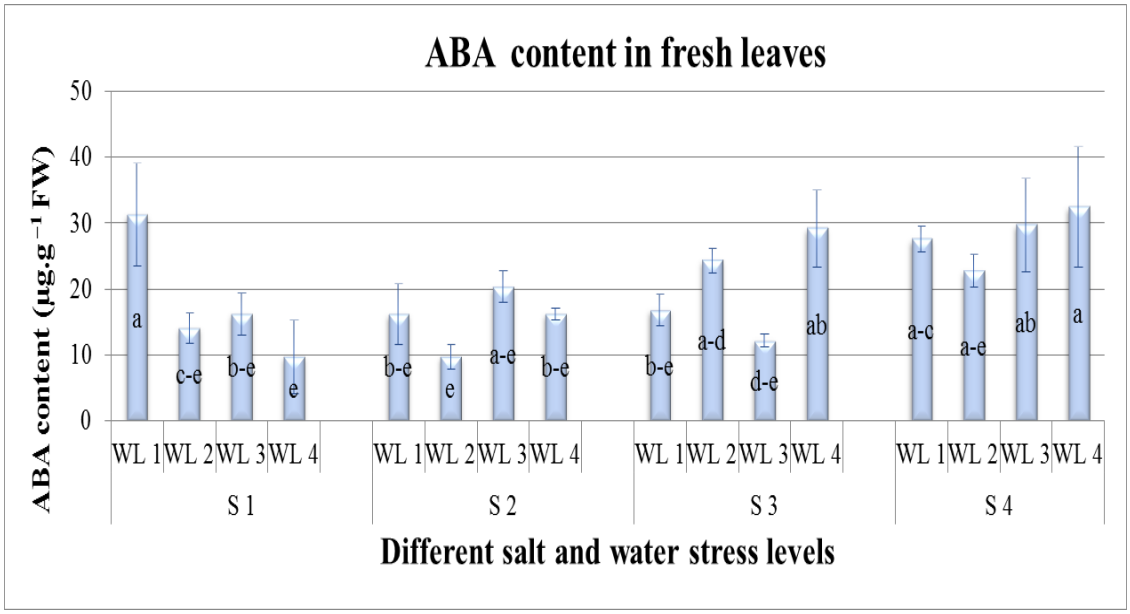

Fig. 4.2.2.15 ABA content (µg.g^{-1} FW) of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.2.16 Proline content (µg.g $^{-1}$ FW)

The ANOVA showed different salt and water stress levels had non-significant effect (P>0.05) on the proline content of *T. mandavillei* (Appendix 4.2.2.16). The interactive effect of salinity and water stress levels was significant (P≤0.05) At lower salinity levels, the POD activity in *T. mandavillei* leaves decreased with increasing water stress. However, at higher salinity levels, POD activity showed the inverse trend. At the higher salinity levels of S3 and S4, POD activity increased with increasing water stress (Fig. 4.2.2.16).

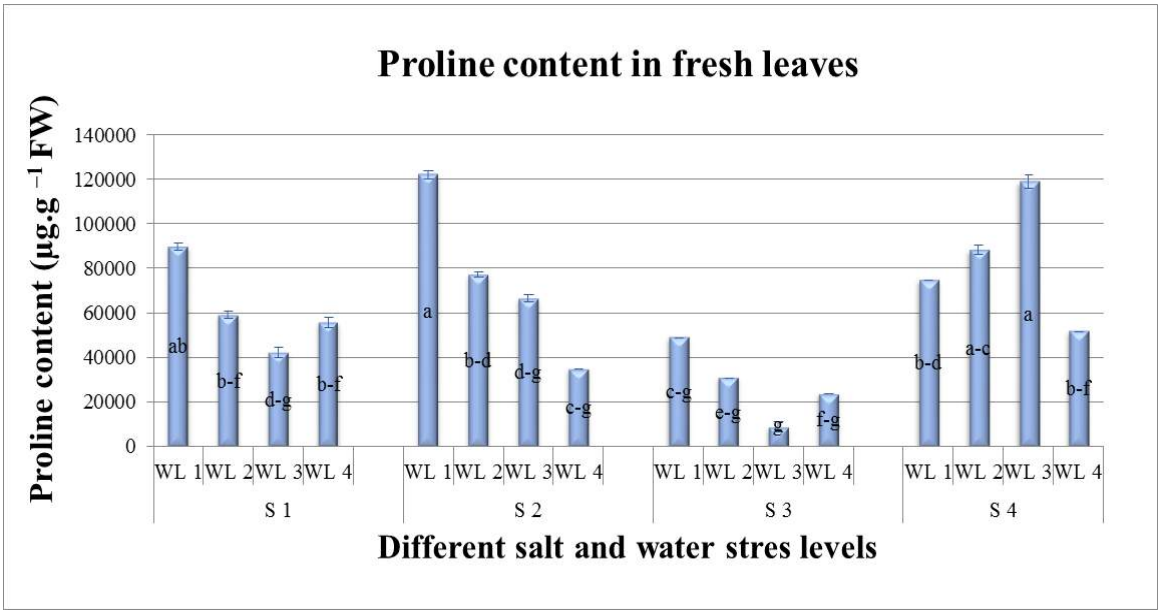

Fig. 4.2.2.16. Proline content (µg.g^{-1}FW) of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.2.17 Catalase activity (units/min/g FW)

ANOVA revealed that different water stress levels had a significant effect ($P \leq 0.05$) on the CAT activity in *T. mandavillei* (Appendix 4.2.2.17). The CAT activity in the leaves of *T. mandavillei* increased with the increasing water stress level. WL1 had the lowest CAT activity of 329 (units/min/g FW) while WL2, WL3 and WL4 had the higher CAT activity of 768, 811 and 949 units/min/g FW, respectively (Fig. 4.2.2.17).

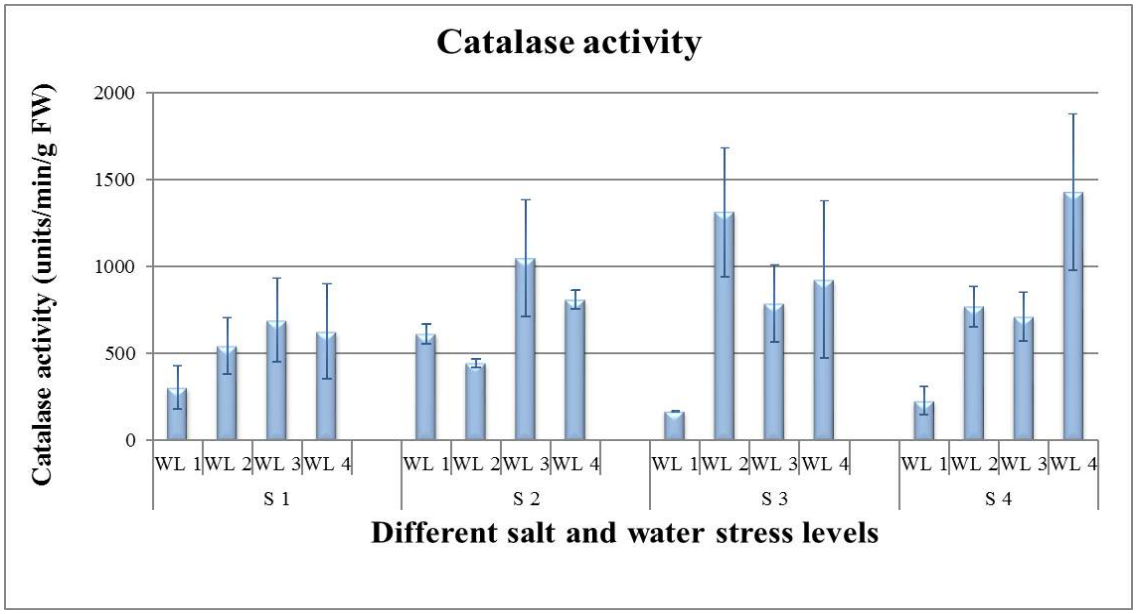

Fig. 4.2.2.17 Catalase activity (units/min/g FW) for *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.2.18 Peroxidase activity (POD) (units/min/g FW)

Analysis of Variance table revealed the significant effect of different water stress levels on POD activity of *T. mandavillei.* Interactive effect of salt and water stress levels on POD activity of *T. mandavillei* was also significant (P≤0.05; Appendix 4.2.2.18). At lower salinity POD activity of *T. mandavillei* in leaves decreased with increasing water stress level. However, at higher salt stress level POD activity had an inverse trend. At higher salinity level of S3 and S4 POD activity increased with increasing water stress level (Fig. 4.2.2.18).

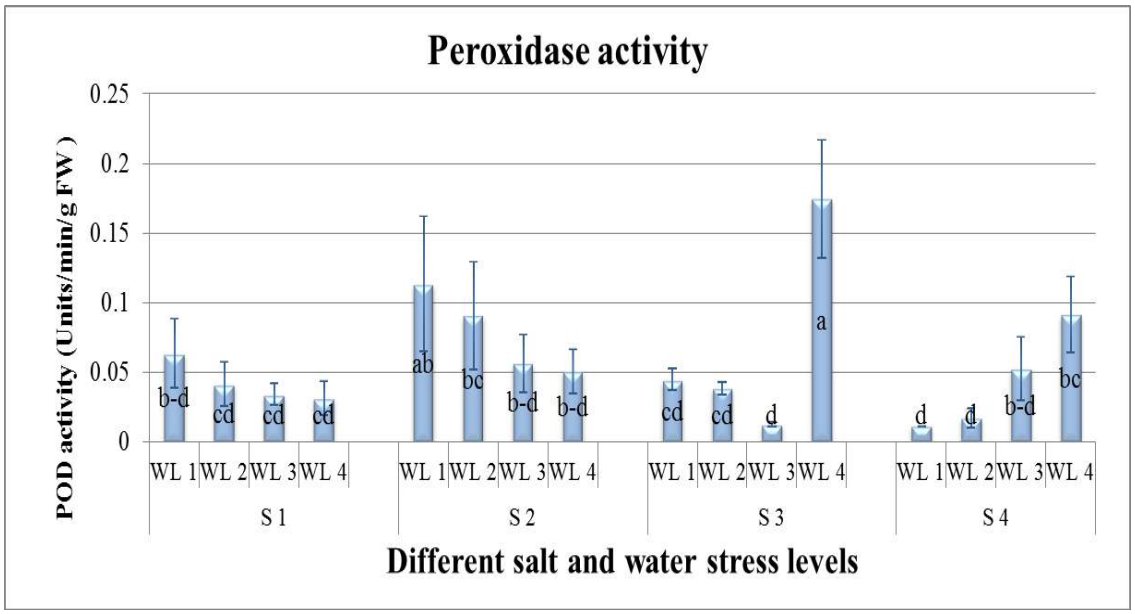

Fig. 4.2.2.18 Peroxidase (POD) activity (units/min/g FW) of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.2.19 Ascorbate peroxidase (APX) activity (units/min/g FW)

Data regarding APX activity of *T. mandavillei* as affected by different levels of salt and water stress is presented in Fig. 4.2.2.19 and their analysis of variance in Appendix 4.2.2.19. Different salt stress levels and the interaction between salt and water stress levels significantly (P≤0.05) affected the APX activity of *T. mandavillei*. Water stress had a non-significant effect (P>0.05) on the APX activity in *T. mandavillei*. At the lower salt stress levels of S1 and S2 APX activity first decreased with increasing water stress from WL1 to WL2 and then increased with increasing water stress. At the higher salt stress levels of S3 and S4, APX activity increased with increasing water stress from WL1-WL2 and then decreased with higher water stress levels of WL3 and WL4.

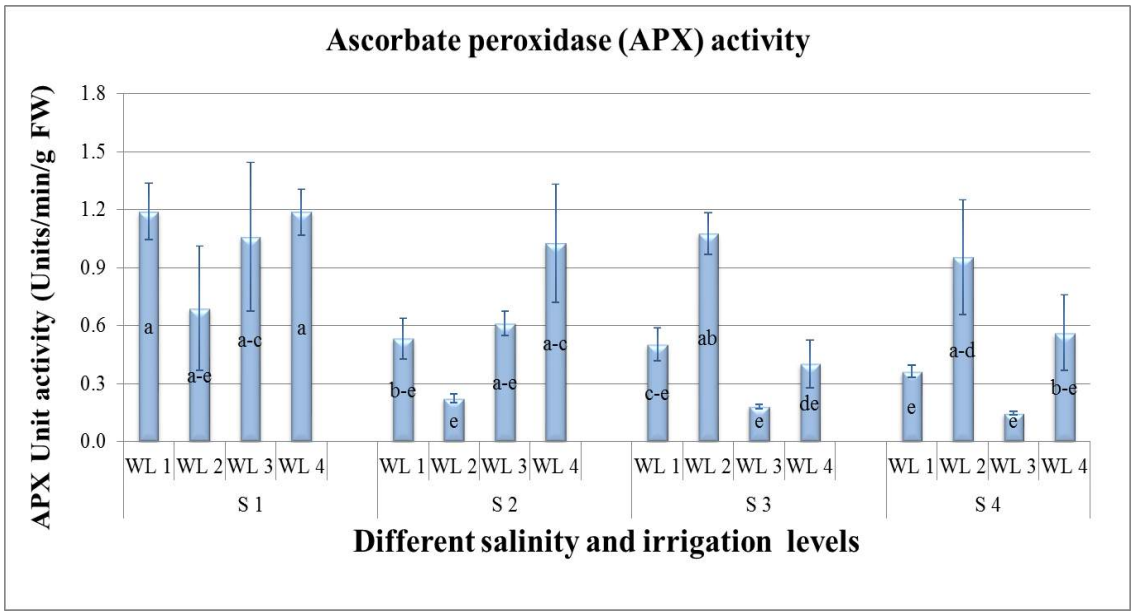

Fig. 4.2.2.19 APX activity of *T. mandavillei* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.3 *Atriplex leucoclada Boiss*

Local/ Arabic name: Ragal, رغل

Mean data regarding different parameters of *A. leucoclada* as affected by different levels of salt and water stress is represented in Fig. 4.2.3.1 - Fig. 4.2.3.19. Analysis of variance (ANOVA) is presented in respective Appendix 4.2.3.1 - 4.2.3.19.

4.2.3.1 Survival percentage (%)

Data regarding survival percentage of *A. leucoclada* as affected by different levels of salt and water stress is presented in Fig. 4.2.3.1 and their analysis of variance in Appendix 4.2.3.1. Both salt and water stress levels and the salt x water stress had the non-significant effect on the survival percentage of *A. leucoclada* ($P>0.05$). Survival percentage data showed that *A. leucoclada* survived on all salt and water stress levels. It is clear from the results of survival percentage that *A. leucoclada* can survive on all studied salt and water stress levels. For most of the treatments survival percentage is between 80-90 %. However, few treatments had lower survival percentage up to 68 % which may be due to environmental effects.

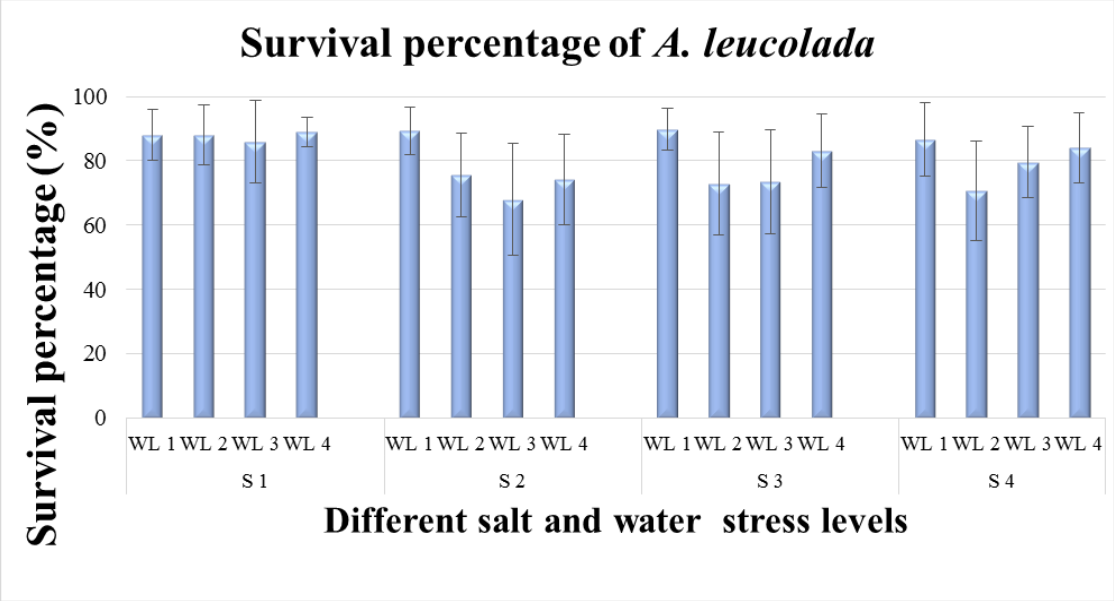

Fig. 4.2.3.1 Survival percentage of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.2 Shoot dry weight (SDW) (g)

Mean effect of salt and water stress on SDW is presented in Fig. 4.2.3.2 and the ANOVA in Appendix 4.2.3.2. Analysis of variance revealed that interaction between different salt and water stress levels significantly ($P \leq 0.05$) affected SDW of *A. leucoclada.* SDW increased during experiment and maximum SDW was recorded for the month of July. Increasing salinity level increased the SDW up to S3 and highest SDW was 26g for S3WL1 in the month of July. While increasing the water stress level decreased the SDW and lowest SDW (2.7g) was recorded for S3WL3 and S1WL2.

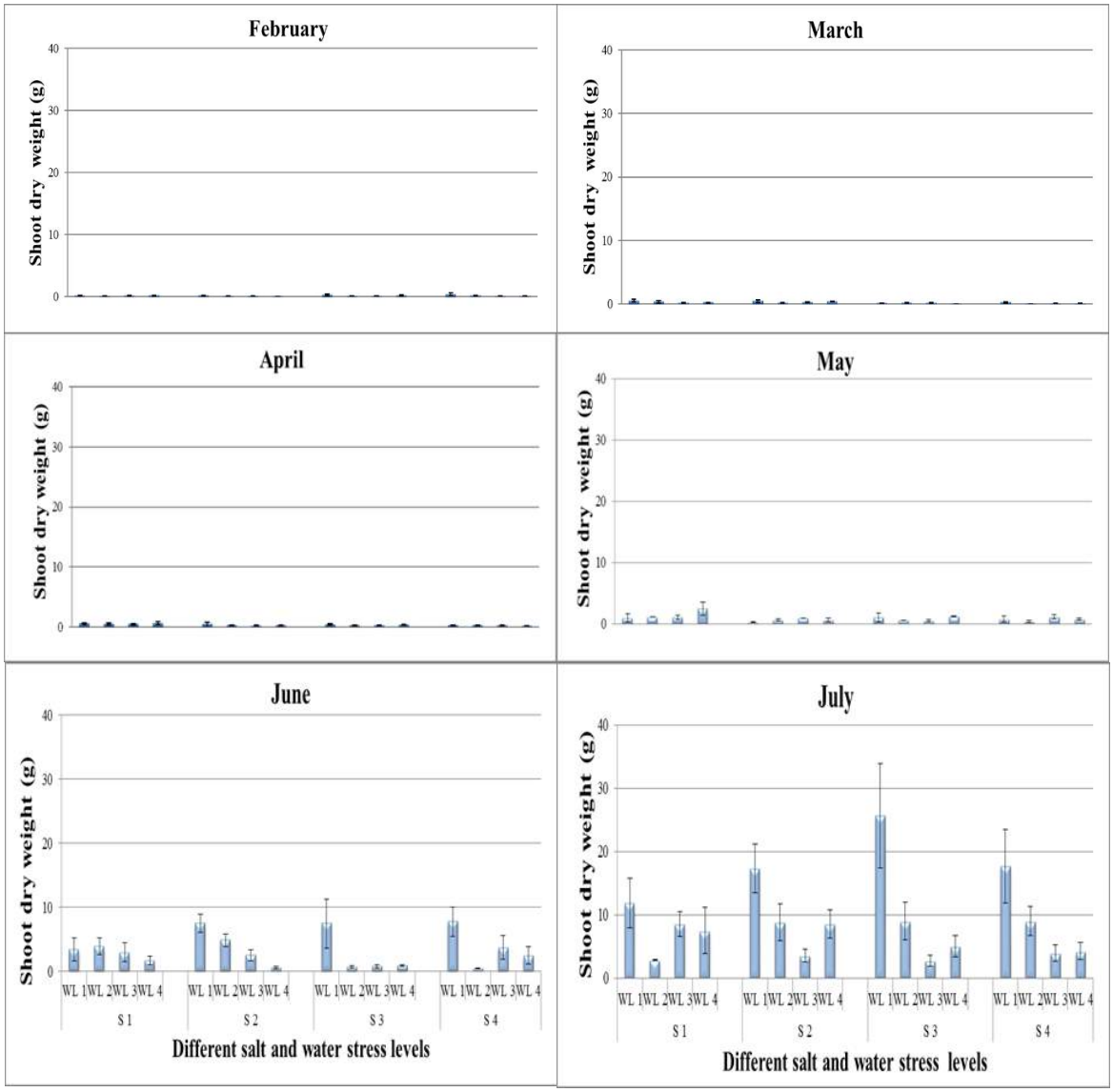

Fig. 4.2.3.2. Shoot dry weight of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.3 Root dry weight (RDW) (g)

Effect of salt and water stress on RDW of *A. leucoclada* is presented in Fig. 4.2.3.3. The ANOVA is in Appendix 4.2.3.3. Analysis of variance showed a significant effect ($P \leq 0.05$) of water stress levels and time interval (month) on RDW of *A. leucoclada*. Increasing water stress increased the RDW of *A. leucoclada*. However, interaction between salt and water stress levels had non-significant ($P > 0.05$) on RDW of *A. leucoclada* (Appendix 4.2.2.3).

RDW was significantly low ($P \leq 0.05$) in the months Feb- May while highest (2.22 g) in the month of July. RDW showed and insignificant decreased with increasing salt stress level. Maximum RDW was measured for WL1 (0.99 g) while other three water levels of WL2, WL3 and WL4 were significantly lower and similar to each other.

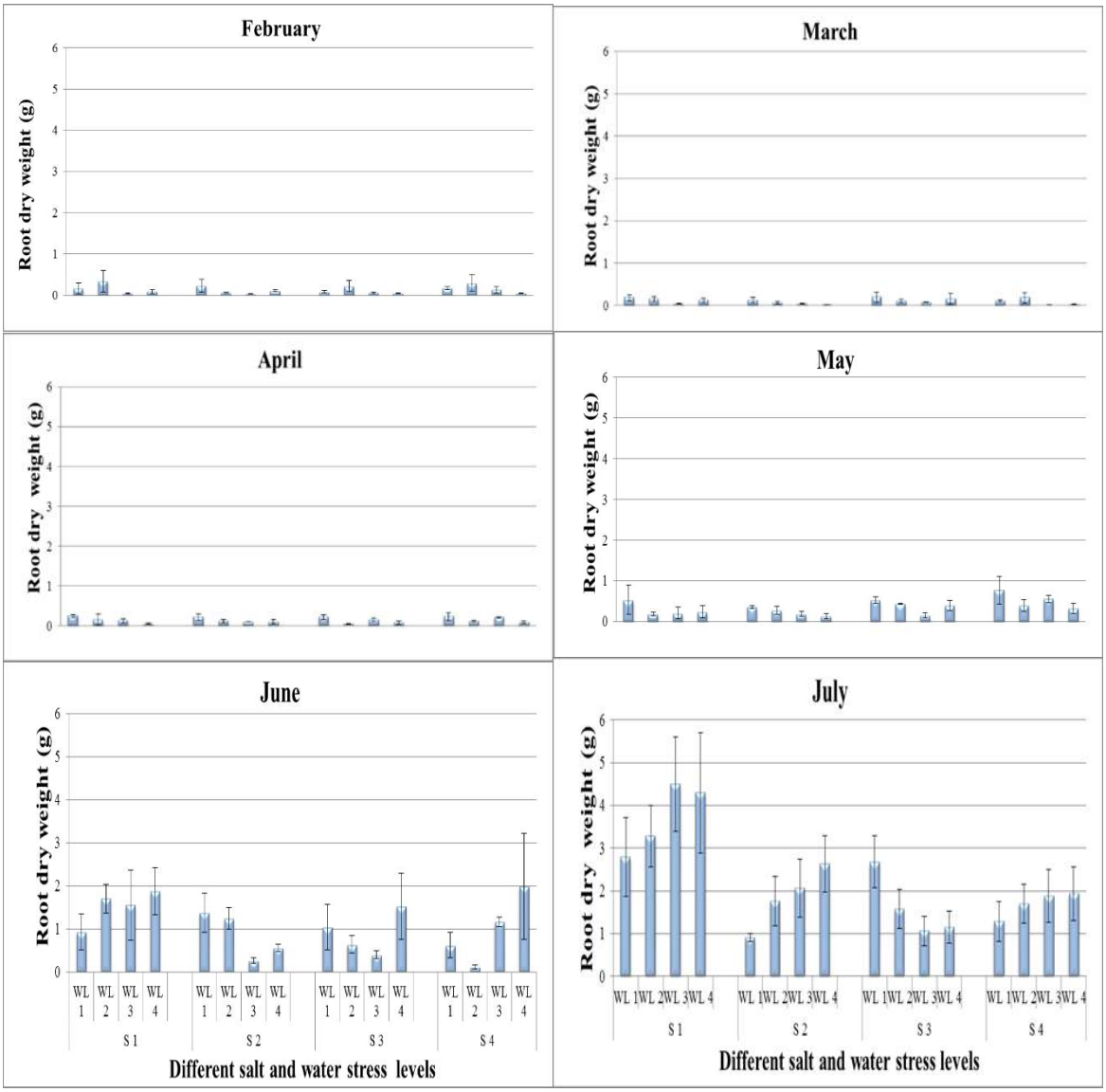

Fig. 4.2.3.3 Root dry weight of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.4 Shoot length (SL) (cm)

Results of the interaction of different salt and water stress levels for SL are presented in Fig. 4.2.3.4. The ANOVA in Appendix 4.2.3.4. Different salinity and water stress levels and the interaction between them showed a significant effect ($P \leq 0.05$) on SL of *A. leucoclada*. SL of *A. leucoclada* increased significantly ($P \leq 0.05$) with the time interval. Therefore, time (month) had significant effect on SL of *A. leucoclada*. Maximum SL was recorded for 60 and 57 cm for S2WL1 and S3WL1 respectively. Lowest SL was 7cm recorded for S4WL3.

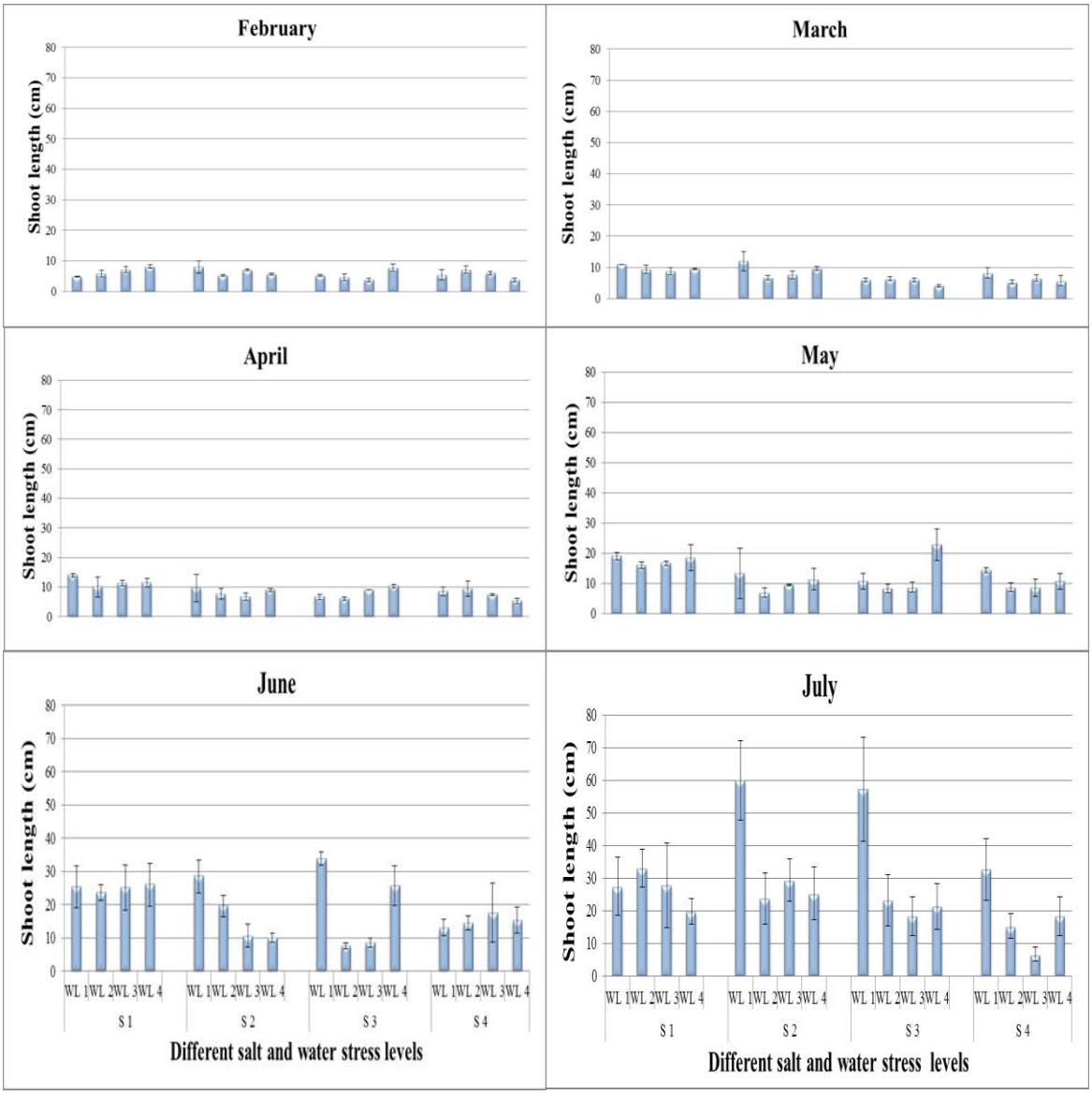

Fig. 4.2.3.4 Shoot length of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.5 Root length (RL) (cm)

Effect of salt and water stress on RL of *A. leucoclada* is presented in Fig. 4.2.3.5 and the ANOVA in Appendix 4.2.3.5. Different salt levels and interaction of salt and water stress levels had significant effect ($P \leq 0.05$) on RL of *A. leucoclada*. RL of *A. leucoclada* was observed maximum (49 cm) for S1WL2 followed by 48 cm for S2WL1. Minimum RL for *A. leucoclada* at the end of experiment was (23 cm) for S3WL3 followed by 24 cm for S4WL2.

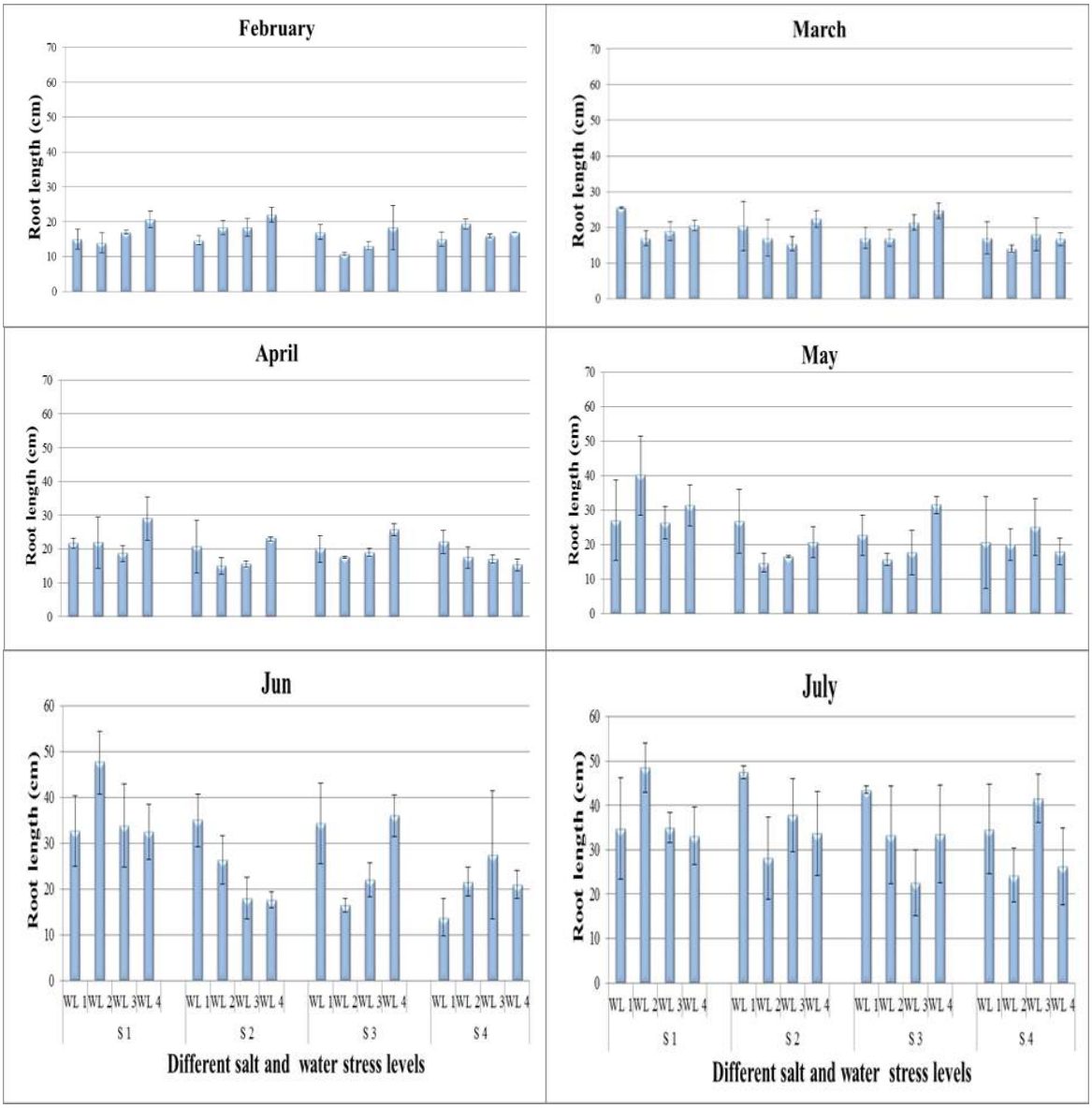

Fig. 4.2.3.5 Root length of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.6 Water use efficiency (WUE) ($g.L^{-1}$)

Results of different salt and water stress levels on WUE are presented in Fig. 4.2.3.6 and the ANOVA in Appendix 4.2.3.6. Analysis of variance revealed that different water stress levels had significant effect ($P \leq 0.05$) on WUE of *A. leucoclada*. WUE first decreased with increasing water stress at WL2 and increased at WL3 and WL4. WL1 had the WUE of 0.23 $g.L^{-1}$ while WL2, WL3 and WL4 had the WUE of 0.13, 0.15 and 0.25 $g.L^{-1}$ respectively.

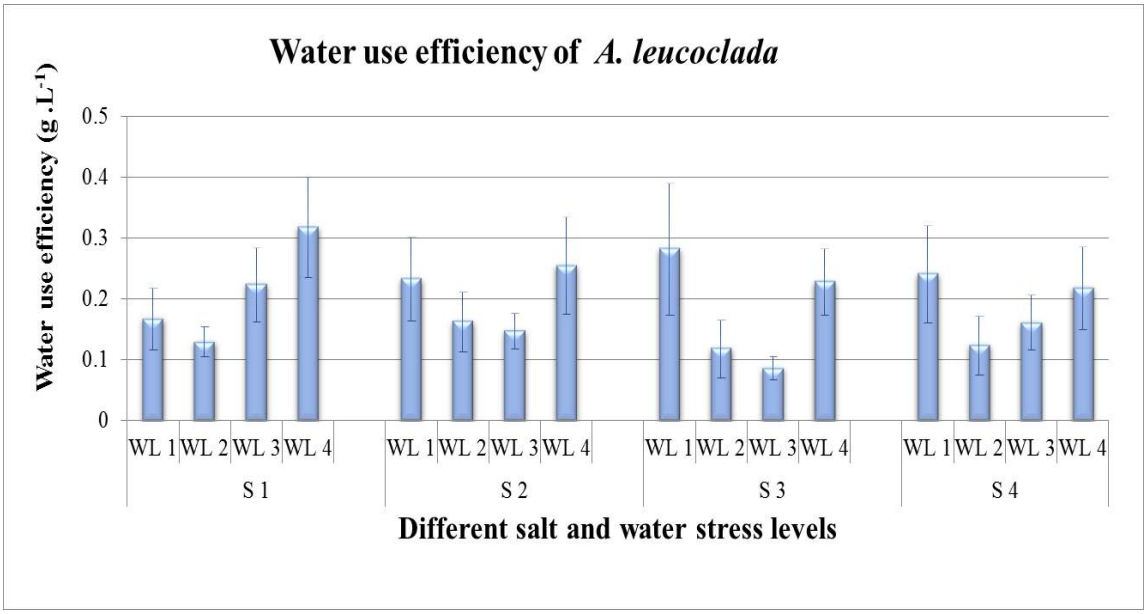

Fig. 4.2.3.6 Water use efficiency of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.7 Chlorophyll index

Results of different salt and water stress on chlorophyll index are presented in Fig. 4.2.3.7 and the ANOVA in Appendix 4.2.3.7. Analysis of variance revealed that different months, water stress levels and interaction between salt and water stress levels significantly ($P \leq 0.05$) affected chlorophyll index of *A. leucoclada*. Both salt and water stress contributed to increase chlorophyll index. However, S2 and S3 decreased the chlorophyll index with increasing water stress level in the months of Feb, March, April and May.

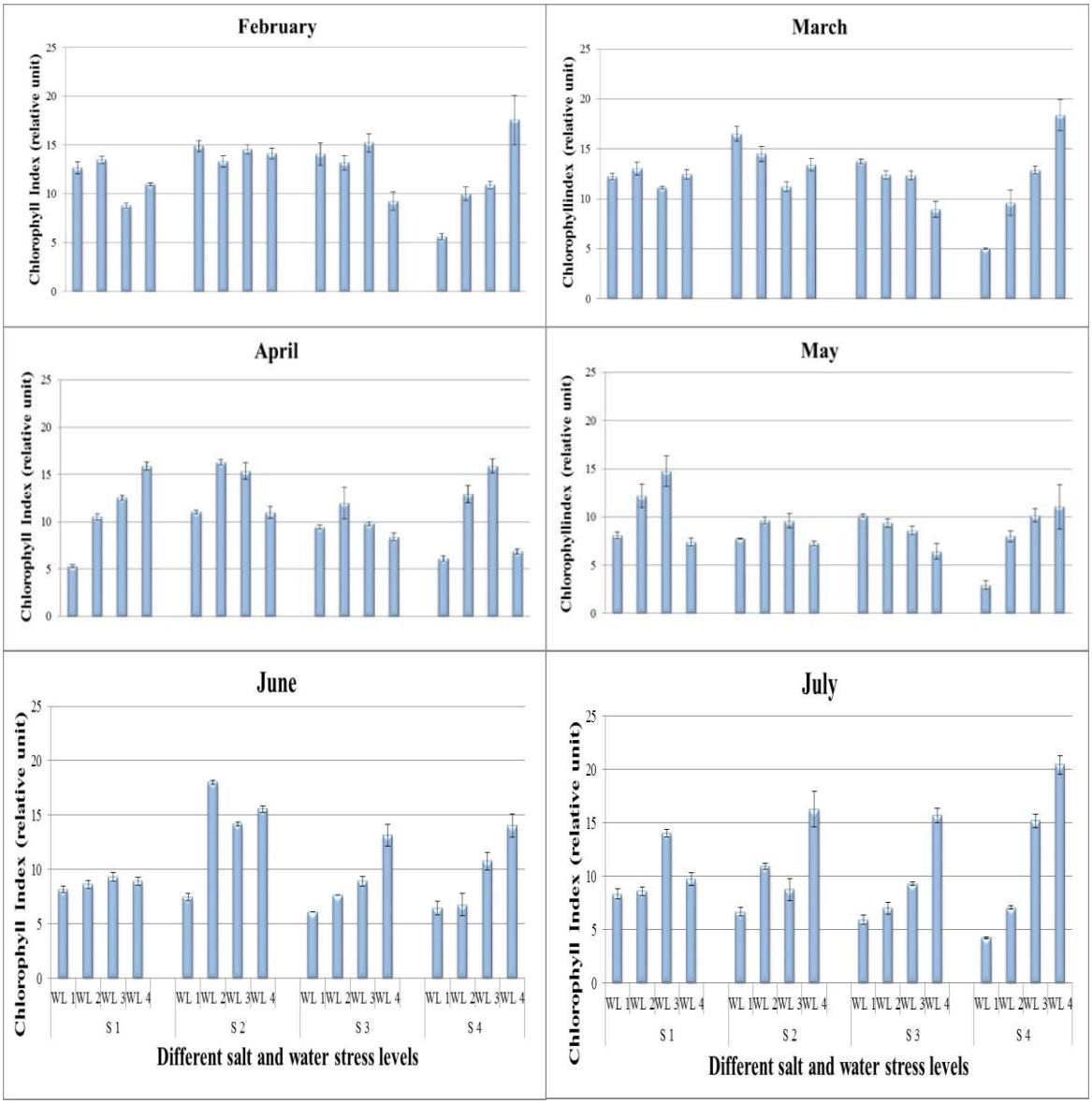

Fig. 4.2.3.7 Chlorophyll index of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.8 Photosynthetic rate (Pr) (µmol/m^2/s)

Data regarding Pr of *A. leucoclada* as affected by different levels of salt and water is presented in Fig. 4.2.3.8 and their analysis of variance in Appendix 4.2.3.8. Different months, salt and water stress levels and interaction of salt by water stress had a significant ($P \leq 0.05$) effect on the Pr of *A. leucoclada*. Pr was higher in start of experiment in the months of February till April. As the temperature increased after April the Pr decreased in the months of May, June and July. Pr had an increasing trend with increasing both salt and water stress. Highest Pr (67 µmol/m^2/s) was recorded during the month of March for S1WL4 while lowest Pr (1.0 µmol/m^2/s) was recorded during the month of May for SL4WL1. Both salt and water stress had interactive effect ($P \leq 0.05$) on Pr of *A. leucoclada*. Salt stress decreases the Pr while water stress increased the Pr significantly ($P \leq 0.05$).

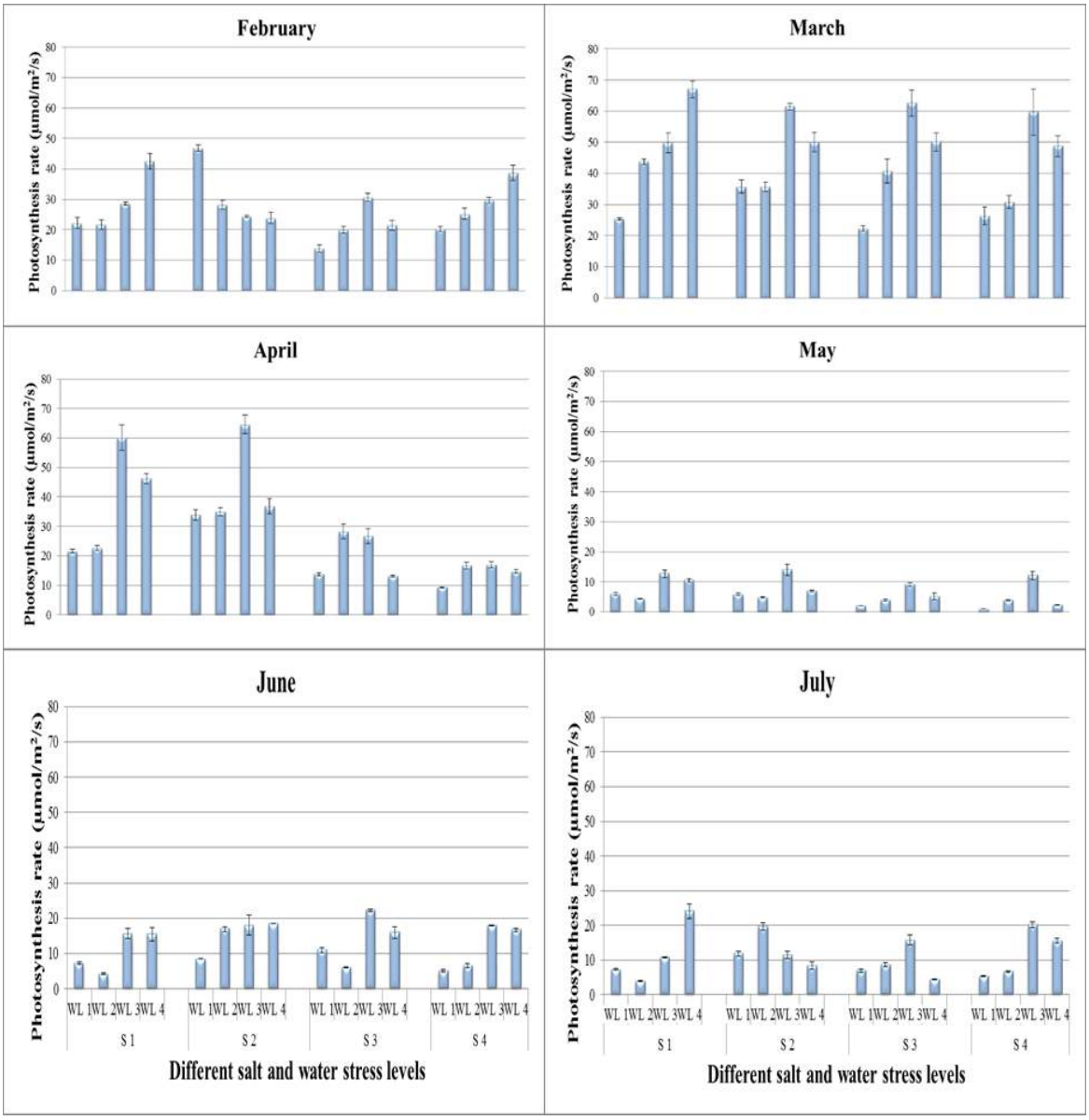

Fig. 4.2.3.8 Photosynthetic rate (Pr) of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.9 Leaf water potential (LWP) (MPa)

A. leucoclada was measured for its leaf water potential at two times, one and five months after the start of treatment application. LWP was decreased significantly by both salt and water stress after one month of treatment application. After one month and five months of treatment application, salt x water stress had significant effect ($P \leq 0.05$) on the leaf water potential of *A. leucoclada* (Appendix 4.2.3.9a. and Appendix 4.2.3.9b.). After one month of treatment application highest LWP (-38 MPa) was recorded for S3WL3 and lowest LWP (-57 MPa) was recorded for S4WL4. After five months of treatment S2WL1 had the maximum LWP of -46 MPa and minimum LWP (-64 MPa) was recorded for S1WL2 (Fig. 4.2.3.9).

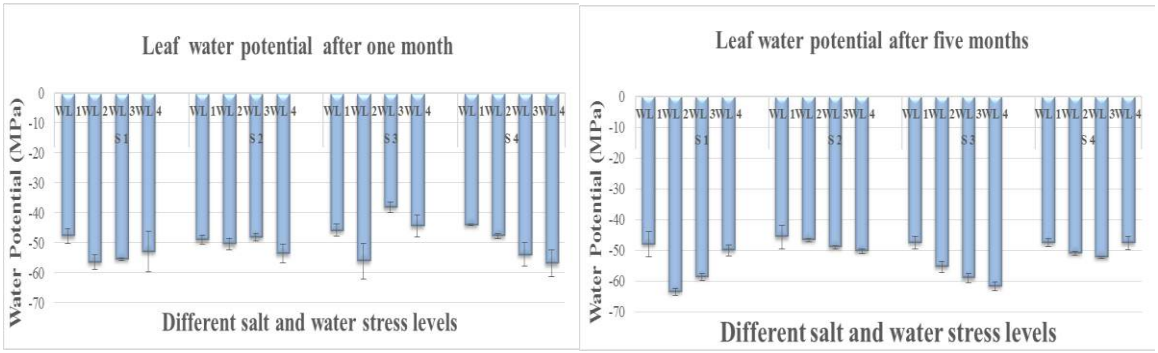

Fig. 4.2.3.9. Leaf water potential of *A. leucoclada* at two different time intervals under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4), and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.10 Plant total nitrogen (%)

Different salt stress levels and the interaction of salt by water stress levels had the significant effect (P≤0.05) on the plant total nitrogen content of *A. leucoclada* (Appendix 4.2.3.10). Maximum plant total nitrogen was recorded as 0.66 % for treatment S4WL3. Lowest plant total nitrogen content (0.33 %) was recorded for S1WL2 and S4WL1 followed by 0.37 % for S1WL1. Plant total nitrogen was lower at low salinity level and increase with increasing salinity stress. Increasing water stress also increased plant total nitrogen content and highest plant total nitrogen content (0.66 %) was measured in S4WL3 (Fig. 4.2.3.10).

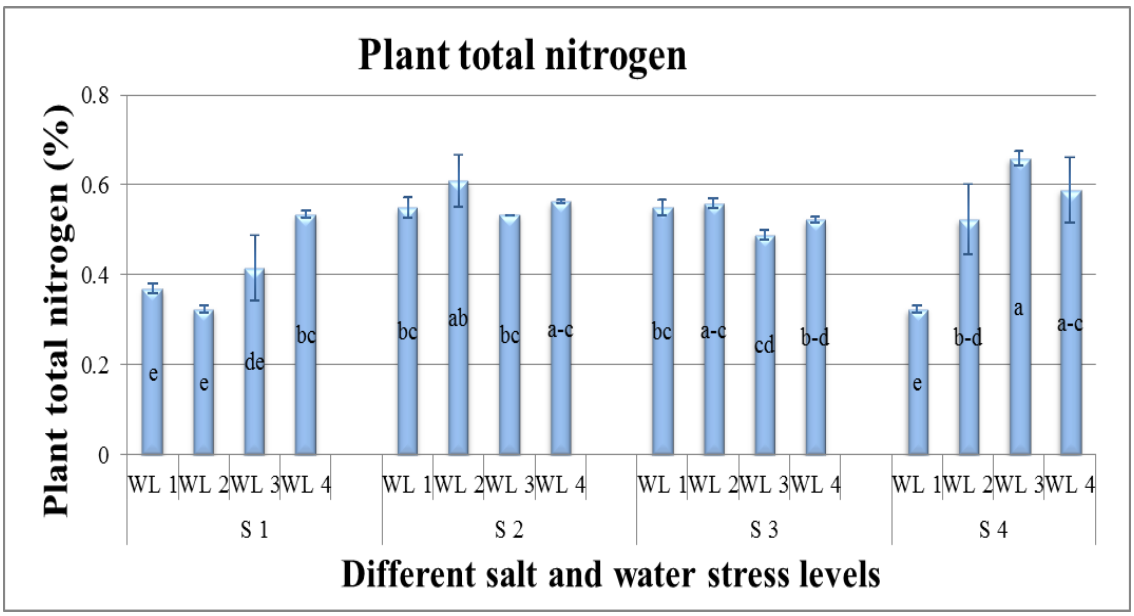

Fig. 4.2.3.10 Plant total nitrogen of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.3.11 Phosphorus content (%)

ANOVA table revealed that plant total phosphorus of *A. leucoclada* was significantly affected (P≤0.05) by the different irrigation and salinity levels and their interaction (Appendix 4.2.3.11). Maximum plant total phosphorus (0.04 %) was recorded for S2WL2. Minimum plant total phosphorus was 0.01 and 0.02 % recorded for S1WL2 and S1WL1 respectively (Fig. 4.2.3.11).

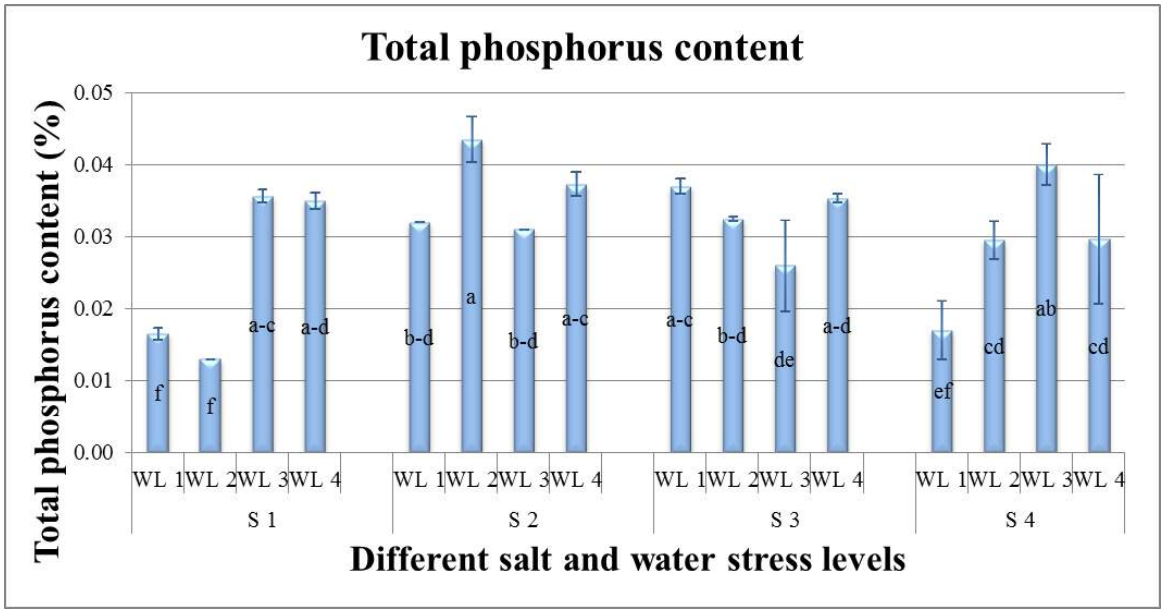

Fig. 4.2.3.11 Plant total phosphorus of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.3.12 Total potassium content (%)

Total potassium content of shoot of *A. leucoclada* had shown significant affect ($P \leq 0.05$) for salt stress (Appendix 4.2.3.12). Total potassium content of *A. leucoclada* decreased with increasing salt stress. Total potassium content of *A. leucoclada* increased at S2 and decreased again at S3 and S4 (Fig. 4.2.3.12).

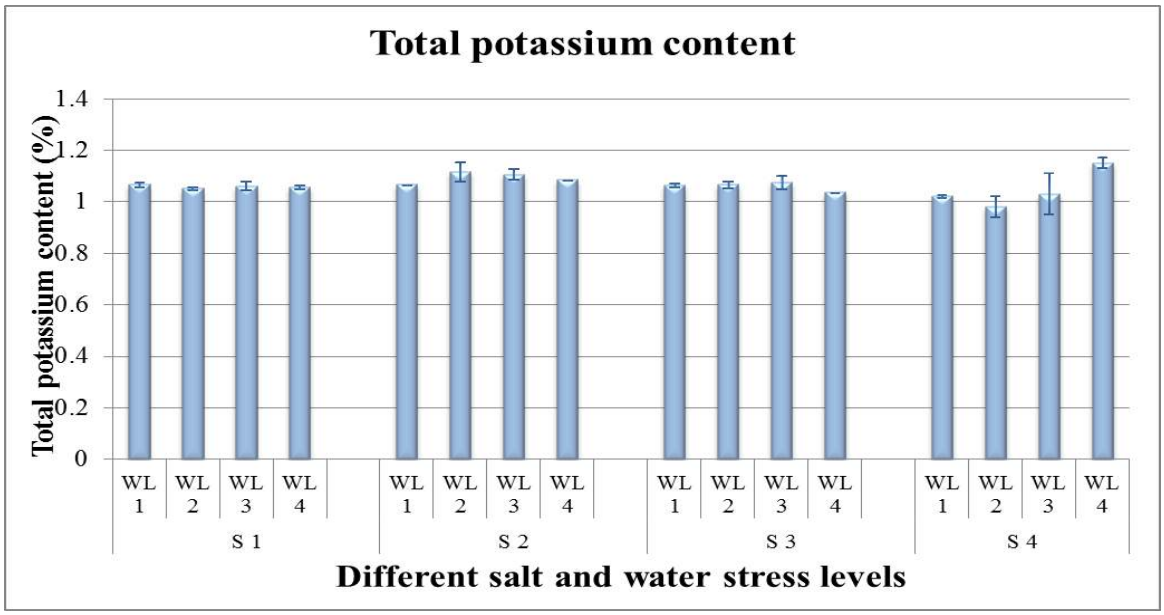

Fig. 4.2.3.12 Plant total potassium content of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.13 Sodium content (µ mole/g)

Na^+ content had an interactive effect ($P \leq 0.05$) for salt and water stress. Na^+ content increased with increasing salinity (Appendix 4.2.3.13). Na^+ content increased not only with increasing salt stress but also with increasing water stress. Na^+ was lower in low salt stress of S1 and lowest Na^+ content was 419 and 435 µ mole/g recorded for S1WL2 and S1WL1 respectively. Na^+ content increased with increasing salinity and water stress and maximum Na^+ content was 524 and 529 µ mole/g reported for S3WL4 and S4WL4 respectively (Fig. 4.2.3.13).

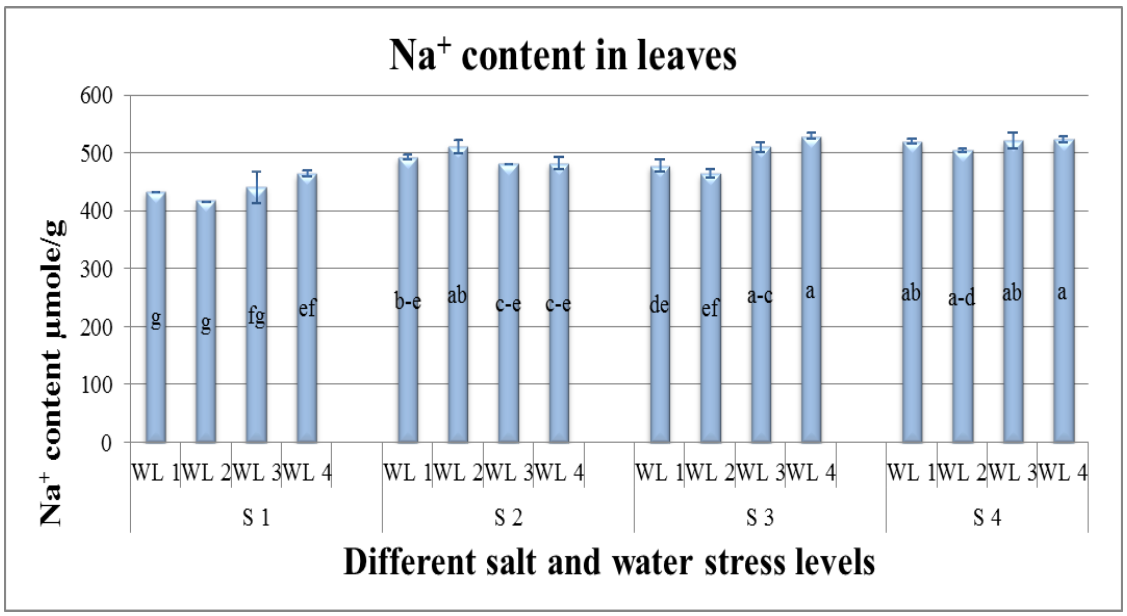

Fig. 4.2.3.13 Na^+ content in leaves of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4), and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.14 Chloride content (µmole/g)

Both salt and water stress had statistically significant interactive effect on the Cl^- content of *A. leucoclada* (Appendix 4.2.3.14). Lowest Cl^- content (34 and 45 µmole/g) was recorded for S1WL1 and S1WL2 followed by 108 µ mole/g in S1WL3. Highest Cl^- content (437 µmole/g) was recorded for S4WL1 followed by 414 µ mole/g for S3WL1. Cl^- content increased with increasing water stress even at low salinity levels when external NaCl concentration was low. However, at highest salt stress level it decreased with increasing water stress (Fig. 4.2.3.14).

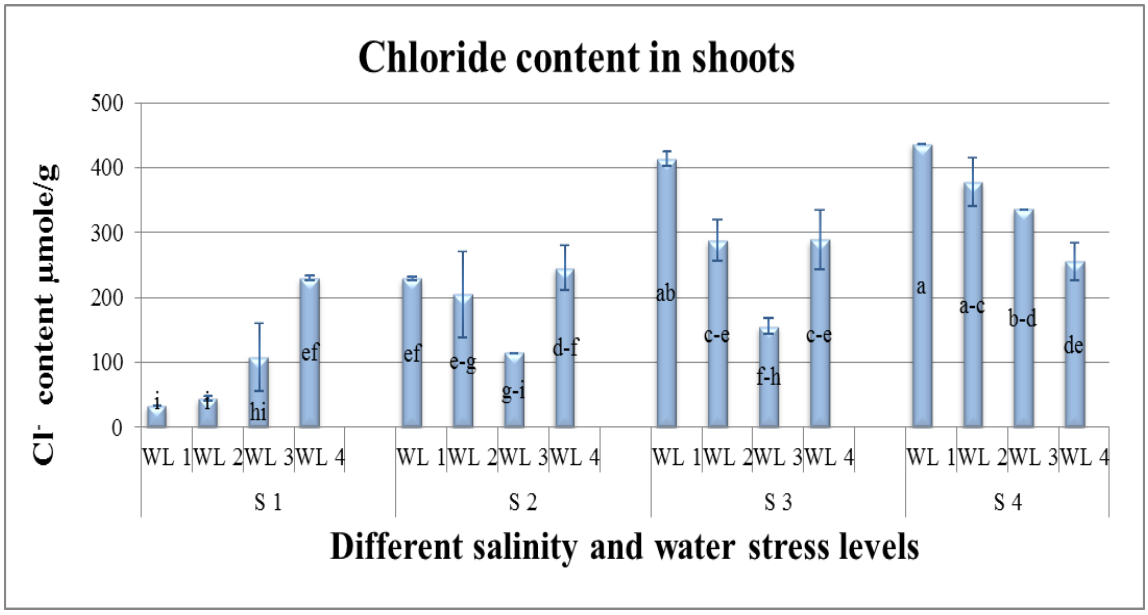

Fig. 4.2.3.14 Cl^- content in leaves of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.3.15 Abscisic acid (µg. g $^{-1}$ FW)

Means of ABA quantified are represented in Fig. 4.2.3.15. Salt and water stress levels had statistically significant (P≤0.05) interaction for ABA production (Appendix 4.2.2.15). ABA production showed an increasing trend with increasing both salt and water stress levels. Maximum ABA content was 92 µg.g^{-1} FW quantified at S4WL4.

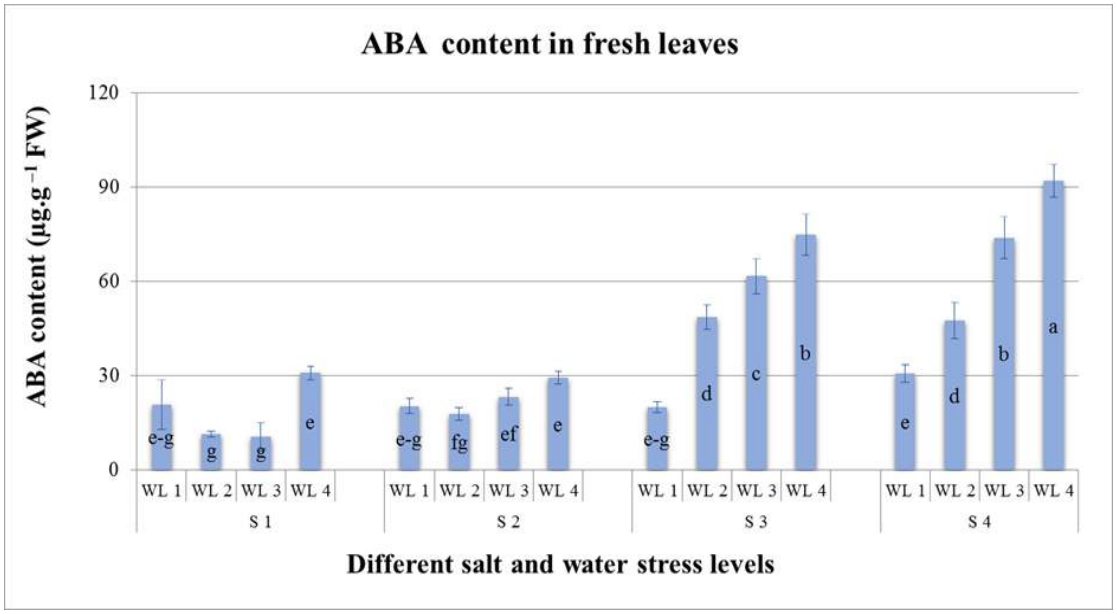

Fig. 4.2.3.15 ABA content (µg. g-1 FW) of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.3.16 Proline content (µg.g $^{-1}$ FW)

ANOVA revealed that different salt and water stress levels had non-significant effect on the proline content of *A. leucoclada* (Appendix 4.2.3.16). Interactive effect of salt and water stress levels was significant (P≤0.05). Proline content in leaves decreased with increasing water stress level at lower salt stress. However, at higher salt stress proline had an inverse trend. Proline content increased with increasing the combine effect of salt and water stress and maximum proline content was 16654 µgg^{-1}FW recorded for S4WL4 (Fig. 4.2.3.16)

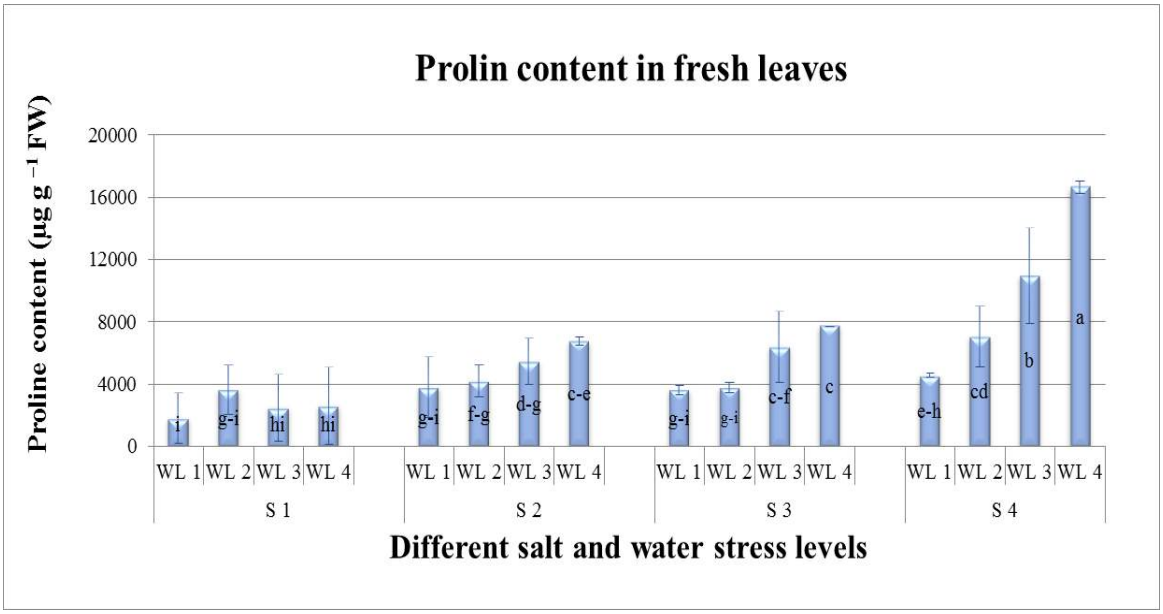

Fig. 4.2.3.16 Proline content (µgg^{-1}FW) of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4), and four irrigation intensities: 100 % of field capacity (Control; WL1) 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.3.17 Catalase activity (units/min/g FW)

ANOVA table revealed that different salt and water stress levels had significant interactive effect (P≤0.05) on the CAT activity of *A. leucoclada* (Appendix 4.2.3.17). CAT activity in leaves of *A. leucoclada* increased with increasing water stress level under S1 and decreased with increasing water stress under S2, S3 and S4. S2WL3 had the lowest CAT activity of 858 (units/min/g FW) while S1WL4 had the highest CAT activity of 6299 units/min/g FW (Fig. 4.2.3.17).

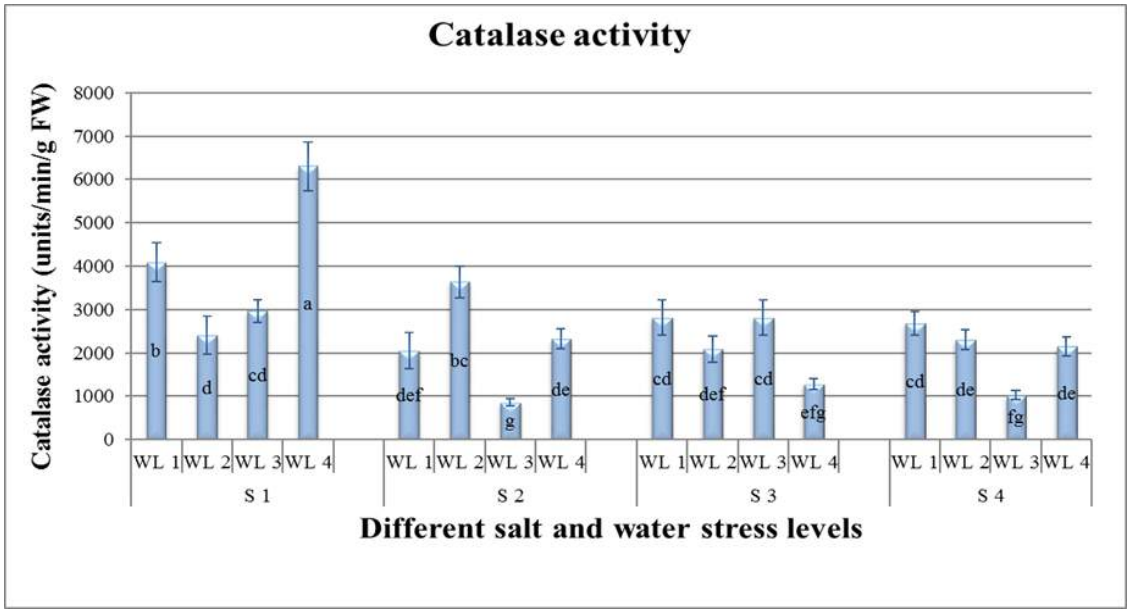

Fig. 4.2.3.17 Catalase activity (units/min/g FW) for *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.3.18 Peroxidase activity (POD) (units/min/g FW)

Variance table revealed the considerable effect of different salt and water stress levels on POD activity of *A. leucoclada* and effect of salinity x water stress levels on POD activity of *A. leucoclada* was significant (P≤0.05; Appendix 4.2.2.18). With increasing water stress level, POD activity of *A. leucoclada* in leaves also increased. Increasing salinity stress level from S1 to S3 also increased POD. However, at higher salinity stress level of S4 POD activity decreased (Fig. 4.2.2.18). Highest POD activity was 0.51 units/min/g FW for S3WL4 and minimum was 0.07 units/min/g FW for S1WL2 treatment.

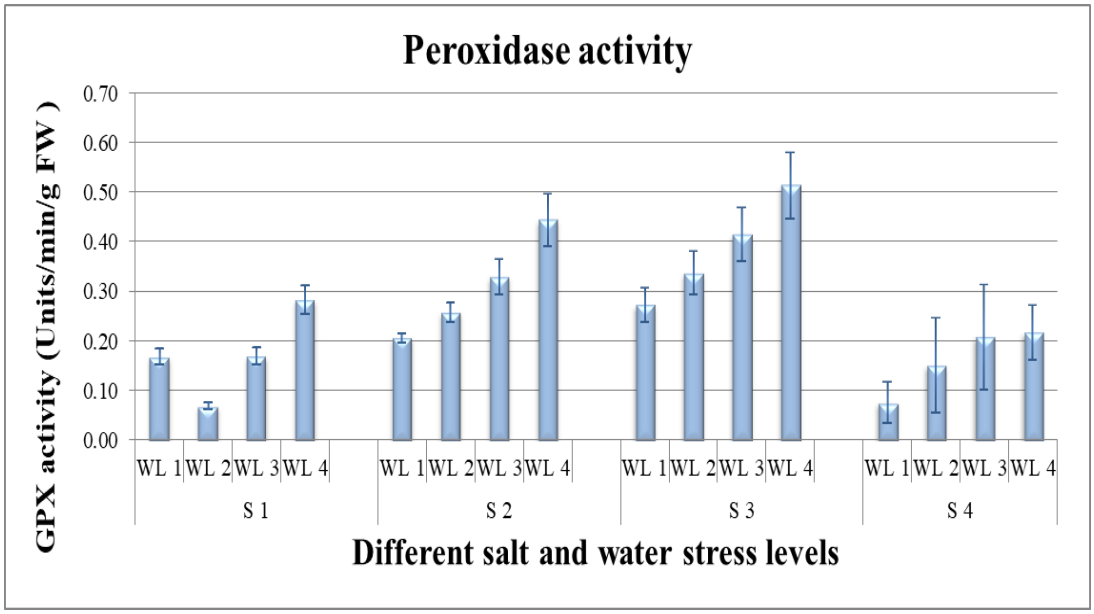

Fig. 4.2.3.18 Peroxidase activity (POD) (units/min/g FW) of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; P ≤ 0.05; mean±SE)

4.2.3.19 Ascorbate peroxidase (APX) activity (units/min/g FW)

Data regarding APX activity of *A. leucoclada* as affected by different levels of salt and water is presented in Fig. 4.2.3.19 and their analysis of variance in Appendix 4.2.3.19. Different salinity and water stress levels significantly ($P \leq 0.05$) affected the APX activity of *A. leucoclada*. The effect of interaction between salinity and water stress levels was non-significant ($P > 0.05$). APX activity in *A. leucoclada* increased with increasing salt stress. Similarly increasing water stress also increased the APX activity in *A. leucoclada.*

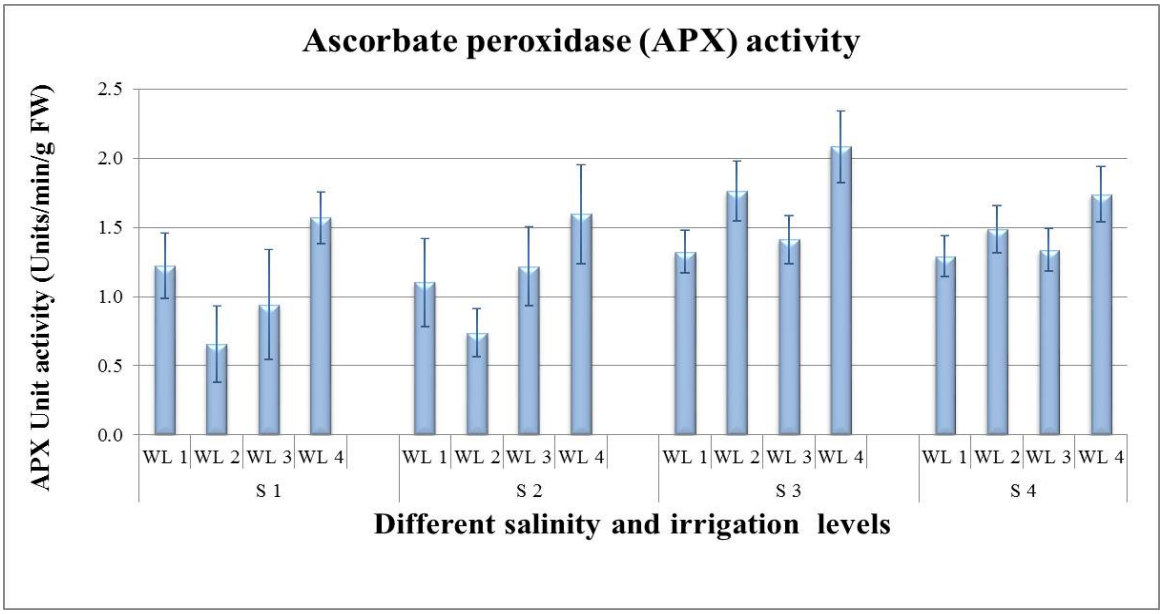

Fig. 4.2.3.19 APX activity of *A. leucoclada* under four salt stress treatments: 5 dSm^{-1} (Control; S1), 10 dSm^{-1} (low salinity level; S2), 15 dSm^{-1} (moderate salinity level; S3) and 20 dSm^{-1} (high salinity level; S4) and four irrigation intensities: 100 % of field capacity (Control; WL1), 80 % of field capacity (low stress; WL2), 60 % of field capacity (moderate stress; WL3) and 40 % of field capacity (severe stress; WL4). Significant differences between treatments are indicated by different lower-case letters (Tukey's HSD post hoc; $P \leq 0.05$; mean±SE)

4.2.4 Discussion

4.2.4.1 Shoot dry weight (SDW)

Salt and water stress are considered as separate and additive factors contributing growth reduction (Bresler and Hoffman, 1986; Cardon and Letey, 1992; Chaves *et al*., 2009; Munns, 2002). This slower growth is a part of plants adaptive mechanism to utilize the cell resources for the stress defense (Zhu, 2001). However, moderate salinity (50 to 250 mM NaCl) can stimulate growth of many halophytes (Flowers *et al*., 1986; Khan *et al*., 2000). Therefore studying salt stress for protective or depressing effect on plant growth and water consumption will avoid excess water application to plant (Ünlükara *et al*., 2015).

Three species under investigation had survived till end of experiment but with different effects of individual and combined salt and water stress. *T. mandavillei* belonging to family *Zygophyllaceae* grew best under lowest stress levels of S1 and WL1 while reduced growth under other three salts and water stress levels with no significant difference between them. In contrast, *S. imbricata* tend to grow optimally under all salt stress and no effect of salt stress was recorded on SDW. *A. leucoclada* had an interactive effect of salt and water stress. SDW even increased with increasing salt stress while increasing water stress decreased SDW under higher salt stress.

NaCl stress severely influenced seedling growth more dramatically compared to germination. Salt stress decreased SL and RL in safflower (*Carthamus tinctorius* L.). Dry matter also increased significantly due to decreased tissue water content under NaCl stress (Farhoudi and Motamedi, 2010; Kaya and Day, 2008) Salt stress have more inhibitory effect on germination than seedling growth (Kaya et al., 2005). Similarly in chickpea drought and salinity have more inhibition effects on seedling growth than the seed germination (kalefetoğlu *et al*., 2009).

Similarly, Hassine *et al.* (2008) also reported 40 mM NaCl can improve plant growth of *Atriplex halimus* however, 160 mM NaCl was harmful for *Atriplex halimus* plants. Same results for 50 mM NaCl were reported by Alla *et al*. (2012). This increase under moderate salinity in growth is increase in water content (Flowers *et al*., 1986).

Similarly increased tissue under water content less than 200 mM external NaCl reported in *Suaeda fruticosa* (L.) Forssk (Khan, *et al*., 2000).

Salt stress resistance mechanism is based on osmotic adjustment by toxic ions (mostly Na^+ and Cl^-) accumulation in the vacuoles (Flowers *et al*., 1986; Zhu 2001; Serrano and Gaxiola, 1994). Anatomical adaptations include increased succulence for accumulation of larger amounts of water (and dissolved ions) in the leaves. *S. imbricata* and *T. mandavillei* have succulent leaves which help them to accumulate more water and ions dissolved in that water (Vicente *et al*., 2004). Therefore, additional succulence increase had not been observed in *S. imbricata* and *T. mandavillei* (Vicente *et al.,* 2004). Here results for *S. imbricata* showed a reduced growth under water stress. These results for *S. imbricata* and *T. mandavillei* are similar to Omami and Hammes (2006) who reported that water stress affect plants more than those subjected to salt stress or salt and water stress together.

Hassine and Lutts (2010) reported results similar to present study in case of *Atriplex halimus* and observed less plant growth in PEG solution as compared to NaCl. Almas *et al.* (2013) also reported that water stress conditions created by PEG decreased seedling growth with higher negative effects compared to NaCl in *Artemisia vulgaris* L. Same negative effects of water stress compared to salt stress are reported for the *Pistacia lentiscus* (Álvarez *et al*., 2018). The present results agree with Okçu, *et al.* (2005) for Pea (*Pisum sativum* L.) triticale (Kaydan and Yagmur, 2008) and soybean (Khan *et al*., 2017). Although soil water potential decreased by saline water irrigation but water flow to the roots remains same. On the other hand, water stress decreases the soil matric potential and decrease water flow to the roots (Homaee *et al*., 2002). This can be the reason that the matric potential during water stress affected the shoot growth of studied species more than that did the osmotic potential (Shainberg and Shalhevet, 2012). These results corroborates with the results of Maggio *et al*. (2005) who reported 22 % less aboveground dry weight in salt stress and 46 % less dry weight in water stress than control.

Higher salinity helped to mitigate the deleterious effects of water stress. In current experiment, higher salinity of S4 did not showed any negative effects of water stress on *S.*

imbricata. Contrary for *A. leucoclada* salt stress up to S4 (20 dSm^{-1}) resulted in lower SDW due to water stress. However, Melo *et al*. (2016) reported for *Atriplex nummularia* that salinity equal to or higher than 30 dSm^{-1} had no significant effect for the water stress. For salt stress below 30 dSm^{-1} water stress reduced the biomass which is in accordance with our results (Melo et al., 2016).

Similarly, *T. mandavillei* had increased growth under combined salt and water stress which is similar to results of Yue *et al*. (2012) who reported that 50 mM NaCl alleviate negative effects of water stress in *Z. xanthoxylum*. Positive effects of NaCl were reported for the halophytic species *Sesuvium portulacastrum* under water stress produced by mannitol. Plant growth increased as twice by addition of 100 mmol L^{-1} NaCl together with mannitol (Slama *et al*., 2007). Similar results were reported for corn (*Zea mays* L.) and melon (*Cucumis melo* L.) yields were affected at low water stress levels while at higher water stress levels, yields were unaffected by the salt stress (Shani and Dudley, 2001). This increased SDW under salt stress was achieved as increasing salt can help to improve the relative water content, and decrease LWP and thus increasing Pr and WUE (Ma *et al.*, 2012; Yue *et al*., 2012).

Higher salt and water stress levels results in decreased transpiration and maximum salt accumulation. Under water stress transpiration is decreased and leaching start after maximum accumulation of salts. Plant root zone is shortened due to salt accumulation in the lower root zone. Salts exit the root zone as a leaching fraction and plant extract water with lowest salinity from the upper part of root zone. Plants self-regulate the irrigation–drainage relationships if water salinity is above tolerance limit eventually avoiding extensive yield loss (Dudley *et al*., 2008). Letey *et al*. (2011) supported same argument and recommended lower leaching requirement under salinity stress.

Salt stress resistance mechanism is based on osmotic adjustment by toxic ions (mostly Na^+ and Cl^-) accumulation in the vacuoles (Flowers *et al*., 1986; Zhu 2001; Serrano and Gaxiola, 1994). Salt-treated plants had developed a NaCl inclusion mechanisms and underwent osmotic adjustment, which could maintain leaf turgor (Rodríguez et al., 2005). Glenn *et al.* (2012) argued that combined salt and water stress

can increase WUE by reduced stomatal conductance which increase water stress tolerance in saline stress conditions. Same is reported for *A. canescens* (Glenn and Brown, 1998), *A. lentiformis* (Meinzer and Zhu, 1999) and *A. halimus* (Alla *et al*., 2011). This improved plant performance under combined salt and water stress may be due to its effect on osmotic adjustment through higher Na^+ and proline accumulation and decrease of K accumulation (Wu *et al*., 2015). NaCl mitigated the deleterious impact of water stress and improve the osmotic adjustment (Martínez *et al*., 2005). Increase Na^+ supply under salt stress can act as fertilizer to encourage plant growth and accumulates a higher concentration of Na^+ than K for osmotic adjustment (Kang *et al*., 2013).

4.2.4.2 Root dry weight (RDW)

Both salt and water stress known to reduce plant growth. However, responses to these stresses depend on the species and even on their accessions. Serrano *et al*. (2017) reported that an accession of pepper showed the minor decrease under water stress while another accession stood out under salt stress. Salt and water stress were mostly studied separately for their effects on crop growth (Hamed *et al.*, 2013). Both of them were considered as separate and additive stress factors for crop yield reduction (Chaves *et al*., 2009; Munns, 2002). Hence only few studies had examined their interactions (Hamed *et al*., 2013). *S. imbricata* had shown no significant effect of salt stress on RDW of *S. imbricata* and *A. leucoclada* and was higher in *T. mandavillei* for lowest salt stress level. On the other hand, increasing water stress had shown major decrease in RDW of all species.

Contrary to our results Maggio *et al*. (2005) stated that increasing irrigation water salinity from 0.5 and 8.5 dSm^{-1} decreased RDW of field-grown cabbage proportionally. However, water stress did not affect root dry mass accumulation. Similarly increasing salt and water stress together had resulted in higher decrease in vegetative growth (Sahin *et al.*, 2018). Same is the case of *Argyranthemum coronopifolium* which had higher growth reductions by salt stress than water stress (Herralde *et al*., 1998; Chaves and Pereira, 1992).

However, for halophytes the growth response is different than the glycophytes. For instance *Anethum graveolens* a moderately tolerant species to salt stress showed the similar

results to present experiment, salt stress had a relatively small effect on plant growth compared to water stress (Tsamaidi *et al*., 2017). Wang *et al.* (2011) testified same results to our study for tamarisk (*Tamarix chinensis* Lour) a highly salt-tolerant plant. Root biomass decreased significantly with the water stress. Under water stress condition root biomass had no significant difference for increasing salt stress although, less than the control irrigation. When water stress was minimum root biomass decreased significantly by increasing salinity from 4-8 $g.kg^{-1}$ while no significant decrease occurs when salt stress level increased more than 8 $g.kg^{-1}$.

Yagmur and Kaydan (2008) also reported greater reduction in RDW of triticale (*Triticosecale* Witm., cv. Presto) due to PEG than the NaCl. Khan *et al*. (2017) reported similar results for soybean RDW. Similarly analyzing Quinoa (*Chenopodium quinoa*), root biomass remain constant for different salt stress treatments while decrease by increasing water stress (Miranda-Apodaca *et al*., 2018).

Increasing salt stress decreases the soil water potential but does not decrease the water flow to the roots. Roots can allow water to move in by osmotically adjusting cortical cells. Therefore, RDW was less affected by salinity as compared to the water stress. These results confirm the findings of Shainberg and Shalhevet (2012) that the matric potential due to water stress affected the root growth more than did the salt stress due to osmotic potential (Khan *et al*., 2015).

4.2.4.3 Shoot length (SL)

Both salt and water stress had an interactive effect on SL of *S. imbricata*. Increasing water stress decreased SL under S1, while in other three salt stress levels (S2, S3, and S4) increasing water stress increased the SL. NaCl in salinity stress had shown a protective effect in water stress conditions for *S. imbricata*. Same protective role was observed in *T. mandavillei* and *A. leucoclada*. However, SL was recorded maximum in the S1 and WL1 only. SL remains statistically same under all other salt and water stress levels.

Shani and Dudley (2001) and Dudley (2008) found protective effect of salt stress on plant growth under water stress. Sahin *et al*. (2018) had results similar to present study in case of *Brassica oleracea* who reported negative impact of water stress on plant height only under low salinity levels. Liu *et al*. (2014) also reported increased plant height of *T. chinensis* under salt stress than control.

Most halophytes had shown same growth pattern under combined stress of salt and water. As examples, sea aster (*Aster tripolium*) (Ueda *et al*., 2003), *Atriplex halimus* (Martínez *et al.,* 2005), *Spartina alterniflora* (Brown *et al.,* 2006), *Sesuvium portulacastrum* (Slama *et al*., 2008; Slama *et al*., 2007), *Bruguiera cylindrica* (Atreya *et al*., 2009), *Zygophyllum xanthoxylum* (Ma *et al*., 2012), *Ipomoea pes-capra* (Sucre and Suarez, 2011).

4.2.4.4 Root length (RL) (cm)

Here, both salt and water stress levels had an interactive effect on the RL of *S. imbricata* and *A. leucoclada.* Water stress decreased the RL of *S. imbricata* under low salt stress. However, as supposed drought stress increases the RL under higher salt stress. RL of *A. leucoclada* also decreased with increasing water stress at lower salt level of S1. However, at higher salt stress levels, RL first decreased with increasing water stress level and increased at maximum water stress level. *T. mandavillei* decreased the RL significantly in response to salt and water stress. Interaction for salt and water stress had non-significant effect on RL. RL was maximum at the lowest salt and water stress level only. While rest of salt and water stress levels had significantly lower RL but similar to each other.

Similarly, Maggio *et al.* (2005) also reported that water stressed plants had greater RL compared to salt stressed plants. Similar results were reported by Liu *et al*. (2014) who decrease RL of *T. chinensis* in salt stress treatment compared to control. Under low soil moisture condition, the main RL increased first and then decreased (Liu *et al*., 2014). The current results are supported by Ünlükara *et al*. (2015) who found that water stress decreased RDWs but did not affect RL for green long pepper.

4.2.4.5 Water use efficiency (WUE)

In the current experiment salt stress had non-significant (P>0.05) effect on WUE of *S. imbricata, T. mandavillei* and *A. leucoclada.* Water stress significantly affected WUE in *S. imbricata* and A. *leucoclada. T. mandavillei* also showed a non-significant increase in WUE with increasing water stress.

These results indicate that under water stress conditions, three species under study can use lower amounts of water per unit of biomass production (Miranda-Apodaca *et al.,* 2018). WUE did not change under saline growth conditions in *Lycopersicon esculentum* (Aranda *et al.,* 2001). Similar results were reported for halophytes like *Chenopodium quinoa.* WUE was maintained under salt stress and increased under water stress conditions (Miranda-Apodaca *et al.*, 2018). Hessini *et al*. (2009) concluded that WUE increased under water stress in *S. alterniflora.* Garcí *et al.* (2004) studied four landscape species under water stress. *Leucophyllum frutescens* increased the water use in relation to leaf area and decreased WUE while *Spiraea vanhouteii*, *Viburnum tinus* and *Arctostaphylos densiflora* decreased the WUE.

Under frequent irrigation, salt stress reduced leaf expansion and carbon gain, but WUE was increased in sorghum (Richardson and McCree, 1985). For *A. halimus*, water stress resistance was associated with higher WUE rather than with a greater osmotic adjustment (Chen *et al*., 2011). In mini-watermelon (*Citrullus lanatus*) plants yield and WUE increased under water stress conditions. This was considered to be associated with mainly with an improvement in nutritional status and higher CO_2 assimilation and water uptake from the soil (Rouphael *et al*., 2008)**.** Yin *et al*. (2005) compared two sympatric species of *Sect. Tacamahaca* Spach, (*Populus).* Drought tolerant *P. przewalskii* employed a conservative water-use strategy by higher WUE under both control and stressed treatments. Whereas, lower drought tolerant *P. cathayana* may employed a prodigal water-use strategy.

WUE increase is related to reduced stomatal conductance. The decrease of stomatal conductance might cause the decrease of transpiration rate (E) and intercellular CO_2 concentration and the increase of WUE under stress (Megdiche *et al*., 2008). Diffusion rate

of CO_2 across stomata resulted changes in internal CO_2 concentration. This decreased internal CO_2 concentration can increase WUE either by increasing CO_2 uptake or by increasing photosynthetic activity (Flexas and Medrano, 2002). This results in slower growth but an increase in the ratio of carbon fixed per unit of water transpired (Glenn and Brown, 1998). Similarly Eisa *et al*. (2012) concluded salt stress improved WUE by decreasing transpiration rate and photosynthesis in *Chenopodium quinoa* Willd. Improved WUE resulted from efficient stomatal control can be related to increased ABA production under stress condition (Hassine and Lutts, 2010; Hassine *et al*., 2009).

Atriplex canescens plants showed enhanced growth performance under salt stress in drying soil by increased organic matter production and WUE (Glenn and Brown, 1998). This increased WUE under salt stress may be due to the reason that Glenn *et al*. (2012) compared salt stress of 85 mol/m^3 with control (0 mol/m^3).

Contrary to our results Tavousi *et al.* (2015) showed that salt and water stress can reduce WUE in pomegranate tree. Other researchers also showed that salt stress caused reduction in WUE. So can point out the research on tomatoes (Azarmi *et al*., 2008) and the corn (Karimi and Naderi, 2007). Drought stress decreased relative water content (RWC) and WUE of *Sophora davidii* seedlings (Wu *et al*., 2008).

WUE may decrease at salt and water stress more than the optimal stress level. Halophytes species *Batis maritima*, *Distichlis spicata*, *Juncus roemerianus*, *Paspalum vaginatum*, *Salicornia bigelovii* and *Spartina alterniflora* have optimum growth under salt stress up to <20 gL^{-1}. Salinity level more than 20 $g.L^{-1}$ leaching fraction of 0·50 is preferable, although the amount of water use will be excessive (El-Haddad and Noaman, 2001). For halophyte *Plantago coronopus* (L.) WUE was only affected if NaCl-saline condition was more than 25 % of sea water salinity (Koyro, 2006). Similarly Behboudian *et al*. (1986) argued salt and water stress decrease leaf water potential of pistachio (*Pistachia vera* L.). Decreasing leaf water potential due to increasing salt stress can decrease photosynthesis activity. However, plants can still continue photosynthesis activity until leaf water potential of as low as -5 MPa. However, plants were less efficient in their water use at the lower range of leaf water potential.

Other possible explanation of this may be that WUE can be improved under water stress only when there is need to balance crop water use against a limited and known soil

moisture reserve. However, under most arid environment plants depend on unpredictable rainfall, plant use maximum soil moisture available as a drought avoidance strategy resulting in lower WUE (Blum, 2005).

4.2.4.6 Chlorophyll index

Photochemical reactions are always highly disturbed by salt and water stresses (Hura *et al*., 2007; Tezara *et al.*, 2005). Salt tolerance is associated with the conservation of Pr and stomatal conductance (Lakshmi *et al*., 1996) and to elevated chlorophyll concentration (Winicov and Seemann 1990; Salama *et al*. 1994). Increasing water stress also reported to decrease the photosynthetic pigments in *Pelargonium odoratissimum* (L.) (Khalid and Cai, 2010).

When salt stress is continued, Rubisco activity is reduced (Delfine *et al.*, 1999). However, there are controversies about Rubisco activity under water stress (Lal *et al.*,1996). According to some researchers during water stress Rubisco activity significantly reduced in the in plants (Maroco *et al*., 2002) while other did not observe any effects of water stress (Delfine *et al*., 2001). The difference in results of these authors may be due to different species studied under different stress intensities (Bota *et al*., 2004).

Chlorophyll content usually increases at low salinity (Winicov, 1991; Locy *et al*., 1996) and degrades at higher salt stress (Salama *et al*., 1994). A report of Zhao *et al*. (2011) stated that exposed seedlings of *Continues coggygria var. cinerea* to water stress significantly increased chlorophyll a content. Increasing salt and water stress results in decreased chlorophyll content (Sahin *et al*., 2018). Irrigation water salinity and quantity noticeably affected the chlorophyll content for cabbage (*Brassica oleracea* var. capitata). Contrary, Jamil *et al*. (2007) reported significant increase in leaf chlorophyll content in cabbage and sugar beet under salt stress. However, Salinity stress did not affect the chlorophyll concentrations in *Atriplex portulacoides* (Redondo-Gómez *et al*., 2007).

4.2.4.7 Photosynthetic rate (Pr)

During experimental period, Pr was measured weekly so Pr varied significantly ($P \leq 0.05$) each month. Pr was maximum during the cooler month of March and start decreasing with increasing temperature in the months after that. Pr was significantly affected by salt x water stress for all three species. Pr showed opposite results of water stress at lowest and highest salt stress level in *Salsola imbricata*. At lower salt stress, Pr decreased with increasing water stress. While, Pr at highest salinity of S4 increased with increasing water stress level. *T. mandavillei* showed same result and Pr usually decreased with increasing water stress. However, at higher salinities Pr increased with increasing water stress. *A. leucoclada* showed different results than other species. Both salt and water stress had interactive effect ($P \leq 0.05$) on Pr of *A. leucoclada*. Salt stress had a protective effect on Pr. Salt stress decreased the Pr while water stress increased the Pr significantly ($P \leq 0.05$).

Under salt or water stress, leaf water potential and thus photosynthetic activity is decreased (Razzaghi *et al*., 2011). This reduction in photosynthesis can be caused by stomatal closure (Goldstein *et al*., 1996), disturbance of photosynthetic activity (Drew *et al*., 1990) or both at low and high salt concentration (Yeo *et al.,* 1991). Ionic imbalance can cause the reduction of chlorophyll content, activity of Rubisco, K in chloroplasts and disintegration of the second photosystem (PSII) (Muhammad, 2004). A report of Zhao *et al*. (2011) stated that exposed seedlings of *Continues coggygria var. cinerea* to water stress significantly reduced the relative growth rate and net photosynthesis rate.

In addition, a reduced photosynthesis may be related to high concentration of sugars in mesophyll cells (Munns *et al*., 1982). On the other hand severe reduction in photosynthesis in case of glycophytes is most probably due to damages in the photosynthetic apparatus and ion toxicity rather than factors affecting stomatal closure (Boughalleb *et al.,* 2009). In hygro-halophyte *A. portulacoides* photosynthesis may decrease through stomatal conductance and hence intercellular CO_2 concentration (Redondo-Gómez *et al.,* 2007). Water stress can inhibit the activity of photosystem II and the rate of CO_2 assimilation (Bloch *et al*., 2006; Monti *et al*., 2006) which in turn could

decrease photosynthesis (Wu *et al.,* 2016). During combined studies of salt and water stress both stresses caused an additive effect on plant growth (Shalhevet, 1994; Shani and Dudley, 2001). Similarly Sun *et al*. (2015) stated that combined stresses caused severe decrease in maize growth. Pr significantly declined when plants were exposed to individual and combine salt and water stress while studying different genotypes of soybean (Khan *et al*., 2017).

However Boughalleb *et al.* (2009) evaluated two xero-halophytes *(Nitraria retusa* and *Atriplex halimus*) and a glycophyte (*M. arborea*). They reported that moderate salinity had a stimulating effect on growth rate and photosynthesis of *Nitraria retusa* and *Atriplex halimus*. While at higher salinities, it decreased significantly. Conversely in *M. arborea* (glycophyte), chlorophyll fluorescence parameters decreased linearly with salinity.

The current results are in line with Wang *et al.* (2011) for *Tamarix chinensis*. At lower salt level, Net Pr decreased with increasing water stress. Net Pr of higher salt treatments was similar for different water stress treatments and lower than control. However, at lower salinity optimal quantum yield of photosystem II of drought treatments were not significantly different from one another ($P>0.05$) (Wang *et al*., 2011). In case of quinoa the highest salt concentration of (500 mM) decreased net Pr by 65 % compared to controls. However, water stress resulted in 77 % lower values for net Pr (Miranda-Apodaca *et al.,* 2018).

4.2.4.8 Leaf water potential (LWP)

Decreasing soil moisture content and water potential during salt stress conditions also reduce the water potential of the plant tissue. This low water potential of leaf tissues is achieved, either through water loss or by adjustments made by the plant to avoid water loss. In response to low water potential additional solutes are accumulated which is referred as osmotic adjustment (OA) (Verslues *et al*., 2006; Zhang *et al*., 1999).

In halophyte species Na^+ is involved in OA. It is supposed that Na^+ largely exists

in the vacuoles occupying about 90% of the total cell volume. Na^+ is reported to contribute up to 27 % OA in calli of *Atriplex halimus* (Martínez *et al*., 2005). Similarly Slama *et al*. (2007) calculated that Na^+ contribute 8 % and 47 % to osmotic adjustment in *S. portulacastrum* plants subjected to salt and water stress (Mozafar and Goodin, 1970).

Ma *et al.* (2012) subjected seedlings of a succulent xerophyte *Zygophyllum xanthoxylum* to individual and combine salt and water stress. Salt and water stress had an additive effect on LWP and leaf osmotic potential reduction. Na^+ contributed 8 % to the total osmotic potential in the control and 13 % in plants subjected to water stress and reach to 28 % in plants subjected to combine salt and water stress (Ma *et al*., 2012). Kusvuran (2012) studying melon (*Cucumis melo* L.) genotypes recorded decreased LWP under salt and water stresses, particularly in sensitive genotypes. LWP decreased more in water stress than that of salt stress. Miranda-Apodaca *et al*. (2018) compared the effect of osmotic and ionic stress on quinoa. It was reported that plants subjected to saline treatment observed a greater capacity for osmotic adjustment up to 0.97 MPa in the 500 mM treatment. In contrast, plants subjected to water stress treatment showed more dehydration. Alian *et al.* (2000) studied genotypic difference for tomato cultivars. Cultivar Fireball was classified as highest salt as it tolerated the highest levels of Na^+ in plant cells although it's fresh and dry weights were the lowest. PEG increased Na^+ accumulation in *Nicotiana glauca* since NaCl strongly increased osmotic adjustment in stressed cells under PEG stress. The water potential diminished significantly due to salt and water stress (González *et al.*, 2012). Álvarez *et al*. (2018) reported decreased water potential for all salt and water stress treatments in *Pistacia lentiscus.*

It was concluded that osmotic adjustment through the uptake of readily available inorganic ions (Na^+ and Cl^-) under salt stress is more efficient than adjustment through the production of organic solutes under water stress (Liu *et al*., 2008; Slama *et al.*, 2008; Sucre and Suarez, 2011; Sara Álvarez *et al*., 2012). Same result for decrease in LWP in mannitol and NaCl combination than the individual were reported by Slama *et al*. (2007) in S. *portulacastrum,* Rodríguez *et al.* (2005) for *A. maritimus* and Wu *et al.* (2015) for Sugar beet (*Beta vulgaris* L.). Herralde *et al.* (1998) submitted plants of *Argyranthemum*

coronopifolium to salt and water stress independently. Water stress promoted significant differences on leaf water potential (-1.76 MPa for Ψw) in stressed plants versus control.

Maggio *et al*. (2005) studied cabbage under salt and water stress. They concluded for salt stress that the yield was correlated to the soil water potential. However, under water stress condition same decrease in soil water potential due soil matric is more harmful to plants. Similar results were previously reported for celery (Pascale *et al.,* 2003). Hassine *et al.* (2008) exposed *Atriplex halimus* plants to 40/160 mM NaCl or 15 % polyethylene glycol. Shoot water potential in plants exposed to PEG remained lower than the plants under highest salt stress. Duarte and Souza (2016) investigated water potentials in *Capsicum annuum* by irrigating with different levels of saline water. Salt stress resulted decrease in LWP. The decrease in the osmotic potential in plant leaves was a mean of saline stress adoption.

Khan *et al.* (2015) compared the leaf water potential of three soybean genotypes subjected to salt stress and the combined salt and water stress conditions. Leaf water potential was highly affected in the combined salt and water stress. Our results are in agreement with the findings of Omami and Hammes (2006) in amaranth under salt and water stress, Mannan *et al.* (2013) for soybean and Khan *et al.,* (2015) for mung bean under salinity stress. It can be concluded that salt stress can help to reduce the negative effects of water stress by osmotic adjustment through Na^+ and proline accumulation.

4.2.4.9 Plant total Nitrogen (N)

Nitrogen is an essential element of many cell components. Therefore, nitrogen deficiency always inhibits plant growth (Hu and Schmidhalter, 2005). In our experiment salt and water stress for all the three species understudy had significant interaction for plant total nitrogen. Plant total nitrogen was reduced with increasing water stress only at low salt stress level. As the salt stress increases negative effect of water stress become minimal.

According to Maksimovic and Ilin (2012) salinity disrupts protein synthesis in plants while water stress disturbs nitrogen metabolism in plant tissues. Salt and water stress

decreased water and mineral uptake and degrade ion homeostasis (Sahin *et al*., 2018; Parida and Das, 2005). Adequate water supply is essential for nitrogen absorption by roots (Costa and Gianquinto, 2002). Even fertilizer N will not increase plant yield without adequate water supply (Haas, and Power, 1965). Water stress conditions decreased the N availability (Tanguilig *et al*., 1987) by decreasing the soil-N mineralization (Bloem *et al*., 1992) and N transport shoots by decrease in transpiration rate (Tanguilig *et al*., 1987). This reduced N uptake under water stress, reduces plant growth (Rouphael *et al*., 2012). Considerable decrease in nitrate reeducates activity had also been reported in leaves of plants exposed to water stress (Ruiz-Lozano and Azcón, 1996).

Salt stress reduces the growth rate that prevents the dilution effect of the N in plants. As a result total N uptake may decreased but N concentration increases or remains unchanged (Munns and Termaat, 1986; Hu and Schmidhalter, 1998a). Contrary many studies showed that total N content may not affected but still salinity reduces the NO_3^- concentration in the leaves (Francois and Clark, 1974; Hu and Schmidhalter, 1998b). NO_3^- concentration decrease under salt stress due to increase in chloride accumulation (Roussos *et al.,* 2007). Nitrate influx is strongly competitive with chloride influx due to NO_3^- /Cl^- antagonism (Hu and Schmidhalter, 1998; Carter *et al*., 2005).

Chakraborty *et al.* (2015) found lower N content in brassica plants under salinity stress. This low N content under salinity stress is due to high Cl^- content (Sahin *et al*., 2018). Similarly, mild salinity of 3 dSm^{-1} administered for 52 days to *A. squamosa* plants decreased nitrogen concentration and increased chloride concentration (Marler and Zozor, 1996). Furthermore, N deficiency due to high salinity is reported in tomato (Pessarakli and Tucker, 1988), lettuce and cabbage (Feigin *et al*., 1991). Yokaş *et al*. (2008) evaluated 3 types of salt concentrations (NaCl, Na_2SO_4 and $CaCl_2$). N concentrations for tomato decreased with increase in any of the salts. Sahin *et al*. (2018) studied the combined effects of salt and water stress on cabbage (*Brassica oleracea* var. capitata). N content decreased with increasing salt and drought stress (Sahin et al., 2018).

As the salt level increase, so does the Cl^- with decrease in nitrogen. It can be concluded that Cl^- may have replaced NO_3^- (Roussos *et al.,* 2007). Khalid and Cai (2011)

concluded that water reduced the harmful effect of salt stress in lemon balm plants. Nitrogen content along with other micronutrients (Mg–Zn–Fe–Mn) decreased by increasing irrigation water salinity and drought individually. However, salinity stress ameliorated this decline induced by water stress. Plants treated with saline irrigation water and mannitol resulted in higher plant growth, and N content than those treated with saline irrigation water alone (Khalid and Cai, 2011).

4.2.4.10 Phosphorus content

Phosphorus is an essential major element required by plants for many physiological functions (Marschner, 2012). In our experiment both salt and water stress had significant interaction on the phosphorus content on all three species studied i.e. *S. imbricata, T. mandavillei* and *A. leucoclada.* For *S. imbricata,* phosphorus content was higher at lowest salinity level of S1 only. At lower salt stress, increasing water stress levels decreased the total phosphorus content significantly. However, at higher salt stress level, water stress had almost no significant effect on phosphorus content of *S. imbricata. T. mandavillei* showed increased phosphorus content at salinity level S1 and S2 with increasing water stress level from WL1 to WL2 and decreased at WL3 and WL4 thereafter. However, at higher salinities of S3 and S4 salt stress had protective effect. Phosphorus content first decreased with increasing water stress and then increases at higher water stress. Similar results were obtained for *A. leucoclada.* Phosphorus content decreased with increasing water stress at lower salt level. However, phosphorus content increased with increasing water stress at highest salinity level. In conclusion for all species salt stress had found to have a protective role against water stress.

It is well known by the earliest studies that water stress restrict P uptake by plants (Pinkerton and Simpson, 1986). P transport to the shoots is severely restricted even under relatively mild water stress (Resnik, 1970). P deficiency appears to be one of the earliest symptoms of water stress (Turner, 1985). Same result were obtained by Kirnak *et al.* (2002) and Sánchez *et al.* (2010) who recorded decrease in P concentration in cherry tomato and watermelon grown in water stress conditions (Ackerson, 1985; Studer, 1993; Garg *et al*., 2004).

In contrast to water stress, salt stress had variable effects depending on plant species and experimental conditions (*Champagnol*, 1979). However, salinity-induced reductions in P concentrations in plant tissues were frequently found (Hu and Schmidhalter, 2005). This reduction in P concentration can be attributed to the activity of ions-antagonists (Gruissem and Jones, 2015).

Grattan and Grieve (1999) reported that Cl^- and SO_4^{-2} salts reduced P uptake in barley and sunflower. Roussos *et al*. (2007) cultured invitro Jojoba (*Simmondsia chinensis*) plants on basil media supplemented with NaCl. Roussos *et al.* (2007) proved by the relative anion concentration results that salinity reduces phosphate uptake. In saline soils P availability is reduced because of ionic-strength effects and low solubility of P minerals (Grattan and Grieve, 1999). Contrary to our results Hu *et al*. (2006) studied effect of salt and water stress on maize crop. Salt stress increased the P concentration by contrast, water stress decreased the P concentration (Hu *et al*., 2006). Khalid and Cai (2011) recorded same results as present study for lemon balm. Increasing irrigation water salinity or water stress decreased the phosphorus quantity. However salinity stress ameliorated this decline induced by water stress. Slama *et al*. (2007) reported increased nutrient accumulation in *S. portulacastrum* plants if grown under water stress by mannitol.

4.2.4.11 Total potassium content

In the current study salt and water stress had an interactive effect on total potassium content of both *S. imbricata* and *T. mandavillei.* Total potassium content decreased with increasing salt stress level. At the same salt stress levels of S1, S2 and S3, total potassium content decreased with increasing water stress level while, at S4 total potassium content did not decreased significantly with increasing water stress level. For *A. leucoclada* only salinity stress levels had a significant effect on total potassium content.

Potassium is an essential plant element for plant growth and a competitor of Na^+ under salt stress (Fournier *et al*., 2005; Kanai *et al*., 2007). K is equally important for maintaining the turgor pressure in plants both under salt and water stress (Marschner, 1995). Moreover, higher K^+: Na^+ ratios also improve the resistance of the plant to salinity

(Asch *et al*., 2000). Under saline conditions, Na^+ in the growth medium might compete with K absorption by the roots (Blumwald, 2000). It is assumed that K uptake and its deposition in tissues by the plant is reduced under salt stress (Lazof and Bernstein, 1999). This reduced potassium concentration in plant tissues grown under salt stress conditions is reported by many authors (Hu and Schmidhalter, 1997; Carter *et al*., 2005; Khan *et al*., 2000; García *et al*., 2004). NaCl induced K deficiencies were reported in a broad range of crops such as spinach (Chow *et al*., 1990), sorghum (Bernstein *et al*., 1995), tomato (Lopez and Satti, 1996; YOKAŞ *et al*., 2008) and maize (Botella *et al*., 1997). Roussos *et al*. (2007) studied invitro effect of salinity on jojoba explant. Potassium concentration was lowest in explants grown in medium supplemented with salt. Same results for nodal segments of jojoba were reported by Mills and Benzioni (1992) previously. This decreased is attributed to antagonistic effects of Na^+ and K ions (Suhayda *et al*., 1990).

Contrary to previous studies many authors reported no significant effect of salt stress on concentration of some basic elements in different plant species (Chen *et al*., 2001a; Lefevre *et al*., 2001a), while other reported a significant alteration due to salt stress (Ghoulam *et al*., 2002; Khan *et al.*, 2000). Studying *A. squamosa* plants mild salinity of 3 dSm^{-1} did not influence potassium and sodium concentrations in plants after 52 days of salinity treatment (Marler and Zozor, 1996). Similarly control and salt stress plants showed similar levels of K in *Bruguiera cylindrica* plants (Atreya *et al.*, 2009).

K plays an important role in osmoregulation under salt and water stress conditions (Blum, 2018). Therefore, plant need to maintain a high level of cytoplasmic K to survive in salt stress environment (Chow *et al*., 1990). Salt tolerant plants absorb less Na^+ and more K through ion selection mechanisms (Cuin *et al*., 2003). Thus selective K uptake over Na^+ is an important physiological process associated with salt tolerance (Poustini and Siosemardeh, 2004; Neill *et al*., 2002). Compartmentalization and distribution of K in relation to stress tolerance is also due to selective K uptake (Carden *et al.*, 2003). Therefore, Na/K ratio is of great importance regarding salt tolerance of a plant. Increasing salt stress can increase the Na/K ratios. There was a negative relationship between Na^+ and K concentration in leaves. Similar results had been observed by Khan *et al.* (1997) and

Goudarzi and Pakniyat (2008). High Na/K ratios negatively affect the plant metabolism and physiology (Yokaş *et al*., 2008)**.** Tolerant genotypes accumulate higher K concretion in tissues as compared to susceptible ones (Blum, 2018).

According to Khan *et al*. (2017) K accumulation in tolerant soybean genotype was higher as compared to susceptible grown in salt and water stress conditions. Similarly transgenic tobacco with mtlD gene accumulated higher K concentration under salt stress (Karakas *et al*., 1997)**.** Similarly Arjenaki *et al.* (2012) revealed for wheat that resistant genotypes had the highest value of K accumulation (Arjenaki *et al*., 2012). Halophytes showed the similar trend for K like glycophyte crops. Salt stress decrease K and increases Na^+ levels in *P. maritima, T. maritima* and to a lesser extent in *H. portulacoides*. In contrast, *L. vulgare* had high level of K in plant tissues (Jefferies *et al.,* 1979). For another halophyte sea aster (*Aster tripolium*), K content showed no significant differences between drought and salt (300 mM NaCl) treatments (Ueda *et al*., 2003). For the halophyte *Salicornia rubra* K concentrations decreased with increasing salt stress (Khan *et al.,* 2001). However, potassium represent levels adequate for growth in the shoots of plants grown at optimal salinity (Peterson, 1974).

Potassium increases the plant's drought resistance by osmotic adjustment (Beringer and Trolldenier, 1979; Marschner, 2012). It also maintains turgor pressure (Mengel and Arneke, 1982) and reduces transpiration under water stress (Andersen *et al*., 1992). Increasing water stress decrease K availability to the plants (Kuchenbuch *et al.*, 1986). However, K accumulation under water stress may be more important than organic solutes production, because osmotic adjustment through K is more energy efficient (Hsiao, 1973). Morgan (1992) revealed that wheat lines showing high osmotic adjustments had a high accumulation of K in their tissues. A recent study by Ma *et al.* (2004) on *Brassica napus* oilseeds showed that K accumulation accounted for about 25 % of drought-induced changes in osmotic adjustment. However, McWilliams (2003) studied the soybeans by increasing irrigation interval from 7 to 11 days. K concentration in leaf tissues increased to 22 % (Aliasgharzad *et al.,* 2009). This indicated that K concentration even raise if duration and intensity of the drought is short and low (McWilliams, 2003).

Only few studies were carried out to evaluate combined effects of salt and water stress most of them are in line with our findings. Khalid and Cai (2011) recorded same results as present study for lemon balm. Potassium quantity decreased by increasing irrigation water salinity and drought individually. However, salinity stress ameliorated this decline induced drought stress by adding mannitol. Slama *et al*. (2007) also reported that nutrients accumulation was increased in *S. portulacastrum* plants under water stress created by using mannitol. Another study was done by Slama *et al*. (2008) on *S. portulacastrum* to study interactive effect of salt and water stress. Potassium concentration was largely restricted in leaf tissues under salt stress while water stress had no significant effect. Moreover, salts stress combined with water stress had similar amount of potassium compared to water stress alone, meaning that water stress had no significant effect on K amount at higher salinity. It was concluded that salt stress alone or combined with water stress increased the potassium use efficiency (KUE) by 50% of *S. portulacastrum* (Slama *et al*., 2008).

Our results are in line with Khan *et al.* (2015) who recorded higher concentration of K under water stress compared to control and decreased with increasing salinity levels for soybean. Still he observes that salt stress had more K accumulation when combined water stress. Similar results were reported by Khan *et al*. (2017)**.** Our results are also in line with Martínez *et al*. (2005) who studied *Atriplex halimus* plant under 15 % PEG and 50 mM NaCl stress. K concentration slightly increased ($P \leq 0.01$) under 15 % PEG while 50 mM NaCl decreased it. Using combine stress of both 15 % PEG and 50 mM NaCl, the K concentration was similar to control without PEG and NaCl (Martínez *et al*., 2005)

4.2.4.12 Sodium content

Na^+ uptake had significant results for the salt and water stress interaction. Na^+ uptake was significantly increased with increasing salt stress for all three species understudy. Water stress was also found to increase Na^+ uptake. Na^+ increased with increasing water stress even at lower salt stress when outer NaCl concentration was low. However, *S. imbricata* and *T. mandavillei* decreased Na^+ uptake with increasing water stress at highest salinity level of S4.

Under salt stress osmotic adjustment is achieved through increased Na^+ and Cl^- uptake. The production of organic osmotica is more energy consuming (Greenway and Munns, 1980). Thus inorganic ion accumulation is an alternative mechanism to adjust osmotic potential and seem to save energy, which enables plant to grow in less favorable conditions (Khalid and Cai, 2011). The shoot acts as a sink for Na^+ ions when plants were grown under salt stress (Jefferies *et al.*, 1979). Cells are able to avoid high levels of salts in the cytoplasm and achieve osmoregulation by increasing salt levels in the vacuoles by intracellular compartmentalization (Khan *et al*., 2000; 2005).

Different species shows different response of ionic concentrations to various salt levels (Jefferies *et al*., 1979). Under salt stress shoots of *P. maritima, T. maritima* and to a lesser extent *H. portulacoides* accumulated high concentration of Na^+ and relatively low concentration of K (Jefferies *et al*., 1979). An important 'salt includer' Jojoba also accumulated significant amounts of sodium under slat stress (Mills and Benzioni, 1992).

Transgenic tobacco plants reported to tolerate the ions in the leaves (Levitt, 1980). However, leaf Na^+ and Cl^- concentrations were increased by salt stress for both plant type of wild and transgenic tobacco (Karakas et al., 1997). Na^+ content in shoots increased sharply across the salt levels in *Atriplex canescens* and had 25 % higher across the salinities (Glenn and Brown, 1998). Na^+ accumulation increased with salt stress in shoots of *Salicornia rubra* (Khan *et al*., 2001). The Na^+ content in salt stress (400 mM NaCl) plants of *Bruguiera cylindricain* was twice that of control (Atreya *et al*., 2009). Na^+ content increased up to five times that of the control and drought under NaCl stress for halophyte sea aster (*Aster tripolium* L) (Ueda *et al*., 2003). Roussos *et al*. (2007) cultured jojoba explants in vitro on a basal medium supplemented with sodium chloride up to 169 mM. Control treatment recorded zero level of sodium, significantly different from all other treatments and increasing the salt treatment increased the Na^+ in the explants.

Our results for water stress are in contrast only with Khalid and Cai (2011) who studied response of lemon balm for different irrigation and salinity levels. Na^+ concentration increased under salt stress but decreased by water stress. However, for halophytic plants like *A. halimus* cultured on MS medium, water stress by PEG increased

Na^+ concentrations (Martínez *et al*., 2005). For another halophyte, *S. portulacastrum* the water stress led to a significant increase in Na^+ concentration in plant tissue (Slama *et al*., 2007). In another study high Na^+ concentration was recorded in plants grown under salt stress and was significantly increased by the combine exposure to salt and water stress (Slama *et al*., 2008).

4.2.4.13 Chloride content

Under salt stress, plants achieve osmotic adjustment by Na^+ and Cl^- uptake. Chloride content is always expected to increase in most of the plant species with increasing irrigation water salinity. Present study revealed a significant salt and water stress interaction that had significant effect on Cl^- uptake of all three species. Increasing salt stress increased Cl^- uptake in all three species. Water stress also increased the Cl^- uptake even at low salinity stress. However, effect of water stress varies under higher salinity levels for each species. For *S. imbricata* under S3 and S4 Cl^- uptake increases with increasing water stress up to WL3 but decreased at WL4. Similarly, for *T. mandavillei* at any salt stress level, Cl^- uptake increased with increasing water stress but decreased at highest water stress levels. *A. leucoclada* response to salt and water stress was different than other two species. Cl^- uptake increased with increasing water stress at lowest salinity level S1 only. At S2 and S3 Cl^- uptake decreased with increasing water stress up to WL3 and increases at WL4. Under highest salinity level of S4 Cl^- uptake decreased with increasing water stress levels.

Non-halophytes had faster uptake of Cl^- than Na^+ (Greenway and Munns, 1980). In glycophyte maize leaves, increasing salinity increased the Cl^- concentration (Martínez *et al.*, 2005). In case of tobacco increasing salinity increased leaf Na^+ and Cl^- concentrations for both wild and transgenic types (Karakas *et al.*, 1997). Halophytic species *Salicornia rubra* was studied by Khan *et al.* (2001). Chloride concentration in shoots increased with increasing irrigation water salinity for *Salicornia rubra*. Another halophyte *Aster tripolium* L was evaluated by Ueda *et al*. (2003) under water stress and NaCl (300 mM) stress. Cl^- content increased three times and Na^+ content increased up to five times in the NaCl-stressed leaves that of the control. However, results of Cl^- content for sea aster contrasted with present findings. Our results are in contrast with Martínez *et al*. (2005)

which reported that drought induced by 15 % PEG had no impact on Cl^-. Furthermore, to avoid toxicity and to achieve osmoregulation jojoba explant adopt intracellular compartmentalization and avoid high levels in cytoplasm (Khan *et al.*, 2000, 2005). Similarly *Sesuvium portulacastrum* and *Arthrocnemum macrostachyum* also reported Na^+ and Cl^- compartmentalization (Messedi *et al*., 2004; Khan *et al*., 2005).

4.2.4.14 Abscisic acid

Salt and water stress induce different effects on plant metabolism (Hassine and Lutts, 2010). These stresses may affect growth hormone and gene expression (Achuo *et al*., 2006). These plant hormones activate acclimation responses under salt and water stress (Schroeder *et al*., 2001; Finkelstein and Gibson, 2002; Zhang *et al*., 2006). One of the most important plant hormone that mediates many stress responses in plants is abscisic acid (ABA) (Finkelstein et al., 2002; Rock, 2000; Zhang et al., 2006). These ABA induced stress responses are important for plant survival during both salt and water stress but effect different physiological processes (Hassine *et al*., 2009). Due to salt and water stress ABA production is triggered in roots which is transported to the shoots (Wilkinson and Davies, 2002). ABA stimulating Na^+ and Cl^- excretion under salt stress and reduce water loss during water stress (Hassine and Lutts, 2010; Walker and Lutts, 2014). Under mild salt stress ABA reduce water loss by transpiration and under high salt stress reduce stomatal density (Adolf *et al*., 2013; Razzaghi *et al*., 2011).

ABA also increased excretion of Na^+ and Cl^- in the external salt-bladders (Hassine *et al*., 2009). Kefu *et al*. (1991) studied barley, cotton and saltbush exposed to salinity. Barley and cotton plants had increased the production of ABA when they were exposed to 75 $molm^{-3}$ NaCl. While saltbush (*Atriplex spongiosa*) plants did not increase ABA on 75 $molm^{-3}$ NaCI salinity but increased at 150 mol m^{-3}. Hassine and Lutts (2010) exposed *Atriplex halimus* plants to iso-osmotic stress of NaCl (160 mM) or PEG (15 %). Hassine and Lutts (2010) reported that ABA accumulated in response to salt (160 mM NaCl). Alla *et al*. (2011) while studying *A. halimus* responses for salt (NaCl) or water stress (PEG) found that salt stress produced more metabolic disturbance than water stress.

ABA is important hormone in controlling water stress responses (Verslues *et al*., 2006). These stress-induced responses include leaf senescence that lead to leaf abscission (Pospíšilová *et al*., 2000). This senescence, decrease the plant's canopy and reduce water loss under salt and water stress (Miller *et al.*, 2010; Taylor and Whitelaw, 2001). Razzaghi *et al*. (2011) observed an increase in ABA with increasing water stress, suggesting it as a signal to regulate stomatal conductance. These results are in accordance with Jacobsen *et al*. (2009). A report of Zhao *et al*. (2011) stated that exposed seedlings of *Continues coggygria var. cinerea* to water stress significantly increased endogenous ABA.

Chen *et al.* (2001b) exposed *Populus euphratica* to salt stress and recorded up to fivefold increase of ABA. He concluded rise of ABA regulated ions uptake and transport under salt stress. Under water stress, ABA improve WUE by closing stomata and reducing water loss through transpiration (Oliveira *et al*., 2013; Waseem *et al*., 2011)**.** ABA act as major signal to regulate transpiration through stomatal pores (Schroeder *et al.,* 2001; Bartels and Sunkar, 2005). ABA-regulated stomatal opening, root growth and conductance (Sharp and LeNoble, 2002; Schroeder, *et al*., 2001) are important in avoidance of low water potential. ABA-induced increase of compatible solutes is important for drought avoidance (Ober and Sharp, 1994). Water stress caused a decrease of shoot growth with increased or unaffected root growth (Van der Weele *et al*., 2000). The relative root and shoot growth is a response to water stress (Hsiao and Xu, 2000) and is the result of regulation of growth by ABA (Sharp and LeNoble, 2002).

4.2.4.15 Proline content

Proline content for *T. mandavillei* and *A. leucoclada* is significantly affected by the salt and water stress and their interaction. *S. imbricata* also had significant effect of salt and water stress interaction on proline content. Increasing water stress at S1 and S2 decreased proline content in *S. imbricata*. Contrary under salt stress levels of S3 and S4 proline content increased with increasing water stress from WL1 up to WL3 and decreased at WL4. Similarly, for *T. mandavillei* proline content decreased with increasing water stress under salt stress level of S1, S2 and S3. However, at S4 proline content increase with increasing water stress from WL1 till Wl3 and decreased at WL4. For *A. leucoclada* at S1

proline content was not significantly affected by water stress levels. However, both salt and water stress had an additive role in increasing proline content. Higher salt stress levels of S2, S3 and S4 increased proline content with increasing water stress level.

Proline accumulation is an important stress resistant mechanism (Hassine *et al*., 2009; Kishor *et al.,* 2005). This proline accumulation is involved in osmotic adjustment and protect cellular structures against salt stress and ROS (Hoque *et al*., 2007). It may act to stabilize the photosystems (Ohnishi and Murata, 2006) and involve in stress signaling (Gong and Bohnert, 2006). Proline is one of the prominent organic solute that is stored in the cytoplasm and organelles to balance the osmotic pressure of the ions in the vacuole under stress conditions (Hasegawa *et al*., 2000). Proline accumulation relates more to the osmotic stress than any specific salt effect (Munns, 2002). Proline accumulation is a preventive metabolic adaptation which act as osmoprotectants and antioxidants and/or free radical scavengers (Larher *et al*., 2009).

Khalid and Cai (2011) reported *M. officinalis* response for proline accumulation applying various levels of salt and water stress. The highest proline content resulted from combine application of salt and water stress. However, Khalid and Cai (2011) concluded that water stress reduced the harmful effect of salt stress in lemon balm plants. Slama *et al*. (2007b) and Blum and Ebercon (1976) regarded proline as a source of energy, nitrogen and carbon for recovering tissues under salt and /or water stress.

Watanabe *et al*. (2000) compared two poplar species i.e. *P. euphratica* and *P. alba* cv. *Pyramidalis* X P. *tomentosa* for proline accumulation under salt and osmotic stress. They concluded Na^+ and proline accumulation had an important role in osmotic adjustment and improves plant performance under osmotic stress. Same results were reported for sugar beet. Proline accumulation increased growth under combine stress of salt and water (Wu *et al*., 2015a).

Errabii *et al.* (2007) investigated the proline concentration of sugarcane under iso-osmotic NaCl and mannitol stress. Increasing NaCl and mannitol stress increase the proline concentration. Their results revealed that salt stress calli accumulated proline more than

mannitol-treated calli. The stress-sensitive one accumulated proline at higher extent than the stress-resistant cultivars. It was suggested that proline accumulation was a symptom of injury rather than a stress resistance trait. Teymouri *et al*. (2009) reported the similar results studying three halophytic *salsola* species (*S. rigida, S. dendroides* and *S. richteri*). Maximum increase in proline concentration under salt stress was recorded for *S. richteri*. However, other two species had no effect of increasing salinity on proline concentration till 400mM and decrease thereafter.

Hassine *et al*. (2008) analyzed proline accumulation in *Atriplex halimus* by using nutrient solution containing 40/160 mM NaCl or 15 % polyethylene glycol. Salt resistance was not related to proline accumulation but was related to lower water-use efficiency. *Atriplex spongiosa* had the similar trend of decreasing proline content in the range for 50 to 300 mol/m^3 but increased rapidly at higher salinities (Storey and Jones, 1979). Same is the case for *Suaeda monoica*, low proline contents were recorded at 500 mol/m^3 NaCl and below. However, a significant increase was detected at high salinities (Storey and Jones, 1979).

Martínez *et al*. (2005) reported same results to that of our experiment. He reported that 0% or 15 % PEG had no impact on the proline concentration at low NaCl (50mM) concentration (Martínez et al., 2005). However, at higher salinities of S2, S3 and S4 in current study both salt and water stress had significant effect on proline.

Atriplex halimus showed similar responses after treating seedlings with either NaCl (50, 300 and 550 mM NaCl) or drought (control and withholding water) (Alla *et al*., 2012). Proline concentration decreased at lower salt stress and increased at higher salt stress. Similarly, water stress also significantly increased the proline accumulation. Proline was significantly increased only by the high salt stress and water stress, nonetheless, combine treatments led to decrease if any (Alla *et al.,* 2012). This significant increase was still in low concentration which was supposed to function osmoprotectant. Similar responses of proline to salinity (Bajji *et al*., 1998) and to osmotic stress (Martinez *et al*., 2003) had been reported. This can be concluded that proline is efficiently only involved in stress tolerance within the first few hours of stress rather than in long term stress tolerance (Hassine *et al.,*

2008).

4.2.4.16 Antioxidant enzymes

Salt and water stress cause an increased production of ROS (Miller *et al.*, 2010a). Overproduction of these ROS under salt and water stress cause oxidative damage (Smirnoff, 1998). These ROS comprises both free radical (O_2, $OH^{\bullet}$, $HO_2^{\bullet}$ and $RO^{\bullet}$) and non-radical (molecular) forms (H_2O_2 and $^1O_2$, singlet oxygen) (Gill and Tuteja, 2010). Plants had developed antioxidant defense mechanism, which can detoxify these ROS (Caverzan *et al*., 2012) and protect plant cells from oxidative damage by scavenging of ROS (Gill and Tuteja, 2010).

This stress tolerance requires an efficient antioxidant system (Esfandiari *et al*., 2007) to detoxify the radicals (Parida and Das, 2005). However, antioxidant responses of plants to salinity vary considerably among species (Hameed *et al.,* 2015). The antioxidant enzyme response to water stress is similar to salt stress (Pan *et al.,* 2006). The most important enzymes were APX and CAT. In particular, APX had a higher affinity for H_2O_2 and reduces it to H_2O utilizing ascorbate as specific electron donor (Caverzan *et al*., 2012; Sofo *et al*., 2015).

In the current study APX activity was significantly affected by salt x water stress in *S. imbricata* and *T. mandaveli. POD* activity was also affected significantly by salt x water stress for all three species understudied. APX-dependent antioxidant enzymes played an important role in salinity tolerance in *Limonium stocksii* (Hameed *et al*., 2015). APX concentrations increased with increasing salinity (Sofo *et al.,* 2015). The increase in APX activity is more distinct in salt-sensitive cultivars than in salt-tolerant cultivars (Chawla *et al.,* 2013).

Antioxidant metabolisms can be different between short and long-term salt treatments (Yıldıztugay *et al.,* 2011). The salt-induced increase in APX activity requires days to become significant and can be considered as a late response (Lopez *et al.,* 1996).

Similarly in rice (*Oryza sativa* L.), APX activity did not showed and change in salt-tolerant cultivar but increased in the salt-sensitive one (Chawla *et al.*, 2013).

A report of Zhao *et al*. (2011) stated that exposed seedlings of *Continues coggygria var. cinerea* to drought significantly reduced the relative growth rate and net photosynthesis rate but increased guaiacol peroxidase and catalase activities. Duarte *et al.* (2013) compared *Halimione portulacoides* and *Sarcocornia.* They concluded that *H. portulacoides* can maintain balance between ROS production and scavenging at maximum salt level. *Cakile maritima* showed improved growth associated with high antioxidant enzyme activities and glutathione content (Amor *et al.*, 2006). Salinity stress in halophytic species *Spartina densiflora* increased the leave Na^+ content accompanied with enhanced activation of POD, APX and CAT activities (Canalejo *et al*., 2014).

In our study, APX activity of *A. leucoclada* increased only under water stress. While effect of salt stress was insignificant. These can be due to higher tolerance of *A. leucoclada* to salt stress which did not showed any negative effects of salt stress on plant growth and antioxidant enzymes. However, water stress significantly reduced the shoot growth of *A. leucoclada.* This could be associated with inadequate increase in antioxidant enzymes and the decreased OH radical scavenging activity. These results are in line with Yıldıztugay *et al*. (2011) who reported higher sensitivity of *Centaurea tuzgoluensis* associated with inadequate increase in CAT, APX and GR activity. *Sorghum bicolor* (C_4) and *Helianthus annuus* (C_3) showed increase levels of antioxidants (APX, CAT, guaiacol peroxidase) in response to drought (Zhang and Kirkham, 1996).

In this experiment response of CAT activity differ for each species under salt and water stresses. *S. imbricata* showed no significant effect ($P>0.05$) of salt or water stress on CAT activity. Although, there was a non-significant ($P>0.05$) increase in CAT activity by increasing water stress. *T. mandavillei* also did not show any significant effect ($P>0.05$) of salt stress on CAT activity.

Many species were reported to have same CAT activity whether grown in presence or absence of salt stress i.e. *Oryza sativa* var. Taipei 309 (Fadzilla *et al.*, 1997).

In *Bruguiera gymnorrhiza* CAT activity was not affected by salt concentrations up to 1000 mM NaCl (Takemura *et al*., 2000). Salt tolerant maize genotypes had no significant effect on CAT activity, but was reduced in salt-sensitive genotype (Neto *et al.,* 2006). In case of rice (*Oryza Sativa* L.) salt-stress increase CAT activity in the sensitive cultivars than in the tolerant cultivars. (Chawla, *et al.,* 2013). Decreased activity of CAT enzyme under water stress in rice also reported by Sharma and Dubey (2005).

Contrary to our findings CAT activity increased significantly with increasing salt stress in a halophyte species *Aeluropus littoralis* (Modarresi *et al*., 2013), *Cakile maritima* (Amor *et al*., 2006), *alfalfa* (*Medicago sativa*) (Wang *et al*., 2009), *Cassia angustifolia* (Agarwal and Pandey, 2004), *A. thaliana* (Ellouzi *et al*., 2011), *Salicornia persica* and *Salicornia europaea* (Aghaleh *et al.*, 2011), *Crithmum maritimum* (Amor *et al*., 2005), *Suaeda nudiflora* Moq. (Cherian and Reddy, 2003) and salt-tolerant relative *L. pennellii* (Corn) D'Arcy (Shalata and Tal, 1998). *Crithmum maritimum* improved plant growth and enhanced the CAT activity at moderate salt levels (Amor *et al*., 2005).

It can be concluded that CAT activity can be increased or decreased depending on concentration and exposure time to salt stress. However, Kalir and Poljakoff-Mayber (1981) reported that CAT activity was stimulated at low concentrations (0-0.5 M) of NaCl but inhibited at concentrations higher than 0.5 M in *Halimione portulacoides.* Salt stress even decreased CAT activity in callus cultures of *Suaeda nudiflora* (Cherian and Reddy, 2003), *Glycyrrhiza uralensis* (Wu and Yu, 2006) and *Lablab purpureus* (Bano *et al*., 2012). CAT activity increased in the seedlings of *Jatropha curcas* L. up to a concentration of 150 mmol NaCl and then decreased (Gao *et al*., 2008). CAT activity increased after 4 hours of treatment in *Cakile maritima* (halophyte) and decreased thereafter (Ellouzi *et al*., 2011). Salinity reduced CAT activity concentration with time dependent manner in Hyacinth bean (*Lablab purpureus*, HA-4 cultivar) leaves (Myrene and Varadahally, 2010).

On the other hand, *A. leucoclada* showed significant effect for salt x water stress. CAT activity decreased with increasing salt stress and increased with increasing water stress for *A. leucoclada.* Increasing water stress increased the CAT activity of *T.*

mandavillei significantly ($P<0.05$). Salt stress on *Atriplex* produce a general increase in antioxidant enzyme activity (Kachout *et al*., 2013). However, for other species of *Atriplex* like *Atriplex hortensis CAT* activity decreased significantly under salt stress. While APX activity was significantly elevated (Kachout *et al*., 2013). Similarly Benzarti *et al.* (2012) investigated the *Atriplex portulacoides* response to salinity (0-1,000 mM NaCl). Leaf APX activity increased by salinity, whereas CAT activity was maximum in the 0-400 mM NaCl range. Boughalleb and Denden (2011) compared *Nitraria retusa* and *Atriplex halimus* for their salt tolerance. *N. retusa* was more tolerant compared to *A. halimus* which was supposed to be due to higher antioxidant activity. Sharma and Dubey (2005) reported that CAT activity declined with increasing levels of drought stress in rice. Zhang and Kirkham (1996) compare antioxidant responses to water stress for *Sorghum bicolor* (C_4) and *Helianthus annuus* (C_3) under either watered or dry conditions. Both species showed increase levels of CAT in response to water stress.

5. SUMMARY

5. SUMMARY

This dissertation explores the potential of native plants to be used in sustainable landscaping under salt and water stress condition. Eco-physiological responses against water stress during germination and field experiments were studied during 2016-18.

Germination responses against salt and water stress were usually studied using different cultivars of individual species to find out optimal stress level of each species. These previous studies showed contradictory negative effects either of NaCl, PEG or both together on different species. In our study, it was concluded that salt or water stress tolerance during germination stress is inherited quality of species. Native species showed variable germination responses to salt and water stress imposed by using NaCl and PEG. Overall most of the species germinated during pre-evaluation studies and nine species were selected for our experiment. A plant species can be resistant to salt or water stress or both irrespective of ionic or osmotic effect of NaCl and PEG. *S. imbricata, T. mandavillei, T. apollinea, A. leucoclada* and *S. italica* stood out best to survive in induced salt and water stress at germination stage.

It is also noteworthy that a species resistant to salt or water stress during germination may or may not give the same result in the field. Out of five selected species in germination experiment only three species survived in the field experiment i.e. *S. imbricata, T. mandavillei* and *A. leucoclada*. On the other hand, *T. apollinea* and *S. italica* although performed well in germination experiment but could not survive under salt stress in the field trial.

Three species i.e. *S. imbricata, T. mandavillei* and *A. leucoclada* were studied in field experiment under salt and water stress conditions for six months. All three species showed morphological and physiological adaptations and both salt and water stress had no negative effect on survival percentage. *S. imbricata* a succulent species from the family *Amaranthaceae* can be classified as obligatory halophyte. *S. imbricata* showed highest growth under lowest water stress and had no effect of salinity stress. NaCl in salt stress had shown a protective effect on SW and SL under water stress. In current study salt stress had

no significant effect on *S. imbricata* and *A. leucoclada.* On the other hand, increasing water stress had shown major decrease in growth parameters. Water stress treatment decreased the RL of *S. imbricata* under low salt stress. However, as supposed water stress increased the RL under higher salt stress. Plant total nitrogen, phosphorus and potassium content were decreased with increasing water stress only at low salt stress level. As the salt stress increases negative effect of water stress become minimal.

Na^+ and Cl^- uptake was significantly increased with increasing salt and water stress. Na^+ content increased with increasing water stress level even at low salt stress level. However, *S. imbricata* decreased Na^+ uptake with increasing water stress at highest salinity level of S4. For S. *imbricata* under S3 and S4 Cl^- uptake increased with increasing water stress up to WL3 but decreased at highest water stress level of WL4. ABA and Proline content in leaves decreased with increasing water stress level at lower salt stress, however at higher salt stress proline had an inverse trend. Salt and water stress levels has an interactive effect on the APX and POD activity of *S. imbricata*. At lower salt stress APX activity increased with increasing water stress up to WL3 and decreased at WL4. At S2, S3 and S4 APX activity first decreased by increasing water stress from WL1 to WL2 and increased thereafter. However, at S4 APX activity reduced at WL4. POD activity of *S. imbricata* in leaves increased with increasing water stress level at lower salt stress. However, at higher salt stress level of S4 POD activity of *S. imbricata* decreased with increasing water stress.

T. mandavillei belonging to family *Zygophyllaceae* can be classified as facultative halophyte. *T. mandavillei* grew well without salt and water stresses and survived under higher salt stress although reduced the growth also. *T. mandavillei* had the maximum SW, RW, SL and RL under S1WL1. In the current study salt and water stress had significant interaction for plant total nitrogen, phosphorus and potassium content. As the salt stress increased negative effect of water stress become minimal on nutrient accumulation. Sodium and chloride content increased not only with increasing salt stress but also with increasing water stress. However, Na^+ uptake decreased with increasing water stress at highest salinity level of S4. ABA and proline content in leaves decreased with increasing

drought level at lower salinity however at higher salinity proline increased with increasing water stress.

CAT activity in leaves of *T. mandavillei* increased with increasing water stress level. Interaction between salt and water stress levels significantly ($P \leq 0.05$) affected the POD and APX activity of *T. mandavillei*. POD activity of *T. mandavillei* in leaves decreased with increasing water stress level at lower salinity. However, at higher salinity POD activity had an inverse trend. At lower salt stress of S1 and S2 APX activity first decreased with increasing water stress from WL1 to WL2 and then increased with increasing water stress. At higher salt stress level of S3 and S4 APX activity first increased with increasing water stress from WL1-WL2 and decreased with increasing water stress level after that.

A. leucoclada could be classified as obligatory halophyte. SDW and SL even increased with increasing salt stress. RDW and RL decreased with increasing salt stress and increased with increasing water stress. Plant total nitrogen was reduced with increasing water stress only at low salt stress level. As the salt stress increased negative effect of water stress become minimal. Phosphorus content increased with increasing water stress level. Potassium content increased significantly with increasing salt stress. Na^+ content increased not only with increasing salt stress but also with increasing water stress. Cl^- uptake in *A. leucoclada* increased with increasing water stress at lowest salinity level S1 only. At salt stress level S2 and S3, Cl^- uptake decreased with increasing water stress up to WL3 and increased at WL4. Under highest salinity level S4 Cl^- uptake decreased with increasing water stress levels. Both salt and water stress had an additive role in increasing ABA and proline content. Higher salt stress levels of S2, S3 and S4 increased proline content with increasing water stress level.

ANOVA table revealed different salt and water stress levels had significant interactive effect ($P \leq 0.05$) on the CAT activity of *A. leucoclada* (Appendix 4.2.3.17). CAT activity in leaves of *A. leucoclada* increased with increasing water stress level under S1 and decreased with increasing water stress under S2, S3 and S4. Variance table revealed the considerable effect of different salt x water stress levels on POD activity of *A. leucoclada*. With increasing water stress level, POD activity of *A. leucoclada* in leaves also

increased. Increasing salinity stress level from S1 to S3 also increased peroxidase. However, at higher salinity stress level of S4 POD activity decreased. APX activity in *A. leucoclada* increased with increasing salt stress. Similarly increasing water stress also increased the APX activity in *A. leucoclada.*

In conclusion *S. imbricata, T. mandavillei* and *A. leucoclada* use salt resistant mechanism to accumulate higher concentration of salts in the cells. They use physiological adaptation using enzymatic and non-enzymatic antioxidant to cope with higher salt stress and ROS (Reactive Oxygen Species) produced. It is also concluded that salt stress had a protective role under water stress condition. These results are more important in determining the irrigation requirements of salt tolerant species.

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APPENDICES

APPENDICES

Appendix 4.1.1: Mean square (from ANOVA) for different germination parameters (IR: Imbibition Rate; GP: Germination Percentage; GI: Germination Index; MDG: Mean Daily Germination; MGT: Mean Germination Time; PI: Promptness Index; GSI: Germination Stress Tolerance Index; CVG: Coefficient of Velocity of Germination; GR: Germination Rate) for *R. stricta*. Significance (*) was assessed at P≤0.05.

Source	Mean Squares								
	IR	GP	GI	MDG	MGT	PI	GSI	CVG	GR
Rep	0.00103	0.00265	0.00005	0.00039	0.00019	0.00151	0.00214	0.09651	0.00039
OL	0.00016	0.44094*	0.02103*	0.0409*	0.0525*	0.13184*	0.82069*	0.29046	0.0409*
Error	0.00051	0.00229	0.00010	0.00035	0.00065	0.00068	0.00283	0.14887	0.00035

Appendix 4.1.2: Mean square (from ANOVA) for different germination parameters (IR: Imbibition Rate; GP: Germination Percentage; GI: Germination Index; MDG: Mean Daily Germination; MGT: Mean Germination Time; PI: Promptness Index; GSI: Germination Stress Tolerance Index; CVG: Coefficient of Velocity of Germination; GR: Germination Rate) for *L. pyrotechnica*. Significance (*) was assessed at P≤0.05.

Source	Mean Squares								
	IR	GP	GI	MDG	MGT	PI	GSI	CVG	GR
OA	0.0000043	0.01816	0.0151*	0.00582*	0.00988*	0.19311*	0.33783*	0.15998*	0.00582*
OL	0.00117	1.39191*	0.07122*	0.16113*	0.0137*	0.81276*	1.05957*	0.12686*	0.16113*
OA*OL	0.0001853	0.31892*	0.00984*	0.02744*	0.01998*	0.09679*	0.25368	0.10407*	0.00216*

Appendix 4.1.3: Mean square (from ANOVA) for different germination parameters (IR: Imbibition Rate; GP: Germination Percentage; GI: Germination Index; MDG: Mean Daily Germination; MGT: Mean Germination Time; PI: Promptness Index; GSI: Germination Stress Tolerance Index; CVG: Coefficient of Velocity of Germination; GR: Germination Rate) for *C. virgatus*. Significance (*) was assessed at P≤0.05.

Source	Mean Squares								
	IR	GP	GI	MDG	MGT	PI	GSI	CVG	GR
Rep	0.00002	0.00349	0.00052	0.00178	0.00038	0.01047	0.01022	0.09748	0.00178
OL	0.00331	0.08061	0.01162*	0.01691	0.00262*	0.12134*	0.70463	0.10958	0.01691
Error	0.00117	0.02035	0.00147	0.00511	0.00039	0.0135	0.10788	0.07898	0.00511

Appendix 4.1.4: Mean square (from ANOVA) for different germination parameters (IR: Imbibition Rate; GP: Germination Percentage; GI: Germination Index; MDG: Mean Daily Germination; MGT: Mean Germination Time; PI: Promptness Index; GSI: Germination Stress Tolerance Index; CVG: Coefficient of Velocity of Germination; GR: Germination Rate) for *A. leucoclada.* Significance (*) was assessed at P≤0.05.

Source	Mean Squares								
	IR	GP	GI	MDG	MGT	PI	GSI	CVG	GR
OA	0.00	1.51593*	0.05563*	0.1068	0.00006	1.10138*	1.67966*	0.00797	0.1068
OL	0.00	0.81203*	0.06165*	0.25413*	0.00515*	1.02941*	0.49127*	0.08533*	0.25413*
OA*OL	0.00	0.25951*	0.00745	0.02521	0.00021	0.16481*	0.08067	0.0051	0.02521

Appendix 4.1.5: Mean square (from ANOVA) for different germination parameters (IR: Imbibition Rate; GP: Germination Percentage; GI: Germination Index; MDG: Mean Daily Germination; MGT: Mean Germination Time; PI: Promptness Index; GSI: Germination Stress Tolerance Index; CVG: Coefficient of Velocity of Germination; GR: Germination Rate) for *S. italica.* Significance (*) was assessed at P≤0.05.

Source	Mean Squares								
	IR	GP	GI	MDG	MGT	PI	GSI	CVG	GR
OA	0.00247	0.06091*	0.01846*	0.02711	0.00441	0.08073*	0.32055	0.00508	0.02711
OL	0.00105	0.155*	0.03694*	0.10119*	0.00836*	0.26074*	0.69694*	0.08672	0.10119*
OA*OL	0.00065	0.03968	0.00273	0.00633	0.00119*	0.04937*	0.20685	0.27513*	0.00633

Appendix 4.1.6: Mean square (from ANOVA) for different germination parameters (IR: Imbibition Rate; GP: Germination Percentage; GI: Germination Index; MDG: Mean Daily Germination; MGT: Mean Germination Time; PI: Promptness Index; GSI: Germination Stress Tolerance Index; CVG: Coefficient of Velocity of Germination; GR: Germination Rate) for *T. glabra*. Significance (*) was assessed at P≤0.05.

Source	Mean Squares								
	IR	GP	GI	MDG	MGT	PI	GSI	CVG	GR
OA	0.00102	0.19734*	0.02833*	0.05138	0.00304	0.25429*	0.86673*	0.56174	0.05138
OL	0.00263	0.52488*	0.07041*	0.12198*	0.02049*	0.57648*	0.35309*	0.26574*	0.12198*
OA*OL	0.00095	0.03909*	0.00638*	0.01084*	0.0282*	0.08766*	0.25981*	0.11643	0.01084*

Appendix 4.1.7: Mean square (from ANOVA) for different germination parameters (IR: Imbibition Rate; GP: Germination Percentage; GI: Germination Index; MDG: Mean Daily Germination; MGT: Mean Germination Time; PI: Promptness Index; GSI: Germination Stress Tolerance Index; CVG: Coefficient of Velocity of Germination; GR: Germination Rate) for *T. apollinea*. Significance (*) was assessed at P≤0.05.

Source	Mean Squares								
	IR	GP	GI	MDG	MGT	PI	GSI	CVG	TGI
OA	0.00	0.01089	0.0026	0.00614	0.00128*	0.04058	0.29459	0.04324	0.00614
OL	0.00217	0.11027*	0.01638*	0.02894*	0.005*	0.19581*	1.01787*	0.02625	0.02894*
OA*OL	0.00439	0.00318	0.00042	0.00196	0.00122	0.00676	0.00635	0.05521	0.00196

Appendix 4.1.8: Mean square (from ANOVA) for different germination parameters (IR: Imbibition Rate; GP: Germination Percentage; GI: Germination Index; MDG: Mean Daily Germination; MGT: Mean Germination Time; PI: Promptness Index; GSI: Germination Stress Tolerance Index; CVG: Coefficient of Velocity of Germination; GR: Germination Rate) for *T. mandavillei*. Significance (*) was assessed at P≤0.05.

Source	Mean Squares								
	IR	GP	GI	MDG	MGT	PI	GSI	CVG	GR
OA	0.00295	0.00073	0.0001889*	0.00143	0.00001	0.00113	3010*	0.02896	0.00143
OL	0.00035	0.0116*	0.00246*	0.02811*	0.00129*	0.01451*	0.10607	0.09439	0.02811*
OA*OL	0.00047	0.00208	0.00213*	0.00134	0.00057	0.00317	0.03564	0.06493	0.00134

Appendix 4.1.9: Mean square (from ANOVA analyses) for different germination parameters (IR: Imbibition Rate; GP: Germination Percentage; GI: Germination Index; MDG: Mean Daily Germination; MGT: Mean Germination Time; PI: Promptness Index; GSI: Germination Stress Tolerance Index; CVG: Coefficient of Velocity of Germination; GR: Germination Rate) for *S. imbricata*. Significance (*) was assessed at P≤0.05.

Source	Mean Squares								
	IR	GP	GI	MDG	MGT	PI	GSI	CVG	GR
OA	0.00295	0.48009*	0.00247*	0.01013*	0.00253*	0.12399*	0.3301*	0.07899*	0.01013*
OL	0.00035	0.18113*	0.01332*	0.01822*	0.00006	0.15021*	0.10607	0.0015	0.01822*
OA*OL	0.00047	0.05817	0.00053	0.00386	0.00034*	0.0243	0.03564	0.00993	0.00386

Appendix 4.2.1.1: Analysis of Variance Table for Survival percentage of *S. imbricata* as affected by salinity and water level

Source	DF	SS	MS	F	P
Rep	6	3019.48	503.247		
SALINITY	3	49.31	16.438	0.48	0.7015
Error Rep*Salinity	18	618.87	34.382		
Water level	3	336.46	112.152	2.23	0.0925
Salinity*Water level	9	427.15	47.461	0.94	0.4948
Error Rep*Salinity*Water level	72	3627.64	50.384		
Total	111	8078.92			

Grand Mean 93.973
CV (Rep*SALINITY) 6.24
CV (Rep*SALINITY*Water level) 7.55

Appendix 4.2.1.2: Analysis of Variance Table for Shoot dry weight of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	25461	12730		
Month	5	2949497	589899	13.39	0.0004
Error Rep*Month	10	440638	44064		
Salinity	3	44662	14887	1.30	0.2909
Month*Salinity	15	80059	5337	0.46	0.9436
Error Rep*Month*Salinity	36	413802	11494		
WL	3	208340	69447	3.72	0.0130
Month*WL	15	367222	24481	1.31	0.2029
Salinity*WL	9	203824	22647	1.21	0.2917
Month*Salinity*WL	45	306555	6812	0.36	0.9999
Error Rep*Month*Salinity*WL	144	2689698	18678		
Total	287	7729757			

Grand Mean 103.70
CV (Rep*Month) 202.42
CV (Rep*Month*SALINITY) 103.39
CV (Rep*Month*SALINITY*WL) 131.79

Appendix 4.2.1.3: Analysis of Variance Table for Root dry weight of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	132.1	66.05		
Month	5	10387.6	2077.53	10.78	0.0009
Error Rep*Month	10	1928.0	192.80		
SALINITY	3	249.4	83.12	1.56	0.2153
Month*SALINITY	15	469.2	31.28	0.59	0.8646
Error Rep*Month*SALINITY	36	1915.0	53.20		
WL	3	1070.5	356.82	4.07	0.0082
Month*WL	15	1548.1	103.21	1.18	0.2952
SALINITY*WL	9	690.4	76.71	0.88	0.5486
Month*SALINITY*WL	45	1196.4	26.59	0.30	1.0000
Error Rep*Month*SALINITY*WL	144	12615.6	87.61		
Total	287	32202.3			

Grand Mean 7.3200
CV (Rep*Month) 189.69
CV (Rep*Month*SALINITY) 99.64
CV (Rep*Month*SALINITY*WL) 127.87

Appendix 4.2.1.4: Analysis of Variance Table for Shoot Length of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	454	227.2		
Month	5	55467	11093.5	27.83	0.0000
Error Rep*Month	10	3987	398.7		
Salinity	3	828	275.9	1.13	0.3486
Month*Salinity	15	2154	143.6	0.59	0.8630
Error Rep*Month*Salinity	36	8762	243.4		
WL	3	620	206.8	0.94	0.4207
Month*WL	15	1100	73.3	0.34	0.9907
Salinity*WL	9	4405	489.4	2.24	0.0229
Month*Salinity*WL	45	3406	75.7	0.35	1.0000
Error Rep*Month*Salinity*WL	144	31517	218.9		
Total	287	112701			

Grand Mean 48.099
CV (Rep*Month) 41.51
CV (Rep*Month*SALINITY) 32.43
CV (Rep*Month*SALINITY*WL) 30.76

Appendix 4.2.1.5: Analysis of Variance Table for Root Length *S. imbricata* as affected by different salt and water stress levels.

Source	DF	SS	MS	F	P
Rep	2	217.3	108.66		
Month	5	22401.0	4480.20	7.26	0.0041
Error Rep*Month	10	6172.9	617.29		
Salinity	3	996.1	332.04	1.14	0.3443
Month*Salinity	15	1363.5	90.90	0.31	0.9905
Error Rep*Month*Salinity	36	10444.6	290.13		
WL	3	503.5	167.82	0.60	0.6173
Month*WL	15	889.3	59.29	0.21	0.9993
Salinity*WL	9	5353.5	594.83	2.12	0.0314
Month*Salinity*WL	45	3215.8	71.46	0.25	1.0000
Error Rep*Month*Salinity*WL	144	40409.9	280.62		
Total	287	91967.4			

Grand Mean 50.800
CV (Rep*Month) 48.91
CV (Rep*Month*SALINITY) 33.53
CV (Rep*Month*SALINITY*WL) 32.98

Appendix 4.2.1.6: Analysis of Variance Table for Water Use Efficiency of S. *imbricata* as affected by different salt and water stress levels.

Source	DF	SS	MS	F	P
Rep	2	317.5	158.732		
SALINITY	3	456.0	151.989	2.03	0.2114
Error Rep*SALINITY	6	449.5	74.921		
WL	3	1608.2	536.066	6.97	0.0002
SALINITY*WL	9	812.5	90.278	1.17	0.3123
Error	264	20313.0	76.943		
Total	287	23956.7			

Grand Mean 7.7083
CV(Rep*SALINITY) 112.29
CV(Error) 113.80

Appendix 4.2.1.7: Analysis of Variance Table for Chlorophyll index of *S. imbricata* as affected by different salt and water stress levels.

Source	DF	SS	MS	F	P
Rep	2	0.0173	0.0087		
Month	5	62.4451	12.4890	84.44	0.0000
Error Rep*Month	10	1.4790	0.1479		
SALINITY	3	0.1645	0.0548	0.76	0.5253
Month*SALINITY	15	3.2030	0.2135	2.95	0.0039
Error Rep*Month*SALINITY	36	2.6061	0.0724		
WL	3	1.3722	0.4574	10.64	0.0000
Month*WL	15	2.9518	0.1968	4.58	0.0000
SALINITY*WL	9	0.6853	0.0761	1.77	0.0785
Month*SALINITY*WL	45	3.8044	0.0845	1.97	0.0014
Error Rep*Month*SALINITY*WL	144	6.1894	0.0430		
Total	287	84.9180			

Grand Mean 0.8224
CV (Rep*Month) 46.76
CV (Rep*Month*SALINITY) 32.72
CV (Rep*Month*SALINITY*WL) 25.21

Appendix 4.2.1.8: Analysis of Variance Table for Photosynthetic rate (Pr) of *S. imbricata* as affected by different salt and water stress levels.

Source	DF	SS	MS	F	P
Rep	2	153.2	76.62		
Month	5	47351.0	9470.20	249.31	0.0000
Error Rep*Month	10	379.9	37.99		
SALINITY	3	357.7	119.24	6.37	0.0014
Month*SALINITY	15	4931.7	328.78	17.56	0.0000
Error Rep*Month*SALINITY	36	674.0	18.72		
WL	3	1264.0	421.32	21.74	0.0000
Month*WL	15	1695.8	113.05	5.83	0.0000
SALINITY*WL	9	1782.1	198.01	10.22	0.0000
Month*SALINITY*WL	45	6167.2	137.05	7.07	0.0000
Error Rep*Month*SALINITY*WL	144	2791.0	19.38		
Total	287	67547.7			

Grand Mean 20.567
CV (Rep*Month) 29.97
CV (Rep*Month*SALINITY) 21.04
CV (Rep*Month*SALINITY*WL) 21.41

Appendix 4.2.1.9a: Analysis of Variance Table for Leaf Water Potential of *S. imbricata* as affected by different salt and water stress levels after one month of treatment application

Source	DF	SS	MS	F	P
Rep	2	6.903	3.4514		
Salinity	3	62.949	20.9830	17.95	0.0021
Error Rep*Salinity	6	7.012	1.1687		
WL	3	21.025	7.0082	4.60	0.0111
Salinity*WL	9	29.819	3.3132	2.18	0.0623
Error Rep*Salinity*WL	24	36.558	1.5233		
Total	47	164.266			

Grand Mean -6.9667
CV (Rep*Salinity) -15.52
CV (Rep*Salinity*WL) -17.72

Appendix 4.2.1.9b: Analysis of Variance Table for Leaf Water Potential of *S. imbricata* as affected by different salt and water stress levels after five month of treatment application

Source	DF	SS	MS	F	P
Rep	2	78.05	39.025		
Salinity	3	1448.19	482.731	14.68	0.0036
Error Rep*Salinity	6	197.29	32.881		
WL	3	496.38	165.461	3.87	0.0218
Salinity*WL	9	180.02	20.003	0.47	0.8819
Error Rep*Salinity*WL	24	1026.59	42.775		
Total	47	3426.52			

Grand Mean -26.030
CV (Rep*SALINITY) -22.03
CV (Rep*SALINITY*WL) -25.13

Appendix 4.2.1.10: Analysis of Variance Table for Plant Total Nitrogen of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.01089	0.00545		
SALINITY	3	0.89294	0.29765	107.28	0.0000
Error Rep*SALINITY	6	0.01665	0.00277		
WL	3	0.08654	0.02885	14.33	0.0000
SALINITY*WL	9	0.11374	0.01264	6.28	0.0001
Error Rep*SALINITY*WL	24	0.04832	0.00201		
Total	47	1.16910			

Grand Mean 0.5573
CV (Rep*SALINITY) 9.45
CV (Rep*SALINITY*WL) 8.05

Appendix 4.2.1.11: Analysis of Variance Table for Phosphorus content of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.00012	0.00006		
SALINITY	3	0.00549	0.00183	94.76	0.0000
Error Rep*SALINITY	6	0.00012	0.00002		
WL	3	0.00079	0.00026	14.67	0.0000
SALINITY*WL	9	0.00102	0.00011	6.34	0.0001
Error Rep*SALINITY*WL	24	0.00043	0.00002		
Total	47	0.00796			

Grand Mean 0.0262
CV (Rep*SALINITY) 16.76
CV (Rep*SALINITY*WL) 16.12

Appendix 4.2.1.12: Analysis of Variance Table for Total Potassium content of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.00030	0.00015		
Salinity	3	0.08286	0.02762	75.36	0.0000
Error Rep*Salinity	6	0.00220	0.00037		
WL	3	0.02394	0.00798	5.64	0.0045
SALINITY* WL	9	0.08112	0.00901	6.37	0.0001
Error Rep*Salinity*WL	24	0.03393	0.00141		
Total	47	0.22435			

Grand Mean 1.1085
CV (Rep*Salinity) 1.73
CV (Rep*Salinity* WL) 3.39

Appendix 4.2.1.13: Analysis of Variance Table for Na^+ content of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	302.2	151.08		
SALINITY	3	12152.2	4050.73	33.42	0.0004
Error Rep*SALINITY	6	727.3	121.21		
WL	3	2428.6	809.52	2.36	0.0968
SALINITY*WL	9	7813.1	868.12	2.53	0.0336
Error Rep*SALINITY*WL	24	8238.3	343.26		
Total	47	31661.5			

Grand Mean 417.98
CV (Rep*SALINITY) 2.63
CV (Rep*SALINITY*WL) 4.43

Appendix 4.2.1.14: Analysis of Variance Table for Cl^- content of *S. imbricata* as ffected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	237.04	118.521		
SALINITY	3	575.85	191.951	4.31	0.0608
Error Rep*SALINITY	6	267.21	44.535		
WL	3	92.23	30.743	0.69	0.5683
SALINITY*WL	9	1147.90	127.544	2.85	0.0194
Error Rep*SALINITY*WL	24	1072.75	44.698		
Total	47	3392.98			

Grand Mean 42.396
CV (Rep*SALINITY) 15.74
CV (Rep*SALINITY*WL) 15.77

Appendix 4.2.1.15: Analysis of Variance Table for ABA content of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	177.7	88.83		
Salinity	3	1647.6	549.21	0.30	0.8229
Error Rep*Salinity	6	10889.5	1814.91		
WL	3	12176.7	4058.91	2.72	0.0668
Salinity* WL	9	38797.7	4310.86	2.89	0.0183
Error Rep*Salinity* WL	24	35811.7	1492.16		
Total	47	99500.9			

Grand Mean 108.41
CV (Rep*Salinity) 39.30
CV (Rep*Salinity* WL) 35.63

Appendix 4.2.1.16: Analysis of Variance Table for Proline content of *S. imbricata* as affected by different salinity and water level

Source	DF	SS	MS	F	P
Rep	2	25700000	12850000		
SALINITY	3	145900000	48620000	7.02	0.0218
Error Rep*SALINITY	6	41560000	6926751		
WL	3	35790000	11920000	1.55	0.2275
SALINITY* WL	9	160100000	17790000	2.31	0.0491
Error Rep*SALINITY* WL	24	184800000	7698890		
Total	47	593800000			

Grand Mean 5112.2
CV (Rep*Salinity) 51.48
CV (Rep*Salinity* WL) 54.28

Appendix 4.2.1.17: Analysis of Variance Table for Catalase activity of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	1752017	876008		
SALINITY	3	4471783	1490594	1.42	0.3271
Error Rep*SALINITY	6	6313694	1052282		
WL	3	2040345	680115	1.52	0.2342
SALINITY*WL	9	6241677	693520	1.55	0.1864
Error Rep*SALINITY*WL	24	10720000	446688		
Total	47	31540000			

Grand Mean 1249.0
CV(Rep*SALINITY) 82.13
CV(Rep*SALINITY*WL) 53.51

Appendix 4.2.1.18: Analysis of Variance Table for Peroxidase activity of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.03816	0.01908		
SALINITY	3	0.13867	0.04622	1.34	0.3474
Error Rep*SALINITY	6	0.20733	0.03455		
WL	3	0.09625	0.03208	1.39	0.2697
SALINITY*WL	9	0.76524	0.08503	3.69	0.0051
Error Rep*SALINITY*WL	24	0.55356	0.02306		
Total	47	1.79919			

Grand Mean 0.2265
CV(Rep*SALINITY) 82.08
CV(Rep*SALINITY*WL) 67.06

Appendix 4.2.1.19: Analysis of Variance Table for APX activity of *S. imbricata* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	1.0162	0.50812		
SALINITY	3	3.1408	1.04694	6.47	0.0261
Error Rep*SALINITY	6	0.9714	0.16190		
WL	3	4.8507	1.61691	6.01	0.0033
SALINITY*WL	9	6.5360	0.72622	2.70	0.0252
Error Rep*SALINITY*WL	24	6.4605	0.26919		
Total	47	22.9756			

Grand Mean 1.5414
CV (Rep*SALINITY) 26.10
CV (Rep*SALINITY*WL) 33.66

Appendix 4.2.2.1: Analysis of Variance Table for Survival Percentage of *T. mandavillei as* affected by salt and water stress levels

Source	DF	SS	MS	F	P
Month	6	1401.5	233.580		
Salinity	3	379.5	126.488	0.92	0.4499
Error Month*Salinity	18	2467.7	137.092		
WL	3	87.8	29.274	0.37	0.7740
Salinity* WL	9	654.7	72.742	0.92	0.5111
Error Month*Salinity* WL	72	5678.0	78.861		
Total	111	10669.1			

Grand Mean 95.839
CV (Rep*Salinity) 12.22
CV (Rep*Salinity* WL) 9.27

Appendix 4.2.2.2: Analysis of Variance Table for Shoot Dry Weight of *T. mandavillei* as affected by salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	592.2	296.12		
Month	5	37818.5	7563.71	51.49	0.0000
Error Rep*Month	10	1469.0	146.90		
Salinity	3	2120.9	706.96	4.66	0.0075
Month*Salinity	15	4921.5	328.10	2.16	0.0293
Error Rep*Month*Salinity	36	5463.2	151.75		
WL	3	2821.7	940.56	4.06	0.0084
Month*WL	15	5435.1	362.34	1.56	0.0910
Salinity*WL	9	576.3	64.03	0.28	0.9802
Month*Salinity*WL	45	2060.1	45.78	0.20	1.0000
Error Rep*Month*Salinity*WL	144	33369.3	231.73		
Total	287	96647.8			

Grand Mean 7.9192
CV (Rep*Month) 153.05
CV (Rep*Month*Salinity) 155.56
CV (Rep*Month*Salinity*WL) 192.22

Appendix 4.2.2.3: Analysis of Variance Table for Root Dry Weight of *T. mandavillei as* affected by salinity and water level

Source	DF	SS	MS	F	P
Rep	2	6.501	3.2507		
Month	5	350.795	70.1589	48.69	0.0000
Error Rep*Month	10	14.408	1.4408		
Salinity	3	15.812	5.2708	4.19	0.0121
Month*Salinity	15	22.988	1.5326	1.22	0.3033
Error Rep*Month*Salinity	36	45.296	1.2582		
WL	3	31.167	10.3889	4.91	0.0028
Month*WL	15	53.366	3.5577	1.68	0.0610
Salinity*WL	9	3.790	0.4211	0.20	0.9940
Month*Salinity*WL	45	22.775	0.5061	0.24	1.0000
Error Rep*Month*Salinity*WL	144	304.949	2.1177		
Total	287	871.847			

Grand Mean 0.9325
CV (Rep*Month) 128.73
CV (Rep*Month*Salinity) 120.29
CV (Rep*Month*Salinity*WL) 156.06

Appendix 4.2.2.4: Analysis of Variance Table for Shoot Length of *T. mandavillei as* affected by salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	99.6	49.79		
Month	5	15874.9	3174.98	83.35	0.0000
Error Rep*Month	10	380.9	38.09		
Salinity	3	1005.8	335.25	11.56	0.0000
Month*Salinity	15	578.0	38.54	1.33	0.2361
Error Rep*Month*Salinity	36	1044.1	29.00		
WL	3	949.1	316.37	5.34	0.0016
Month*WL	15	541.7	36.12	0.61	0.8638
Salinity*WL	9	356.5	39.61	0.67	0.7366
Month*Salinity*WL	45	1246.0	27.69	0.47	0.9980
Error Rep*Month*Salinity*WL	144	8535.6	59.28		
Total	287	30612.2			

Grand Mean 13.565
CV (Rep*Month) 45.50
CV (Rep*Month*Salinity) 39.70
CV (Rep*Month*Salinity*WL) 56.76

Appendix 4.2.2.5: Analysis of Variance Table for Root Length of *T. mandavillei as* affected by salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	199.0	99.48		
Month	5	38216.4	7643.27	111.61	0.0000
Error Rep*Month	10	684.8	68.48		
Salinity	3	1539.2	513.05	7.87	0.0004
Month*Salinity	15	463.5	30.90	0.47	0.9386
Error Rep*Month*Salinity	36	2346.1	65.17		
WL	3	2675.7	891.91	7.70	0.0001
Month*WL	15	846.9	56.46	0.49	0.9439
Salinity*WL	9	1271.1	141.24	1.22	0.2872
Month*Salinity*WL	45	1233.6	27.41	0.24	1.0000
Error Rep*Month*Salinity*WL	144	16672.3	115.78		
Total	287	66148.6			

Grand Mean 23.595
CV (Rep*Month) 35.07
CV (Rep*Month*Salinity) 34.21
CV (Rep*Month*Salinity*WL) 45.60

Appendix 4.2.2.6: Analysis of Variance Table for Water Use Efficiency of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	2.014	1.00686		
SALINITY	3	9.858	3.28596	3.19	0.1056
Error Rep*SALINITY	6	6.188	1.03129		
WL	3	8.504	2.83471	1.94	0.1242
SALINITY*WL	9	3.060	0.33999	0.23	0.9896
Error	264	386.624	1.46448		
Total	287	416.247			

Grand Mean 0.5548
CV(Rep*SALINITY) 183.06
CV(Error) 218.14

Appendix 4.2.2.7: Analysis of Variance Table for Chlorophyll index of *T. mandavillei as* affected by salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.3962	0.19809		
Month	5	8.1048	1.62096	33.81	0.0000
Error Rep*Month	10	0.4794	0.04794		
SALINITY	3	2.2539	0.75131	7.75	0.0004
Month*SALINITY	15	1.5079	0.10053	1.04	0.4432
Error Rep*Month*SALINITY	36	3.4900	0.09694		
WL	3	0.0300	0.01000	0.11	0.9546
Month*WL	15	2.0603	0.13736	1.50	0.1125
SALINITY*WL	9	1.7960	0.19956	2.18	0.0267
Month*SALINITY*WL	45	5.5136	0.12252	1.34	0.1016
Error Rep*Month*SALINITY*WL	144	13.1901	0.09160		
Total	287	38.8223			

Grand Mean 0.9239
CV (Rep*Month) 23.70
CV (Rep*Month*SALINITY) 33.70
CV (Rep*Month*SALINITY*WL) 32.76

Appendix 4.2.2.8: Analysis of Variance Table for Photosynthetic rate (Pr) of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	4798	2399.2		
Month	5	140349	28069.9	30.57	0.0000
Error Rep*Month	10	9182	918.2		
SALINITY	3	24945	8314.9	28.40	0.0000
Month*SALINITY	15	20918	1394.5	4.76	0.0001
Error Rep*Month*SALINITY	36	10540	292.8		
WL	3	14980	4993.2	12.19	0.0000
Month*WL	15	13572	904.8	2.21	0.0084
SALINITY*WL	9	17331	1925.7	4.70	0.0000
Month*SALINITY*WL	45	31623	702.7	1.72	0.0089
Error Rep*Month*SALINITY*WL	144	58988	409.6		
Total	287	347226			

Grand Mean 52.990
CV (Rep*Month) 57.19
CV (Rep*Month*SALINITY) 32.29
CV (Rep*Month*SALINITY*WL) 38.20

Appendix 4.2.2.9a: Analysis of Variance Table for Leaf Water Potential of *T. mandavillei* as affected by different salt and water stress levels after one month of treatment application

Source	DF	SS	MS	F	P
Rep	2	32.610	16.3052		
Salinity	3	188.982	62.9939	10.14	0.0092
Error Rep*Salinity	6	37.277	6.2128		
WL	3	56.117	18.7055	8.49	0.0005
Salinity*WL	9	45.093	5.0104	2.27	0.0524
Error Rep*Salinity*WL	24	52.898	2.2041		
Total	47	412.977			

Grand Mean -7.0300
CV (Rep*Salinity) -35.46
CV (Rep*Salinity*WL) -21.12

Appendix 4.2.2.9b: Analysis of Variance Table for Leaf Water Potential of *T. mandavillei* as affected by different salt and water stress levels after five month of treatment application

Source	DF	SS	MS	F	P
Rep	2	25.931	12.965		
Salinity	3	366.396	122.132	18.87	0.0019
Error Rep*Salinity	6	38.830	6.472		
WL	3	20.187	6.729	0.79	0.5106
Salinity*WL	9	49.634	5.515	0.65	0.7451
Error Rep*Salinity*WL	24	204.040	8.502		
Total	47	705.018			

Grand Mean -12.429
CV (Rep*Salinity) -20.47
CV (Rep*Salinity*WL) -23.46

Appendix 4.2.1.10: Analysis of Variance Table for Plant Total Nitrogen of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.01032	0.00516		
Salinity	3	0.63117	0.21039	71.82	0.0000
Error Rep*Salinity	6	0.01758	0.00293		
WL	3	0.08124	0.02708	2.61	0.0744
Salinity* WL	9	0.27829	0.03092	2.98	0.0156
Error Rep*Salinity*WL	24	0.24862	0.01036		
Total	47	1.26721			

Grand Mean 0.5099
CV (Rep*Salinity) 10.62
CV (Rep*Salinity* WL) 19.96

Appendix 4.2.2.11: Analysis of Variance Table for Phosphorus content of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.00012	0.00		
Salinity	3	0.00139	0.00	45.61	0.0002
Error Rep*Salinity	6	0.00006	0.00		
WL	3	0.00012	0.00	1.00	0.4107
Salinity* WL	9	0.00086	0.00	2.33	0.0472
Error Rep*Salinity* WL	24	0.00098	0.00		
Total	47	0.00353			

Grand Mean 0.0302
CV (Rep*Salinity) 10.56
CV (Rep*Salinity* WL) 21.17

Appendix 4.2.2.12: Analysis of Variance Table for Total Potassium content of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.00101	0.00051		
Salinity	3	0.05673	0.01891	9.55	0.0106
Error Rep*Salinity	6	0.01188	0.00198		
WL	3	0.02869	0.00956	5.13	0.0070
Salinity*WL	9	0.04885	0.00543	2.91	0.0176
Error Rep*Salinity*WL	24	0.04474	0.00186		
Total	47	0.19189			

Grand Mean 1.0850
CV (Rep*Salinity) 4.10
CV (Rep*Salinity*WL) 3.98

Appendix 4.2.2.13: Analysis of Variance Table for Na^+ content of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	726	363.2		
Salinity	3	78318	26105.9	65.01	0.0001
Error Rep*Salinity	6	2409	401.6		
WL	3	4468	1489.3	2.56	0.0783
Salinity*WL	9	78842	8760.2	15.08	0.0000
Error Rep*Salinity*WL	24	13937	580.7		
Total	47	178701			

Grand Mean 583.46
CV (Rep*Salinity) 3.43
CV (Rep*Salinity*WL) 4.13

Appendix 4.2.2.14: Analysis of Variance Appendix for Cl^- content of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	640	319.8		
Salinity	3	100881	33627.0	17.50	0.0023
Error Rep*Salinity	6	11532	1922.0		
WL	3	83352	27783.9	7.22	0.0013
Salinity* WL	9	107334	11926.0	3.10	0.0129
Error Rep*Salinity* WL	24	92333	3847.2		
Total	47	396072			

Grand Mean 384.76
CV (Rep*Salinity) 11.39
CV (Rep*Salinity*WL) 16.12

Table 4.2.2.15: Analysis of Variance Appendix for ABA content of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	64.81	32.406		
Salinity	3	1077.07	359.022	6.83	0.0232
Error Rep*Salinity	6	315.43	52.571		
WL	3	197.62	65.875	0.98	0.4192
Salinity* WL	9	1446.93	160.770	2.39	0.0429
Error Rep*Salinity*WL	24	1615.32	67.305		
Total	47	4717.18			

Grand Mean 20.620
CV (Rep*Salinity) 35.16
CV (Rep*Salinity* WL) 39.79

Appendix 4.2.2.16: Analysis of Variance Table for Proline content of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	315400000	157700000		
SALINITY	3	21370000000	7125000000	36.33	0.0003
Error Rep*SALINITY	6	1177000000	196100000		
WL	3	10930000000	3643000000	5.44	0.0053
SALINITY*WL	9	14000000000	1556000000	2.32	0.0480
Error Rep*SALINITY*WL	24	16070000000	669500000		
Total	47	63870000000			

Grand Mean 62406
CV (Rep*SALINITY) 22.44
CV (Rep*SALINITY*WL) 41.46

Appendix 4.2.2.17: Analysis of Variance Table for Catalase activity for *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	567997	283999		
SALINITY	3	503808	167936	0.60	0.6370
Error Rep*SALINITY	6	1672922	278820		
WL	3	2591859	863953	6.16	0.0030
SALINITY*WL	9	2513458	279273	1.99	0.0863
Error Rep*SALINITY*WL	24	3368337	140347		
Total	47	11210000			

Grand Mean 714.34
CV (Rep*SALINITY) 73.92
CV (Rep*SALINITY*WL) 52.44

Appendix 4.2.2.18: Analysis of Variance Table for Peroxidase activity of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.00846	0.00423		
SALINITY	3	0.01134	0.00378	2.83	0.1291
Error Rep*SALINITY	6	0.00803	0.00134		
WL	3	0.01606	0.00535	3.47	0.0318
SALINITY*WL	9	0.05384	0.00598	3.88	0.0038
Error Rep*SALINITY*WL	24	0.03702	0.00154		
Total	47	0.13474			

Grand Mean 0.0578
CV (Rep*SALINITY) 63.30
CV (Rep*SALINITY*WL) 67.96

Appendix 4.2.2.19: Analysis of Variance Table for APX activity of *T. mandavillei* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.05663	0.02831		
SALINITY	3	2.15622	0.71874	5.16	0.0424
Error Rep*SALINITY	6	0.83566	0.13928		
WL	3	0.59112	0.19704	1.90	0.1567
SALINITY*WL	9	3.26780	0.36309	3.50	0.0068
Error Rep*SALINITY*WL	24	2.49008	0.10375		
Total	47	9.39751			

Grand Mean 0.6699
CV (Rep*SALINITY) 55.71
CV (Rep*SALINITY*WL) 48.08

Appendix 4.2.3.1: Analysis of Variance Table for Survival percentage of *A. leucoclada as* affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Month	6	43708.3	7284.72		
SALINITY	3	2372.3	790.77	2.28	0.1145
Error Month*SALINITY	18	6255.1	347.51		
Water	3	3295.2	1098.40	2.01	0.1217
SALINITY*Water	9	2280.9	253.43	0.46	0.8938
Error	64	35015.8	547.12		
Total	103				

Grand Mean 80.103
CV (Month*Salinity) 23.24
CV (Month*Salinity*WL) 29.20

Appendix 4.2.3.2: Analysis of Variance Table for Shoot Dry Weight of *A. leucoclada* as affected by salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	50.48	25.240		
Month	5	2994.26	598.852	43.92	0.0000
Error Rep*Month	10	136.34	13.634		
SALINITY	3	3.64	1.214	0.18	0.9090
Month*SALINITY	15	70.71	4.714	0.70	0.7669
Error Rep*Month*SALINITY	36	242.18	6.727		
WL	3	421.46	140.485	21.93	0.0000
Month*WL	15	1107.37	73.825	11.52	0.0000
SALINITY*WL	9	112.68	12.520	1.95	0.0488
Month*SALINITY*WL	45	399.19	8.871	1.38	0.0774
Error Rep*Month*SALINITY*WL	144	922.47	6.406		
Total	287	6460.78			

Grand Mean 2.3344
CV (Rep*Month) 158.17
CV (Rep*Month*SALINITY) 111.11
CV (Rep*Month*SALINITY*WL) 108.42

Appendix 4.2.3.3: Analysis of Variance Table for Root Dry Weight of *A. leucoclada as* affected by salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	3.913	1.9566		
Month	5	169.931	33.9862	42.12	0.0000
Error Rep*Month	10	8.069	0.8069		
SALINITY	3	0.706	0.2352	0.66	0.5827
Month*SALINITY	15	5.924	0.3950	1.11	0.3852
Error Rep*Month*SALINITY	36	12.850	0.3569		
WL	3	10.226	3.4088	9.70	0.0000
Month*WL	15	30.053	2.0035	5.70	0.0000
SALINITY*WL	9	3.418	0.3798	1.08	0.3807
Month*SALINITY*WL	45	19.938	0.4431	1.26	0.1547
Error Rep*Month*SALINITY*WL	144	50.618	0.3515		
Total	287	315.646			

Grand Mean 0.6691
CV (Rep*Month) 134.25
CV (Rep*Month*SALINITY) 89.29
CV (Rep*Month*SALINITY*WL) 88.61

Appendix 4.2.3.4: Analysis of Variance Table for Shoot Length of *A. leucoclada as* affected by salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	543.0	271.51		
Month	5	16226.5	3245.30	45.38	0.0000
Error Rep*Month	10	715.1	71.51		
SALINITY	3	1195.1	398.38	5.60	0.0029
Month*SALINITY	15	1875.4	125.03	1.76	0.0827
Error Rep*Month*SALINITY	36	2561.3	71.15		
WL	3	2048.8	682.93	12.89	0.0000
Month*WL	15	3613.7	240.91	4.55	0.0000
SALINITY*WL	9	1085.3	120.59	2.28	0.0205
Month*SALINITY*WL	45	3276.1	72.80	1.37	0.0823
Error Rep*Month*SALINITY*WL	144	7627.9	52.97		
Total	287	40768.2			

Grand Mean 13.649
CV (Rep*Month) 61.96
CV (Rep*Month*SALINITY) 61.80
CV (Rep*Month*SALINITY*WL) 53.33

Appendix 4.2.3.5: Analysis of Variance Table for Root Length of *A. leucoclada as* affected by salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	1493.3	746.65		
Month	5	10799.0	2159.81	32.44	0.0000
Error Rep*Month	10	665.7	66.57		
SALINITY	3	1807.2	602.41	7.40	0.0006
Month*SALINITY	15	1498.1	99.88	1.23	0.2974
Error Rep*Month*SALINITY	36	2930.5	81.40		
WL	3	468.6	156.20	1.79	0.1514
Month*WL	15	760.7	50.72	0.58	0.8853
SALINITY*WL	9	2180.6	242.28	2.78	0.0050
Month*SALINITY*WL	45	3353.1	74.51	0.85	0.7247
Error Rep*Month*SALINITY*WL	144	12553.7	87.18		
Total	287	38510.6			

Grand Mean 23.518
CV (Rep*Month) 34.69
CV (Rep*Month*SALINITY) 38.36
CV (Rep*Month*SALINITY*WL) 39.70

Appendix 4.2.3.6: Analysis of Variance Table for Water Use Efficiency of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.2528	0.12641		
SALINITY	3	0.0406	0.01354	0.32	0.8081
Error Rep*SALINITY	6	0.2503	0.04171		
WL	3	0.7367	0.24556	3.59	0.0143
SALINITY*WL	9	0.3838	0.04265	0.62	0.7771
Error	264	18.0735	0.06846		
Total	287	19.7377			

Grand Mean 0.1936
CV(Rep*SALINITY) 105.49
CV(Error) 135.15

Appendix 4.2.3.7: Analysis of Variance Table for Chlorophyll index of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	106.10	53.048		
Month	5	419.22	83.844	7.09	0.0045
Error Rep*Month	10	118.21	11.821		
SALINITY	3	217.57	72.525	3.65	0.0215
Month*SALINITY	15	271.00	18.066	0.91	0.5628
Error Rep*Month*SALINITY	36	716.26	19.896		
WL	3	534.61	178.203	8.99	0.0000
Month*WL	15	605.98	40.398	2.04	0.0164
SALINITY*WL	9	598.80	66.533	3.36	0.0009
Month*SALINITY*WL	45	953.82	21.196	1.07	0.3743
Error Rep*Month*SALINITY*WL	144	2854.35	19.822		
Total	287	7395.91			

Grand Mean 10.954
CV (Rep*Month) 31.39
CV (Rep*Month*SALINITY) 40.72
CV (Rep*Month*SALINITY*WL) 40.64

Appendix 4.2.3.8: Analysis of Variance Table for Photosynthetic rate (Pr) of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	463.1	231.55		
Month	5	48565.4	9713.08	110.58	0.0000
Error Rep*Month	10	878.4	87.84		
SALINITY	3	2760.6	920.19	17.42	0.0000
Month*SALINITY	15	4925.2	328.35	6.22	0.0000
Error Rep*Month*SALINITY	36	1901.8	52.83		
WL	3	8210.8	2736.92	20.52	0.0000
Month*WL	15	4798.6	319.90	2.40	0.0040
SALINITY*WL	9	2591.9	287.99	2.16	0.0282
Month*SALINITY*WL	45	5439.3	120.87	0.91	0.6409
Error Rep*Month*SALINITY*WL	144	19208.4	133.39		
Total	287	99743.4			

Grand Mean 21.884
CV (Rep*Month) 42.83
CV (Rep*Month*SALINITY) 33.21
CV (Rep*Month*SALINITY*WL) 52.78

Appendix 4.2.3.9a: Analysis of Variance Table for Leaf Water Potential of *A. leucoclada* as affected by different salt and water stress levels after one month of treatment application

Source	DF	SS	MS	F	P
Rep	2	133.05	66.526		
SALINITY	3	305.96	101.987	3.38	0.0954
Error Rep*SALINITY	6	181.15	30.191		
WL	3	279.14	93.046	3.27	0.0387
SALINITY*WL	9	717.36	79.706	2.80	0.0212
Error Rep*SALINITY*WL	24	683.12	28.463		
Total	47	2299.78			

Grand Mean -50.067
CV (Rep*SALINITY) -10.97
CV (Rep*SALINITY*WL) -10.66

Appendix 4.2.3.9b: Analysis of Variance Table for Leaf Water Potential of *A. leucoclada* as affected by different salt and water stress levels after five month of treatment application

Source	DF	SS	MS	F	P
Rep	2	31.60	15.800		
SALINITY	3	558.53	186.178	14.32	0.0038
Error Rep*SALINITY	6	77.99	12.998		
WL	3	423.46	141.154	14.69	0.0000
SALINITY*WL	9	487.29	54.143	5.63	0.0003
Error Rep*SALINITY*WL	24	230.65	9.610		
Total	47	1809.52			

Grand Mean -52.120
CV (Rep*SALINITY) -6.92
CV (Rep*SALINITY*WL) -5.95

Appendix 4.2.3.10: Analysis of Variance Table for Plant Total Nitrogen of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.01289	0.00645		
Salinity	3	0.15951	0.05317	17.55	0.0023
Error Rep*Salinity	6	0.01818	0.00303		
WL	3	0.07008	0.02336	5.66	0.0044
Salinity*WL	9	0.20900	0.02322	5.63	0.0003
Error Rep*Salinity*WL	24	0.09898	0.00412		
Total	47	0.56864			

Grand Mean 0.5080
CV (Rep*Salinity) 10.84
CV (Rep*Salinity*WL) 12.64

Appendix 4.2.3.11: Analysis of Variance Table for Phosphorus content of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.00008	0.0000392		
Salinity	3	0.00080	0.0002658	31.42	0.0005
Error Rep*Salinity	6	0.00005	0.00000846		
WL	3	0.00055	0.0001848	4.99	0.0079
Salinity*WL	9	0.00204	0.0002268	6.12	0.0002
Error Rep*Salinity*WL	24	0.00089	0.00003706		
Total	47	0.00441			

Grand Mean 0.0307
CV (Rep*Salinity) 9.48
CV (Rep*Salinity*WL) 19.84

Appendix 4.2.3.12: Analysis of Variance Table for Potassium content of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.00097	0.00048		
Salinity	3	0.01449	0.00483	9.92	0.0097
Error Rep*Salinity	6	0.00292	0.00049		
WL	3	0.00666	0.00222	0.79	0.5124
Salinity*WL	9	0.04893	0.00544	1.93	0.0961
Error Rep*Salinity*WL	24	0.06766	0.00282		
Total	47	0.14164			

Grand Mean 1.0636
CV (Rep*Salinity) 2.07
CV (Rep*Salinity*WL) 4.99

Appendix 4.2.3.13: Analysis of Variance Table for Na^+ content of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	1283.6	641.8		
Salinity	3	39430.2	13143.4	41.25	0.0002
Error Rep*Salinity	6	1912.0	318.7		
WL	3	4147.6	1382.5	5.22	0.0065
Salinity*WL	9	9259.8	1028.9	3.88	0.0038
Error Rep*Salinity*WL	24	6362.6	265.1		
Total	47	62395.8			

Grand Mean 486.59
CV (Rep*Salinity) 3.67
CV (Rep*Salinity*WL) 3.35

Appendix 4.2.3.14: Analysis of Variance Table for Cl^- content of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	97	49		
Salinity	3	416649	138883	56.66	0.0001
Error Rep*Salinity	6	14708	2451		
WL	3	65613	21871	7.52	0.0010
Salinity*WL	9	190044	21116	7.26	0.0000
Error Rep*Salinity*WL	24	69773	2907		
Total	47	756884			

Grand Mean 235.39
CV (Rep*Salinity) 21.03
CV (Rep*Salinity*WL) 22.91

Appendix 4.2.2.15a: Analysis of Variance Table for ABA content of *A. leucoclada as* affected by different salinity and water stress levels

Source	DF	SS	MS	F	P
Rep	2	616.3	308.15		
SALINITY	3	15884.9	5294.97	106.24	0.0000
Error Rep*SALINITY	6	299.0	49.84		
WL	3	7711.2	2570.41	63.08	0.0000
SALINITY*WL	9	4956.0	550.67	13.51	0.0000
Error Rep*SALINITY*WL	24	978.0	40.75		
Total	47	30445.5			

Grand Mean 38.396
CV(Rep*SALINITY) 18.39
CV(Rep*SALINITY*WL) 16.63

Appendix 4.2.3.16: Analysis of Variance Table for Proline content of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	6012837	3006419		
SALINITY	3	320200000	106700000	52.08	0.0001
Error Rep*SALINITY	6	12290000	2049916		
WL	3	168100000	56030000	41.91	0.0000
SALINITY*WL	9	139000000	15440000	11.55	0.0000
Error Rep*SALINITY*WL	24	32080000	1336723		
Total	47	677700000			

Grand Mean 5747.7
CV(Rep*SALINITY) 24.91
CV(Rep*SALINITY*WL) 20.12

Appendix 4.2.3.17: Analysis of Variance Table for Catalase activity for *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	1027140	513570		
SALINITY	3	28530000	9510111	13.96	0.0041
Error Rep*SALINITY	6	4088712	681452		
WL	3	8808429	2936143	12.48	0.0000
SALINITY*WL	9	38820000	4313169	18.33	0.0000
Error Rep*SALINITY*WL	24	5647974	235332		
Total	47	86920000			

Grand Mean 2614.9
CV (Rep*SALINITY) 31.57
CV (Rep*SALINITY*WL) 18.55

Appendix 4.2.3.18: Analysis of Variance Table for Peroxidase activity of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.07910	0.03955		
SALINITY	3	0.42081	0.14027	6.35	0.0273
Error Rep*SALINITY	6	0.13263	0.02211		
WL	3	0.24966	0.08322	50.40	0.0000
SALINITY*WL	9	0.05069	0.00563	3.41	0.0078
Error Rep*SALINITY*WL	24	0.03963	0.00165		
Total	47	0.97251			

Grand Mean 0.2578
CV (Rep*SALINITY) 57.67
CV (Rep*SALINITY*WL) 15.76

Appendix 4.2.3.19: Analysis of Variance Table for APX activity of *A. leucoclada* as affected by different salt and water stress levels

Source	DF	SS	MS	F	P
Rep	2	0.9365	0.46824		
SALINITY	3	2.3807	0.79356	4.08	0.0676
Error Rep*SALINITY	6	1.1676	0.19460		
WL	3	2.6524	0.88413	5.88	0.0037
SALINITY*WL	9	1.3031	0.14479	0.96	0.4933
Error Rep*SALINITY*WL	24	3.6101	0.15042		
Total	47	12.0503			

Grand Mean 1.3443
CV (Rep*SALINITY) 32.81
CV (Rep*SALINITY*WL) 28.85