In the Name of God,
the supremely Merciful, the most Kind.
Alleviating the adverse effects of salinity in eggplant (Solanum melongena L.) by using plant growth enhancer.

By
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Regd. No. 2005-ag-1765
M.Sc. (Hons.) Horticulture

A thesis submitted in the partial fulfillment of the requirement for the degree of
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In
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To

The Controller of Examinations,
University of Agriculture,
Faisalabad.

We the supervisory committee, certify that the contents and form of thesis submitted by Mr. Zaid Mustafa, Regd. No. 2005-ag-1765, have been found satisfactory and recommend that it be processed for evaluation by external examiner(s) for the award of degree.

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DECLARATION

“I hereby declare that the content of the thesis, entitled “Alleviating the adverse effects of salinity in eggplant (Solanum melongena L.) by using plant growth enhancer.” are product of my own research and no part has been copied from any published source (except the references, standard mathematical or genetic models/equation/formulae etc.). I further declare that this work has not been submitted for award of any other diploma/degree. The university may take action if the information provided is found inaccurate at any stage”.

Zaid Mustafa
Regd. No. 2005-ag-1765
Dedicated
To
My Beloved
Parents,
Brothers &
Sisters
WHO ALWAYS RAISED THEIR HANDS FOR MY SUCCESS
AND HAPPINESS, SO MUCH OF WHAT
I AM TODAY IS BECAUSE OF YOU AND I WANT TO TELL YOU
THAT
I THANK YOU,
APPRECIATE YOU
AND LOVE YOU
FROM THE CORE OF MY HEART, EVER AND FOREVER
ACKNOWLEDGEMENTS

To Allah, who has created this wonderful world that inspires immensity, awe, wonder, challenges, helplessness, creativity and is at the base of all human departments art, culture, science and technology and to Hazrat Muhammad (peace be upon him), the Prophet of Allah, who held human wellbeing very dear to his heart.

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ABSTRACT

Eggplant (*Solanum melongena* L.) locally known as ‘brinjal’, ‘baengan’ or ‘bataon’, is a high value horticultural crop, playing important role in increasing income of farming community. Biotic and abiotic factors contribute in yield loss of eggplant but the saline underground water is the main reason and the cultivation of salt sensitive eggplant genotypes also yield. The objective of this research was to characterize the eggplant genotypes against salinity stress and to induce the salt tolerance in eggplant by foliar application of chitosan and to study the physiological, biochemical and ionic changes in eggplant in response to NaCl salinity and chitosan.

Pot culture experiments were carried out in lath (screen) house of Institute of Horticultural Sciences, University of Agriculture, Faisalabad, to accomplish the investigation. Study comprised of two phases, each phase with two experiments. In first experiment 13 locally grown eggplant genotypes were exposed to different salinity levels [(control, 3, 6, 9, 12 and 15 dS m\(^{-1}\) (decisiemens per meter)]. Different growth (plant height, root length, shoot fresh weight, root fresh weight and plant dry matter) and ionic attributes (sodium and potassium) were recorded. All the eggplant genotypes showed a considerably variable response under salinity stress. ‘Saadia’ was found salt tolerant, while ‘Black Beauty’ was salt sensitive genotype. In the second experiment growth, physiological, biochemical, and ionic attributes of Saadia-tolerant and Black Beauty-sensitive eggplant genotypes (screened out in first experiment) were investigated under different salinity levels (control, 3, 6, 9, 12 and 15 dS m\(^{-1}\)) of NaCl. Results of second experiment showed that salt stress caused the reduction in growth (plant height, root length, shoot and root fresh weight, shoot and root dry weight), water relations (leaf water potential, leaf osmotic potential, leaf turgor pressure and RWC), physiological (photosynthesis rate, transpiration rate and stomatal conductance, except WUE) and biochemical (chlorophyll contents) traits of both tolerant and non-tolerant genotypes. But tolerant genotype (Saadia) showed less reduction in above traits in comparison to sensitive ones (Black Beauty). The enzymatic (SOD, POD and CAT) activities, proline and glycinebetaine were substantially increased in both eggplant genotypes under salt stress conditions. But tolerant (Saadia) showed the maximum increase. Among the ionic traits, Na\(^+\) and Cl\(^-\) were increased while Ca\(^{2+}\) and K\(^+\) significantly decreased in response to salt stress. Whereas, tolerant maintained the high concentration of Ca\(^{2+}\) and K\(^+\) ions and the least amounts of toxic ions (Na\(^+\) and Cl\(^-\)).
In the third experiment diverse levels of chitosan i.e. 75, 100, 125, 150, 175 and 200 mg L\(^{-1}\) were supplemented as a foliar spray on tolerant (Saadia) and sensitive (Black Beauty) genotypes grown under 9 dS m\(^{-1}\). Chitosan 150 mg L\(^{-1}\) was found to be the optimum dose to increase eggplant growth attributes (plant height, root length, shoot fresh weight, root fresh weight and plant dry matter). Whereas, in fourth experiment Saadia (tolerant) and Black Beauty (non-tolerant) eggplant genotypes were grown under saline and non-saline conditions, with and without chitosan. Results showed that chitosan improved the physiological, turgor pressure, enzymatic activities (SOD, POD and CAT), biochemical attributes (proline and glycinebetaine) and beneficial ions (Ca\(^{2+}\) and K\(^{+}\)) and decreased the toxic ions (Na\(^{+}\) and Cl\(^{-}\)), lipid peroxidation and osmotic potential as well as water potential in both tested eggplant genotypes. Among the yield parameters chitosan increased the number of fruits, fruit diameter, average fruit weight and yield per plant in both eggplant genotypes under stressed and non-stressed environments. Overall, it can be concluded that salt stress reduced the eggplant growth and productivity. Foliar application of chitosan induced salt tolerance in eggplant and improved yield attributes.
Chapter 1

INTRODUCTION

Eggplant (Solanum melongina L.) is a member of the solanaceae family; its sub-family is solanoideae, tribe solaneae, genus Solanum and subgenus leptostemonum (Dun.) Bitt., with more than 450 species distributed in Europe, Asia, America and Africa. The edible part of eggplant is a fruit known as brinjal, garden egg, aubergine, guinea squash, patlican or melanzana. The fresh weight of the eggplant fruit is composed of 1.4% protein, 0.3% minerals, 0.3% fat, 1.3% fiber, 92.7% water and the remaining 4% consisting of various carbohydrates and vitamin A, B and C (Khan, 1979). It originated in the Indian subcontinent with many primitive cultivars of eggplant, still grown in Asian countries. Eggplant has many cultivars which differ in their physiological attributes, biochemical and morphological characters including shape (round, long, oval, oblong, pear shape), size (very small to large), color (white, green, pink, purple, brown, blue) and is cultivated in tropical, subtropical and temperate zones (D’Arcy, 1972; Sihachakr et al., 1993). In recent years, consumer interest in the quality of vegetable products has tremendously increased particularly for the positive effects of vegetables on human health (Kaplan et al., 2002). Quality includes odor, flavor, nutritional value, health related anti-oxidative compounds and physical appearance of vegetables. Vegetable quality depends on genetic, environmental and cultural factors and their interactions; on cultural factors such as growing medium (soilless or in soil) and water quantity and quality, mineral nutrition and salinity (Huyskens and Schreiner, 2003; Gruda, 2005; Rouphael et al., 2012). Eggplant is consumed almost worldwide due to its medical and nutritional properties (Kittas et al., 2006; Raig on et al., 2008). Due to its phenolic constituents, eggplant has oxygen radical scavenging capacity and is ranked in the top ten nutritionally important vegetables (Grubben, 1977; Cao et al., 1996; Vinson et al., 1998; Hanson et al., 2006). Okmen et al. (2009) reported that different cultivars of eggplant vary in their water soluble antioxidant activity ranging from 2664 to 8247 mmol Trolox kg\(^{-1}\) and also in their total phenolic contents production from 615 to 1376 mg/kg. Chun et al. (2005) observed that a 100 g serving of eggplant provides significant quantities of phenolics (7.7-13.5% of the projected daily intake) and the minerals like potassium (3.3-5.9% of the recommended dietary allowance; RDA) (Institute of medicine; IM, 2004), phosphorus (2.8-6.2% of RDA) (IM, 2000), and Cu (4.3-9.7% of RDA) (IM, 2001).
Eggplant is rich in vitamins and minerals like calcium, iron, phosphorus, potassium, fat and carbohydrates. Health benefits of eggplant include anti-constipation, lowering cholesterol level and colitis pain, helpful in stomach ulcers and nervous conditions. Anthocyanins and nasunins, found in eggplant peel, characteristic of red or purple colored fruits, are the main phenolic compounds in eggplant (Vinson et al., 1998; Kayamori and Igarashi, 1994; Noda et al., 2000; Mazza et al., 2004). According to FAO (2013), more than 9044 hectares are under eggplant cultivation in Pakistan with total production of 91126 MT, but the average yield per ha is 1 to 3 fold low compared to the rest of world. The yield loss in eggplant is associated with biotic as well as abiotic stresses (Shahbaz and Ashraf, 2013). Biotic factors involved in yield loss of eggplant are soil borne nematodes (Dhawan and Sethi, 1976; Sonawane and Darekar, 1984), bacterial (Ano et al., 1991; Goth, 1991), and viral diseases (Dombrovsky et al., 2012 and 2013); insects including mites (Srinivasan, 2009), fruit borer (Adiroubane and Raghu raman, 2008; Mainali, 2014), white fly (Dombrovsky et al., 2013), jassids ( Rao et al., 1968; Bindra and Mahal, 1981) and beetles (Raj and Kumaraswami, 1979; Arpaia et al., 1997; Hamilton et al., 1997; Jelenkovic et al., 1998). Temperature (Nothmann and Koller, 1975; Nothmann et al., 1979), drought (Byari and Rabighi, 1996; Bafeel and Moftah, 2008; Karam et al., 2011; Amiri et al., 2012), heavy metal toxicity (Kiran et al., 2014) and salinity (Zipelevish et al., 2000; Khalid et al., 2012; Hanachi et al., 2014) are abiotic factors involved in yield loss of eggplant. Salinity is the major abiotic factor which restricts crop productivity in all agricultural crops (Roy et al., 2014). Crop production decreases due to increased soil degradation, water scarcity, organic matter depletion, acidity, poor drainage, nutrient depletion and salinization (Cakmak, 2002; Ok et al., 2007; Jung et al., 2011; Roy et al., 2014). Salinization is the excessive accumulation of soluble salts in root zone of the plant. Sodium and chloride ions cause salt stress. Soils with excessive sodium contents have a sodicity problem and are called sodic, while soils with excessive soluble salts have a salinity problem and are called saline. Salinity is a global agricultural issue; as half of irrigated lands and 20% of cultivated area are saline in the world (Ghassemi et al., 1995; Edelstein et al., 2011). Moldakimova et al. (2012) reported that production areas with salinity issues have doubled between 2001 and 2011 and approximately one third of world total cultivated area which provides 40% of world food is salt affected (UNO, 2011). Countries like Australia, Egypt, China, United States of America, India and Pakistan have salinity problems ranging from 15 to 36% of irrigated lands (Schwabe et al., 2006; Chaves et al.,
Due to salinity, 40,000 hectares (ha) of land become unfertile in our globe annually (Ghafoor et al., 2004). Geological surveys reveal that Pakistan has arid and semiarid climatic conditions mainly. Pakistan has about 23 million hectares (m ha) total cultivated lands at present, out of which 6.2 m ha lands are salt affected where 2.8 m ha salt effected land is under cultivation with low yield whereas, 3.4 m ha is completely uncultivable. Additionally, modern canal irrigation system is used to irrigate 75% of farmlands which is the major cause of salinization in Pakistan (Khan, 2007). Pakistan is facing severe problem of salinity with an annual loss of about Rs.20 billion reported due to reduction in crop yield in salt affected areas of Indus Basin (Anonymous, 2001).

Sources of salt accumulation in the Indus Basin include irrigation application, ground water and mineral weathering. Salt accumulation in the top soil is due to high evapotranspiration and shallow depth of the ground water which allow salts to move up with moisture through capillary action (Shah et al., 2011). Besides, high evapotranspiration rate due to hot climatic conditions of arid and semiarid zone is less than atmospheric precipitation. Annual precipitation is not sufficient to flush salt out of rhizosphere which results into more and more accumulation of salt in root zone (Maksimovic and Ilin, 2012). Salinity is an alarming abiotic stress for arable land and fresh water resources (streams, wetlands, lakes and rivers) worldwide, mainly in arid and semi-arid areas.

Although, information regarding crop salt tolerance for over 130 crop species is documented, yet there are many vegetable crops which lack reliable salt tolerance data. Understanding salt tolerance of vegetables is more important than field crops because the cash value of vegetable crops is usually high (Shannon and Grieve, 1999). Eggplant is economically important crop in Asia, Africa, Europe, subtropics, Central America and some warm temperate regions (Mediterranean area, South of the USA) (Collonnier et al., 2001; Sihachakr et al., 1993; Kantharajah and Golegaonkar, 2004).

Excessive soil salinity causes physiological drought (osmotic effect) initially leading to reduced plant growth rate, poor and spotty stand of crop, uneven and stunted growth, smaller leaves, sometimes fewer leaves and poor yield due to excessive concentration and absorption of harmful ions, a clear proof of salinity toxicity to the plants as it reduces the absorption of other essential plant nutrients in eggplant (Munns and Termaat, 1986; Jacoby, 1994; Chen and Jiang, 2009; Bybordi, 2010; Sahu et al., 2010). Severity in reduction of growth is directly proportional to the increase in toxic ions concentration (Greenway and Munns, 1980). Salt stress causes specific ion toxicity, oxidative and
osmotic stress, ionic and hormonal imbalance which alter the metabolic activities operating at the cellular level. All these stresses (outcome of salinity stress) have adverse effects on photosynthesis, leaf senescence and other biochemical processes which result in reduced plant growth and development (Nonami and Boyer, 1990). All important crop plants including vegetables are susceptible to abiotic stresses and their growth suppression varies with species. Salinity is one of the major abiotic stress which causes decreased productivity in various crops of arid and semi-arid regions including eggplant. At 8.5 dSm\(^{-1}\) salinity level, 50% growth reduction has been reported by Shalhevet et al. (1983) in eggplant. Moreover, salinity affects fruit yield and quality of eggplant (Moldakimova et al., 2012) by decreasing germination percentage and vegetative growth and affecting physiology (Ye et al., 2013). Eggplant growth has been shown to be sensitive (Bresler et al., 1982) or moderately sensitive (Maas, 1984) to salt stress. Variation in response may be due to cultivar differences and experimental conditions. However, the molecular, biochemical and physiological mechanism of salt tolerance in plants are not completely understood. Due to insufficient knowledge of these mechanisms, the development of salt tolerant crop is slow (Yamaguchi and Blumwald, 2005; Munns et al., 2006). Limitation in crop salt tolerance development by genetic means led to use other techniques to partially mitigate the adverse effects of salinity in plants.

Plant biostimulants include diverse substances or microorganisms when applied to plants or the rhizosphere induce natural process to enhance nutrient efficiency, growth, abiotic stress tolerance and quality of crop, regardless of the presence of nutrients in the product (European Biostimulants Industry Council; EBIC, 2012a). Biostimulants improve growth and development of plant from seed germination to maturity. They increase yield and quality by improving the efficiency of plant’s metabolism, assisting nutrient assimilation, increasing water use efficiency, enhancing quality attributes of produce, including color, sugar contents, fruit setting etc., increasing tolerance and recovery to abiotic stresses and enhancing certain physiochemical properties of soil (EBIC, 2012b). The world market for biostimulants has been estimated to accomplish $2,241 million by 2018 and to have a total annual growth rate of 12.5% from 2013 to 2018 (Anonymous, 2013). Reports of ‘The European Biostimulants Industry Council’ showed that in 2012 over 6.2 m hac were treated with biostimulants in Europe. The largest market for biostimulants in 2012 was Europe (EBIC, 2013).
Both, long and short-term approaches can be used to mitigate salt stress in eggplant. It is obligatory to use these approaches particularly short-term (chitosan usage) to overcome the yield loss in eggplant due to salinity. Chitosan is a linear polysaccharide, derived from chitin. It is present naturally in crustacean’s shell, fungus cell wall and insect skeleton. It is the second most abundant biopolymer in nature next to cellulose. Chitosan got more attention in agriculture due to its application as seed coating, foliar application, bio-pesticide, organic fertilizer and growth promoter, etc. (Gornik et al., 2008). Its biological functions are antimicrobial, growth inhibitors of some pathogens; enhancement of plant immune system (Andres et al., 2007) and alleviation of abiotic stresses (drought, heat, heavy metal toxicity, salinity) (Bittelli et al., 2001; Farouk et al., 2011). Exogenously applied chitosan enhances plant growth and induces biotic stress tolerance as well as abiotic stress tolerance including low and high temperature stress, drought and salinity by stimulating the physiological and biochemical processes. Considering the aforementioned information, eggplant is moderately sensitive to salinity and chitosan had a positive role in salinity mitigation. Additionally, evaluation of local eggplant genotypes against salinity had not been reported yet. So, a comprehensive study was planned with the following objectives.

- Evaluation of the local eggplant genotypes against salinity.
- Understanding the morpho-physiological, biochemical and ionic changes in eggplant under salinity stress.
- Optimization of best chitosan level for the enhancement of salinity tolerance in eggplant.
- Investigation of chitosan treatment on growth, water relation, physiological, biochemical and ionic changes in eggplant under salinity stress.
Chapter 2

REVIEW OF LITERATURE

2.1. Effects of salinity on plants

Salinity influences plants in a variety of ways such as nutritional disorders, specific osmotic effect and specific ion toxicity. Salinity reduced the percentage of seed germination in globe artichoke and an increase of salt concentration delayed germination (Mauromicale and Licando, 2002). While salinity delayed seed germination and emergence, the newly growing seedlings were more susceptible to, hypocotyls and cotyledon, injury under saline environment (Esechie et al., 2002). Salinity reduced plant density (Goyal et al., 1999), reduced seed germination, growth and fruit yield in melon cultivars (Nerson and Paris, 1984).

It is well recognized that salinity affects plant growth by numerous ways at various stages of development (Bernstein and Hayward, 1958). It was observed that in early stages, salinity decreased seed germination and emergence while at later growth stages it reduced vegetative and reproductive growth (Maas and Poss, 1989). Savvas and Lenz (2000) reported that salinity had no significant effects on number of flowers and fruits of eggplant but decreased crop yield due to reduction in fruit size.

Salinity affects primary processes of plant growth and photosynthesis (Munns et al., 2006). Photosynthesis is extremely affected by salinity or osmotic stresses by a variety of means because these stresses limit CO$_2$ diffusion (Flexas et al., 1999). Extreme salt stress conditions restrict photochemical system and apparatus (Souza et al., 2005) or damage photosynthetic metabolism (Lawlor and Cornic, 2002). Intercellular CO$_2$ level is restricted by indirect result of stomatal closure which increases the susceptibility to photochemical damages because low CO$_2$ assimilation steps up light energy at photosystem-II as a consequence of photorespiration (Tezara et al., 2005). Osmotic homeostasis and leaf water relations are also altered by salinity (Munns, 2002). When plants are exposed to prolonged severe salinity they undergo permanent photosynthetic apparatus damage which is linked with decrease of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity (Delfine et al., 1999).

Plant maintains a balance between the reactive oxygen species (ROS) production and the quenching activity of antioxidants under normal physiological conditions. However, when plant experiences stress conditions, this balance is upset and results in oxidative
stress (Weckx and Clijster, 1997). When plants are exposed to salt stress, production of excessive ROS is observed, which disturbs the metabolic processes of the cell and they experience oxidative stress (Munns and Tester, 2008). Carbohydrate oxidation, lipid peroxidation of cell membrane, protein denaturation, pigment breakdown, DNA damage and distortion of enzymatic activity are the responses of reactive oxygen species (Scandalios, 1993; Noctor and Foyer, 1998). Under oxidative stress plants produce number of enzymatic and non-enzymatic antioxidants to combat the reactive oxygen species. Main antioxidative enzymes are glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX), while non-enzymatic ROS scavengers are glutathione and ascorbate (Colville and Smirnoff, 2008). In tomato, higher levels of enzymatic antioxidants have been reported for salt tolerant genotypes than salt susceptible (Dogan et al., 2010).

2.2. Effects of salinity on eggplant
Eggplant is sensitive to salt stress, whether it is grown openly in the field (Heuer et al., 1986), in greenhouse soil (Sonneveld, 1988; Chartzoulakis and Loupassaki, 1997), hydroponically (Savvas and Lenz, 1994; Islam et al., 2010) or in any substrate (Hamdy et al., 2002), regardless of the salt type (Savvas and Lenz, 2000). Sonneveld, (1988) reported that eggplant is more sensitive to salt stress than tomato.

2.2.1 Seed germination of eggplant under salinity
Flowering plants like eggplant produce their seed by sexual means for their next generation. The survival and propagation of these plant species depends highly on the successful seed germination and establishment of a normal seedling. Germination is a process in which dry mature seed uptake water from surrounding environment under favorable conditions and the protrusion of radical root takes place. Moreover, Nonogaki et al. (2010) described that seed germination comprises three phases; imbibition (phase 1), metabolic process (phase 2) and radical emergence (phase 3). Germinating seeds are vulnerable to disease, injury and environmental stresses and seed germination is considered as the most critical stage in a plant life cycle (Nonogaki et al., 2010, Rajjou et al., 2012). Apart from light, moisture, temperature and dormancy factors, seed germination in eggplant is also affected by physiological quality of the seed, physical abnormalities in seed structure such as free spaces in seed, seed damage (Silva et al., 2012), and salinity (Chartzoulakis and Loupassaki, 1997; Saeed et al., 2014).

Chartzoulakis and Loupassaki, (1997) observed the effects of six different NaCl salinity levels (0, 1, 2.5, 5, 10 and 15 d Sm⁻¹) on seed germination of eggplant hybrid ‘Delica’ in
sand-perlite (1:3) growing medium. Germination was delayed at low level (5 d Sm⁻¹) and reduced germination percentage was observed at higher salinity levels (10 dS m⁻¹ and 15 dS m⁻¹). Demir et al. (2003) checked different seed lots (42, 45, 50, 55, 60, 70 and 80 days after anthesis) of eggplant against four different salinity levels (0, 3.5, 7 and 14 dS m⁻¹). It was observed that NaCl inhibited seed germination and emergence in all lots. At higher salinity level (14 dS m⁻¹) seedling abnormalities were observed e.g cotyledon unfolding, chlorosis, and necrotic areas. It was also concluded that seed harvested between 50-60 days after anthesis gave better seed germination percentage under saline conditions than those harvested earlier.

Akinci et al. (2004) found similar results regarding eggplant seed germination. Higher salinity level not only delayed germination but also decreased its percentage. They also found that salt tolerance was higher in ‘Kemer’ cultivar than ‘Pala’ and ‘Aydin Siyahi’ in terms of seed germination rate and percentage and concluded that different cultivars of eggplant had different tolerances to salinity.

Recent findings by Basalah, (2010), Abbas et al. (2010), Selvaraj, (2011) and Saeed et al. (2014) on seed germination of eggplant under saline regimes depict that eggplant is sensitive to salinity at seed germination and early growth stages. Moreover, variations in salt tolerance of eggplant cultivars exist. However, germination stage in eggplant is critical to salinity stress. The resultant decrease in germination percentage after exposure to salinity is also reported in tomato (Jones, 1986), carrot (Schmidhalter and Oertli, 1991), sugar beet (Ghoulam and Fares, 2001) and chili pepper (Mustafa et al., 2014). Ayers (1952) reported that salinity delayed germination rate in almost all crop plants including eggplant.

Soil salinity causes a decrease in seed germination in eggplant either by osmotic effect in which plant is unable to absorb water and ions from external environment which imbalance the hormonal and enzymatic activities of seed (Dumbroff and Cooper, 1974; Greenway and Munns, 1980).

Some plants are relatively tolerant to salinity during the germination stages but are more susceptible during emergence and early seedling development (Rhoades, 1990). Eggplant can be directly seeded into the field. Direct seeding has a lot of disadvantages (weeds, non-uniform crop stand, time, etc.) over transplanting. In Pakistan, eggplant seedlings are prepared on raised beds either in protected or open conditions and then transplanted to the field. Eggplant is normally transplanted into the green house or field after initial growth
and development in the nursery. So, our main focus is to review the effects of salinity on the eggplant vegetative growth and development after germination.

### 2.2.2. Salinity and growth of eggplant

Functions of root are to anchor the plant in the soil, absorb water and supply available mineral nutrition to the plant. In salt containing growing media, exposure to salt stress begins at the root level. Roots of different species of plants show different morphological, physiological and growth behaviors to salt stress. Under severe saline conditions, roots are unable to absorb adequate water and the uptake of excess Na\(^+\) and Cl\(^-\) ions instead of necessary minerals leads to physiological and biochemical changes in whole plant.

Pessarakl and Tucker, (1988) observed a significant decrease in root and shoot dry weight in eggplant under salinity. Moreover, they observed that roots were less affected by salinity in early stages of seedling development in eggplant than to shoots but later on, no differences in root and shoot dry weight reduction were observed. Pascale et al. (1995) determined that 1% soil salinity reduced the root length of eggplant by up to 50% and additionally noted that root density was less than control. Various organs of eggplant and kidney bean (*Phaseolus vulgaris* L.) were cultured in vitro and the effect of salinity on morphogenesis was studied. It was observed that callus cultures obtained from different organs expressed variations in salt tolerance. Explant (piece of tissues) of different organ differed in regeneration and survival potential under salinity stress. Moreover variation of explants, obtained from different organs, due to salinity threshold level was an indication of organ specificity to salt tolerance. Furthermore, rhizogenesis was severely affected by salinity stress and sensitive cultivars were more affected (Yusufov and Alieva, 2002).

Detailed studies by Akinci *et al.* (2004) on eggplant cultivars ‘Kemer’, ‘Pala’ and ‘Aydın Siyahi’ showed that the decrease in hypocotyls and radicle length, fresh and dry weights of roots started at 5 dS m\(^{-1}\) level of salinity in all varieties. Eggplant cultivar ‘Xianlvqie’ was tested against different salinity levels and a significant decrease in root fresh weight was observed (Baoli *et al.*, 2010). Wu *et al.* (2012b) reported that salt stress of 9 dS m\(^{-1}\) reduced eggplant root fresh weight by 38%. Moreover, roots of eggplant were 20% less affected compared to shoots (Wu *et al.*, 2012b). Recently, Shaheen *et al.* (2013) observed that eggplant cultivar ‘New Noble’ expressed significant decrease in root length, fresh and dry weight at 5 dS m\(^{-1}\) salinity level but maximum reduction of these characters was observed at higher salinity levels (10 and 15 dS m\(^{-1}\)).

Pascale *et al.* (1995) observed that eggplant plant height was reduced 30% by irrigation with 17 dS m\(^{-1}\) saline water. Salinity reduced plant height and leaf area even at very low
level of 2.5 dS m\(^{-1}\) in eggplant (Chartzoulakis and Loupassaki, 1997). They also observed that in leaf tissue, concentration of Cl\(^-\) was higher than Na\(^+\) and the growth of leaves was severely affected by NaCl salinity and photosynthetic rate of older leaves was inversely proportional to the concentration of Na\(^+\) and Cl\(^-\) ions, while newly expanding leaves were less affected even at higher salinity levels. The reductions in leaf area, shoot length and shoot fresh and dry weight of eggplant under salinity was also confirmed by Akinci \textit{et al.} (2004). They also noted that varieties differed in their salt tolerance potential. Similar observations were made by Abbas \textit{et al.} (2010) on two cultivars of eggplant under salt stress. Selvaraj, (2011) observed a reduction in stem diameter, plant height and shoot fresh weight in eggplant.

Three cultivars of eggplant (‘round Indian’, ‘Jahromian’ and ‘long Dezfolian’) were tested against different levels of salinity and natural growing conditions by Sadeghi \textit{et al.} (2012). They observed decrease in leaf number, plant height, and shoot dry weight under saline conditions. Moreover, shoot/root ratio was negatively affected by salt stress and ‘round Indian’ was more tolerant to salt stress than other cultivars while ‘Dezfolian’ was found to be most sensitive to NaCl salinity. Moreover; Chartzoulakis and Loupassaki, (1997) observed that leaf growth of eggplant was more sensitive to salinity as compared to other growth parameters.

Pascale \textit{et al.} (1995) found a 40% decrease in plant biomass at higher salinity level while under same saline condition leaf area was reduced up to 55% in eggplant. Eggplant biomass and leaf area was significantly reduced at 2.5 dS m\(^{-1}\) salinity level. Akinci \textit{et al.} (2004) reported that the decrease in leaf area of eggplant under saline conditions was due to necrosis and shrinkage of leaf mesophyll cells. It was also observed that seedling fresh and dry weights were decreased under saline conditions in eggplant. Salinity negatively affected eggplant leaf area index, leaf water potential and plant water contents which lead to reduced growth and a subsequent decrease in plant biomass (Leone \textit{et al.}, 2007). Abbas \textit{et al.} (2010) and Wu \textit{et al.} (2010) also observed a decrease in fresh and dry biomass of eggplant under saline environment.

Rud \textit{et al.} (2011) checked the effect of salinity on fresh weight of eggplant leaves and noted that at 15 dS m\(^{-1}\) salinity level leaf fresh weight was decreased by 70%. Similarly, salinity decreased plant biomass by reducing fresh and dry weights of root and shoot (Wu \textit{et. al.}, 2012b). Different cultivars of eggplant showed declining trend in fresh biomass production under increasing salt stress but maximum decrease in fresh biomass was observed in ‘Black Beauty’ (87.8%) and ‘Adriatica’ (86.6%) at 16 dS m\(^{-1}\), while it was
minimum in ‘Galine’ and ‘Bonica’ i.e. 35.9% and 36.9%, respectively, under same saline conditions (Hanachi et al., 2014). Decrease in eggplant biomass due to salinity was related to a decrease in plant height and stem diameter, root length and density, leaf number and leaf area, and shoot and root biomass (Hegazi et al., 2014). The above findings indicated that salinity played a major role in decreasing fresh and dry biomass of eggplant.

2.2.3. Mineral uptake and ionic contents of eggplant under salinity

Initially, salt stress in eggplant imposes two effects: an osmotic stress that inhibits water intake and/or water efflux of root cells and ion toxicity of Na\(^+\) and Cl\(^-\) (Yasar et al., 2006; Munns and Tester, 2008). At higher salinity level, eggplants accumulate Cl\(^-\) which causes chlorosis, necrosis and burning of leaf margins (Unlukara et al., 2010). High accumulation of Na\(^+\) in root and leaves due to salinity also causes nutrient deficiency in eggplant (Tester and Davenport, 2003; Dekoum et al., 2013; Shaheen et al., 2013). Nutrient deficiency is due to competitive interaction of ions or by changing the ion selectivity of membrane which results in salt induced calcium ion (Ca\(^{2+}\)) and potassium ion (K\(^+\)) deficiencies (Romero et al., 1997; Yasar et al., 2006). Ca\(^{2+}\) deficiency further induces magnesium ion (Mg\(^{2+}\)) deficiency in eggplant which leads to physiological disorders which effect plant growth (Sonneveld and Welles, 1988; Dekoum et al., 2013). Ca\(^{2+}\) deficiency under salt stress has been also observed in tomato and pepper (Rouphael et al., 2012).

Salinity decreases nitrogen (N) and water uptake in eggplant (Oliveira et al., 2011) which lead to a decrease in dry matter production (Pessarakli and Tucker, 1988; Kaswala et al., 2012). N deficiency is due to chloride (Cl\(^-\)) and nitrate (NO\(_3\)) ion interactions (Savvas and Lenz, 2000). Different eggplant cultivars have different potential to uptake N under saline regimes and salinity decreased N mineralization in soil (Savvas and Lenz, 1994). Salinity also reduced phosphorus (P) uptake in eggplant (Kaswala et al., 2012). Due to NaCL salinity plants of eggplant were unable to uptake potassium (K\(^+\)) from the soil (Oliveira et al., 2011; Shaheen et al., 2013; Dekoum et al., 2013) and K\(^+\) uptake was more affected compared to N and P (Unlukara et al., 2010; Kaswala et al., 2012). K\(^+\) uptake was reduced by Na\(^+\) interaction (Grattan and Grieve, 1999). Moreover, Ding et al. (2012) and Abbas et al. (2013) reported that leaf K\(^+\)/Na\(^+\), Ca\(^{2+}\)/Na\(^+\) ratios decreased significantly in eggplant under saline conditions. Salinity reduced N, P and K\(^+\) contents in vegetative part and in fruits of eggplant (Savvas and Lenz, 2000; Magio et al., 2007).
2.2.4. Fruit quality and quantity of eggplant grown under saline conditions

Salinity reduced number of flowers in eggplant and also delayed flowering which contribute to total fruit yield loss (Sadeghi and Rassoli, 2013). Shalhevet et al. (1983) concluded that a decrease in individual fruit weight of eggplant was due to stress imposed by salinity which contributed to yield reduction of crop. Pascale et al. (1995) observed a yield loss in eggplant under saline conditions. Sifola et al. (1995) attributed the decrease in fruit length to inhibitory effect of salt stress on vegetative and reproductive parts of eggplant. Yield reduction of 23% in eggplant was observed at 2.5 dS m\(^{-1}\) and 88% at 15 dS m\(^{-1}\). The yield loss in eggplant under saline conditions was due to a decrease in fruit size and number per plant (Chartzoulakis and Loupassaki, 1997). Savvas and Lenz, (2000) and Hamdy et al. (2002) reported that salinity level up to 6.1 dS m\(^{-1}\) severely effectd eggplant vegetative growth and early fruit yield. The loss in fruit yield was due to a decrease in mean fruit yield associated with low water contents of fruit. Flowering and fruit set stages are very sensitive to salinity which should be less than 4 dS m\(^{-1}\) at these stages to get high fruit yield in commercial farming (Akinci et al., 2004; Hamdy et al., 2002).

Significant decrease in fruit yield of 63% was observed at 7 dS m\(^{-1}\) in ‘Kemer’ cultivar of eggplant. Threshold value of soil salinity for fruit yield of eggplant were determined as <1.5 dS m\(^{-1}\) and for vegetative dry weight it was 6.7 dS m\(^{-1}\) which indicates that fruit yield is more sensitive to salinity as compared to vegetative growth in eggplant (Unlukara et al., 2010). Naik et al. (2011) found similar results for fruit yield in eggplant. Kaswala et al. (2012) also reported that salinity at early stages reduced eggplant fruit yield. Number of fruits and individual fruit weight of ‘Dilnasheen’ and ‘Bemisal’ cultivars of eggplant declined with 9 dS m\(^{-1}\) salt stress (Abbas et al., 2013). Moura and Carvalho, (2014) noted that high salinity caused 30-54% commercial production loss in ‘Cica’ hybrid of eggplant. Moreover, salinity reduced fresh and dry weight of eggplant fruit which ultimately lead to a decrease in total fruit yield (Hegazi et al., 2014). Silva et al. (2013) reported that every 1 dS m\(^{-1}\) increase in soil salinity above the 1.71 dS m\(^{-1}\) threshold caused 8.65% reduction in eggplant yield.

Salinity and water deficiency decrease fruit quality of eggplant (Kirnak et al., 2001). Pascale et al. (1995) reported that 45% of marketable quality of eggplant was affected by 1% soil salinity. Salinity decreased mesocarp firmness and polyphenol and ascorbic acid in eggplant fruit (Sifola et al., 1995).
Quality of vegetable fruits is severely affected by salinity. Salt stress caused Ca\(^{2+}\) deficiency which led to physiological disorders such as blossom end rot in pepper (Geraldson, 1957) and in tomato (Spurr, 1959), vitrescence in melon (Baptiste et al., 1999), and soft rot in eggplant (Kreij, 1990). Soft spots on eggplant fruits are due to Ca\(^{2+}\) deficiency and characterized as blossom end rot (Cerda et al., 1979). In blossom end rot, Ca\(^{2+}\) concentration is below 20 mmol kg\(^{-1}\) in the dry matter of fruit (Chiu and Bould, 1976; Kreij, 1990; Morley et al., 1993). Calyx browning of fruit is a disorder related to high Ca\(^{2+}\) contents in fruit of eggplant (Maaswinkel, 1988). Savvas and Lenz, (1994) also observed a decrease in Ca\(^{2+}\) contents in eggplant fruit due to salinity which decreased fruit shelf life. Salinity also reduced the marketable yield of eggplant fruit by 45% (Maggio et al., 2007).

Islam et al. (2010) reported that very low salinity levels were beneficial to fruit yield and quality of eggplant. Irrigation applied with 2% saline sea water to eggplant cultivar ‘Ryoma’, improved fruit yield and quality by 14% and 23%, respectively. Harvested fruits were rich in sugar, minerals and dry matter also.

### 2.2.5. Physiological changes in eggplant due to salt stress

Abiotic stresses such as salinity affect crop production adversely by inducing different physiological responses in photosynthesis, osmotic adjustment, water relation and gas exchange characteristics in eggplant (Poltronieri et al., 2011). Agronomic attributes are not reliable when studying the salt stress tolerance of eggplant because they can be the combined effects of environmental and genetic factors. There is little information available on the effect of salt tolerance on agronomic characters of individual crops; however, physiological criteria are more reliable information (Ashraf, 2004).

Photosynthetic apparatus is the most susceptible physiological component of eggplant to salt stress (Wu et al., 2012b). Photosynthesis was decreased by 52% at 1% salinity level (Pascale et al., 1995). Photosynthesis is inversely related to the concentrations of Na\(^{+}\) and Cl\(^{-}\) ions in eggplant (Chartzoulakis and Loupassaki, 1997). A decrease in photosynthetic rate in eggplant under salt stress is strongly correlated with the reduction of chlorophyll contents and salt stress reduces both chlorophyll a and b contents in eggplant (Shaheen et al., 2013). Salinity inhibits gas exchange capacity of eggplant (Pascale et al., 1995). Decrease in stomatal conductance by 30% was reported at 4.9 dS m\(^{-1}\) salinity level in eggplant by Magio et al. (2007). Wu et al. (2012b) reported that salt stress decreased net photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO\(_2\) concentration in eggplant. Two cultivars of eggplant, ‘L-888’ and ‘Round’ were tested at
15 dS m\(^{-1}\) and results indicated that salinity reduced CO\(_2\) assimilation rate, transpiration rate and water use efficiency of both cultivars (Shahbaz et al., 2013). Similar results regarding gas exchange characteristics were reported by Shaheen et al. (2013) on cultivar ‘New Noble’ of eggplant under salt stress. Abbas et al. (2013) found that different cultivars of eggplant had a variable physiological response to salinity, but a linear decreasing trend was common.

Salt stress causes ionic imbalance in eggplant. Concentration of Cl\(^-\) in leaf tissue of eggplant was higher than Na\(^+\) under saline regimes (Chartzoulakis and Loupassaki, 1997). Pascale et al. (1995) observed a decrease in water potential of -0.43 to -0.77 MPa in roots and -0.97 to -1.42 MPa in shoots. Overall, water potential of eggplant decreased under salt stress and was associated with the production of osmolyts and accumulation of inorganic ions from external saline environment which lead to a decrease in osmotic potential (Magio et al., 2007). Salinity reduced leaf relative water contents of eggplant (Hegazi et al., 2014). Salt stress also reduced osmotic and leaf water potential of eggplant. Whereas, increase in leaf turgor potential was observed in eggplant as salinity level increased in the root zone (Shaheen et al., 2013). A higher salinity level caused electrolyte leakage in eggplant (Ding et al., 2012). These physiological changes end up into physiological leaf senescence in eggplant under saline regimes (Magio et al., 2007).

**2.2.6. Biochemical alterations in eggplant under salt stress**

Salt stress imposes various physiological and biochemical changes in eggplant. To cope with this stress, plants adopt several mechanisms at the cellular, metabolic and whole plant level. Such mechanisms include stress signaling, osmotic regulation, ion homeostasis, and antioxidant production. In fact, it is hard to find single criteria suitable to study the salt stress tolerance of eggplant. It is obvious that physiological and biochemical attributes are more helpful for better understanding of salt tolerance in eggplant as compared to growth indicators (Ashraf, 2004). Eggplant salt stress tolerance depends on multiple biochemical pathways which help in the acquisition of water by plant, maintenance of ion homeostasis, and protection of the photosynthetic apparatus. Essential pathways are involved in the synthesis of reactive oxygen species (ROS) scavenging enzymes, osmotically active metabolites, and specific proteins which support ion and water flux (Parida and Das, 2005; Shaheen et al., 2013).

Leaves of eggplants show significant increase in malondialdehyde (MDA) contents, electrolyte leakage, hydrogen peroxide contents, and hydroxyl radical and superoxide production at higher salinity levels (McCord, 2000; Yasar et al., 2006; Arora et al., 2008;
Ding et al., 2012; Shaheen et al., 2013). Salinity increases Na⁺, Cl⁻, and ROS level in eggplant and results in cell toxicity, plasma membrane leakage and cell death (Wang et al., 2003). The end product of lipid peroxidation is MDA which is a potential indicator of oxidative damage to the cell membrane. So, MDA is the potential indicator that determines the magnitude of lipid damage by ROS. MDA is less produced in salt tolerant cultivars of eggplant compared to sensitive ones (Jain et al., 1987). Eggplant produces enzymatic and non-enzymatic ROS scavengers to cope with salt induced oxidative stress (Shaheen et al., 2013). Salt stress increases super oxide dismutase (SOD), catalase (CAT), ascorbate per oxidase (APX) and glutathione reductase (GR) enzymes activity in eggplant to minimize the ROS damage (Masood et al., 2006; Dai et al., 2009; Ding et al., 2012; Manar et al., 2013). SOD enzyme converts superoxide into H₂O₂ which is further scavenged by APX and CAT (Wu et al., 2012b). Alpha-amylase activity decreases at higher salinity levels in eggplant (Basalah, 2010). Wild genotypes of eggplant produce more antioxidant enzymes and less MDA under salt stress conditions, thus, high enzymatic activity means more tolerance to salinity (Yasar et al., 2013). Non enzymatic antioxidants such as total phenols and tannins have shown an increase in eggplant in response to salinity (Hegazi et al., 2014). Due to salinity, a decrease in total soluble proteins (Shaheen et al., 2013) and an increase in total soluble sugars (TSS), proline and glycine betaine have been observed in eggplant (Shaheen et al., 2013; Hegazi et al., 2014).

In relation to the physiology of osmotic adjustment in saline soils, there is the issue of metabolic costs for osmotic adjustment. Growing or surviving in a saline soil imposes some costs; the cost of excluding salt, of intracellular compartmentation and of excreting it through salt glands. This cost however, is relatively small in relation to that needed to synthesize organic solutes for osmotic adjustment (Yeo, 1983; Raven, 1985). The number of moles of ATP needed to use one mole of NaCl as an osmoticum is approximately 4 in root cells and 7 in leaf cells whereas, the number required to synthesize an organic compound is an order of magnitude higher (Raven, 1985). The ATP requirement for the synthesis or accumulation of solutes in leaves was assessed by Raven (1985) as 3-5 for Na⁺, 34 for mannitol, 41 for proline, 50 for glycinebetaine and about 52 for sucrose. (These values assume a production of 0.5 mole of ATP per photon and nitrate as the source of N).
2.3 Possible approaches to alleviate the drastic effect of salinity in eggplant

Abiotic stresses such as drought, flooding, high or low temperature, nutrient deficiency, heavy metal toxicity, and salinity restrict crop productivity worldwide. Salinity can be more problematic in developing countries. The major negative aspects can be food insecurity, unemployment and poverty in rural population. Whereas, salt stress and nutrient stress render meager human nutrition. Thus, abiotic stresses are major contributors to poverty for rural communities in developing countries. In these circumstances, multiple approaches should be adopted to maximize crop yield and economic returns in an unfavorable environment. Possible approaches currently tested to alleviate the drastic effects of salinity in eggplant include use of appropriate fertilizer, seed priming, plant growth promoting rhizobacteria (PGPR), grafting, exogenous application of plant growth substances, biotechnological and breeding techniques.

2.3.1 Fertilization

Salinity damage can be overcome, to some extent, by foliar application of micro and macronutrients in eggplant (Padem et al., 1999). Savvas and Lenz (2000) observed that an increase in NaCl led to a decrease in Mg concentration in growth of eggplant leaves grown in rockwool. The decrease in Mg concentration can be overcome by adding Mg in the irrigation water. Azarpour et al. (2012) reported that N fertilizer application and foliar spray of humic acid (50 mg L⁻¹) enhanced vegetative and reproductive yield of eggplant. Kiran et al. (2014) reported that humic acid application in irrigation water can alleviate the drastic effect of abiotic stresses (heavy metal, drought and salinity stress) on growth and development of eggplant. Moreover, Semida et al. (2014) reported that a soil amendment consisting of a mixture of humic acid, green waste compost, and elemental sulphur designated as organo-mineral fertilizer reduced the adverse effects of salt stress in eggplant by increasing total porosity and water holding capacity of soil.

K⁺ is an important inorganic solute that plays major roles associated with osmoregulation, stomatal behavior, protein synthesis, enzyme activity, membrane polarization, neutralization of non-diffusible negatively charged ions and cell expansion (Elumalai et al., 2002). In addition, K⁺ lowers the ROS production by supporting photosynthetic electron transport and reduces the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Cakmak, 2005). Villora et al. (2003) reported that K⁺ controls the status and distribution of NO₃ and molybdenum (Mo) in aerial parts of eggplant, and higher concentration of K⁺ improved the quality of eggplant fruit by
lowering the level of Mo and NO₃ in fruit. However, an increase in NaCl concentration in irrigation water or an increase in soil salinity was accompanied by a decrease in K⁺ contents and K⁺/Na⁺ ratio in eggplant (Akinci et al., 2004). Addition of different K fertilizers to the soil or in irrigation water reduces the adverse effect of salinity (Kaya et al., 2007; Khayat et al., 2007). Elwan (2010) checked the effect of K₂HPO₄ spray on eggplant under saline regimes. It was observed that spray of K fertilizer significantly alleviated the effect of salinity in eggplant by lowering Na⁺ contents in fruit and by increasing vegetative growth, fruit yield, and K⁺ and Ca²⁺ contents.

Marques et al. (2011) reported that excessive application of KCl and K₂SO₄ fertilizer decreased fruit yield and increased proline contents in the leaf of eggplant. Foliar application of K₂SO₄ (2.5 g L⁻¹) during flowering stage enhanced fruit yield and quality of eggplant (Fawzy et al., 2007). K₂SO₄ was reported as a better source of K⁺ for eggplant cultivation (Marques et al., 2012) while, KCl reduced quality of eggplant fruit (Zipelevish et al., 2000). Potassium fertilizers that are used in eggplant production can contribute to an increase in electrical conductivity (EC) of soil, KCl has a more tendency to salinize soil compared to K₂SO₄ (Marques et al., 2014).

Aminifard et al. (2010) reported that 100 kg ha⁻¹ N is required for successful production of eggplant. In non-saline conditions, N increased fruit yield of eggplant by increasing number of fruits per plant (Pal et al., 2002; Aujla et al., 2007). Sharma (1980) observed that the yield of eggplant cultivated in saline soils can be increased by the addition of 40 Kg ha⁻¹ N. Sharma (1980) also reported that calcium ammonium nitrate (NO₃ as a source of N) is better than diammonium sulphate (NH₄ as a source of N) in saline soils for eggplant production. Time and method of fertilizer application are also important for successful production. Sharma (1980) also reported that foliar application of 3% N along with basal dose of 20 kg ha⁻¹ N can improve the growth and production of eggplant under saline conditions. Excessive dozes of N delayed flowering in eggplant (Sat and Saimbhi, 2003). It was reported that N is more important than P to increase yield of eggplant (Lima et al., 2014).

P is a component of many organic compounds and is used in many important metabolic reactions in plants (Awad et al., 1990). Phosphorus enhances fruit quality and number of fruits per plant in eggplant (Zipelevish et al., 2000). Salinity also causes P deficiency in several crops (Kaya et al., 2003). Transport of P is affected by salinity in plants which can be alleviated by foliar application of monopotassium phosphate (Kaya et al., 2001,
Ameliorative effects of P nutrition on salinity stress in crop plants have been studied less than other mineral nutrients (Plaut et al., 2013).

### 2.3.2 Seed priming

Primin is a controlled pre-soaking technique that allows seed to complete pre-germinative physiological and biochemical activities but restrict germination/radical protrusion (Bradford, 1986; Moradi and Younesi, 2009; Mathews et al., 2011). Different seed priming techniques are used in agricultural crops to enhance germination and to improve stress tolerance at early germinating stages. Hydropriming, osmopriming, drum priming, matric priming and hormonal priming are such examples (Basra et al., 2003). Seed priming improved seed vigor, uniformity, germination and emergence leading to a healthy seedling stand establishment which could compete with weeds and environmental stresses including salinity and an increase in plant yield (Hosseein et al., 2011).

Primin eggplant seeds with hormones or vitamins (salicylic acid and ascorbic acid) lowered the EC of leachates. Lower EC of leachates is an indication of enhanced membrane repair under controlled hydration. Membrane integrity of primed seeds was better than unprimed seeds of eggplant (Rudrapal and Nakamura, 1988). The lower EC in chelate induced by hormonal priming was accompanied by higher germination emergence and germination index, and lower mean germination time. This implies that seed priming guarantee membrane and genetic repair and trigger metabolic activities in eggplant (Trigo and Trigo, 1999). Osmopriming improved seed germination of eggplant; moreover, priming with KNO₃ salt was effective in increasing seed germination (Shahlaei et al., 2009). Older seeds have less germination potential, and priming with GA₃ and KNO₃ was shown to increase germination of aged eggplant seeds (Demir et al., 1994). Nascimento (2005) reported that priming seeds of eggplant cv. ‘Cica’ improved germination. Demir et al. (2009) also reported that priming of aged eggplant seeds with karrikinolide enhanced germination and emergence. Solid-matrix priming for 72 hours with 80% water holding capacity improved seed vigor in eggplant (Venkatasubramanian and Umarani, 2007). Reis et al. (2012) reported that priming with water or KNO₃ enhanced vigor of eggplant seed without affecting seed viability. Chemical priming agents are used effectively to enhance seed germination, emergence and seedling uniformity in a number of plant species including eggplant. Recently, Mavi (2014) reported that an organic priming agent (a decoction obtained from dried flower of marigold species rich in lutein and gallic acid) increased both germination and emergence and decreased both mean germination and emergence times in eggplant.
These reports addressed the effects of seed priming on germination percentage, rate, seed vigor and viability under controlled condition. However, the effects of priming on eggplant seeds under saline conditions have been less studied.

2.3.3 Plant growth promoting rhizobacteria and fungi

Potential use of beneficial microorganisms is another approach to mitigate the effects of different environmental stresses such as salinity, drought, or poor fertility (Azcon-Aguilar and Barea, 1997; Abdel-Rahman et al., 2011). Paul and Harshad (2014) observed that plants inoculated with plant growth promoting rhizobacteria (PGPRs) have beneficial bacteria in their rhizosphere that counteract osmotic stress caused by salinity which improve hydration, nutrient uptake, root and shoot growth, chlorophyll contents and resistance to disease. The PGPRs have been reported to mitigate the adverse effects of salinity on the growth of horticultural crop plants such as eggplant (Bochow et al., 2001), tomato (Mayak et al., 2004), bean (Yildirim and Taylor, 2005), artichoke (Saleh et al., 2005), squash (Yildirim et al., 2006), lettuce (Barassi et al., 2006), radish (Yildirim et al., 2008) and strawberry (Karlidag et al., 2013).

*Bacillus megaterium* (phosphate solubilizing bacteria) and *Bacillus mucilaginosus* (potassium solubilizing bacteria) enhance the nutrient availability to eggplant grown in nutrient deficient and saline soils (Han and Lee, 2005). PGPRs have been isolated and characterized for eggplant growth promotion potential by Godinho et al. (2010). Three PGPRs strains *Enterobacter aerogenes* BM10, *Xanthobacter autotrophicus* BM13, and *Bacillus brevis* FK2 were isolated from salt-affected kidney bean and maize fields. *B. brevis* increased K+/Na+ ratio and K+-Na+ selectivity in eggplant shoots and was the most useful strain for reducing the damage caused by salt stress (El-Azeem et al., 2012). Inoculation with PGPRs can lessen the salinity damage in eggplant.

Some vesicular-arbuscular mycorrhizal (VAM) fungi have been reported to enhance eggplant growth (Ortas et al., 2011) and alleviated salt stress (Al-Karaki, 2000; He et al., 2007; Abdel-Latef and Chaoxing, 2011) by improving water uptake and maintaining hormonal balance (Danneberg et al., 1992; Ruiz-Lozano and Azcon, 1995) in eggplant (Yilmaz, 2005). Mohammad and Mittra (2013) reported that the *G. deserticola* (VAM) mitigate the drastic effect of heavy metal and salinity in eggplant. Oztekin et al. (2013) reported that both mycorrhizal inoculation and grafting technique are beneficial to mitigate the effects of salinity in eggplant.
2.3.4. Grafting

It is a horticultural technique in which vegetative parts (buds or shoot) from one plant are inserted to other so that the two sets of vascular tissues may join together. Grafting technique was originated in Japan and Korea to overcome soil borne diseases and adverse environmental factors such as low soil temperature and soil salinity, and to increase total yield in greenhouse vegetable production (Lee et al., 2010). In 20th century, grafting technology was introduced to Europe and North America with improved methods suitable for commercial production (Burelle et al., 2008). Grafting enhanced resistance to heat (Abdelmageed and Gruda, 2009), water logging (Black et al., 2003), alkalinity (Colla et al., 2010b), flooding (Romero et al., 1997; Yetisir et al., 2006), soil borne diseases (Goth, 1991) and limited the negative effect of boron, cadmium, copper and manganese toxicity (Edelstein et al., 2007; Rouphael et al., 2008b; Savvas et al., 2009). Oda (1999) reported that eggplant was the first record of a member of solanaceae family to be grafted in 1950 with Solanum integrifolium used as rootstock (Yamakawa, 1982), other commercially grafted members include tomato and pepper (Ioannou, 2001; Davis et al., 2008). Salt stress caused osmotic effect and specific ion effect in plants (Hajiboland et al., 2010). Grafting was effective in overcoming salinity stress in vegetable crops (Romero et al., 1997; Santa-Cruz et al., 2002; Ozbek et al., 2006; Colla et al., 2006). The maximum benefits of grafting were gained by using resistant rootstocks (Ozbek et al., 2009; Colla et al., 2010a; Ozbek et al., 2013). Grafting reduced cadmium accumulation in the fruit of eggplant grown in cadmium polluted soils (Arao et al., 2008). Eggplant ‘Suqiqie’ were grafted on ‘Torvum Vigor’ (a salt resistant commercial Japanese rootstock) and grown in 8 dS m⁻¹ salt level in a hydroponic medium (Liu et al., 2007). Grafted seedlings were 8.8%, 31.1%, and 8% higher in chlorophyll contents, photosynthetic rate and dry mass. In addition, super oxide (*O₂), hydrogen peroxide (H₂O₂) and MDA contents were significantly lower and anti-oxidative enzymatic activities (SOD, CAT, POD and GR) were higher in grafted plant than self-rooted seedling of eggplant (Liu et al., 2007). Wei et al. (2009) also used the same rootstock in 8 dS m⁻¹ CaNO₃ and observed that grafted eggplant significantly improved enzymatic activities, gas exchange characteristics, free insoluble bound and conjugated polyamines and dry biomass. Wei et al. (2009) also observed a decrease in *O₂, MDA contents, H₂O₂ level, electrolyte leakage, polyamine oxidase (PAO, EC 1.5.3.3) and diamine oxidase (DAO, EC 1.4.3.6). Similar results were reported in grafted tomato under CaNO₃ stress (Zhang et al., 2008). Other species such as S. macrocarpon L. and S. aethiopicum which are resistant to abiotic stresses such as
salinity, can also be used as rootstocks for eggplant (Schippers, 2000). Gisbert et al. (2011) reported that fruit quality, earliness, and total yield can be improved by developing interspecific hybrid rootstocks such as *S. melongena* with *Solanum incanum* L. (SI×SM) for cultivar ‘Black Beauty’ of eggplant. Eggplant can also be grafted on other members of *Solanaceae* family such as tomato salt-resistant rootstocks (Giuffrida et al., 2014).

Grafting is less practiced in vegetables than fruits due to high labour cost. In the 1990s, several agricultural industries invented robots for grafting to reduce the cost of grafted seedling yet models are limited and less flexible (Burelle et al., 2008). Scientists are interested in developing more flexible grafting robots to assist the greenhouse industry (Lee et al., 2010).

### 2.3.5 Plant breeding

There is variation in salinity tolerance between and within species. Breeding approaches to improve plant salt tolerance include (1) screening of germplasm against salinity, (2) salt tolerant hybrid development and (3) selection of salt tolerant plants with desired characteristics. Relatively tolerant commercial cultivars can be developed by taking advantage of genetic variability that exists within a species (Munns, 2002). A wide genetic diversity has been observed in *Solanum* species regarding biotic and abiotic stress tolerance, agronomic, and nutritional quality traits (Magioli and Mansur, 2005; Raigon et al., 2008). It is important to know the eggplant genetic resources for successful development of an eggplant breeding program (Sekara et al., 2007). Major focus of eggplant breeders is to improve resistance to diseases and pests, adaptation to different environmental conditions and, improvement in yield and quality (Bargato et al., 2007). Cultivation acreage and consumption of eggplant has been increased due to breeding of eggplant cultivars and development in production technology (Sekara et al., 2007).

Akinci et al. (2004) reported variability in response of eggplant cultivars to salinity tolerance depending on the growth stage. Selvaraj (2011) characterized 20 eggplant genotypes and reported that IC112589, IC126784, IC203585, IC90774 and IC112960 were tolerant to salinity at the germination stage. Okmen et al. (2009) investigated 26 eggplant cultivars for phenolics contents and total water soluble antioxidant activity and observed a 2.2 and 3.1 fold differences among cultivars. Their study provided useful information to breeders for the development of salt tolerant cultivars of eggplant with high antioxidant activity. Vinatoru et al. (2013) reported that hybrids of eggplant were more productive and more tolerant to biotic and abiotic stresses under greenhouse and
field conditions. Unfortunately, reports regarding eggplant salt tolerance improvement with the help of breeding approaches are scanty.

Evaluation of genetic variability of eggplant germplasm is important for useful application such as breeding objectives and for preservation of genetic resources. Therefore, germplasm is important for cultivar improvement and gene exploitation such as abiotic stress tolerance genes. It is crucial to identify polymorphism along within cultivars and lines for breeding purpose. Due to autogamous nature of eggplant, low frequency of polymorphism in cultivars and lines has been reported (Nunome et al., 2003; Stagel et al., 2008). Furthermore, germplasm availability is not a problem for breeders. The difficulty lies in screening of large number of individuals and how to quantify gene interaction with salinity. A number of genes are involved in salinity tolerance and gene expression is influenced by environment (Munns, 2002). Due to sexual incompatibilities, crossing of eggplant with wild relatives have limited success. But, eggplant ability to regenerate in tissue culture, has received an interest through the application of biotechnology, especially somatic hybridization, haploidisation, somaclonal variation, and genetic transformation (Collonier et al., 2001).

2.3.5 Biotechnological approaches

Conventional breeding has some limitations in vegetable stress tolerance improvements (Rajam et al., 1998). For example, hybrid incompatibilities restrict the success of crossing eggplant with wild relatives. On the other hand, the use of biotechnological approaches, particularly gene transfer, has great application to overcome reproductive issues of eggplant and other family members (Collonier et al., 2001). Various traits such as ion exclusion, osmotic tolerance, and tissue tolerance can be introduced to improve salinity tolerance in eggplant (Chinnusamy et al., 2005; Roy et al., 2014). Polyamines (putrescine, spermidine and spermine), proline, glycine betaine, and mannitol are compounds that play an important role against biotic and abiotic stresses including salinity, and expression/incorporation of stress-associated gene can lead to salt tolerance in plants (Rajam, 1997; Kumar et al., 2006). Availability of salinity tolerant genes for vegetable improvement is a key factor for gene transfer (Collonier et al., 2001). Solanum species have wide genetic diversity (Magioli and Mansur, 2005) and wild relatives of eggplant have been assessed for resistance against severe biotic as well as abiotic stresses (Blestosos et al., 1998). For example, Solanum torvum L. a commonly-used eggplant rootstock, is a positive genetic source of biotic and abiotic stresses (Dauny et al., 1991; Wei et al., 2009). Solanum grandiflorum Ruiz & Pavon, S. khasianum Clarke, S.
*mammosum* L. and *S. viarum* Dun. are species of eggplant that are resistant to frost (Baksh and Iqbal, 1979). Moreover, *S. macrocarpon* L. and *S. linnaeanum* Hepper & Jaeger are tolerant to drought and salinity, respectively (Daunay *et al.*, 1991). Using RAPD and RFLP markers, two genetic linkage maps of eggplant have been constructed (Collonnier *et al.*, 2001). Eggplant responds well to tissue culture which allows the application of biotechnology in eggplant, especially somaclonal variation and genetic transformation for gene transfer. Somaclonal variation can be used to obtain salt resistant lines (Collonier *et al.*, 2001). Tolerance to abiotic stress can be incorporated in plants by genetic engineering (Grover *et al.*, 1999; Bohnert and Shen, 1999). *Agrobacterium tumefaciens* has been commonly used to perform genetic engineering. With the help of *A. tumefaciens*, genes of interest can be transferred from wild relatives to cultivated vegetable species (Sihachakr *et al.*, 1994). In addition, genetic engineering needs to incorporate exact combination of genes into leading cultivars and their evaluation in field to determine the effect on salt tolerance and yield increment (Roy *et al.*, 2014).

Jain *et al.* (1987) developed a salt tolerant line through tissue culture mutation which was tolerant to almost 17 dS m⁻¹ salinity. Prabhavathi and Manchikatla (2007) introduced a key gene oat arginine decarboxylase (ADC, EC 4.1.1.19) through *Agrobacterium*-mediated transformation to enhance the biosynthesis of polyamines in eggplant. The transgenic eggplant was more tolerant to low and high temperature, drought, heavy metal and salinity due to the increased level of polyamines. A gene (HAL1) from yeast was isolated and transferred to eggplant var. PKM1 with the help of *Agrobacterium*. In vitro at 15 dS m⁻¹, callus weight from the transgenic population was not significantly different from control population. In addition, in vivo at 5 dS m⁻¹, total dry weight of transgenic eggplant was not significantly different from non-transgenic eggplant grown under non saline conditions (Kumar *et al.*, 2014). In another experiment, bacterial mannitol-1-phosphodehydrogenase (*mtlD*) gene was transferred into eggplant by *Agrobacterium*-mediated transformation. Seeds of transgenic eggplant tolerated 20 dS m⁻¹ salinity at germination stages while seeds of untransformed control eggplant failed to germinate at the same level. Transgenic leaf explants was more capable of regeneration under salt-amended MS medium than non-transgenic control leaf explants. Furthermore, transgenic lines of eggplant were more tolerant to drought and chilling stresses than control non-transgenic lines (Prabhavathi *et al.*, 2002). Salt tolerant genes have been isolated from wild eggplant cultivar (*Solanum torvum* Swartz) by Chen *et al.* (2012a). These genes can
be used for genetic improvement of eggplant using transgenic technology. Some miRNAs are also involved to regulate gene expression in *Solanum linnaeanum* response to salt stress and can help when making strategies for genetic improvement of *Solanaceae* crops (Zhuang et al., 2014). Genetic variations are limited within the *Solanum melongena* L. Hence, incorporation of resistant genes from the wild relatives *Solanum torvum* and *Solanum sisymbriifolium* to commercial cultivars are required. In general, genetic engineering and optimum and proper use of growth regulators can considerably increase eggplant yield and improve the fruit quality under saline conditions (Pessarakli and Dris, 2003).

### 2.3.7. Use of plant growth promoting substances.

Abiotic stresses limit plant growth and yield, and salinity is one of the most important abiotic stress that contribute to yield loss. Phytohormones and plant growth regulators are produced within plants and play an important role in the ability of plants to tolerate various stresses. These are the most essential endogenous substances in plants involved in salt stress tolerance (Fahad et al., 2014). Phytohormones alter physiological processes and biochemical events of plants under salt stress, and the exogenous application of phytohormones has been proposed as a pragmatic approach to manage salinity (Fatma et al., 2013; Iqbal et al. 2014). Exogenous application of phytohormones had a fair degree of success in salt stress alleviation (Iqbal and Ashraf, 2013; Amjad et al., 2014). Judicious exogenous application of plant growth regulators improved growth and yield under stressful conditions in eggplant (Wojdyla, 2001; Tanaka et al., 2013).

Cytokinins (CTK) are also phytohormones which regulate various physiological processes like seed germination, cotyledon expansion, cell expansion and differentiation, leaf and chloroplast senescence (Thomas, 1992; Iqbal and Ashraf, 2005; Rashotte et al., 2005; Dong et al., 2009) as these are antagonistic to abscisic acid (ABA) (Blackman and Davies, 1984). Increased ABA level, due to salinity, decreased CTK level in plant and led to stunted growth and poor yield (Iqbal et al., 2006; Javid et al., 2011). The antioxidant enzymatic activities of APX, CAT, SOD and POD increased in the leaves of eggplant, under salt stress, by a foliar application of the cytokinin 6-benzyladenine (6-BA). Furthermore, 6-BA reduced superoxide production and MDA contents in cell membranes of eggplant under salt stress and increased chlorophyll contents, net photosynthetic rate, transpiration rate, and intercellular CO₂ level. Therefore, Wu et al. (2012a) concluded that 6-BA alleviated the effect of salinity in eggplant.
Brassins are a new class of phytohormones that play an important role in biotic and abiotic stress alleviation such as salinity (Krishna, 2003; Nunez et al., 2003; Ozdemir et al., 2004; Arora et al., 2008). 24-Epibrassinolide (EBR) is an active brassinosteroid and has been investigated to alleviate salt stress in eggplant. EBR at 100 nM increased chlorophyll concentration, stomatal conductance, and net photosynthetic rate in eggplant under saline regimes (Wu et al., 2012b). Exogenous application of EBR also increased height, stem diameter, root and shoot fresh biomass of eggplant under saline conditions (Wu et al., 2012b). EBR decreased electrolyte leakage, MDA contents, superoxide and hydrogen peroxide production, and increased enzymatic activities in eggplant under salt stress. Furthermore, EBR decreased Na\(^+\) and Cl\(^-\), and increased K\(^+\) and Ca\(^{2+}\) under saline conditions (Ding et al., 2012).

Plants produce jasmonic acid (JA) naturally which induces salt tolerance mechanisms. Manar et al. (2013) reported that eggplant embryos, pretreated with 10 μM JA and grown under salinity stress, showed relatively better development and an increase in enzymatic activities (APX, CAT and SOD).

Seaweed extract (SWE) is a bio-stimulant which augmented chlorophyll contents, root and shoot growth, flower and fruit set, nutrient uptake, delayed senescence in plants and improved shelf life of fruit (Khan et al., 2009; Briceno-Dominguez et al., 2014; Stirk et al., 2014). Seaweed (Ascophyllum nodosum) extract (5 cm\(^3\) L\(^{-1}\)) alleviated salt stress in eggplant completely at 5.5 dS m\(^{-1}\) salinity level and partially at higher salinity level by enhancing enzymatic activities (APX and SOD) and K\(^+\) uptake (Hegazi et al., 2014). SWE at 20-40 % improved germination of eggplant. A study on eggplant cultivar ‘Pusa Purple Long’ revealed that SWE could be used as an alternative of phytohormones in tissue culture (Satish et al., 2014). Plants physiologically produce metabolites such as proline and glycinebetaine which are involved in water and ion homeostasis under salt stress. Foliar application of these metabolites also alleviated adverse effects of salinity in eggplant (Abbas et al., 2010; Shahbaz et al., 2013).

However, the extent of salt stress alleviation is low when an individual approach is adopted due to the complex mechanism of salt tolerance in eggplant. A combination of these possible approaches may have a better chance of success. There could be multifaceted approaches to handle salinity issue. Out of these applications, use of plant growth promoter is proving more effective than others.
2.4 Chitosan, a natural biopolymer

Chitosan is N-deacetylated derivative of chitin which is an amino polysaccharide and the second most abundant biopolymer in nature after cellulose (Allan and Hadwiger 1979; Sandford, 1989; Lamiaa and Barakat, 2011). Chitosan was discovered in 1850 (Kim and Rajapakse, 2005). Chitin is a component of insect cuticles, exoskeleton of arthropods and shells of crustaceans such as shrimps and crabs, also of many fungal cell walls (Araki and To, 1975; Bartnicki-Garcia, 1968; Hadwiger and Beckman, 1980; Domard and Domard, 2002). Chitosan is composed of β-(1,4)-amino-2-deoxy-D-glucose and β-(1,4)-2-acetamido-2-deoxy-D-glucose units (Bautista-Banos et al., 2006). It differs from cellulose due to presence of nitrogen (Freepons, 1991). Chitosan is a new useful material and has become of great interest due to its versatile applications (Majeti and Kumar, 2000). Chitosan activates the plant defense system against pathogens (Iriti et al., 2009). As an elicitor as well as nontoxic biodegradable material, chitosan has become a new class of plant protectants, with a beneficial role in sustainable agriculture (Bautista-Banos et al., 2006). Chitosan is safe for the environment with unique properties such as bioactivity as well as biocompatibility (Dias et al., 2013). Elicitor activity of chitosan depends on its chain length (Walker-Simmons and Ryan, 1984), degree of deacetylation, conformational structure and distribution of acetyl groups (Tsai et al., 2002). Chitosan is soluble in diluted organic acids (Cheah et al., 1997). Hien (2004) reported that chitosan stimulated plant growth and triggered defensive mechanisms of plants by stimulating enzymatic activities. Whereas, Guan et al. (2009) suggested that chitosan increased plant growth by improving water uptake and essential nutrients through cell osmotic adjustments. Furthermore, chitosan role in growth, morphogenesis, and development seemed similar to phytohormones (Cote and Hahn, 1994).

Versatile functional properties of chitosan are due to its positive charge which make it possible to be utilized in many industries such as cosmetics (Majeti & Kumar, 2000; Lang and Clausen, 1989), biomedicine (Kulpinsky et al., 1997; Nishimura, 1997; Felt et al., 1998), agriculture (Yamada, et al., 1993; Hoagland and Parris, 1996; Ren et al., 2001), food (Shahidi and Synowiecki, 1991; Fang et al., 1994; Roller and Covill, 1999; Benjakul et al., 2000), biotechnology (Sandford, 1989), as an antioxidant in sausages (Xie et al., 2001), clarifying agent in juices (Boguslawski et al., 1990; Soto-Peralta et al., 1989), enzymatic browning inhibitor (Sapers, 1992; Dornenburg and Knorr, 1997), waste water management (Jeuniaux, 1986) and environmental protection (Peniche-Covas et al., 1987). In agriculture, chitosan has been used as a fertilizer, foliar spray, seed priming and
coating, leaf, fruit and vegetable coatings and in controlled agrochemical release, to increase plant growth and yield (New et al. 2004; Farouk et al. 2008 and 2011), to protect plants against microorganisms (Farouk et al. 2008) and against oxidative stress (Guan et al. 2009).

2.4.1 Effects of chitosan on germination, growth, physiology and biochemistry of plants

Seed treated with chitosan, improved germination rate of pumpkin, cucumber, chili and cabbage (Chandrkrachang, 2002). Seeds of pearl millet primed with chitosan showed improved seedling vigor and enhanced germination (Manjunatha et al., 2008). Sui et al. (2002) reported that seed soaked with chitosan improved germination, length and weight of radicle and hypocotyl in rapeseed. Similarly, chitosan increased growth in soybean sprouts (No et al., 2003). Chitosan improved seed germination in rice (Ruan and Xue, 2002) and elicited vigor of maize seedlings (Shao et al., 2005). Foliar application of chitosan enhanced vegetative growth (Farouk et al. 2008) and had a positive effect on leaf, root and shoot growth of radish and sweet pepper (Ghoname et al. 2010, Farouk et al. 2011). Chitosan application increased fresh and dry weight of leaves as well as number of leaves, plant height and yield component in strawberry (Abdel-Mawgoud et al., 2010). Chitosan enhanced leaf area, root and shoot length, fresh and dry weight of roots and shoots as well as chlorophyll contents in bean leaves (Sheikha and Al-Malki, 2011). Moreover, increased length, thickness, and weight in the hypocotyls of sunflower were observed due to chitosan treatment (Cho et al., 2008). Kim et al. (2005) reported that sweet basil treated with chitosan effectively induced phytochemicals in plants and significantly produced 17% more weight and 12% more height. Chitosan application improved growth in soybean sprouts (Lee et al., 2005) and cabbage (Hirano, 1988). Chitosan increased yield in other agricultural crops as well (Mondal et al., 2012). Amendments of chitosan in a growing substrate caused early flowering in ornamental plants (Ohta et al., 2004). Chitosan increased number of flowers in gladioli (Ramos-Garcia et al., 2009), gerbera (Wanichpongpan et al., 2001) and lisanthius (Ohta et al., 1999). *Dendrobium* treated with chitosan produced more inflorescences as well as number of flowers (Limpanavech et al., 2008). Moreover, Win et al. (2005) reported that foliar application of chitosan significantly increased the inflorescence in *Dendrobium*. Chitosan-treated corms of ‘Gompey’ freesia produced more leaves, flowers and corms, flowered earlier and had more shoots compared to non-treated plants (Salachna and Agnieszka, 2014).
Chitosan improved carbohydrate and chlorophyll contents in cucumber (Farouk et al., 2008) and radish (Farouk et al., 2011). Seeds of peanut soaked with chitosan showed an increase in germination percentage, energy of germination, lipase activity, indole acetic acid and gibberellic acid (GA₃) concentration (Zhou et al., 2002). The mode of action of chitosan on growth enhancement of plants is not yet clear. Chitosan may induce signals to synthesize gibberellins and auxins to enhance growth and development (Uthairatanakij et al., 2007). Chitosan stimulates fundamental processes of plants on every level, from single cells to tissues, through physiological and biochemical processes, through changes in the molecular level linked to gene expression (Limpanavech et al., 2008). Growth stimulating effects of chitosan on plant are due to enhanced availability and uptake of water and essential nutrients by adjusting cell osmotic pressure and by restricting the accumulation of ROS by increasing enzymatic activities and antioxidants (Guan et al., 2009). In addition, chitosan has positive effects on phosphorus uptake of plants (Farouk and Amany, 2012). Increase in net photosynthetic rates, transpiration rate and stomatal conductance was observed in soybean and maize with foliar application of chitosan without any effect on intercellular CO₂ concentration (Khan et al., 2002).

Chitosan has antioxidant activity (Park et al., 2004; Guo et al., 2005). It might have a potential as a free radical quencher (Kim and Thomas, 2007). The antioxidant activity of chitosan is illustrated by numerous mechanisms (Muzzarelli et al., 1997). Chitosan has been reported to have DNA protective properties (Prashanth et al., 2007) because it can scavenge OH and O₂⁻ radicals due to its structural features like a large number of amino groups as well as hydroxyl groups to inactivate ROS (Li et al., 2002; Yin et al., 2002; Sun et al., 2004; Feng et al., 2009). Hexanoyl chitin and N-benzoylhexanoyl chitosan can trap peroxide radicals in an organic solvent after the radical chain reaction initiated by 2,4-dimethylvaleronitrile (Xie et al., 2001). Chitosan derivatives reduced benzoyl peroxide and t-butylhydroperoxide-induced lipid peroxidation (Matsugo et al., 1998). Carboxymethyl chitosan and hydroxypropyl chitosan sodium possess scavenging activities against hydroxyl radicals (Xue et al., 1998). Feng et al. (2007) reported that chitosan derivatives scavenge superoxide anion radicals, α,α-diphenyl-b-picryl-hydrazyl (DPPH) radicals and hydrogen peroxide radicals. They also noted that low molecular weight chitosan had more reducing potential as compared to high molecular weight chitosan.

Ma et al. (2014) reported that presoaking of wheat seeds with chitosan improved wheat growth by increasing photosynthetic rate, chlorophyll contents, stomatal conductance and
enzymatic activities of CAT, peroxidase (POD), and SOD. Chitosan improved catalase and peroxidase activities and decreased malondialdehyde in maize (Guan et al., 2009). The activities of catalase and peroxidase enzymes were increased when chitosan was exogenously applied at fruit set and fruit growing stage of tomato (Ortega-Ortiz et al., 2007). Xu et al. (2007) found a decrease in malondialdehyde contents in Hydrilla verticillata when treated with chitosan. Hexamers and pentamers of chitosan induced enzymatic activities in soybean leaf tissues (Khan et al., 2003). Osman et al. (2013) reported that enzymatic activities (peroxidase, chitinase and polyphenol oxidase) were improved in eggplant when treated with chitosan either by root dipping or by foliar application under nematode stress.

2.4.2 Role of chitosan in abiotic stress alleviation in plants

Chitosan is helpful in mitigating stresses induced by unfavorable conditions such as low or high temperatures (Lizarraga-Pauli et al., 2011), drought (Pongprayoon et al., 2013) and salinity (Jabeen and Ahmad, 2013). Guan et al. (2009) reported that maize (Zea mays L.) seeds primed with chitosan were tolerant to low temperature stress.

2.4.2.1 Drought

Bittelli et al. (2001) reported that chitosan effectively reduced transpiration as well as water use by 26-43% while sustaining growth and yield in pepper under drought stress condition. Whereas, Farouk and Amany (2012) reported that foliar applied chitosan at the rate of 250 mg L\(^{-1}\) improved growth, yield, as well as physiological constituents of cowpea shoots under drought stress and non-stress conditions. Similarly, Sharifa and Abu-Muriefah (2013) concluded that foliar application of 200 mg L\(^{-1}\) chitosan solution enhanced growth, yield, and quality as well as physiological attributes under drought stressed and non-stressed common bean plants. Foliar application of chitosan reduced water loss of plants by closing of stomata after film formation on leaf surface, making leaves resistant to water vapor loss under drought stress (Tambussi and Bort, 2007). Zeng and Liu (2012) demonstrated that coating wheat seeds with chitosan improved growth, yield, chlorophyll contents and activities of SOD, CAT and POD enzymes and reduced MDA contents under drought stress conditions. Pretreatment of apple leaves with 100 mg L\(^{-1}\) chitosan solution decreased MDA contents and increased SOD and CAT enzymes activity under drought stress (Yang et al., 2009). Mahdavi et al. (2011) reported that a low concentration of chitosan reduced oxidative damage in safflower caused by drought stress. Drought stress in crops is linked with oxidative damage. Foliar application of chitosan at 250 mg L\(^{-1}\) effectively reduced H\(_2\)O\(_2\) production, lipid peroxidation and
membrane permeability and elevated the antioxidant enzyme activities in the leaves of cowpea under drought stressful conditions indicating that chitosan coped with oxidative damage caused by drought stress in cowpea plants (Farouk et al., 2013). Benavides-Mendoza et al. (2004) reported that chitosan induced drought and salt stress tolerance in lettuce and onion.

2.4.2.2 Salinity

Mahdavi (2013) reported that salinity inhibited growth of Isabgol (*Plantago ovata* Forsk) and that seeds of Isabgol soaked in 0.2% chitosan solution prior to sowing partially improved its salt tolerance and ameliorated the adverse effects of salinity on its growth. Similarly, Mahdavi and Rahimi (2013) concluded that seeds of Ajowan treated with 0.2% chitosan were tolerant to salinity stress in terms of germination rate, germination percentage, seedling vigor and length and dry weight of radicle and hypocotyl in comparison to non-treated seeds. Chitosan increased height and biomass of wheat plants under salt stress (Lianju et al., 2011). Chitosan showed positive effects on salt stress alleviation in safflower and sunflower (Jabeen and Ahmad, 2013).

Ma et al. (2011) reported that pretreatment of 0.0625% oligochitosan of wheat under salt stress significantly increased dry weight, root and shoot length, photosynthetic rate, stomatal conductance, and chlorophyll contents. Furthermore, in the same study, oligochitosan reduced MDA contents and increased SOD, CAT, and POD activities and stepped up proline accumulation. Foliar applied chitosan at the rate of 150 mg L⁻¹ reduced MDA contents but increased leaf proline and chlorophyll contents, besides enhancing SOD and CAT activities in tomato plants grown under 20 dSm⁻¹ NaCl stress (Gu, 2012). Chitosan could hinder lipid per oxidation by chelating metal ions or by intermingling with lipids where osmotic stress is limiting plant growth and development (Xue et al., 1998).
Chapter 3

MATERIALS AND METHODS

Abiotic factors such as cold, high temperature, heavy metals, UV-B, excess water, drought, senescence, desiccation and salinity stress contribute to reduced yield and production of crops. Among these factors, salt stress is the most important one. Summer vegetable crops including eggplant require heavy irrigation during their growing season, due to the deficiency of canal water in Pakistan and utilization of brackish underground water for irrigation purposes, salts level is being elevated in vegetable growing fields. Eggplant also experiences reduction in growth and yield under saline conditions. So, a comprehensive study was designed under the title of “alleviating the adverse effects of salinity in eggplant by using plant growth enhancer”. The study comprised of two phases. First aspect was to assess the response of eggplant genotypes against different salinity levels. Thirteen different eggplant genotypes were categorized into salt tolerant and salt sensitive genotypes on the basis of stress tolerance indices and ionic responses, one salt tolerant and salt sensitive eggplant genotypes were studied in detail for growth, water relation, physiological, biochemical and ionic attributes under different salinity levels. The other aspect of this study was to optimize the chitosan (growth enhancer) level exogenously supplemented at various concentrations and to determine the role of chitosan in enhancement of salt stress tolerance in eggplant on the basis of fruit yield, water relations, physiological, biochemical and enzymatic attributes. Chitosan is a linear polysaccharide derived from chitin material found in crustacean shell, fungal cell wall and exoskeleton of insects. Chitosan is a second abundant biopolymer in nature and consisted of β-1, 4-linked 2-amino-D-glucose units.

![Chemical Structure of Chitosan](image)

The studies were carried out at the Institute of Horticultural Sciences (IHS), in collaboration with stress physiology laboratory, Department of Agronomy and analytical biochemistry laboratory, Department of Biochemistry, University of Agriculture, Faisalabad. The study comprised of the following experiments.
3.1. Experiment-1

Screening of eggplant genotypes for salt tolerance on the basis of plant growth salt tolerance indices and ionic analysis

<table>
<thead>
<tr>
<th>Location</th>
<th>Lath house (IHS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity levels</td>
<td>6 (control, 3, 6, 9, 12 and 15 dS m(^{-1}) of NaCl)</td>
</tr>
<tr>
<td>No. of genotypes</td>
<td>13 (table 3.1.1)</td>
</tr>
<tr>
<td>Replications</td>
<td>4</td>
</tr>
<tr>
<td>Growth medium</td>
<td>Sand culture</td>
</tr>
<tr>
<td>Nutrient Solution</td>
<td>Half strength Hoagland solution (table 3.1.2)</td>
</tr>
</tbody>
</table>

3.1.1 Experimental details:

Ten seeds of each eggplant genotype were sown in plastic pots containing seven Kg of sand rinsed with distilled water. After emergence of first true leaf (15 days after germination), the number of plants per pot were adjusted to five by thinning out the week and less vigorous ones. Salt solutions at different concentrations (control, 3, 6, 9, 12 and 15 dS m\(^{-1}\) of NaCl) were applied to develop salinity one month after sowing time. To avoid the osmotic shock, NaCl concentration was adjusted by gradually increasing 3 dS m\(^{-1}\) every two days interval until desired concentration was reached. Half strength Hoagland solution was used as nutrient solution. The selected salinity levels were maintained during the trial by testing the EC of the growing medium with the assistance of EC meter and any increase or decrease in desired salinity level was adjusted with the aid of buffer solution. Each pot containing 5 seedlings was considered as one replicate and four pots were considered one treatment. Fifty days after seed sowing plants were harvested and data was recorded for following parameters.

- Plant height stress tolerance index
- Root length stress tolerance index
- Shoot fresh weight stress tolerance index
- Root fresh weight stress tolerance index
- Dry matter stress tolerance index
- Leaf potassium (K\(^+\)) contents (%) 
- Leaf sodium (Na\(^+\)) contents (%)
Table 3.1 Eggplant genotypes used in 1st experiment.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Genotype</th>
<th>Sr. No.</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Qaisar</td>
<td>8</td>
<td>Nirala</td>
</tr>
<tr>
<td>2</td>
<td>Saadia</td>
<td>9</td>
<td>VRIB-9901</td>
</tr>
<tr>
<td>3</td>
<td>Black Beauty</td>
<td>10</td>
<td>Black Oval</td>
</tr>
<tr>
<td>4</td>
<td>Dilnashin</td>
<td>11</td>
<td>WER</td>
</tr>
<tr>
<td>5</td>
<td>Black Boll</td>
<td>12</td>
<td>P.P.R</td>
</tr>
<tr>
<td>6</td>
<td>Bemisal</td>
<td>13</td>
<td>VRIB-9501</td>
</tr>
<tr>
<td>7</td>
<td>New Noble</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2 Composition of Hoagland nutrient solutions (used as nutrient medium)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stock (g/L)</th>
<th>ML of stock soln. for 10L ½ conc.</th>
<th>ML of stock soln. for 200L ½ conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macro nutrients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>136</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>KNO₃</td>
<td>101</td>
<td>25</td>
<td>500</td>
</tr>
<tr>
<td>Ca(NO₃)₂.4H₂O</td>
<td>236</td>
<td>25</td>
<td>500</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>246</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>Micro nutrients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>2.86</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>MnCl₂.4H₂O</td>
<td>1.81</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>ZnSO₄.7H₂O</td>
<td>0.22</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>CuSO₄.5H₂O</td>
<td>0.08</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>H₂MoO₄.H₂O</td>
<td>0.02</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Fe-EDTA</td>
<td>37.33</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

3.1.2. Data collection/sampling

Shoot and root samples were collected for the estimation of various indicators of salt stress after fifty days of seed sowing. These indicators comprised of shoot length, root length, shoot and root fresh weight, plant dry weight and chemical analysis of leaf sodium and potassium contents.

3.1.3 Measurement of shoot lengths (cm)

The seedlings were up rooted and washed with distilled water to remove the particles of sand. Shoot length of five seedlings from each replicate were measured in centimeters.
(cm) from the base of hypocotyls to the tip of the shoot with the help of meter rod. The average of each replication was calculated.

3.1.4 Measurement of root lengths (cm)
The seedlings were up rooted and washed with distilled water to remove the particles of sand. Root length of five selected seedlings from each replicate was measured in centimeters (cm) from the base of hypocotyls to the tip of the longest root with the help of meter rod. The average of each replication was calculated.

3.1.5 Measurement of root and shoot fresh weight (g)
After measuring the root and shoot lengths, the seedlings were wiped out with filter paper in order to eliminate any water present on their leaves and shoots. A digital balance was used to calculate the shoot and root fresh weights and average fresh weight of each replicate was recorded.

3.1.6 Measurement of plant dry biomass (g)
After calculating fresh weights, the five plants from each replicate were taken in paper bags and then placed in oven (Memmert-110, Schawabach, Germany) and were dried at 70 °C for 72 hours. The dry weights were taken by using digital balance and average dry weight of each replicate was recorded.

3.1.7 Calculation of salt tolerance indices
The plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), shoot fresh weight stress tolerance index (SFWSI), root fresh weight stress tolerance index (RFWSI) and dry matter stress tolerance indices (DMSI) were obtained using the formulae described by Ashraf et al. (2008).

\[
PHSI = \frac{\text{Plant height of stressed plant}}{\text{plant height of control plant}} \times 100
\]

\[
RLSI = \frac{\text{Root length of stressed plant}}{\text{root length of control plant}} \times 100
\]

\[
SFWSI = \frac{\text{Shoot fresh weight of stressed plant}}{\text{shoot fresh weight of control plant}} \times 100
\]

\[
RFWSI = \frac{\text{Root fresh weight of stressed plant}}{\text{root fresh weight of control plant}} \times 100
\]

\[
DMSI = \frac{\text{Dry matter of stressed plant}}{\text{dry matter of control plant}} \times 100
\]

3.1.8 Ion determination
Following Allen et al. (1986), dried ground leaf material (0.1 g) from each replicate was digested separately in 2 mL of digestion mixture (0.42 g of Se and 14 g of LiSO₄·2H₂O were added to 350 mL of H₂O₂ and were mixed well and 420 mL of conc. H₂SO₄ were added slowly to it, keeping it in an ice bath). All flasks containing plant samples and the digestion mixture were heated up to 200 °C on a hot plate. Each digested sample was
diluted up to 50 mL and used for the determination of Na\textsuperscript{+} and K\textsuperscript{+} using a flame photometer (Jenway, PFP-7).

3.1.9 Calculation of leaf K\textsuperscript{+} and Na\textsuperscript{+} percentage (%)
Increase in leaf Na\textsuperscript{+} and decrease in K\textsuperscript{+} percentage was calculated using the following formulas.

\[
\text{% Decrease in potassium} = \frac{\text{Leaf potassium (control)} - \text{Leaf potassium (salinity)}}{\text{Leaf potassium (control)}} \times 100
\]

\[
\text{% Increase in sodium} = \frac{\text{Leaf sodium (control)} - \text{Leaf sodium (salinity)}}{\text{Leaf sodium (control)}} \times 100
\]

3.1.10. Experimental design and statistical analysis
Experimental unit having four replications and six treatments was designed in a Complete Randomized Design (CRD) with two factor factorial arrangements. Analysis of variance (ANOVA) and multiple comparison tests (Tukey test) were computed using Statistix 8.1 computer package. Differences among treatments were considered significant at \( p \leq 0.05 \) after statistical analysis.

3.2. Experiment-II
Effect of salinity on growth, physiological, water related, biochemical and ionic attributes of salt sensitive and salt tolerant eggplant genotypes

**Genotypes:** 2 (Saadia salt tolerant and Black Beauty salt sensitive screened out in 1\textsuperscript{st} experiment)

**Replications:** 4

**Treatments:** 6

- \( T_1 = \) Control
- \( T_2 = 3 \text{ dS m}^{-1} \) (NaCl)
- \( T_3 = 6 \text{ dS m}^{-1} \) (NaCl)
- \( T_4 = 9 \text{ dS m}^{-1} \) (NaCl)
- \( T_5 = 12 \text{ dS m}^{-1} \) (NaCl)
- \( T_6 = 15 \text{ dS m}^{-1} \) (NaCl)

3.2.1 Experimental details:
Seeds of salt tolerant (Saadia) and sensitive (Black Beauty) genotypes identified in first experiment were sown in plastic pots containing 7 Kg of sand. After emergence of first true leaves (15 days after germination), the number of plants per pot were adjusted to five by thinning out the week and less vigorous ones. Six salt stress levels i.e. (control), 3, 6, 9, 12 and 15 dS m\textsuperscript{-1} of NaCl were applied 30 days after sowing. Data was collected after
fifty days of seed sowing to check the effect of salinity on specific parameters and to find out the optimum salinity level for further studies on which almost fifty percent growth reduction was observed.

3.2.2 List of parameters studied in 2\textsuperscript{nd} experiment:

**Growth parameters**
- Plant height (cm)
- Root length (cm)
- Shoot fresh weight (g)
- Shoot dry weight (g)
- Root fresh weight (g)
- Root dry weight (g)

**Physiological attributes**
- Stomatal conductance ($g_s$) by (IRGA) mmol m\textsuperscript{-2} s\textsuperscript{-1}
- Net photosynthesis rate ($Pn$) by (IRGA) µmol CO\textsubscript{2} m\textsuperscript{-2} s\textsuperscript{-1}
- Transpiration rate ($E$) by (IRGA) mmol H\textsubscript{2}O m\textsuperscript{-2} s\textsuperscript{-1}
- Water use efficiency ($Pn/E$)
- Chlorophyll index (SPAD value)

**Water Relations**
- Leaf osmotic potential ($\Psi_s$) (-MPa)
- Leaf water potential ($\Psi_w$) (-MPa)
- Leaf turgor potential ($\Psi_p$) (MPa)
- Relative water contents (%)

**Biochemical attributes**
- Super oxide dismutase (SOD) (U mg\textsuperscript{-1} protein)
- Catalase (CAT) (U mg\textsuperscript{-1} protein)
- Peroxidase (POD) (U mg\textsuperscript{-1} protein)
- Proline (µmol g\textsuperscript{-1} fwt)
- Glycinebetaine (µmol g\textsuperscript{-1} fwt)
- Malondialdehyde (MDA) (nmol g\textsuperscript{-1} fwt)

**Ionic analysis of leaf**
- Ca\textsuperscript{2+} contents (mg g\textsuperscript{-1} dwt)
- K\textsuperscript{+} contents (mg g\textsuperscript{-1} dwt)
- Na\textsuperscript{+} contents (mg g\textsuperscript{-1} dwt)
• Cl⁻ (mg g⁻¹ dwt)

3.2.3 Measurement of growth parameters
These indicators were observed as described in experiment number one under section 3.1.3 to 3.1.7.

3.2.4 Photosynthetic rate, transpiration rate and stomatal conductance
For the measurement of physiological attributes such as photosynthetic rate (µmol m⁻² s⁻¹), transpiration rate (mmol m⁻² s⁻¹) and stomatal conductance to water, three young fully developed and healthy leaves per plant (two plants from each experimental unit) were selected. These selected leaves were placed one by one in the chamber of portable apparatus termed as Infra-Red Gas Analyzer (IRGA) (LCi-SD, ADC Bio-scientific UK). All the readings of above mentioned physiological attributes were taken at day time from 10.00 a.m. to 12.00 a.m. at atmospheric pressure 99.9 kPa, molar flow of air per unit leaf area 403.3 mmol m⁻² s⁻¹, PAR (photosynthetically active radiation) at leaf surface was maximum up to 1711µmol m⁻² s⁻¹, water vapor pressure in the chamber ranged from 6.0 to 8.9 mbar, surrounding CO₂ concentration was 352 µmol mol⁻¹ and atmospheric temperature ranged from 22.4 to 27.9°C (Zekri, 1991; Abbas et al., 2013).

3.2.5 Water use efficiency:
Water use efficiency (WUE) (µmol CO₂ mmol⁻¹ H₂O) is the ratio between photosynthesis (Pn) and the amount of water transpired (E) which was measured as:

\[
Water \ use \ efficiency \ (WUE) = \frac{Photosynthetic \ rate \ (A)}{Transpiration \ rate \ (E)}
\]

3.2.6 Chlorophyll index (Chl. meter) (SPAD value)
Chlorophyll index was measured by using chlorophyll meter (CCM-200 plus bio scientific USA).

3.2.7 Leaf water potential (Ψw) (-M Pa)
At the end of experiment, a razor was used to cut the fully expanded leaves and was placed in the gasket of pressure chamber (Model, 615, USA) to estimate the Ψw. The readings taken were in the morning before 7.00 am.

3.2.8 Leaf osmotic potential (Ψs) (-M Pa)
The same leaf that was used in pressure chamber for Ψw was placed in a plastic bag and kept at low temperature (~20 °C) in a freezer for a week. The frozen leaf material was then thawed at room temperature for half an hour and cell sap was extracted with the help of a disposable syringe. The 10 µl of extracted sap was placed on osmometer (Wescor Model-5500) with the help of plastic syringe and Ψs measurement was taken.
3.2.9 Leaf turgor potential (Ψp)
The difference between Ψw and Ψs is regarded as turgor potential (Ψp), therefore in this study Ψp was measured by using the following formula.

\[ (\Psi_p) = (\Psi_w) - (\Psi_s) \]

3.2.10 Relative water contents (%)
For relative water content’s (RWR) measurements of three leaves (flag leaf) from each treatment were taken. Fresh weight (FW) of each sample was recorded using a digital electrical balance (Chyo, MK-500C) and leaves were dipped in distilled water for 24 hours. Then leaves were taken out, wiped with the tissue paper and the turgid weight (TW) was recorded. The samples were dried at 65°C for 72 h and dry weight (DW) of each sample was recorded. Relative water contents were calculated using the formula given by Yang et al. (1996).

\[ \text{RWC} = \frac{\text{(Fresh weight of leaf} - \text{Dry weight of leaf)}}{\text{(Turgid weight of leaf} - \text{Dry weight of leaf)}} \times 100 \]

3.2.11 Proline determination
The proline was determined according to the Bates et al. (1973) method. Fresh leaf material of 0.5 g was ground in 10 mL of 3% sulfo-salicylic acid. The sample material was filtered by using Whatman No. 40 filter paper. Two mL of the filtrate was taken in a 25 mL test tube and reacted with 2 mL acid ninhydrin solution (acid ninhydrin solution was prepared by dissolving 1.25 g ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M orthophosphoric acid) and 2 mL of glacial acetic acid and test tubes were heated for 1 h at 100°C. Reaction was terminated in an ice bath, the reaction mixture was extracted with 10 mL toluene which formed a chromophore. Continuous air stream was passed vigorously for 1-2 minutes in the reaction mixture to separate aqueous phase from the chromophore containing toluene. Isolated colored phase was allowed to stand for 2-3 minutes room temperature and its absorbance was noted at 520 nm using above mentioned model of spectrophotometer. Toluene was used as a blank. The proline concentration was calculated by using a standard curve developed by Analar grade proline and calculated on fresh weight (FW) basis as follows:-

\[ \text{µmole proline g}^{-1} \text{FW} = \frac{[(\text{mg of proline mL}^{-1}) \times (\text{mL of toluene})]}{\text{(wt. of sample}/5)/115} \]

3.2.12 Leaf glycinebetaine (GB)
GB in leaf tissues was determined following Grieve and Grattan (1983). Fresh leaf material (1.0 g) from each replicate was shaken for 5 min in 10 mL of 0.5% toluene solution and filtered. After filtration, 1 mL of the extract was mixed with 1 mL of 2N
H₂SO₄. Then 0.5 mL of this mixture was taken in a glass tube and 0.2 mL of potassium tri-iodide (KI₃) solution was added. Then 2.8 mL ice cooled distilled water and 6 mL of 1-2 dichloroethane (cooled at 48 °C) were added to the mixture. The upper aqueous layer was discarded and optical density (OD) of the organic layer measured at 365 nm.

3.2.13 Malondialdehyde (MDA)

MDA was estimated following Carmak and Horst (1991). Fresh leaf tissue (1.0 g) from each replicate was ground in 5 mL of 1% TCA under cold conditions and centrifuged at 15,000 rpm for 10 min. In a test tube, 3 mL of a mixture of TCA and TBA (0.5% thiobarbituric acid in 20% TCA) was added to 0.5 mL of the supernatant. This mixture was then incubated at 58 °C in a shaking water bath for 50 min. The reaction was finally stopped by placing the test tubes on ice and optical densities measured at 532 and 600 nm.

3.2.14 Activities of antioxidant enzymes

The fresh leaf material (0.5 g) from each replicate was ground well in a grinder. Then 5 mL of cooled phosphate buffer (50 mM; pH 7.8) was added to it. Then the homogenate was vortex and centrifuged at 15,000 rpm for 15 min at 48 °C. The supernatant was separated and used for the assays of antioxidative enzymes. The SOD activity was determined following Giannopolitis and Ries (1977) by appraising the photoreduction of nitroblue tetrazolium (NBT) by the enzyme. The reaction mixture contained riboflavin (1.3 mM), NBT (50 mM), EDTA (75 nM), methionine (13 mM), phosphate buffer (20 mM) and enzyme extract which were homogenized in a test tube. This solution was irradiated for 15 minutes under white fluorescent light (15W lamp) at 80 mmol m⁻²s⁻¹. Then the OD of the solutions was read using a spectrophotometer (IRMECO U2020) at 560 nm. The amount of enzyme required to inhibit half of NBT photoreduction was considered equal to one unit of SOD activity. The protocol of Chance and Maehly (1955) was followed for the appraisal of the activities of POD and CAT. The changes in absorbance were recorded at 470 nm after every 30 second. A change in the absorbance per minute was considered equal to one unit of POD activity. Three mL of reaction solution used for the determination of CAT activity contained 5.9 mM H₂O₂, 50 mM phosphate buffer having pH 7.8 and 0.1 mL enzyme extract. For the determination of CAT activity, the reaction was initiated by adding the enzyme extract. The changes in absorbance of the reaction solution after every 20 second were measured at 240 nm. A change of 0.01 units per minute in absorbance was considered equal to one unit CAT.
activity. The activity of each enzyme was calculated and expressed on the basis of total protein. Protein concentration of the extract was measured following Bradford (1976).

3.2.15 Ion determination

Na\(^+\), K\(^+\) and Ca\(^{2+}\) were determined as described in experiment one under section 3.1.8.

3.2.16 Determination of Cl\(^-\)

Chloride ions in the plant samples were extracted by heating the material in water. Dried ground leaf sample (0.1 g) was taken in a test tube and 10 mL of distilled water were added to it and then incubated it overnight at 25 °C. The tubes were then heated at 80 °C in a digestion block until the volume in the test tubes remained half of the original volume. After cooling, distilled water was added to each test tube to maintain the volume up to 10 mL again and Cl\(^-\) concentration in the leaf and root extracts determined using a chloride analyzer (Model 926, Sherwood, Cambridge, UK).

3.2.17 Experimental design and statistical analysis

Experimental unit having four replications and six salinity treatments was designed in a Complete Randomized Design (CRD) with two factor factorial arrangements. Analysis of variance (ANOVA) and multiple comparison tests (Tukey test) were computed using Statistix 8.1 computer package. Differences among treatments were considered significant at p ≤ 0.05 after statistical analysis.

3.3. Experiment-III

Optimization of chitosan levels for the enhancement of salinity tolerance in eggplant

Genotypes: 2 (Saadia and Black Beauty)
Replications: 4
Treatments:

\[
\begin{align*}
T_1 &= \text{Control} \\
T_2 &= \text{Salinity (9 dS m}^{-1}\text{)} \\
T_3 &= \text{Ch (075 mg L}^{-1}\text{)} + \text{Salinity (9 dS m}^{-1}\text{)} \\
T_4 &= \text{Ch (100 mg L}^{-1}\text{)} + \text{Salinity (9 dS m}^{-1}\text{)} \\
T_5 &= \text{Ch (125 mg L}^{-1}\text{)} + \text{Salinity (9 dS m}^{-1}\text{)} \\
T_6 &= \text{Ch (150 mg L}^{-1}\text{)} + \text{Salinity (9 dS m}^{-1}\text{)} \\
T_7 &= \text{Ch (175 mg L}^{-1}\text{)} + \text{Salinity (9 dS m}^{-1}\text{)} \\
T_8 &= \text{Ch (200 mg L}^{-1}\text{)} + \text{Salinity (9 dS m}^{-1}\text{)}
\end{align*}
\]
3.3.1 Experimental details:
An optimization experiment of chitosan was conducted to find out the best chitosan dose that will be useful under saline conditions. Chitosan (35 KDa) was imported from Chinese company, Zhengzu Sigma Co. One salt tolerant (Saadia) and one salt sensitive (Black Beauty) genotypes identified in first experiment were used. The experiment was carried out in pots containing sand. Half strength Hoagland solution was applied as nutrient solution. Seeds were sown in pots, salinity level (9 dS m\(^{-1}\)) optimized in second experiment was applied. Seven levels of chitosan (0, 75, 100, 125, 150, 175 and 200 mg L\(^{-1}\)) were applied as foliar spray by making solutions with one percent acetic acid and distill water after ten days of salinity exposure. Fifteen days after chitosan application (60 days after seed sowing), growth parameters were assessed. Experiment was planned in CRD and replicated four times. Data was recorded for following parameters.

- Plant height (cm)
- Root length (cm)
- Shoot fresh weight (g)
- Shoot dry weight (g)
- Root fresh weight (g)
- Root dry weight (g)

3.3.2 Measurement of growth parameters
These indicators were observed as described in experiment number one under section 3.1.3 to 3.1.7.

3.3.3 Experimental design and statistical analysis:
Experimental unit having four replications and eight treatments was designed in a Complete Randomized Design (CRD) with two factor factorial arrangements. Analysis of variance (ANOVA) and multiple comparison tests (Tukey test) were computed using Statistix 8.1 computer package. Differences among treatments were considered significant at p ≤ 0.05 after statistical analysis.
3.4 Experiment-IV

Effects of chitosan on growth, physiological, water related, biochemical, ionic and yield attributes of salt sensitive and salt tolerant eggplant genotypes.

Genotypes: 2 (Saadia and Black Beauty)
Replications: 4
Salinity levels: 2 (control & 9 dS m\(^{-1}\))
Chitosan Levels: 2 (control & 150 mg L\(^{-1}\))

Treatments:

\(T_1\) = Control (without salinity and chitosan)
\(T_2\) = Control + chitosan=150 mg L\(^{-1}\) (optimized in experiment # 3)
\(T_3\) = Saline (9 dS m\(^{-1}\))
\(T_4\) = Chitosan (150 mg L\(^{-1}\)) + saline (9 dS m\(^{-1}\))

3.4.1 Experimental details:

Two genotypes of eggplant one salt tolerant (Saadia) and one salt sensitive (Black Beauty) were sown (ten seeds of each) in pots and were thinned out to five at first true leaf stage. One salt stress level i.e. 9 dS m\(^{-1}\) (optimized in second experiment) was applied after 30 days of seed sowing in splits of 3 dS m\(^{-1}\) to avoid osmotic shock. Half strength Hoagland solution was applied as a nutrient solution. Optimized concentration (150 mg L\(^{-1}\)) of chitosan from third experiment was applied to both control and saline treatment after ten days of salinity application. Fifteen days after chitosan application (60 days after seed sowing) gas exchange characteristic and water relations were measured and next day four plants were carefully uprooted for biochemical and ionic attributes. One plant in each pot was left for yield attributes.

3.4.2 List of parameters studied in this experiment:

**Physiological attributes**

- Stomatal conductance \((g_s)\) by (IRGA) mmol m\(^{-2}\)s\(^{-1}\)
- Net photosynthesis rate \((Pn)\) by (IRGA) \(\mu\)mol CO\(_2\) m\(^{-2}\)s\(^{-1}\)
- Transpiration rate \((E)\) by (IRGA) mmol H\(_2\)O m\(^{-2}\)s\(^{-1}\)
- Water use efficiency \((Pn/E)\)
- Chlorophyll index (SPAD value)

**Water Relations**

- Leaf osmotic potential \((\Psi_s)\) (-MPa)
- Leaf water potential \((\Psi_w)\) (-MPa)
- Leaf turgor potential (Ψp) (MPa)
- Relative water contents (%)

**Biochemical attributes**
- Super oxide dismutase (SOD) (U mg\(^{-1}\) protein)
- Catalase (CAT) (U mg\(^{-1}\) protein)
- Peroxidase (POD) (U mg\(^{-1}\) protein)
- Proline (µmol g\(^{-1}\) fwt)
- Glycinebetaine (µmol g\(^{-1}\) fwt)
- Malondialdehyde (MDA) (nmol g\(^{-1}\) fwt)

**Ionic analysis of leaf**
- Ca\(^{2+}\) contents (mg g\(^{-1}\) dwt)
- K\(^{+}\) contents (mg g\(^{-1}\) dwt)
- Na\(^{+}\) contents (mg g\(^{-1}\) dwt)
- CI\(^{-}\) (mg g\(^{-1}\) dwt)

**Yield Attributes**
- Number of fruits per plant
- Average fruit weight (g)
- Fruit diameter (cm)
- Average yield per plant (g)

**3.4.3 Measurement of attributes**
All attributes were observed as described in experiment number two under section 3.2.4 to 3.2.16.

**3.4.4 Experimental design and statistical analysis:**
Experimental units replicated four times having two genotypes, two salinity levels and two chitosan treatments were designed in a CRD with three factor factorial arrangements. Analysis of variance (ANOVA) and multiple comparison tests (Tukey test) were computed using Statistix 8.1 computer package. Differences among treatments were considered significant at p ≤ 0.05 after statistical analysis.
Chapter 4

RESULTS

4.1 Experiment-I: Screening of eggplant genotypes for salt tolerance on the basis of plant growth salt tolerance index and ionic analysis

In this experiment 13 eggplant genotypes were grown in plastic pots containing sand as growing media and ½ strength Hoagland was used as a nutrient solution. Plants were subjected to six different salinity levels i.e. control, 3, 6, 9, 12 and 15 dS m\(^{-1}\) of NaCl after 30 days of seed sowing. Plants were harvested after 50 days of seed sowing to study the growth salt tolerance indices and ionic analysis. Results regarding these indices are given below.

4.1.1 Plant height stress tolerance index (PHSI)

The shoot length of controlled and salt stressed seedlings was used to calculate the plant height stress tolerance index (PHSI) in 13 eggplant genotypes. PHSI of different eggplant genotypes was significantly affected with salinity stress (Fig 4.1.1). Significant interaction was expressed by eggplant genotypes and salinity stress (Appendix 1). The maximum PHSI (92%) was observed in Saadia with 3 dS m\(^{-1}\) salinity level, while minimum PHSI (41%) was in case of Black beauty under 15 dS m\(^{-1}\) salinity level. In general, the performance of Saadia in terms of higher PHSI (76.7%) under salinity stress was best, statistically similar with Qaisar (74%). Black Beauty (65%) showed poor performance, statistically at par with Dilnashin (67%). A significant decrease in PHSI was observed with increasing salinity level in all eggplant genotypes.

4.1.2 Root length stress tolerance index (RLSI)

Increasing salinity stress significantly decreased root length stress tolerance index (RLSI) in all eggplant genotypes. The maximum decrease in RLSI was observed at 15 dS m\(^{-1}\) (Fig. 4.1.1). The maximum RLSI was noted in Saadia (82.8%) which was not statistically different with Qaisar (82.6%), Bemisal (81.9%) and Black Oval (81.3%). While the minimum RLSI was observed in Black Beauty (74.4%) statistically at par with WER (75.1%), Dilnashin (75.5%), Black Boll (75.7%) and PPR (76.8%). The interaction between salinity and genotypes was non-significant (Appendix 1).

4.1.3 Shoot fresh weight stress tolerance index (SFWSI)

A significant decrease in shoot fresh weight stress tolerance index (SFWSI) was noted due to increase in salinity in all eggplant genotypes. The maximum SFWSI was noted at 3 dS m\(^{-1}\) and the minimum at 15 dS m\(^{-1}\). Genotypes responded differently with increasing
Figure 4.1.1 Plant height stress tolerance index (PHSI), root length stress tolerance index (RLLSI) and shoot fresh weight stress tolerance (SFWSI) index of thirteen eggplant genotypes as affected by different salinity levels at \( P \leq 0.05 \). The values are means of four replicates + standard error (SE).
salinity stress. Performance of Saadia was better by keeping highest SFWSI (77.6%) statistically alike with Qaisar (76.5%) and Bemisal (75.6%) under salinity stress. Black Beauty showed poor performance by retaining lowest SFWSI (64.5%), followed by WER (66.9%) and Dilnashin (67.1%). Interactive effect of salinity and genotypes was significant for SFWSI (Appendix 1). The maximum SFWSI (94.5%) was noted in Saadia under 3 dS m\(^{-1}\) and the minimum (44.4%) was in Black Beauty under 15 dS m\(^{-1}\) salinity (Fig. 4.1.1).

### 4.1.4 Root fresh weight stress tolerance index (RFWSI)

Root fresh weight stress tolerance index (RFWSI) was significantly decreased with increasing salinity stress in eggplant genotypes. Significant variation among genotypes was observed as Saadia was at top by sustaining the maximum RFWSI (84.2%) statistically similar with Bemisal (82.9%) and Black Beauty (76.1%) at bottom statistically at par with Dilnashin (77.1%) and Black Boll (77.3%) under saline conditions. The interaction between genotypes and salinity treatment was significant for RFWSI (Appendix 1). The maximum value for RFWSI (93.6%) was noted in Saadia under 3 dS m\(^{-1}\) salinity stress and the minimum (69.1%) in Black Beauty under 15 dS m\(^{-1}\) salinity stress (Fig. 4.1.2).

![Figure 4.1.2 Root fresh weight stress tolerance index (RFWSI) of thirteen eggplant genotypes as affected by different salinity levels at \(P\leq0.05\). The values are means of four replicates \(\pm\) standard error (SE).](image-url)
4.1.5. Plant dry matter stress tolerance index (PDMSI)

Plant dry matter stress tolerance index (PDMSI) was significantly reduced with increasing salinity stress in all eggplant genotypes. All the genotypes showed significant variations in response to salinity stress regarding PDMSI. The maximum PDMSI (75.7%) was observed in Saadia followed by Qaisar (73.1%), Bemisal (72.2%) and Black Oval (71.9%) and the minimum PDMSI (64.1%) in Black Beauty statistically at par with Dilnashin (65.7%) and Black Boll (65.9%) under saline conditions. The interaction between genotypes and salinity treatments was significant for PDMSI (Appendix 1). The maximum PDMSI (95.1%) was recorded under 3 dS m\(^{-1}\) salinity stress in Saadia and the minimum (42.7%) in Black Beauty with 15 dS m\(^{-1}\) salinity stress (Fig. 4.1.3).

4.1.6. Leaf sodium (Na\(^+\)) contents (%)

Leaf sodium (Na\(^+\)) contents (%) were significantly increased with increasing salinity levels in all eggplant genotypes. Significant variation among eggplant genotypes was noted in term of leaf Na\(^+\) contents %. In general the maximum leaf Na\(^+\) percentage was observed in Black Beauty (150%) statistically similar with Black Boll (143%) and the minimum was noted in Saadia (100%) followed by Black oval (109%) and Qaisar (117%) under saline conditions. The interactive effect of genotype and salinity was significant for leaf Na\(^+\) contents % (Appendix 2). Higher leaf Na\(^+\) accumulation 238% was noted in Black Beauty at 15 dS m\(^{-1}\) and the minimum in Saadia 33% at 3 dS m\(^{-1}\) (Fig. 4.1.4).

Figure 4.1.3 Plant dry matter stress tolerance index (PDMSI) of thirteen eggplant genotypes as affected by different salinity levels at \(P \leq 0.05\). The values are means of four replicates ± standard error (SE).
4.1.7 Leaf potassium (K⁺) contents (%)
Increasing salinity stress significantly reduced the leaf K⁺ percentage (%) in all eggplant genotypes. The highest K⁺ reduction was observed at 15 dS m⁻¹ and the least at control. The maximum percent reduction in leaf K⁺ was recorded in Black Beauty (58%), followed by black Boll (56%) and the minimum was in Saadia (44%), followed by Black Oval (47%) Bemisal (47%) and P.P.R (48%) (Fig.4.1.4). Interaction between genotypes and salinity treatment was found non-significant (Appendix 2).

Figure 4.1.4 Percent increase in leaf sodium (Na⁺) contents (%) and percent decrease in leaf potassium (K⁺) contents (%) of thirteen eggplant genotypes as affected by different salinity levels at $P \leq 0.05$. The values are means of four replicates $\pm$ standard error (SE).
4.1.8 Correlation matrix among various growth and ionic attributes of eggplant genotypes

Correlation among different variables is given in table 4.1.1. Plant dry matter stress tolerance index (PDMSI) exhibited positive correlation with shoot fresh weight stress tolerance index (SFWSI), plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), root fresh weight stress tolerance index (RFWSI) and potassium contents (K⁺) while negative correlation with sodium contents (Na⁺). SFWSI revealed positive correlation with PHSI, RLSI, RFWSI and K⁺ whereas negative correlation with Na⁺. In the same way, PHSI presented positive and significant correlation with RLSI, RFWSI and K⁺ while negative correlation with Na⁺. A highly positive correlation of RLSI was noted in case of RFWSI and K⁺ but negative correlation was observed with Na⁺. RFWSI had positive correlation with K⁺ but negative with Na⁺. K⁺ also had negative correlation with Na⁺ but positive with all other parameters. On the other hand, Na⁺ had negative correlation with all studied attributes. These results may indicate that Na⁺ negatively affect growth attributes and K⁺. It can be postulated that use of salt tolerance indices and K⁺ as selection criteria may help breeders in selection of the genotypes with better salt tolerance.

Table 4.1.1 Correlation matrix among various attributes of different studied eggplant genotypes

<table>
<thead>
<tr>
<th></th>
<th>PDMSI</th>
<th>SFWSI</th>
<th>PHSI</th>
<th>RLSI</th>
<th>RFWSI</th>
<th>K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFWSI</td>
<td>0.974</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHSI</td>
<td>0.974</td>
<td>0.974</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RLSI</td>
<td>0.857</td>
<td>0.862</td>
<td>0.856</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RFWSI</td>
<td>0.923</td>
<td>0.926</td>
<td>0.912</td>
<td>0.893</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>0.935</td>
<td>0.930</td>
<td>0.926</td>
<td>0.806</td>
<td>0.886</td>
<td>1</td>
</tr>
<tr>
<td>Na⁺</td>
<td>-0.957</td>
<td>-0.949</td>
<td>-0.952</td>
<td>-0.832</td>
<td>-0.901</td>
<td>-0.930</td>
</tr>
</tbody>
</table>

Values indicate Pearson’s correlation coefficient; Values in bold are different from 0 with a significance level alpha=0.05

PDMSI (plant dry matter stress tolerance index), SFWSI (shoot fresh weight stress tolerance index), PHSI (plant height stress tolerance index), RLSI (root length stress tolerance index), RFWSI (root fresh weight stress tolerance index), Na⁺ (sodium contents) and K⁺ (potassium contents).
4.1.9 Categorization of thirteen eggplant genotypes against salt stress on the basis of salt tolerance indices and ionic contents.

Mean values of salt tolerance indices and ions percentages (Na$^+$ and K$^+$) for each genotype were divided by ten and given as marks. Finally, the marks of every genotype were summed up to categorize them. The genotype which got maximum score was marked as salt tolerant while genotype with lowest score considered as salt sensitive. The ranking of eggplant genotypes on the basis of their performance under salt stress showed that Saadia was at first position followed by Qaisar, Black Oval and Bemisal while Black Beauty was considered as salt susceptible (Table 4.1.2).

**Table 4.1.2 Ranking of eggplant genotypes on the basis of performance under salt stress**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Genotypes</th>
<th>PHSI (10)</th>
<th>RLSI (10)</th>
<th>SFWSI (10)</th>
<th>RFWSI (10)</th>
<th>PDMSI (10)</th>
<th>Na$^+$ (-15)</th>
<th>K$^+$ (10)</th>
<th>Cumulative (45)</th>
<th>Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Qaisar</td>
<td>7.5</td>
<td>8.3</td>
<td>7.7</td>
<td>8.2</td>
<td>7.3</td>
<td>11.7</td>
<td>6.6</td>
<td>33.9</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Saadia</td>
<td>7.7</td>
<td>8.3</td>
<td>7.8</td>
<td>8.5</td>
<td>7.6</td>
<td>10.1</td>
<td>7.2</td>
<td>37</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Black Beauty</td>
<td>6.5</td>
<td>7.4</td>
<td>6.4</td>
<td>7.6</td>
<td>6.4</td>
<td>15</td>
<td>6</td>
<td>25.3</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>Dilnashin</td>
<td>6.7</td>
<td>7.5</td>
<td>6.7</td>
<td>7.7</td>
<td>6.6</td>
<td>12.4</td>
<td>6.6</td>
<td>29.4</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Black Boll</td>
<td>6.8</td>
<td>7.6</td>
<td>6.9</td>
<td>7.7</td>
<td>6.6</td>
<td>14.4</td>
<td>6.2</td>
<td>27.4</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>Bemisal</td>
<td>7.4</td>
<td>8.2</td>
<td>7.6</td>
<td>8.3</td>
<td>7.2</td>
<td>13.6</td>
<td>6.8</td>
<td>31.9</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>WEL</td>
<td>7.1</td>
<td>7.7</td>
<td>7.2</td>
<td>7.9</td>
<td>6.8</td>
<td>12.2</td>
<td>6.7</td>
<td>31.2</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>Nirala</td>
<td>7.1</td>
<td>7.8</td>
<td>7.2</td>
<td>8</td>
<td>6.8</td>
<td>13.3</td>
<td>6.6</td>
<td>30.2</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>VRIB 9901</td>
<td>7</td>
<td>7.8</td>
<td>7.2</td>
<td>8</td>
<td>6.7</td>
<td>12.9</td>
<td>6.6</td>
<td>30.4</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>Black Oval</td>
<td>7.3</td>
<td>8.1</td>
<td>7.5</td>
<td>8.1</td>
<td>7.1</td>
<td>11</td>
<td>7</td>
<td>34.1</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>WER</td>
<td>6.8</td>
<td>7.5</td>
<td>6.7</td>
<td>7.8</td>
<td>6.6</td>
<td>13.1</td>
<td>6.4</td>
<td>28.7</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>P.P.R.</td>
<td>7.1</td>
<td>7.7</td>
<td>7.1</td>
<td>8</td>
<td>6.8</td>
<td>13.2</td>
<td>6.8</td>
<td>30.3</td>
<td>7</td>
</tr>
<tr>
<td>13</td>
<td>VRIB 9501</td>
<td>7</td>
<td>7.7</td>
<td>7</td>
<td>8</td>
<td>6.9</td>
<td>13.1</td>
<td>6.5</td>
<td>30</td>
<td>9</td>
</tr>
</tbody>
</table>

**Conclusion**

Eggplant genotypes had variable response against salinity stress. PHSI, RLSI, SFWSI, RFWSI, PDMSI and ionic contents (Na$^+$ and K$^+$) are the effective screening tools for salt tolerance in eggplant.
4.2 Experiment-II: Effect of salinity stress on growth, physiological, water relations, biochemical and ionic attributes of salt sensitive and salt tolerant eggplant genotypes

The objective of this experiment was to compare growth, physiological, water related, biochemical and ionic changes that occur with in salt tolerant (Saadia) and salt sensitive (Black Beauty) eggplant genotypes under different NaCl salinity levels.

4.2.1 Effect of salinity stress on growth attributes

4.2.1.1 Effect of salinity stress on plant height (cm)

Plant height was significantly reduced in both eggplant genotypes with increasing salinity levels; however, the maximum reduction was noted under 15 dS m\(^{-1}\), followed by 12, 9, 6 and 3 dS m\(^{-1}\). The interactive effect of genotype (G) × salinity (S) was statistically significant (Appendix 3), and salt tolerant genotype-Sadia maintained higher plant height than salt sensitive Black Beauty under all salinity levels. Poor response of both the eggplant genotypes was observed with higher salinity level of 15 dS m\(^{-1}\). The maximum plant height was measured in Saadia (33.3 cm) with control (no salinity) and minimum in Black Beauty (12.76 cm) with salinity level of 15 dS m\(^{-1}\). Overall, performance of Saadia genotype in terms of plant height was higher than Black Beauty under salinity stress (Fig. 4.2.1.1).

4.2.1.2. Effect of salinity stress on root length (cm)

Root length of both eggplant genotypes differed significantly with respect to salinity levels; however, maximum reduction in root length was observed with 15 dS m\(^{-1}\), followed by 12, 9, 6 and 3 dS m\(^{-1}\) (Fig. 4.2.1.1). The eggplant genotype, Saadia maintained higher root length compared to Black Beauty. The significant interaction was noted between genotype (G) and salinity (S), as maximum root length (10.05 cm) was measured in Saadia with control (no salinity) and minimum (5.42 cm) in Black Beauty with salinity level of 15 dS m\(^{-1}\) (Appendix 3).

4.2.1.3 Effect of salinity stress on shoot fresh weight (g)

The shoot fresh weight (SFW) of both the genotypes decreased significantly by varying salinity levels while the maximum decrease was observed at 15 dS m\(^{-1}\) pursued by 12, 9, 6 and 3 dS m\(^{-1}\). The significant effect was observed between genotypes in terms of SFW. The genotype-Sadia maintained higher SFW than that of black beauty. The interactive effect between G×S was significant (Appendix 3), as maximum SFW (17.74 g) was
recorded in Saadia with control (no salinity) and minimum in black beauty (7.59 g) at 15 dS m\(^{-1}\) (Fig. 4.2.1.2).

### 4.2.1.4 Effect of salinity stress on root fresh weight (g)

The root fresh weight (RFW) of both the genotypes reduced significantly by increasing salinity levels, whereas maximum reduction was observed at 15 dS m\(^{-1}\), followed by 12, 9, 6 and 3 dS m\(^{-1}\). The eggplant genotypes also differed significantly in terms of RFW. The salt tolerant genotype-Saadia maintained higher RFW as compared to salt sensitive genotype-Black beauty. The interaction between G×S was significant (Appendix 4) as maximum RFW (2.75 g) was recorded in Saadia at control and minimum in black beauty (2.15 g) at 15 dS m\(^{-1}\). Increase in RFW was observed by both genotypes with decreasing salinity stress and the maximum was at control level. Poor response of both eggplant genotypes was observed with higher salinity level of 15 dS m\(^{-1}\) (Fig. 4.2.1.1).

### 4.2.1.5 Effect of salinity stress on shoot dry weight (g)

The different salinity levels significantly reduced the shoot dry weight in both the eggplant genotypes, however maximum reduction was observed at 15 dS m\(^{-1}\) pursued by 12, 9, 6 and 3 dS m\(^{-1}\). The eggplant genotypes also differed significantly with respect to shoot dry weight. The genotype Saadia retained significantly higher shoot dry weight compared to Black Beauty. The interaction between G×S was significant (Appendix 4), while maximum shoot dry weight (3.52 g) was recorded in Saadia with control and minimum (1.69 g) in Black Beauty at 15 dS m\(^{-1}\). Increase in shoot dry weight was observed in both genotypes with decreasing salinity stress and the maximum was at control level. Poor response of both eggplant genotypes were observed in respect to shoot dry weight with increasing salinity level of 15 dS m\(^{-1}\) (Fig. 4.2.1.2).

### 4.2.1.6 Effect of salinity stress on root dry weight (g)

The root dry weight of both the genotypes decreased significantly by increasing salinity level, whereas maximum suppression was observed at 15 dS m\(^{-1}\) followed by 12, 9, 6 and 3 dS m\(^{-1}\). The eggplant genotypes also differed significantly in terms of root dry weight. The salt tolerant genotype, Saadia maintained higher root dry weight as compared to salt sensitive genotype-Black beauty. The interaction between G×S was significant (Appendix 4); maximum root dry weight (0.42 g) was recorded in Saadia at control and minimum (0.27 g) in Black beauty at 15 dS m\(^{-1}\). Increase in root dry weight was observed by both genotypes with decreasing salinity stress and the maximum was at control level. Poor response of both eggplant genotypes was observed with higher salinity level of 15 dS m\(^{-1}\) (Fig. 4.2.1.2).
Figure 4.2.1.1 Effect of different salinity levels on plant height, root length and root fresh weight of salt tolerant (Saadia) and salt sensitive (Black Beauty) eggplant genotypes.
Figure 4.2.1.2 Effect of different salinity levels on shoot fresh weight, root and shoot dry weight of salt tolerant (Saadia) and salt sensitive (Black Beauty) eggplant genotypes.
4.2.2 Effect of salinity stress on physiological attributes

4.2.2.1 Effect of salinity stress on net photosynthetic rate (Pn)

The net photosynthetic rate (Pn) was significantly affected by varying salinity levels. The maximum Pn was observed in control (no salinity) which gradually decreased by increasing salinity stress levels of 3, 6, 9, 12 and 15 dS m$^{-1}$. The eggplant genotypes also differed significantly in terms of Pn. The salt tolerant genotype (Saadia) maintained higher Pn than that of salt sensitive genotype (Black Beauty). The significant interaction was observed between G×S (Appendix 5), as maximum Pn was recorded in Saadia (6.28 μmol CO$_2$ m$^{-2}$s$^{-1}$) at control and minimum in Black beauty (1.73 μmol CO$_2$ m$^{-2}$s$^{-1}$) at 15 dS m$^{-1}$. Increase in Pn was recorded by both genotypes with decreasing salinity stress levels and the maximum was at control level. Poor response of both eggplant genotypes was observed with higher salinity level of 15 dS m$^{-1}$ (Fig. 4.2.2.1).

4.2.2.2 Effect of salinity stress on stomatal conductance (gs)

The stomatal conductance (gs) in both the genotypes reduced significantly by increasing salinity levels whereas, maximum reduction was observed at 15 dS m$^{-1}$, followed by 12, 9, 6 and 3 dS m$^{-1}$. The eggplant genotypes also differed significantly in terms of gs. Salt tolerant genotype-Saadia maintained higher gs as compared to salt sensitive genotype-Black beauty. The interaction between G×S was significant (Appendix 5), maximum gs were recorded in Saadia (0.157 mmol m$^{-2}$s$^{-1}$) at control and minimum in Black beauty (0.08 mmol m$^{-2}$s$^{-1}$) at 15 dS m$^{-1}$. Increase in gs was observed by both genotypes with decreasing salinity stress and the maximum was at control level. Poor response of both eggplant genotypes in terms of gs was observed with higher salinity stress level of 15 dS m$^{-1}$ (Fig. 4.2.2.1).

4.2.2.3 Effect of salinity stress on transpiration rate (E)

The transpiration rate (E) of both the genotypes declined significantly by varying salinity levels whereas, maximum reduction was observed at 15 dS m$^{-1}$ pursued by 12, 9, 6 and 3 dS m$^{-1}$. The significant effect was observed between genotypes in terms of E. The salt tolerant genotype-Saadia maintained higher E than that of salt sensitive genotype-Black beauty. The significant interaction was observed between G×S (Appendix 5), maximum E was recorded in Saadia (2.88 mmol H$_2$O m$^{-2}$s$^{-1}$) with control (no salinity) and minimum in black Beauty (0.645 mmol H$_2$O m$^{-2}$s$^{-1}$) at 15 dS m$^{-1}$. Increase in E was observed by both genotypes with decreasing salinity stress and the maximum was at control level. Poor response of both eggplant genotypes in terms of E was observed with higher salinity stress level of 15 dS m$^{-1}$ (Fig. 4.2.2.1).
Figure 4.2.2.1 Effect of different salinity levels on gas exchange characters of salt tolerant (Saadia) and salt sensitive (Black Beauty) eggplant genotypes.
4.2.2.4 Effect of salinity stress on water use efficiency (WUE)

WUE showed an increasing trend under increasing salinity stress, however, maximum increase was observed at 15 dS m\(^{-1}\) and gradually decreased by 12, 9, 6 and 3 dS m\(^{-1}\). The eggplant genotypes also showed changing trend in terms of WUE. The salt tolerant genotype Saadia exhibited significantly higher WUE as compared to salt sensitive genotype-Black Beauty. The interaction between G×S was significant (Appendix 6), maximum WUE (3.647) was recorded in salt tolerant genotype (Saadia) at 15 dS m\(^{-1}\) and minimum in Black beauty (2.09) at control (Fig. 4.2.2.1). Positive response of both eggplant genotypes was observed in terms of WUE with higher salinity stress (15 dS m\(^{-1}\)).

4.2.2.5 Effect of salinity stress on chlorophyll index (SPAD values)

Chlorophyll index was significantly reduced in both the eggplant genotypes with increasing salinity stress; however, the maximum reduction was noted under 15 dS m\(^{-1}\), followed by 12, 9, 6 and 3 dS m\(^{-1}\). The eggplant genotypes also showed varying trend in terms of Chlorophyll index. The salt tolerant genotype-Saadia maintained significantly higher Chlorophyll index than that of salt sensitive-Black beauty. The significant interaction between G×S was observed (Appendix 6), however maximum Chlorophyll index (38.7 SPAD value) maintained by genotype-Saadia at control and minimum in Black beauty (12.9 SPAD value) at 15 dS m\(^{-1}\). Poor response of both eggplant genotypes was observed in terms of chlorophyll index with higher salinity level of 15 dS m\(^{-1}\). Overall, performance of Saadia genotype in terms of increased Chlorophyll index was better than Black Beauty under salinity stress (Fig. 4.2.2.2).

![Figure 4.2.2.2 Effect of different salinity levels on chlorophyll index of salt tolerant (Saadia) and salt sensitive (Black Beauty) eggplant genotypes.](image-url)
4.2.3 Effect of salinity stress on water relations

4.2.3.1 Effect of salinity stress on leaf water potential (Ψ<sub>W</sub>) (-M Pa)

Leaf water potential was significantly reduced in both the genotypes with increasing salinity levels. Variable response was observed for leaf water potential under salt stress between salt tolerant (Saadia) and salt sensitive (Black Beauty). Saadia displayed better performance in term of leaf water potential under salinity stress. A significant interaction was observed between salinity levels and genotypes (Appendix 7). The maximum water potential was noted in Saadia (-0.16 M Pa) followed by Black Beauty (-0.21 M Pa) under control and the minimum in Black Beauty (-1.25 M Pa) at 15 dS m<sup>-1</sup> salinity level (Fig. 4.2.3).

4.2.3.2 Effect of salinity stress on leaf osmotic potential (Ψ<sub>S</sub>) (-M Pa)

Increasing salinity stress (3, 6, 9, 12 and 15 dS m<sup>-1</sup>) substantially decreased the osmotic potential of eggplant genotypes. Salt tolerant saadia maintained high osmotic potential as compared to sensitive (Black Beauty) under saline conditions (Fig. 4.2.3). Interaction between salinity and genotypes was recorded as significant (Appendix 7). Both the genotypes displayed higher osmotic potential under control but the lowest (-1.3 M Pa) was noted in Black Beauty under highest salinity level of 15 dS m<sup>-1</sup>.

4.2.3.3 Effect of salinity stress on leaf turgor potential (Ψ<sub>P</sub>) (M Pa)

Leaf turgor potential was decreased significantly by increasing salinity stress; however the maximum leaf turgor potential was recorded at control and gradually decreased by increasing salinity stress of 3, 6, 9, 12 and 15 dS m<sup>-1</sup>. The eggplant genotypes also differed significantly in terms of leaf turgor potential. The salt tolerant genotype (Saadia) demonstrated higher leaf turgor potential than that of salt sensitive genotype (Black beauty). The significant interaction was observed between G×S (Appendix 7); the maximum leaf turgor potential was recorded in Saadia (0.584 M Pa) at control (no salinity) and the minimum in Black Beauty (0.05 M Pa) at 15 dS m<sup>-1</sup>. Negative response of both the eggplant genotypes was observed in terms of leaf turgor potential with increasing salt stress level of 15 dS m<sup>-1</sup> (Fig. 4.2.3).

4.2.3.4 Effect of salinity stress on relative water contents (RWC)

The increasing salinity stress significantly reduced the relative water contents (RWC) in both the eggplant genotypes, however maximum reduction was observed at 15 dS m<sup>-1</sup> pursued by 12, 9, 6 and 3 dS m<sup>-1</sup>. The eggplant genotypes also differed significantly with
Figure 4.2.3 Effect of different salinity levels on water relations of salt tolerant (Saadia) and salt sensitive (Black Beauty) eggplant genotypes.
respect to RWC under saline conditions. The genotype Saadia retained significantly higher RWC compared to Black Beauty. The interaction between G×S was significant (Appendix 7), while maximum RWC (85.4%) was recorded in Saadia, followed by Black Beauty (82.86%) with control and minimum in Black Beauty (39.3%) by 15 dS m⁻¹. Increase in RWC was observed in both the genotypes with decreasing salinity stress and the maximum was at control level. Poor response of both eggplant genotypes were observed in respect to RWC with increasing salinity level of 15 dS m⁻¹ (Fig. 4.2.3)

4.2.4 Effect of salinity stress on antioxidants enzymes

4.2.4.1 Effect of salinity stress on activity of superoxide dismutase (SOD)
The activity of SOD was significantly affected by varying salt stress levels. The maximum SOD activity was recorded at salt stress of 15 dS m⁻¹ and gradually decreased by decreasing salinity stress levels of 12, 9, 6 and 3 dS m⁻¹. The eggplant genotypes also differed significantly in terms of SOD activity. The salt tolerant genotype (Saadia) exhibited higher SOD activity than that of salt sensitive genotype (Black beauty) (Fig. 4.2.4). The significant interaction was observed between G×S (Appendix 8), maximum SOD enzyme activity (4.6 U mg⁻¹ Protien) was recorded in Saadia at salt stress level of 15 dS m⁻¹ and minimum in Black beauty (2.85 U mg⁻¹ Protien) at control (no salinity). Positive response of both eggplant genotypes was observed in terms of SOD activity with increasing salt stress level of 15 dS m⁻¹.

4.2.4.2 Effect of salinity stress on activity of catalase (CAT)
The CAT activity was increased significantly by increasing salinity stress; however, maximum CAT activity was recorded at salinity stress of 15 dS m⁻¹ and gradually decreased by decreasing salinity stress of 12, 9, 6, and 3 dS m⁻¹ (Fig. 4.2.4). The eggplant genotypes also differed significantly in terms of CAT activity. The salt tolerant genotype (Saadia) demonstrated higher CAT activity than that of salt sensitive genotype (Black beauty). Significant interaction was observed between G×S (Appendix 8); maximum CAT activity was recorded in Saadia (0.33 U mg⁻¹ Protien) at salt stress level of 15 dS m⁻¹ and minimum in Black beauty (0.15 U mg⁻¹ Protien) at control (no salinity). Positive response of both eggplant genotypes was observed in terms of CAT activity with increasing salt stress level of 15 dS m⁻¹.

4.2.4.3 Effect of salinity stress on activity of peroxidase (POD)
The POD activity showed an increasing trend under salinity stress; however, maximum increase was recorded at salinity stress of 15 dS m⁻¹ and gradually decreased by
Figure 4.2.4 Effect of different salinity levels on enzymatic activities of salt tolerant (Saadia) and salt sensitive (Black Beauty) eggplant genotypes.
decreasing salinity stress of 12, 9, 6 and 3 dS m⁻¹ (Fig. 4.2.4). The eggplant genotypes also varied significantly in terms of POD activity. The salt tolerant genotype-Saadia displayed higher POD activity than that of salt sensitive genotype-Black beauty. The significant interactions were observed between G×S (Appendix 8); maximum POD activity was recorded in Saadia (2.71 U mg⁻¹ Protien) at salinity stress level of 15 dS m⁻¹ and minimum in Black beauty (0.57 U mg⁻¹ Protien) at control. Positive response of both eggplant genotypes was observed in terms of POD activity with increasing salinity stress of 15 dS m⁻¹.

4.2.5 Effect of salinity stress on biochemical attributes

4.2.5.1 Effect of salinity stress on proline

The proline contents of both genotypes increased significantly by increasing salinity stress; however maximum increase was observed at 15 dS m⁻¹ and gradually decreased by 12, 9, 6 and 3 dS m⁻¹ (Fig. 4.2.5). The eggplant genotypes also differed significantly in terms of leaf proline contents. The salt tolerant genotype-Saadia accumulated significantly higher free proline as compared to salt sensitive genotype-Black beauty. The interaction between G×S was significant (Appendix 9); maximum proline contents (25.17 µg g⁻¹ f.wt.) were recorded in salt tolerant genotype (Saadia) at 15 dS m⁻¹ and minimum in salt sensitive-Black beauty (2.87 µg g⁻¹ f.wt.) at control. Healthy response of both eggplant genotypes was observed in terms of free proline accumulation with higher salinity stress (15 dS m⁻¹).

4.2.5.2 Effect of salinity stress on glycinebetaine

The glycine betaine (GB) is a major organic osmolyte that accumulate in a variety of plants in response to various environmental stresses like salinity. The GB contents of both the genotypes differed significantly by salinity stress; though maximum accumulation was observed at 15 dS m⁻¹ and gradually decreased by 12, 9, 6, 3 and 0 dS m⁻¹ (Fig. 4.2.5). The eggplant genotypes also varied significantly in terms of GB accumulations. The salt tolerant genotype-Saadia accumulated significantly higher GB as compared to salt sensitive genotype-Black beauty. The significant interaction was observed between G×S (Appendix 9), maximum GB contents were quantified in Saadia (5.12 µmol g⁻¹ f.wt.) at 15 dS m⁻¹ and minimum in Black beauty (0.92 µmol g⁻¹ f.wt.) at control. Positive response of both eggplant genotypes was observed in terms of GB contents with increasing salinity stress level of 15 dS m⁻¹.
Figure 4.2.5 Effect of different salinity levels on biochemical attributes of salt tolerant (Saadia) and salt sensitive (Black Beauty) eggplant genotypes.
4.2.5.3 Effect of salinity stress on malondialdehyde (MDA) contents

MDA contents exhibited an increasing trend under salinity stress, whereas maximum was recorded at 12 dS m\(^{-1}\) which was statistically similar with 15 and 9 dS m\(^{-1}\) but significantly higher over 3 dS m\(^{-1}\) and control (Fig. 4.2.5). The eggplant genotypes also differed significantly in terms of MDA contents. The salt tolerant genotype (Saadia) showed higher MDA contents than that of salt sensitive genotype (Black beauty). The significant interactions were observed between G×S (Appendix 9); maximum MDA contents (4.15 nmol g\(^{-1}\) f.wt.) were recorded in Black beauty at salinity stress level of 15 dS m\(^{-1}\) and minimum in Saadia (2.27 nmol g\(^{-1}\) f.wt.) at control. Positive response of both eggplant genotypes was observed in terms of MDA contents with increasing salinity stress of 15 dS m\(^{-1}\).

4.2.6 Effect of salinity stress on ionic attributes

4.2.6.1 Effect of salinity stress on leaf calcium (Ca\(^{2+}\)) contents

The Ca\(^{2+}\) contents showed decreasing trend under salinity stress, whereas, the maximum reduction was noted under 15 dS m\(^{-1}\), followed by 12, 9, 6 and 3 dS m\(^{-1}\). The eggplant genotypes also differed significantly in terms of Ca\(^{2+}\) contents. The genotype Saadia maintained significantly higher Ca\(^{2+}\) contents than that of Black beauty. The interaction between G×S was significant (Appendix 10), maximum Ca\(^{2+}\) contents were recorded in genotype Saadia (15.1 mg g\(^{-1}\) d.wt.) at control (no salinity) and minimum in Black beauty (4.5 mg g\(^{-1}\) d.wt.) at 15 dS m\(^{-1}\) (Fig. 4.2.6). Poor response of both eggplant genotypes was observed in terms of Ca\(^{2+}\) with higher salinity stress (15 dS m\(^{-1}\)).

4.2.6.2 Effect of salinity stress on leaf potassium (K\(^{+}\)) contents

The K\(^{+}\) contents reduced significantly by increasing salinity stress, whereas, the maximum reduction was observed under 15 dS m\(^{-1}\) followed by 12, 9, 6 and 3 dS m\(^{-1}\). The eggplant genotypes also differed significantly in terms of K\(^{+}\) contents. The genotype Saadia maintained significantly higher K\(^{+}\) contents than that of Black beauty. The interaction between G×S was significant (Appendix 10); maximum K\(^{+}\) contents (50.75 mg g\(^{-1}\) d.wt.) were recorded in genotype-Sadia at control (no salinity) and minimum in Black beauty (16.5 mg g\(^{-1}\) d.wt.) at 15 dS m\(^{-1}\). Poor response of both eggplant genotypes was observed in terms of K\(^{+}\) contents with higher salinity stress (15 dS m\(^{-1}\)) (Fig. 4.2.6).
Figure 4.2.6 Effect of different salinity levels on ionic attributes of salt tolerant (Saadia) and salt sensitive (Black Beauty) eggplant genotypes.
4.2.6.3 Effect of salinity stress on leaf sodium (Na⁺) contents

Leaf sodium (Na⁺) contents were significantly increased by increasing salinity stress. The maximum Na⁺ contents were recorded at salinity stress of 15 dS m⁻¹ and gradually decreased by decreasing salinity stress of 12, 9, 6, and 3 dS m⁻¹. The eggplant genotypes also varied significantly in terms of leaf Na⁺ accumulation. The salt sensitive genotype (Black Beauty) absorbed maximum Na⁺ contents in its leaf than that of salt tolerant genotype (Saadia). The significant interaction was observed between G×S (Appendix 10), maximum leaf Na⁺ absorbance was recorded in Black Beauty (30 mg g⁻¹ d.wt.) at salinity stress level of 15 dS m⁻¹ and minimum in Saadia (9.5 mg g⁻¹ d.wt.) at control. Positive response of both eggplant genotypes was observed in terms of leaf Na⁺ absorbance with increasing salinity stress of 15 dS m⁻¹ (Fig. 4.2.6).

4.2.6.4 Effect of salinity stress on leaf chloride (Cl⁻) contents

Mean comparisons of salinity stress levels indicated significant effect on Cl⁻ contents whereas, the maximum increase was observed under 15 dS m⁻¹, followed by 12, 9, 6 and 3 dS m⁻¹. The eggplant genotypes also differed significantly in terms of Cl⁻ contents. The genotype Black Beauty maintained significantly higher Cl⁻ contents than that of Saadia. The significant interaction was observed between G×S (Appendix 10), maximum Cl⁻ contents (28.8 mg g⁻¹ d.wt.) were recorded in genotype-Black Beauty at 15 dS m⁻¹ and minimum in Saadia (9.8 mg g⁻¹ d.wt.) at control. Positive response of both eggplant genotypes was observed in terms of Cl⁻ contents with higher salinity stress (15 dS m⁻¹) (Fig. 4.2.6).

4.2.7 Correlation matrix among various growth, water relations, physiological, biochemical and ionic attributes of eggplant genotypes

A correlation matrix among different attributes is given in table 4.2. It is elaborating that there is a highly positive and significant correlation between plant height (PH) and shoot dry weight (SDW), root dry weight (RDW), chlorophyll index (chl.), photosynthetic rate (Pn), stomatal conductance (gs), relative water contents (RWC), turgor pressure (Ψp), potassium contents (K⁺) and calcium contents (Ca²⁺). On the other hand, a highly negative correlation of PH with water potential (Ψw), osmotic potential (Ψs), glycinebetaine (GB), proline, melondialdehyde (MDA), sodium contents (Na⁺) and chloride (Cl⁻) was observed. There is a highly positive and significant correlation between SDW and RDW, chl., Pn, gs, RWC, Ψp, K⁺ and Ca²⁺ while negative correlation with Ψw, Ψs, GB, proline, MDA, Na⁺ and Cl⁻. Moreover RDW also showed positive correlation
Table 4.2 Correlation matrix among various attributes of tested eggplant genotypes

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Values indicate Pearson’s correlation coefficients; Values in bold are different from 0 with a significance level alpha=0.05

PH (plant height), SDW (shoot dry weight), RDW (root dry weight), Chl. (chlorophyll), Pn (photosynthetic rate), gs (stomatal conductance), RWC (relative water contents), Ψp (turgor pressure), Ψw (water potential), Ψs (osmotic potential), GB (glycinebetaine), MDA (melondialdehyde), K⁺ (potassium contents), Ca²⁺ (calcium contents), Na⁺ (sodium contents) and Cl⁻ (chloride).
with chl., Pn, gs, RWC, Ψp, K⁺ and Ca²⁺ while negative with Ψw, Ψs, proline, MDA, Na⁺ and Cl⁻. There is highly positive correlation between chlorophyll and Pn, gs, RWC, Ψp, K⁺ and Ca²⁺ whereas negative with Ψw, Ψs, Gb, proline, MDA, Na⁺ and Cl⁻. Pn had positive and significant correlation with gs, RWC, Ψp, K⁺ and Ca²⁺ but negative with Ψw, Ψs, MDA, Na⁺ and Cl⁻. Moreover gs had significantly positive correlation with RWC, Ψp, K⁺ and Ca²⁺ while negative with Ψw, Ψs, Gb, proline, MDA, Na⁺ and Cl⁻. However, RWC had positive correlation with Ψp, K⁺ and Ca²⁺ but negative with Ψw, Ψs, Gb, proline, MDA, Na⁺ and Cl⁻. Ψp also had positive correlation with K⁺ and Ca²⁺ while negative with Ψw, Ψs, Gb, proline, MDA, Na⁺ and Cl⁻. Na⁺ revealed positive correlation with Ψw, Ψs, Gb, proline, MDA and Cl⁻ and highly negative with PH, SDW, RDW, chl., Pn, gs, RWC, Ψp, K⁺ and Ca²⁺. It can be postulated that Na⁺ had negative correlation with growth, physiological, water relations (RWC and Ψp) and ionic attributes and positive with biochemical attributes.

**Conclusion**

Salinity stress had significant depressing effect on growth, water relations, gas exchange characteristics and biochemical parameters of eggplant. Salinity stress had a positive effect on WUE, Proline and glycinebetaine. Salinized plants of both eggplant cultivar exhibited maximum enzymatic activities (SOD, CAT and POD) at higher salinity levels. Salt tolerance potential of eggplant had direct link with the concentration of antioxidants and compatible organic solutes accumulations as Saadia (tolerant) exhibited maximum concentrations of these indicators. Salt tolerance potential had strong relationship with osmotic adjustment as salt tolerant (Saadia) cultivar demonstrated efficient osmotic adjustment than non-tolerant (Black Beauty) cultivar under saline environments. Sensitive (Black Beauty) accumulated high ratios of toxic ions (Na⁺ and Cl⁻) whereas low concentration of beneficial ions (K⁺ and Ca²⁺) in leaves than tolerant (Saadia) cultivar. Hence it could be concluded that, tolerant plants had ability to cope with excessive salts in soil solution. Salt stress had significant positive effect on proline contents, water use efficiency, antioxidant enzyme activities and ionic (Na⁺ and Cl⁻) contents. Hence these attributes could be marked as indicators of salt stress.
4.3 Experiment-III: Optimizing the foliar application of chitosan levels for the enhancement of salinity tolerance in eggplant

4.3.1 Plant height (cm)
Diverse chitosan levels were applied as a foliar spray to salt tolerant (Saadia) and salt sensitive (Black Beauty) eggplant genotypes under salinity stress conditions to find out the best level of chitosan for salt tolerance induction in eggplant. The interaction effect among chitosan treatments and genotypes was found to be significant for plant height (Appendix 11). The maximum plant height was observed under no salinity stress in Saadia as well as in Black Beauty genotype. The minimum plant height was observed in plants of Black Beauty (salt sensitive genotype) under 9 dS m\(^{-1}\) salinity stress with no chitosan application. Among the chitosan treatments, increase in plant height was observed in both the eggplant genotypes under saline conditions, as the maximum plant height was noted in Saadia (salt tolerant genotype) with the application of chitosan treatment @ 150 mg L\(^{-1}\) under 9 dS m\(^{-1}\) salinity stress (Fig. 4.3.1).

4.3.2 Root length (cm)
Interaction between chitosan treatments and genotypes was statistically significant in case of root length (Appendix 11). The maximum root length was noted under controlled conditions in Saadia (salt tolerant genotype) which was statistically similar with Black Beauty (salt sensitive genotype). The minimum root length was noted in Black Beauty (salt sensitive genotype) under saline conditions. Similarly, an increase in root length was observed with the application of different chitosan treatments under saline conditions in both the eggplant genotypes, while Saadia genotype gave the maximum root length with the application of 150 mg L\(^{-1}\) chitosan which was statistically similar with other chitosan levels (Fig. 4.3.2).

4.3.3 Shoot fresh weight (g)
Shoot fresh weight of Saadia (salt tolerant genotype) was the maximum under control treatment which was statistically same with Black Beauty (salt sensitive genotype) and the lowest was in Black beauty (salt sensitive genotype) under saline conditions. A significant interaction was expressed by eggplant genotypes and chitosan treatments (Appendix 11). Chitosan @ 150 mg L\(^{-1}\) was effective to mitigate the effect of salinity by exhibiting the maximum shoot fresh weight in both tolerant and sensitive eggplant genotypes (Fig 4.3.1).
Figure 4.3.1 Effect of chitosan foliar application on plant height, root length and shoot fresh weight of eggplant genotypes under salinity stress at $P \leq 0.05$. The values are means of four replicates $\pm$ standard error (SE).
4.3.4 Root fresh weight (g)

The chitosan treatments exhibited significant variations for root fresh weight. Control treatment was at top regarding the maximum root fresh weight. The lowest root fresh weight was observed under saline conditions in sensitive Black Beauty. Increase in root fresh weight was noted in both the eggplant genotypes in response to chitosan under saline conditions. The maximum root fresh weight was observed in tolerant Saadia with the application of 150 mg L\(^{-1}\) of chitosan under saline conditions of 9 dS m\(^{-1}\) which was statistically similar with salinity and other chitosan treatments (100, 125, 175 and 200 mg L\(^{-1}\)). Sensitive Black beauty also gave maximum root fresh weight with 150 mg L\(^{-1}\) foliar chitosan treatment under saline conditions which was statistically similar with 175 mg L\(^{-1}\) chitosan treatments, but different from other treatment (Fig. 4.3.1). Variation between the both genotypes was highly significant as tolerant Saadia had significantly higher root fresh weight than sensitive Black Beauty (Appendix 12). The interaction between genotypes and chitosan treatments was significant.

4.3.5 Shoot dry weight (g)

The interactive effect of genotypes and chitosan treatments was significant for shoot dry weight (Fig. 4.3.2). Tolerant Saadia exhibited the maximum shoot dry weight under control conditions which was statistically similar with sensitive Black Beauty and the minimum shoot dry weight was observed in sensitive Black Beauty under saline conditions. Tolerant Saadia and sensitive Black Beauty gave the maximum shoot dry weight with the application of 150 mg L\(^{-1}\) chitosan under saline conditions in comparison to their chitosan and salinity treatments (Appendix 12).

4.3.6 Root dry weight (g)

Significant interaction was observed in genotypes and chitosan treatments for root dry weight (Appendix 12). Variation between the both genotypes was highly significant as tolerant Saadia had significantly higher root dry weight than sensitive Black Beauty. The highest root dry weight was recorded in tolerant Saadia followed by sensitive Black Beauty under control conditions and the lowest was recorded in sensitive Black Beauty under saline conditions. Chitosan @ 150 mg L\(^{-1}\) gave the highest root dry weight in both eggplant genotypes (Fig. 4.3.2).

Conclusion

Chitosan @ 150 mg L\(^{-1}\) was found to be the best for salt tolerance induction in eggplant. Salt tolerant cultivar was more responsive to chitosan application than sensitive.
Figure 4.3.2 Effect of chitosan foliar application on root fresh weight, shoot and root dry weight of eggplant genotypes under salinity stress at $P \leq 0.05$. The values are means of four replicates ± standard error (SE).
4.4 Experiment-IV Effects of chitosan on physiological, water relations, biochemical, ionic and yield attributes of salt sensitive and salt tolerant eggplant genotypes under saline and non-saline conditions.

4.4.1 Leaf water relations

4.4.1.1 Leaf water potential
Salt stress considerably reduced the water potential (Ψw) of salt-tolerant and sensitive eggplant genotypes (Fig. 4.4.1). The noticeably lower Ψw was noted in plants supplied with chitosan (Ch) under normal and salt stress conditions. The genotype Saadia showed considerably higher Ψw than Black Beauty. A highly significant interaction was recorded between genotype (G) and salinity stress (S), maximum Ψw was noted in genotype Saadia under normal condition and minimum in genotype Black Beauty exposed to salt stress. Significant interaction (S × Ch) was recorded and considerably lower Ψw was observed in plants exposed to chitosan under salt stress. The two way interaction (G × Ch) was also found to be significant (Appendix 13) and minimum Ψw was observed in genotype black beauty supplied with chitosan, however maximum was observed in untreated genotype Saadia.

4.4.1.2 Leaf osmotic potential
Osmotic potential (Ψs) of both the genotypes considerably (P≤0.001) reduced by salinity stress. Application of chitosan also reduced Ψs of salt tolerant and sensitive genotypes under both normal and salt stress conditions (Fig. 4.4.1). The genotype Saadia maintained considerably higher Ψs than that of Black Beauty. A highly significant three way interaction (S × Ch × G) was recorded (Appendix 13). A maximum Ψs was noted in genotype Saadia grown under normal conditions without chitosan application and minimum in genotype Black Beauty exposed to chitosan under salt stress. The considerably higher Ψs was observed in genotype Saadia supplied with chitosan under salinity stress as compared untreated genotype Black Beauty.

4.4.1.3 Leaf turgor potential
Plants exposure to salt stress showed significant reduction in turgor potential (Ψp) as compared to normal conditions. The significantly higher Ψp was noted in plants exposed to chitosan as compared to untreated control. Both the eggplant genotypes varied significantly in terms of Ψp. The considerably higher Ψp was noted in salt-tolerant genotype Saadia than in salt sensitive Black Beauty. The significant interaction was recorded among salinity stress (S), genotype (G) and chitosan (Ch) for this variable.
Figure 4.4.1 Effect of chitosan foliar application on water relations of eggplant genotypes under saline and non-saline conditions at $P \leq 0.05$. The values are means of four replicates $\pm$ standard error (SE).
A maximum $\Psi_p$ was observed in genotype Saadia exposed to chitosan under normal conditions; however minimum was noted in salt stressed Black Beauty without chitosan supply. Considerably higher $\Psi_p$ was also noted in genotype Saadia foliarly treated with chitosan under salt stress (Fig. 4.4.1).

### 4.4.1.4 Relative water contents

Statistical analysis regarding relative water contents (RWC) indicated a highly significant effect of salinity stress on salt-tolerant and sensitive eggplant genotypes. Salt stress markedly reduced the plant RWC as compared to normal conditions whereas, chitosan application substantially improved the water status of both the genotypes under normal and salinity stress (Fig. 4.4.1). Genotype Saadia maintained considerably higher RWC than that of Black Beauty. The non-significant interaction was recorded among all of the treatment combinations (Appendix 13).

### 4.4.2 Gas exchange characteristics

#### 4.4.2.1 Net photosynthetic rate ($P_n$)

The net photosynthetic rate ($P_n$) of salt tolerant and sensitive eggplant genotypes was significantly reduced by salinity stress, while chitosan application markedly improved the $P_n$ rate under both normal and salt stress conditions (Fig. 4.4.2). Salt tolerant genotype Saadia exhibited significantly higher $P_n$ than that of salt sensitive Black Beauty. Significant interaction was recorded between salinity stress (S) and chitosan (Ch) for $P_n$ (Appendix 14). The maximum $P_n$ was recorded in plants supplied with chitosan under normal conditions, however minimum was observed in untreated salt stress. The significantly higher $P_n$ was also observed in plants supplied with chitosan under salt stress as compared to control.

#### 4.4.2.2 Stomatal Conductance ($g_s$)

Salt stress significantly reduced the stomatal conductance ($g_s$) of both the genotypes, whereas application of chitosan considerably improved the $g_s$ of salt sensitive and tolerant genotypes under both normal and salt stress conditions (Fig. 4.4.2). The highest order interaction was significant among salinity stress (S), chitosan (C) and genotypes (G) for stomatal conductance (Appendix 14). Maximum $g_s$ was recorded in genotype Saadia sprayed with chitosan under normal conditions, significantly lower was observed in salt sensitive genotype Black Beauty exposed to salt stress without chitosan application. The genotype Saadia and Black Beauty performed equally under salt stress supplied with chitosan.
Figure 4.4.2 Effect of chitosan foliar application on gas exchange characteristics of eggplant genotypes under saline and non-saline conditions at $P \leq 0.05$. The values are means of four replicates ± standard error (SE).
4.4.2.3 Transpiration rate (E)
Data regarding transpiration rate (E) indicated a highly significant effect of salt stress on both genotypes. Plants exposed to salt stress significantly reduced the E, however application of chitosan notably improved this variable in both the genotypes (Fig. 4.4.2). Genotype Saadia showed pronounced increase in E as compared to Black Beauty under normal and salt stress conditions. Two way interactions (G × S) was found to be significant for transpiration rate (Appendix 14). Both genotypes performed equally under normal conditions, however significantly higher E was recorded in salt tolerant genotype Saadia as compared to salt-sensitive Black Beauty.

4.4.2.4 Water use efficiency (WUE)
Water use efficiency (WUE) of salt-tolerant and sensitive eggplant genotypes was significantly increased by salt stress. Foliar treatment of chitosan also significantly improved the WUE of both genotypes under normal and salt stress conditions. Both the genotypes also differed significantly for WUE and markedly higher were observed in genotype Saadia under both normal and salt stress conditions (Fig. 4.4.2). The significant interaction (S × Ch × G) was recorded for this variable (Appendix 15) and maximum WUE was observed in Black Beauty foliarly treated with chitosan under salt stress and minimum in untreated Black Beauty.

4.4.2.5 Chlorophyll index (SPAD values)
Measurement of chlorophyll index is proportionate to the amount of chlorophyll contents present in the leaf. The chlorophyll index of both the eggplant genotypes was considerably reduced by salt stress. Chitosan application noticeably improved the amount of chlorophyll in both the genotypes under normal and salt stress conditions. Chlorophyll index of both the eggplant genotypes did not influence significantly, however significant interaction was recorded between genotype (G) and salt stress (S) (Appendix 15). Maximum chlorophyll index was observed in Black Beauty under normal conditions and minimum was observed in similar genotype exposed to salinity stress (Fig. 4.4.3).

4.4.3 Osmoprotectants
Accumulation of low molecular weight organic osmolytes viz. proline (PRO) and glycinebetaine (GB) in plant leaf tissues suggest that osmoregulation may increase salt tolerance of eggplant genotypes.
4.4.3.1 Proline
Data regarding proline accumulation indicated a highly significant effect of salinity stress. Its accumulation increased fivefold in both the genotypes under salt stress conditions. Foliar chitosan markedly improved the accumulation of proline in salt-tolerant and sensitive genotypes of eggplant under normal and salt stress conditions (Fig. 4.4.3). Salt tolerant genotype (Saadia) showed marked increase in accumulation of proline than salt sensitive Black Beauty. Two (S × G, S × Ch, G × Ch) and three way (S × Ch × G) interactions were found to be significant for leaf proline contents (Appendix 16). Maximum proline accumulation was recorded in the genotype Saadia foliar treated with chitosan under salt stress, however minimum was observed in untreated Black Beauty.

4.4.3.2 Glycinebetaine (GB)
Accumulation of glycinebetaine (GB) greatly increased in both the eggplant genotypes exposed to salt stress. The significantly higher GB contents were also recorded in both salt tolerant and sensitive eggplant genotypes exposed to chitosan under normal and salt stress conditions (Fig. 4.4.3). Genotype Saadia maintained considerably higher contents of endogenous-GB than Black Beauty. Significant interactions were recorded for leaf GB contents (Appendix 16) and maximum accumulation was noted in genotype Saadia foliarly treated with chitosan under salt stress, nevertheless minimum was observed in untreated Black Beauty.

4.4.3.3 Lipid peroxidation
The accumulation of malondialdehyde (MDA) contents is effective indicator of lipid peroxidation and considerably increased by salinity stress. Foliar chitosan treatment considerably reduced the MDA contents (Fig. 4.4.3). Both the genotypes were also different in terms of MDA contents and considerably higher were noted in salt sensitive genotype Black Beauty as compared to Saadia. Significant interaction (S × Ch × G) was recorded for MDA contents (Appendix 16) and considerably lower was recorded in genotype Saadia exposed to chitosan under normal conditions which was statistically at par with genotype Black Beauty and Saadia with or without chitosan supply. The maximum MDA contents were recorded in untreated genotype Black Beauty grown under salinity conditions (Fig. 4.4.3).
Figure 4.4.3 Effect of chitosan foliar application on chlorophyll index and biochemical attributes of eggplant genotypes under saline and non-saline conditions at $P \leq 0.05$. The values are means of four replicates $\pm$ standard error (SE).
4.4.4 Antioxidants
The activation of antioxidant defense system in plants to combat oxidative impairment indicates stress-tolerance potential in plants.

4.4.4.1 Superoxide dismutase (SOD)
The activity of SOD was significantly increased by salinity stress. Application of chitosan also notably improved the SOD activity of both genotypes under normal and salt stress conditions (Fig. 4.4.4). Significant interaction was recorded between genotype (G) and chitosan (Ch) for SOD activity and maximum was observed in genotype Saadia supplied with chitosan. The significantly higher activity of this enzyme was also observed in genotype Saadia as compared to Black Beauty. Two way interactions (S × G) were significant for SOD activity (Appendix 17) and maximum was noted in genotype Saadia exposed to salt stress and minimum in Black Beauty under normal conditions.

4.4.4.2 Catalase (CAT)
Plants exposure to salinity stress showed significant increase in CAT activity. Foliar chitosan supply also considerably improved the activity of this enzyme in both the genotypes under normal and salt stress conditions. Considerably higher CAT activity was noted in genotype Saadia than in Black Beauty (Fig. 4.4.4). The significant interaction (S × Ch × G) was observed for this variable (Appendix 17). The significantly higher CAT activity was observed in genotype Saadia exposed to chitosan under salt stress.

4.4.4.3 Peroxidase (POD)
Imposition of plants to salinity stress showed considerable improvement in peroxidase activity. Foliar application of chitosan notably improved the POD activity in salt tolerant and sensitive eggplant genotypes under control and saline conditions (Fig. 4.4.4). Genotype Saadia showed pronounce increase in POD activity as compared to Black Beauty. Two and three way interactions were found to be significant among all of the treatment combinations (Appendix 17). Saadia genotype is more responsive to chitosan supply under salt stress and showed maximum POD activity, however, minimum was noted in untreated Black Beauty. The sensitive eggplant genotype (Black Beauty) treated with chitosan even performed better under salt stress.
Figure 4.4.4 Effect of chitosan foliar application on enzymatic activity of eggplant genotypes under saline and non-saline conditions at $P \leq 0.05$. The values are means of four replicates ± standard error (SE).
4.4.5 Accumulation of inorganic ions

4.4.5.1 Leaf calcium

Data about leaf calcium (Ca\(^{2+}\)) contents indicated a highly significant effect of salt stress. Salt stress significantly reduced the uptake of Ca\(^{2+}\), however chitosan application considerably improved its concentration in leaf tissues of both the eggplant genotypes (Fig. 4.4.5). Both the genotypes also differed significantly for leaf Ca\(^{2+}\) contents and noticeably higher were noted in genotype Saadia as compared to Black Beauty. Significant interaction (G × S, G × Ch, S × Ch) was noted for this variable (Appendix 18). Genotype Saadia performed well under salinity stress and chitosan supply in terms of Ca\(^{2+}\) uptakes as compared to Black Beauty. Plants supplied with chitosan under salinity stress also improved the leaf calcium contents as compared to untreated salt-stressed plants.

4.4.5.2 Leaf potassium (K\(^{+}\)) contents

Leaf potassium (K\(^{+}\)) contents of salt-tolerant and sensitive eggplant genotypes were significantly decreased by salt stress; however chitosan treatment considerably improved its concentration in both the genotypes (Fig. 4.4.5). Both the genotypes also varied significantly for leaf K\(^{+}\) contents and markedly higher were noted in genotype Saadia as compared to Black Beauty. Two way interactions (G × S, S × Ch) were found to be significant for this variable (Appendix 18). Genotype Saadia showed improved leaf K\(^{+}\) contents under salinity stress as compared to Black Beauty. Plants supplied with chitosan under salinity stress also improved the uptake of K\(^{+}\) as compared to untreated control.

4.4.5.3 Leaf chloride

Leaf chloride (Cl\(^{-}\)) contents of both the eggplant genotypes were significantly reduced by salt stress; however chitosan application considerably reduced the uptake of Cl\(^{-}\) in both the genotypes (Fig. 4.4.5). Significant variation was also recorded in both the genotypes for leaf Cl\(^{-}\) contents and considerably lower were noted in genotype Saadia as compared to Black Beauty. A higher order interaction (G × S × Ch) was found to be significant for this variable (Appendix 18). Maximum leaf Cl\(^{-}\) concentrations were maintained by the genotype Black Beauty and minimum was noted Saadia treated with chitosan under normal conditions.

4.4.5.4 Leaf sodium (Na\(^{+}\)) contents

Plants exposure to salt stress showed significant increase in leaf sodium (Na\(^{+}\)) contents, however foliar treatment of chitosan significantly reduced the uptake of Na\(^{+}\) in both the
Figure 4.4.5 Effect of chitosan foliar application on leaf ionic contents of eggplant genotypes under saline and non-saline conditions at $P \leq 0.05$. The values are means of four replicates ± standard error (SE).
genotypes (Fig. 4.4.5). Significant difference was also noted in both genotypes for leaf Na\(^+\) contents and substantially lower were noted in genotype Saadia as compared to Black Beauty. Three way interactions (G × S × Ch) were found to be significant for this variable (Appendix 18). Considerably lower leaf Na\(^+\) concentration was maintained by the genotype Saadia exposed to chitosan under normal conditions and maximum was observed in untreated salt stressed Black Beauty.

4.4.6 Yield attributes

4.4.6.1 Number of fruits per plant
Salinity has no influence on number of fruit in both the eggplant genotypes (Fig 4.4.6). Chitosan application significantly improved the number of fruits in both salt tolerant and sensitive eggplant genotypes. Genotypes varied among themselves in term of fruit number. Higher numbers of fruits were noted in genotype Saadia as compared to Black Beauty. Non-significant interactions (two and three way) were noted for this variable (Appendix 19).

4.4.6.2 Fruit diameter (cm)
Fruit diameter of both the eggplant genotypes was significantly decreased by salt stress and significantly increased by chitosan application (Fig 4.4.6). Both the genotypes also varied significantly for fruit diameter and noticeably higher were noted in genotype Saadia as compared to Black Beauty. Two way interactions (S × Ch) were found to be significant for this variable (Appendix 19). The maximum root fruit diameter was noted under normal conditions with chitosan application and the minimum was noted under salt stress without chitosan application.

4.4.6.3 Average fruit weight (g)
Average fruit weight of salt tolerant and sensitive eggplant genotypes was significantly reduced by salt stress, but chitosan application considerably improved the average fruit weight in both the genotypes (Fig 4.4.6). Significant difference was also noted in both the genotypes for average fruit and considerably lower was noted in genotype Black Beauty than Saadia. Significant interaction (S × Ch) was noted for this variable (Appendix 19). The highest average fruit weight was maintained under normal conditions with chitosan application and the lowest was noted under stressed conditions without chitosan supply.
Figure 4.4.6 Effect of chitosan foliar application on yield attributes of eggplant genotypes under saline and non-saline conditions at $P\leq0.05$. The values are means of four replicates ± standard error (SE).
4.4.6.4 Yield per plant (g)
Plants under salt stress exhibited substantial decrease in yield, however foliar supply of chitosan considerably improved the yield per plant in both the genotypes (Fig 4.4.6). Significant difference was also noted in both the genotypes for fruit yield and markedly higher was noted in genotype Saadia as compared to Black Beauty. Two way interactions (S × Ch) were found to be significant for this variable (Appendix 19). Considerably lower fruit yield per plant was maintained by both the genotypes under salinity stress without chitosan application and the higher yield per plant was observed with chitosan supply under normal conditions.

4.4.7 Correlation matrix among various attributes of salt tolerant and salt sensitive eggplant genotypes

The correlation matrix table is explaining that yield had positive correlation with AFW (average fruit weight), FD (fruit diameter), chl. (chlorophyll), Pn (photosynthetic rate), gs (stomatal conductance), RWC (relative water contents), Ψp (turgor pressure), K+ (potassium contents) and Ca2+ (calcium contents) while negative correlation with Ψw (water potential), MDA (melondialdehyde), Na+ (sodium contents) and Cl− (chloride). Moreover Pn has positive correlation with yield, chl., gs, RWC, Ψp, K+ and Ca2+ and negative with MDA, Na+ and Cl−. Likewise RWC had positive correlation with yield, chl., gs, Ψp, K+ and Ca2+ while negative with Ψw, MDA, Na+ and Cl−. Na+ had positive correlation with Ψw, proline, MDA and Cl− whereas negative with yield, chl., Pn, gs, RWC, Ψp, K+ and Ca2+. It can be postulated from correlation matrix table that chl., Pn, gs, RWC, Ψp, K+ and Ca2+ might be directly linked with growth and yield attributes. Likewise it can be concluded that salt stress significantly affected various physiological, water relations, ionic and biochemical variables either positively or negatively. On the other hand it may be assumed that proline, Ψw, MDA and Cl− are involved in stress tolerance indication as they are directly proportional to salt stress.
Table 4.4 Correlation matrix among various attributes of salt tolerant and salt sensitive eggplant genotypes

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<th></th>
<th>Yield</th>
<th>AFW</th>
<th>FD</th>
<th>Chl.</th>
<th>Pn</th>
<th>Gs</th>
<th>RWC</th>
<th>Ψp</th>
<th>Ψw</th>
<th>Ψs</th>
<th>Proline</th>
<th>GB</th>
<th>MDA</th>
<th>K⁺</th>
<th>Ca²⁺</th>
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Values indicate Pearson’s correlation coefficients;
Values in bold are different from 0 with a significance level alpha=0.05
AFW (average fruit weight), FD (fruit diameter), chl. (chlorophyll), Pn (photosynthetic rate), gs (stomatal conductance), RWC (relative water contents), Ψp (turgor pressure), Ψw (water potential), Ψs (osmotic potential), GB (glycinebetaine), MDA (melondialdehyde), K⁺ (potassium contents), Ca²⁺ (calcium contents), Na⁺ (sodium contents) and Cl⁻ (chloride).
Conclusion

Chitosan improved the salt tolerance potential of both eggplant cultivars by improving the water relations via increasing turgor pressure and relative water contents. Chitosan also induced salt resistance in tested eggplant cultivars by increasing enzymatic activities (SOD, CAT and POD) and high osmolyte accumulation. Chitosan improved the yield of eggplant cultivars by increasing number of fruits, fruit diameter and fruit weight under saline and non-saline environments. Salt stress reduced the yield of eggplant cultivars by reducing fruit diameter and average fruit weight. Salinity stress (9 dS m\(^{-1}\)) had no influence on number of fruits of salt sensitive (Black Beauty) and salt tolerant (Saadia) eggplant genotypes.
DISCUSSION

Eggplant is a very important crop around the globe but salt stress severely affects its productivity. All agricultural crops and especially vegetable crops are at serious risk due to increasing salts level of irrigated farm lands. Although, Pakistan has world’s largest canal irrigation system but still our farmers use underground brackish water to cope crop water requirements in summer. Both canal and tube well water used for irrigation purpose adds up the salts in the farming lands. The outcomes of current investigations may help vegetable growers of Pakistan by minimizing salinity related yield losses of eggplant in moderately saline farming lands/marginal lands. Salt tolerant genotypes can be cultivated in salt affected fields and foliar application of chitosan (150 mg L$^{-1}$) alleviates the adverse effects of salinity.

**Experiment 1**

The attributes like plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), shoot fresh weight stress tolerance index (SFWSI), root fresh weight stress tolerance index (RFWSI) and plant dry matter stress tolerance index (PDMSI), leaf sodium (Na$^{+}$) and potassium (K$^{+}$) contents were observed to estimate the effects of salinity stress on eggplant genotypes. Stress tolerance indices are worthy tools to understand stress tolerance potential of plants (Ashraf et al., 2008; Kausar et al., 2012). Early growth stages of eggplant are more susceptible to salinity (Akinci et al. 2004). Genotypes were considered tolerant and sensitive ones on the basis of growth and ionic attributes. Screening for salinity tolerance at seedling stage has many benefits such as being rapid and efficient, not more expensive, less laborious and highly reliable than investigations at advance growth stages (Dasgan et al., 2002).

The results of the present study revealed that genotypes behaved differently in all the attributes studied under different salinity stress levels and eggplant genotypes differed for salt tolerance potential. It was observed that most of genotypes did not show satisfactory performance at higher salinity levels (12 and 15 dS m$^{-1}$) in contrast to tolerant. The findings of this study indicated that response of genotypes was highly variable for salt tolerance indices. Significant decrease in all salt tolerance indices was observed with increasing salinity levels. The maximum PHSI was displayed by Saadia, followed by Qaisar, while the minimum PHSI was exhibited by Black Beauty and Dilkashin under saline regimes. The highest RLSI was noted in Saadia, followed by Qaisar, Bemisal and
Black Oval, while the lowest RLSI in case of Black Beauty, WER, Dilnashin, Black Boll and PPR under saline conditions. Similarly, Saadia performed better in case of SFWSI, followed by Qaisar and Black Beauty performed poor. The performance of Saadia, followed by Bemisal for RFWSI was adequate, while the minimum RFWSI was occupied by Black Beauty, Dilnashin and Black Boll. Moreover, the results for PDMSI of genotypes were alike to RFWSI. These huge differences reflect the ability of salt tolerant genotypes to produce more biomass under salt stress as compared to sensitive genotypes. Decline of growth is a common effect of salinity stress in most of plants (Akram et al., 2012). These findings are in accordance with results reported by Akinci et al. (2004) Unlukara et al. (2010) and Shaheen et al. (2013) for growth attributes of eggplant under saline environments. All plants including vegetable crops reduce growth, with corresponding development of small and stunted leaves and reduced plant height (Shannon and Grieve 1999). Genotype with higher salt tolerance indices than others under salinity stress depicts their maximum salt tolerance potential than sensitive ones.

Moreover, leaf sodium (Na⁺) contents were significantly increased with increasing salinity stress in all eggplant genotypes. Significant variation among eggplant genotypes was noted in term of leaf Na⁺ contents. The maximum leaf Na⁺ percentage was observed in Black Beauty, followed by Black Boll and the minimum in Saadia under saline conditions. Increasing salinity stress significantly reduced the leaf K⁺ percentage in all eggplant genotypes. The highest percentage reduction in leaf K⁺ was recorded in Black Beauty, followed by black Boll and the least in Saadia, followed by Black Oval, Bemisal and PPR Differences in leaf Na⁺ and K⁺ of eggplant genotypes might be due to their genetic variability and root permeability for these ions. The results revealed that genotypes that were poor in growth performance accumulated more Na⁺ and less K⁺ contents in leaf under saline regimes in comparison to best performing genotypes in term of growth attributes. Results also indicate that Na⁺ inhibited K⁺ accumulation in leaves. So, it can be concluded that higher level of Na⁺ ions in irrigation water/rhizosphere may reduce growth attributes and hinder leaf K⁺ contents in eggplant genotypes. Moreover, increasing salinity stress negatively affected all above discussed attributes except Na⁺ contents of leaves. It is concluded that high leaf K⁺ and low leaf sodium designated the salt tolerance potential of eggplant genotypes so these can be used as effective characterizing tool (Magio et al., 2007). Hence, the tested eggplant genotypes were also ranked into salt tolerant and non-tolerant on the basis of these important ionic indicators of salinity stress. Potassium ion (K⁺) deficiency might be due to competitive interaction
of ions or by changing the ion selectivity of membrane in eggplant under salinity stress (Romero et al., 1997; Yasar et al., 2006). These findings are in agreement with Savvas and Lenz (1994).

The outcomes of the study revealed Saadia as the most tolerant genotype. Qaisar, Black Oval, and Bemisal also performed better than other genotypes under salt stress conditions and might be considered as moderately salinity tolerant genotypes. Black Beauty was found to be the most sensitive genotype under saline environments (Table 4.1.3). The variation in response among eggplant genotypes under saline conditions may be due to genetic variability (Selvaraj 2011). These variations in eggplant genotypes under salt stress condition can be helpful in development of new genotypes (Sekara et al., 2007).

**Experiment 2**

Another experiment was conducted after screening thirteen genotypes of eggplant for salt tolerance, for understanding the changes in physiological, biochemical, enzymatic, ionic and growth related attributes of salt tolerant (Saadia) and sensitive (Black Beauty) genotypes. Both the genotypes of eggplant were tested against different salinity levels.

Results indicated that salt stress substantially reduced growth indicators such as plant height, root length, shoot and root fresh weights (Fig. 2.2.1.1) as well as root and shoot dry weights (Fig. 2.2.1.2) separately in both the genotypes. Black Beauty was found more sensitive to salt stress. Plant height is purely under genetic control but abiotic stresses such as salinity has negative influence on the growth as it is evident from the results (Fig. 2.2.1.1). A negative correlation was observed between growth attributes and increasing salinity stress in both the genotypes. The poor performance of Black Beauty in these attributes may be due to failure in osmotic adjustment, salt induced nutritional deficiencies, disturbances in metabolic pathways and ion toxicity. Sodium and chloride ions also damage photosynthetic apparatus by reducing photosynthetic enzymes activities. Salinity also reduces cell division and elongation due to its osmotic effect which result into stunted growth. Salt tolerant Saadia was less affected by increasing salinity stress in terms of growth parameters. It is because tolerant (Saadia) was able to accumulate less toxic ions in its upper parts. Black Beauty was incapable to maintain its water status by keeping down its turgor potential under stressful conditions. Saadia was found to be efficient in osmotic adjustment. Reports of Baoli et al. (2010) and Wu et al. (2012b) on eggplant under salinity stress are in agreement with this study.

Salt stress leads to osmotic stress which disrupts plant water relations and same is the outcome of present study. As salinity causes osmotic stress to the plant therefore it is
important to maintain the turgor potential of cell. Salinity stress considerably inhibited the water potential ($\Psi_w$), osmotic potential ($\Psi_s$), turgor potential ($\Psi_p$) and relative water contents (RWC) in both tested eggplant genotypes (Fig. 4.2.3). Findings of Poltronieri et al. (2011) and Hegazi et al. (2014) showed that salinity stress initiated the marked reduction in eggplant water relation and RWC; results of current study proved the former reports. As tolerant genotype (Saadia) deposited less concentration of toxic ions in its leaves, so exhibited lowest decrease in its water relations and maintained highest $\Psi_p$. Whereas, high amount of toxic ions were accumulated in the leaves of sensitive genotype (Black beauty), which obviously decreased the $\Psi_w$, $\Psi_s$, $\Psi_p$ and RWC. As concentration of salts increased in root zone of plants, $\Psi_w$ was decreased. The decline in $\Psi_w$ of soil may cause a condition called as physiological drought (Zrig et al., 2015). In this condition plant can’t uptake the available water from its root zone. Findings of present study illustrate that decrease in $\Psi_w$, $\Psi_s$ and $\Psi_p$ of plant under salinity stress might be due to either increase in dissolved solutes or water depletion or combination of both. The $\Psi_w$ of eggplant genotypes was decreased, might be due to increase in osmolytes such as glycinebetaine and proline observed in this study for osmotic adjustment. Osmolytes play a necessary role in osmotic adjustment by maintaining $\Psi_p$ of plants under saline regimes (Gao et al., 2014). The $\Psi_p$ helps guard cell to work normally in plants and efficiently normalize the gaseous exchange under salinity stress. Osmolytes regulate stomatal conductance by increasing or decreasing the $\Psi_w/\Psi_s$ in stressed plant (Ashraf and Foolad, 2007; Perez-Perez et al., 2009). Capacity of osmotic adjustment depends upon stress intensity and varies among species and genotypes. Saadia was better in osmotic adjustment and proved to be salt tolerant than Black Beauty. Leaf water potential is connected with plant productivity (Vasquez et al., 2006), turgor potential ($\Psi_p$) and osmotic potential ($\Psi_s$) which specifies that plant genotypes having low $\Psi_s$ and high turgor pressure gave good yield (Ginestar and Castel, 1996). Outcomes of this investigation also showed that eggplant genotype with less decrease in $\Psi_s$ exhibited more fresh and dry weights and higher photosynthesis rate under saline conditions. There are several reports that indicate that lowering of $\Psi_s$ has implication in maintaining the turgor in salinized plants (Hui et al., 2004; Demetriou et al., 2007).

The relative water contents (RWC) are an attribute among water relations, which describes the water status of a plant, basically it indicates the absolute amount of water which is needed by the plant to achieve full artificial saturation. Salt stress caused the marked reduction in RWC of both the eggplant genotypes but tolerant-Saadia experienced
minimum reduction than sensitive Black Beauty. High RWC in the leaves of Saadia showed that it is less suffered from physiological drought due to the better osmotic adjustment potential, so it maintained ample amounts of water in leaves. Whereas, low RWC in the leaves of Black Beauty displayed that it experienced high osmotic stress. Outcomes of this study confirmed the findings of Hegazi et al. (2014) who found significant decrease in RWC in plants growing under saline environments.

Salinity stress substantially reduced photosynthetic rate (Pn), transpiration rate (E) and stomatal conductance (gs) (Fig. 4.2.2.1) as well as chlorophyll index (Fig. 4.2.2.2) of both tolerant and sensitive genotypes but the maximum drastic effect of salinity was observed in Black Beauty (sensitive). The decrease in photosynthetic activity might be attributed to lower stomatal conductance and reduction in transpiration rate. This accumulation distresses the photosynthetic activity either directly or indirectly by reducing the availability of CO₂ in crop plants (Flexas et al., 2007). Salts also affect the photosynthesis by making fluctuations in leaf water relations and osmotic adjustment, which ultimately lead to inhibition in growth. Since, in this study salinity also negatively affected the water relation, so reduction in photosynthetic activity may be attributed to decrease in water potential, osmotic potential and turgor potential. Therefore, in present study, it is apparent that tolerant Saadia genotype with high turgor potential demonstrated the highest photosynthetic rate compared to sensitive Black Beauty. Low salinity stress causes the osmotic stress while higher salinity stress cause Na⁺ and Cl⁻ toxicity which damages the plant cells reported by Munns (2002). Salt stress also influences the photosynthetic activity by adversely disturbing the working of photochemical apparatus (Hura et al., 2007). Reduction in photosynthesis in tested eggplant genotypes could be due to the ionic imbalance, because the availability of K⁺ and Ca²⁺ got decreased due to the antagonistic effect between Na⁺ and K⁺ under saline environments. The K⁺ regulates the turgidity of guard cells for opening and closing of stomata so it has great importance for stomatal conductance. Hence, K⁺ deficiency in leaves, indirectly affect the photosynthetic activity. Thus, higher photosynthetic activity of tolerant Saadia genotype might be positively related with the maximum availability of K⁺ in its leaf tissues, which enabled the efficient gaseous exchange. Whereas, sensitive Black Beauty genotype was high in leaf Na⁺ and low in K⁺ contents and its photosynthetic rate was reduced. Above facts depict that salt induced high osmotic stress, high ion toxicity and less availability of K⁺ are the key factors that may be the cause of reduced photosynthetic activity in tested eggplant genotypes. Thus, low osmotic stress, low ion toxicity, efficient gaseous exchange and
sufficient availability of K\(^+\) made the Saadia genotype, to maintain the highest photosynthetic activity (\(Pn\)) and exhibited high biomass production than sensitive Black Beauty under saline environments. Liu \(et\ al.\) (2007) and Wu \(et\ al.\) (2012b) have also reported similar results regarding photosynthetic rate in eggplant under saline environment.

Stomatal conductance (\(gs\)) is also an essential physiological attribute which is closely linked with photosynthesis and plant biomass. Salinity also severely influences \(gs\) along with other physiological characteristics. Mainly \(gs\) is the speed at which vapor evaporates through stomata; as evaporation increases, leaf conductance will increase. Outcomes of current study presented that NaCl stress reduced \(gs\) in both the eggplant genotypes but the highest reduction was observed for Black Beauty than Saadia. Moisture status of the plant tissues influences \(gs\). A positive relationship was perceived between \(gs\) and \(Pn\) in this study. The genotype Saadia with the high \(gs\) displayed the maximum photosynthetic activity while the genotype Black Beauty with least \(gs\) has low photosynthesis rate.

As, in present investigation salt stress reduced the water status of both the eggplant genotypes so, it may be the cause of declined stomatal conductance (\(gs\)), due to less availability of internal moisture. Saadia maintained higher water status and was also high in \(gs\) characteristic.

Low leaf K\(^+\) concentration is also measured as the reason for reduction in \(gs\) because K\(^+\) regulates the opening and closing of stomata (Matsumoto \(et\ al.\), 2005) and in present study salinity stress significantly reduced the leaf K\(^+\) contents of both the tested genotypes. Consequently, the decline in \(gs\) might be due to the reduction in leaf K\(^+\) contents. As, salinized plants of Saadia demonstrated the minimum decrease in leaf K\(^+\) contents so they sustained the maximum \(gs\) while stressed plants of Black Beauty failed to keep up the leaf K\(^+\) contents, resultantly experienced the maximum reduction in \(gs\).

Salinity stress had a reducing influence on the various physiological aspects of eggplant including \(gs\) (Magio \(et\ al.\), 2007; Poltronieri \(et\ al.\), 2011; Wei \(et\ al.\), 2009).

Meanwhile, in present investigation, salt stress reduced \(gs\) by decreasing RWC and leaf K\(^+\) contents, so the rate of transpiration declined because less water vaporized from the stomata due to reduced moisture status of plant. Salinity reduced moisture availability and RWC in both the tested genotypes due to osmotic stress particularly in case of salt sensitive genotype hence, high reduction of transpiration rate in Black Beauty might be associated with reduced water contents than salt tolerant Saadia. Findings of this study
regarding the transpiration rate are in accordance with the results of Wu et al. (2012b) and Shahbaz et al. (2013).

The amount of water expended for per unit dry biomass production is termed as water use efficiency (WUE) (Monclus et al., 2006). Both the genotypes displayed the improvement in WUE in present study. Tolerant genotype (Saadia) exhibited the maximum WUE than sensitive (Black Beauty). Maintenance of efficient gs and chlorophyll index could be the best suited reason of high WUE in salt tolerant eggplant genotype. As, tolerant Saadia genotype presented the minimum decrease in stomatal conductance and chlorophyll index compared to sensitive Black Beauty so, it has efficient gaseous exchange mechanism, which accelerated the working potential of photosynthetic apparatus Due to enhanced working efficiency, photosynthetic apparatus expeditiously converted the absorbed nutrients and water into food materials which finally increased the plants WUE (Zekri, 1987). Though, the outcomes of this study are disagreeing to the findings of Tattini et al. (1995), they reported that WUE has no relationship with physiological attributes such as photosynthesis rate (Pn), stomatal conductance (gs) and transpiration rate (E). The results of present study, are also contrary with the reports of Katerji et al. (2003), who found non-significant effect of salt stress on WUE in salt tolerant plants while significant reduction in case of sensitive crops. Similarly, Shahbaz et al. (2013) analyzed the eggplant under salt stress and reported a depressing effect of salinity on WUE. But the results of this study are in accordance with the findings of Wu et al. (2012b), who noted a significant increase in WUE in eggplant under salinity stress.

Salinity stress considerably reduced chlorophyll index in both the tested eggplant genotype. Many aspects are involved in the reduction or degradation of chlorophyll under stressed conditions and lipid per oxidation is one of them (Hassine and Lutts, 2010). The decline in chlorophyll index in both tolerant and sensitive eggplant genotypes can be endorsed to high lipid per oxidation under saline regimes. As salt tolerant Saadia has the least lipid peroxidation (MDA), so chlorophyll index was less degraded. Salt sensitive Black Beauty genotype has less chlorophyll due to high lipid per oxidation in its leaves. An enzyme, chlorophyllase is also responsible for declining in the chlorophyll contents and salt stress enhances the activity of this enzyme (Li et al., 2015). Therefore, chlorophyll reduction may also be due to the increased activity of this enzyme in current investigation.

The degradation of chloroplast membrane due to the specific toxicity of Na+ and Cl− cannot be disregarded, since higher the toxic ions in the leaf tissues then more will be the
decrease in chlorophyll contents. Liu et al. (2007) and Shoresh et al. (2011) also reported that decrease in chlorophyll contents in eggplant under salt stress is strongly correlated with the increase in toxic ions into its leaves. Therefore, the variations in chlorophyll index between salt tolerant and sensitive genotypes could be because of this aspect in the current investigation. Therefore, the maximum photosynthetic rate (Pn) of Saadia under saline environments might be due to the less reduction in chlorophyll index.

Salt stress caused an ionic and osmotic effect which results in to oxidative stress and then reduced supply of CO_2 results in carbon reduction in Calvin cycle and ultimately reduction in electron acceptor (NADP^+) in photosynthesis under stressed conditions. The reduction in ferrodoxin during electron transmission in photosynthesis, affects in the generation of superoxide radicals due to the transfer of electrons to oxygen from PS-I by a mechanism, termed as Mehler reaction (Hsu and Kao, 2003). Superoxide radical starts a chain of reactions that generates reactive oxygen species (ROS), which disrupts the metabolic processes inside the cell by oxidative degradation of lipid, nucleic acids and proteins (Munns and Tester, 2008). The ROS also degrade the cell membranes by lipid peroxidation (LPO) in plants under saline regimes (Wang et al., 2003; Wu et al., 2012b). A compound named as malondialdehyde (MDA) is formed during LPO which is end product of LPO and is used for oxidative damage determination (Hajlaoui et al., 2010). Though, salinity stress positively influenced the LPO in both the tested eggplant genotypes, but the maximum LPO was noted for salt sensitive Black Beauty than tolerant Saadia (Fig. 4.2.5). The maximum MDA contents in sensitive genotype is the indication of maximum oxidative injury while, the tolerant genotype resisted the ROS generation, so presented less MDA and consequently minimum LPO. Findings of present study proved that LPO has strong negative relationship with salt tolerance. Findings of present study are in accordance with the reports of Yasar et al. (2013) regarding the LPO in eggplant under salt stress regimes.

Therefore, the generation of ROS species such as superoxide radical (\(^{·}\)O_2), hydroxyl radical (OH\(^·\)) and hydrogen peroxide (H_2O_2) is the common phenomena under salt stress conditions. Subsequently, ROS species cause harm to the photosynthesis and other important macromolecules (Ding et al., 2012; Manar et al., 2013) and disrupt the cellular structure, so their quenching is the common mechanism to reduce the oxidative stress injury (Dai et al., 2009; Wu et al., 2012b) Plants have developed an antioxidant defense system based on many antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) etc. to reduce the oxidative loss under stressed
surroundings especially salt stress (Masood et al., 2006). Antioxidant system of plant maintains the ROS species to at less toxic level inside the cell. The salinized plants of the tested genotypes displayed an improved values of antioxidant enzymes (SOD, CAT and POD) than non-stressed, but the maximum enzymatic activities were recorded in tolerant genotype (Saadia) while it was minimum in sensitive (Black Beauty) (Fig. 4.2.4) in present investigation. Higher antioxidant enzymes (AOE) activities in tolerant Saadia showed that it is well adapted to the saline condition by reducing the ROS. Whereas, Black Beauty failed to adjust itself under salinity stress because of less AOE activities, resulting in high ROS production which lead to the maximum lipid peroxidation and the minimum photosynthesis. A strong correlation was found between AOE activities and salt stress tolerance in this study. Results of this study confirmed the findings of Hegazi et al. (2014). Several former reports are also in agreement with the findings of current investigation (Colville and Smirnoff, 2008; Dai et al., 2009).

Plants have the potential to keep their water absorption ability under stressed conditions such as drought, salinity, cold and high temperature. Excessive salts in soil solution lead to salt induced osmotic stress and ion toxicity. High salts level also cause reduction in the water potential of soil solution and this lower water potential accelerates the synthesis of low molecular weight organic solutes for osmotic adjustment (OA) in the plants under salinity stress conditions (Farkhondeh et al., 2012; Bojorquez-quintal et al., 2014). Eggplant genotypes suffered from osmotic stress under saline environments and salinized plants adopted OA mechanism by attaining different organic osmolytes (proline and glycinebetaine etc) in their tissues. Genotype Saadia with more accumulation of proline and glycinebetaine showed high salt tolerance potential because these osmolytes are involved in the maintenance of turgor potential under salinity stress which regulates the numerous metabolic processes inside plant body. Plants with maximum concentration of these osmolytes are high in OA potential. Glycinebetaine (GB) is a very important osmolyte that plays a vital role in cellular OA and GB performs its role in OA of leaf chloroplasts and protects the thylakoid membranes from the severe influence of salt stress (Chen and Murata, 2011; Kamil et al., 2014).

Proline is another osmolyte, which promotes the deposition of useable nitrogen and improves the membrane stability under saline conditions. Outcomes of present investigation demonstrated that stressed plants of both the tested eggplant genotypes had high ratios of osmoprotectants (proline and glycinebetaine) with respect to non-stressed
plants (Fig. 4.2.5). The salt tolerant genotype (Saadia) exhibited high amount of proline and glycinebetaine contents while it was less in case of sensitive (Black Beauty). The maximum accumulation of osmoprotectants in tolerant genotype is the indication of efficient OA while the minimum increase in osmolytes in salt sensitive genotype is a sign of low OA potential under saline surroundings. It might be also the reason of high salt tolerance of Saadia than Black Beauty. A positive relationship was recognized between amount of osmoprotectants and salt tolerance. Similar findings have been reported by Rajam (1997), Kumar et al. (2006), Abbas et al. (2010) and Shahbaz et al. (2013).

Salt stress also employed a substantial effect on various ionic traits of investigated eggplant genotypes (Fig. 4.2.6). In this study Na$^+$, K$^+$, Ca$^{2+}$, and Cl$^-$ accumulation in leaves were recorded. It was noticed that salinity stress elevated Na$^+$ and Cl$^-$ in leaves but a declining pattern was recorded in case of Ca$^{2+}$ and K$^+$. Both tolerant and sensitive genotypes displayed marked variations regarding ionic aspects. From the results it is evident that salt tolerant genotype (Saadia) displayed the lowest ratios of Na$^+$ and Cl$^-$ in its leaves while it was maximum in the leaves of salt sensitive (Black Beauty). Salt tolerant plants contain less amount of toxic ions in upper parts by adopting a mechanism of deposition of toxic ions in to their roots (Dekoum et al., 2013; Shaheen et al., 2013). Less accumulation of Na$^+$ and Cl$^-$ in leaves of tolerant genotype (Saadia) might be due to aforementioned reason. Toxic ions substantially reduced the photosynthesis rate (Pn) stomatal conductance (gs) and transpiration rate (E) etc in non-tolerant Black Beauty. A negative association was found between concentration of toxic ions (Na$^+$ and Cl$^-$) and dry matter production, and physiological attributes (Pn, gs, E). Although, both tolerant and sensitive genotypes presented the substantial reduction in their K$^+$ and Ca$^{2+}$ ions but tolerant genotype maintained the high concentration of these ions in leaves than sensitive one. Actually, an antagonistic effect subsists between Na$^+$ and beneficial ions (K$^+$ and Ca$^{2+}$) (Unlukara et al., 2010; Kaswala et al., 2012; Farooq et al., 2015), under this influence Na$^+$ inhibits the entry of essential ions from soil solution to the roots, resulting in the reduction of these ions in plant leaves. So, this antagonistic effect may be the cause of reduction in beneficial ions (K$^+$ and Ca$^{2+}$) in tested eggplant genotypes. Outcomes of present study are in agreement with the reports of Elwan et al. (2010) and Oliveira et al. (2011).

**Experiment 3**

Different types of economical and feasible techniques are used to alleviate the harsh effects of salinity stress (Oztekin et al., 2009; Oztekin et al., 2013). These methods
consist of seed priming, plant growth promoting rhizobacteria (PGPR), grafting, exogenous application of plant biostumulants, foliar applications of antioxidants, phytohormones and osmo-protectant (Fahad et al., 2014; Mavi et al., 2014; Semida et al., 2014). Among the osmoprotectants chitosan have taken considerable consideration for tolerance induction against abiotic stresses. In present investigation various concentrations of chitosan (35 KDa) were applied as a foliar spray to eggplant genotypes, grown under salt stressed environments. It was found that chitosan considerably reduced salt induced deleterious effects by enhancing growth in eggplant. Chitosan improved the plant height, root length, root and shoot, fresh and dry weights separately under saline conditions. Parallel results were reported by Ma et al. (2011), wheat exhibited maximum growth with chitosan treatments under salt stress. Chitosan acts on plants as a phytohormone like compounds in regulation processes of morphogenesis, growth and development (Cote and Hahn 1994). Chitosan may motivate the defensive systems of plants, induces certain enzymes such as glucanases, pectinases and chitinases, and enhances growth of eggplant (Hien 2004). Guan et al. (2009) suggested that chitosan promotes plant growth by improving the availability and uptake of water with vital nutrients through adjusting cell osmotic potential. The positive effect of chitosan on plant growth might be ascribed to the promoting effects on nutrients uptake and nutritional status (Farouk and Amany, 2012).

Considering the results of present study, it is clear that foliar application of chitosan can mitigate the injurious effects of salt stress on growth of eggplant. The foliar application of chitosan @ 150 mg L\(^{-1}\) at seedling stage considerably enhanced the vegetative growth of both the tested eggplant genotypes in comparison to other chitosan level under saline regimes (Fig. 4.3.1 and 4.3.2). Similar results were reported by Farouk and Amany (2012), they observed that chitosan @ 250 mg L\(^{-1}\) improved vegetative growth of cowpea under drought stress. Sharifa and Abu-Muriefah (2013) also concluded that foliar application of 200 mg L\(^{-1}\) chitosan solution enhanced growth of common bean plants under stressed and non-stressed conditions.

**Experiment 4**

After optimization trial, optimized dose of chitosan with 150 mg L\(^{-1}\) was sprayed for salt tolerance induction on tolerant and sensitive eggplant genotypes, grown under saline (9 dS m\(^{-1}\)) as well as non-saline conditions. It is apparent from aforementioned findings that salt stress considerably reduced the growth attributes in both the tested eggplant genotypes whereas, chitosan improved these attributes by partially ameliorating the
depressing effect of salinity. Salt stress caused alterations in physiological, biochemical, ionic and water relevant attributes leading to decrease in both the tested eggplant genotypes. Hence, the aim of this trial was to find the effect of chitosan on water relations, physiological, biochemical, ionic and yield attributes of salt tolerant and salt sensitive eggplant genotypes. In this investigation, results indicated that chitosan facilitated the salt stressed plants to a diverse degree in the reversal of reduced growth, gas exchange characteristics and water status of eggplant. The adverse effect of salinity (9 dS m$^{-1}$) was substantially alleviated when eggplant seedlings were sprayed with 150 mg L$^{-1}$ chitosan solution.

The plants under saline condition, showed a substantial reduction in chlorophyll index (SPAD value), Pn, and gs (Fig. 4.4.3). Furthermore, closure of stomata caused by decline in K$^+$ in guard cells and reduced availability of internal CO$_2$ might lead to Pn reduction. Khan et al. (2010) also reported similar results. In this research, it is also apparent that chitosan improved Pn and gs of eggplant under stressed and non-stressed circumstances. The outcomes of current study also demonstrated that there was the highest photosynthetic activity with the increase in stomatal conductance with the application of chitosan under non stressed condition and both (Pn and gs) had positive correlation with each other. These findings were in accordance with the results recorded in mustard (Wani et al. 2011). The chlorophyll index was also improved in return to foliar application of chitosan under saline as well as non-saline conditions in both the tested eggplant genotypes. The increase in chlorophyll index with the application of chitosan might be due to the stability of protein complexes and protection of chlorophyll by reduced activity of chlorophyllase (chlorophyll-degrading enzyme). The increase in photosynthetic rate by the application of chitosan might be due to higher chlorophyll index under saline and normal conditions. The outcomes of present study suggested that exogenous application of chitosan had a positive effect on photosynthesis of both the tested eggplant genotypes under stressed and non-stressed conditions. Hence, growth and yield enhancement was evidently attributed to the elevation of carbon fixation as reflected by gs, Pn and chlorophyll index. Results also showed that Saadia (tolerant) genotype was high in these variables under stressed and normal condition with chitosan application. So, it can be concluded that Saadia was more responsive to chitosan application than sensitive (Black Beauty) genotype.

It is evident from the results, that chitosan increased the transpiration rate (E) and water use efficiency (WUE) in both the tested eggplant genotypes with chitosan application.
under stressed and normal conditions. It is might be due to efficient osmotic adjustment and water regulation caused by chitosan under salinity. Similarly, increase in transpiration rate was observed in soybean and maize with foliar application of chitosan by Khan et al. (2002). However, the eggplant genotypes showed a substantial effect of chitosan on their WUE under saline conditions but the maximum increase was noted under saline environments. Above discussion reveals that chitosan has increased the chlorophyll index and improved the plants capacity to transform the available water and CO\(_2\) into food materials, hence plants exhibited better WUE in response to foliar applied chitosan in this study.

The water related attributes (leaf water potential, osmotic potential, turgor potential and relative water contents) were drastically affected in salinized plants of both the eggplant genotypes in comparison to control plants. But the foliar application of chitosan assisted the osmotic adjustment of salinized plants by inducing a more reduction in leaf water potential (\(\Psi_w\)) and osmotic potential (\(\Psi_s\)) in both the tested eggplant genotypes (Fig. 4.4.1). The present study revealed that chitosan might be strengthened the mechanism of osmotic adjustment (OA) by increasing the organic osmolytes (proline and GB) and reducing the accumulation of inorganic toxic ions. The results of this study verified the findings of Tester and Davenport (2008), who suggested that osmotic adjustment and salt tolerance in eggplant are linked with the concentration of inorganic ions like Na\(^+\) and K\(^+\). Similar kinds of observations are reported by Dekoum et al. (2013). But in present study, chitosan enhanced the growth in salinized eggplant plants of both the tested genotypes by decreasing \(\Psi_s\) and \(\Psi_w\) which was a step toward osmotic adjustment. Therefore, growth enhancement in response to chitosan in stressed plants might be due to the aspects other than OA like conservation of leaf turgor potential (\(\Psi_p\)). While leaf \(\Psi_p\) is very important factor for plant growth because it aids the cell elongation and cell expansion by forcing the cell wall outward (Horie et al., 2012). In present study chitosan ascertained its role for enhancing \(\Psi_p\) in both salt stressed and non-stressed plants. Raza et al. (2016) reported that \(\Psi_p\) has great importance in plant growth. Decrease in \(\Psi_s\) lead to increase in \(\Psi_p\) as it is apparent from the findings of this study.

Salinity significantly reduced the RWC of both the tested eggplant genotypes. These findings are in concordance with the report of Hegazi et al. (2014). RWC were substantially increased with the application of chitosan under saline as well as non-saline conditions in this study. As it is apparent from this study, it can be suggested that chitosan might have improved RWC due to high accumulation of osmolytes such as proline and
GB which in turn reduced osmotic potential and assisted attracting water in order to sustaining turgor potential, improving cell water status under saline environments. Chitosan also increased the leaf K⁺ contents which in turn activating enzymes, stoma movement, membrane polarization, regulating osmotic pressure and consequently balancing osmotic and turgor potential as presented in Figures 4.4.1. Moreover, tolerant (Saadia) responded more efficiently comparative to sensitive eggplant genotype (Black Beauty). Farouk et al. (2011) reported that chitosan improved RWC of radish under cadmium stress.

Salt stress lead to oxidative damage in plants, ROS production is accountable for NaCl-induced injury to macromolecules and cellular organizations (Oukarroum et al., 2015). Plants with high levels of antioxidants under stressed environments have been described to have great tolerance to oxidative injury. One of the defensive mechanisms is the enzymatic antioxidant system, which includes the consecutive and concurrent action of a number of enzymes. SOD, CAT and POD are major enzymes used to show the status of antioxidant aptitude. SOD is the principal line of defense, which converts superoxide radical to H₂O₂ and acts as a defender against salt (Houmani et al., 2015). In the present study, SOD activity was higher in leaves (Fig. 4.4.4) of both the tested eggplant genotypes under salt stress than control. Rise in SOD activity under salt stress might be due to high level of ROS, which causes increase in the expression of gene responsible for SOD (Xing et al., 2015). Higher activity of SOD in stressed plants is a sign of effective detoxification of superoxide radical. Salt-tolerant plants had defensive mechanism against ROS through the enhanced activity of SOD enzyme under salt stress. Increase in SOD activity was reported to be related with the tolerance of plants to salinity stress (Ahmad et al., 2015). The amount of H₂O₂ produced by SOD in the cell is removed afterward by POD. POD activity was increased in the leaves of both the tested eggplant genotypes under NaCl stress than control (Fig. 4.4.4). Apart from POD, CAT is also an enzyme of scavenging H₂O₂ from cells (Vuleta et al., 2015). CAT activity was also increased under salt stress in both the tested eggplant genotypes (Fig.4.4.4). CAT and POD exhibited parallel level of activity in the current study in both the tested eggplant genotypes under salinity stress. Therefore, it can be concluded that both POD and CAT are might be equally essential in detoxifying H₂O₂ under salinity stress in eggplant. When the plants were exposed to chitosan (150 mg L⁻¹) application under saline as well as normal conditions, SOD, POD and CAT activities were substantially improved (Fig. 4.4.4). So, chitosan had capacity to alleviate the drastic effects of salinity in eggplant by
elimination of ROS due to enhancement in enzymatic activities (SOD, POD and CAT). These findings are in accordance with Ma et al. (2012), who reported an increase in SOD, POD and CAT activity in wheat with chitosan treatment grown under salt stress. Chitosan, that was alike to plant growth enhancer used to increase plant resistance as signaling molecule, might be its role as signals that elicit the enzymes synthesis and trigger stress-response gene expression in NaCl stress.

MDA is a sign of lipid peroxidation and shows the degree of oxidative stress in plants (Shi et al., 2015). These findings showed that MDA contents in leaves were significantly increased under salt stress in comparison with the control, indicating the ROS injury due to oxidative stress (Fig. 4.4.3). When plants were exposed to chitosan application, MDA content displayed a considerable reduction in the leaves of the both the tested eggplant genotypes under salinity stress. Chitosan decreased MDA content in stressed plant, it is might be due to higher activities of antioxidant enzymes in response to its application. It indicates that exogenous application of chitosan had a shielding effect on salt persuaded membrane damage. So, findings of present study suggest that growth enhancement in tested eggplant genotypes with the application of chitosan might be due its protective effect against oxidative injury.

Osmolytes such as proline and glycinebetaine was increased in leaves of salinized plants than control (Fig. 4.4.3). The elevated level of proline and glycinebetaine in both the tested genotypes under salinity stress might be due to the induction of their biosynthesis or reduction in oxidation of proline to glutamate or decline in its consumption in protein synthesis (Procházková et al., 2015; Roychoudhury et al., 2015). Proline and glycinebetaine substantially increased when the stressed and non-stressed plants were exposed to chitosan foliar application. It is acknowledged that proline is a source of nitrogen that rescues plants under stress (Iqbal et al., 2015), acts as an osmolyte which decreases the osmotic potential of the cells and the uptake of lethal ions, and also performs a principal role in defending plants from osmotic stress (Terzi et al., 2015). In this study, our findings also showed that the chitosan enhanced the osmolytes (proline and glycinebetaine) in both the eggplant genotypes under salinity and control conditions. Role of chitosan in salt stress alleviation might be due to its regulatory role in proline. Plant under normal conditions gave poor response to chitosan as compared to those under saline conditions. Chitosan substantially enhanced the accumulation of osmolytes (proline and glycinebetaine). Increase in accumulation of these osmolytes in present investigation is the suggestion of osmotic adjustment under the effect of chitosan. It was noted that salt
sensitive (Black Beauty) genotype showed more effective response to chitosan under saline environments than tolerant-Saadia. It is apparent that chitosan improved more accumulation of proline in salt sensitive genotype whereas increased the glycinebetaine in tolerant genotype under salt stress. Therefore, it can be concluded that chitosan indirectly enhanced plant growth and development by reducing the osmotic stress. Instead of osmotic adjustment, these osmolytes perform several other functions such as water uptake, nutrient balance, maintenance of cell turgor and integrity. Therefore, efficient response of tolerant eggplant genotype in present study might be due to above mentioned facts. Gu, (2012) reported that chitosan improved the proline contents of salinized tomato plants. It can be suggested that stress alleviation effect of chitosan might be due to its positive effect on osmolytes which assisted the plant for efficient OA under saline environments.

Salinity stress exposed an increasing effect on leaf Na$^+$ and Cl$^-$ whereas depressing effect on Ca$^{2+}$ and K$^+$ contents in both the tested eggplant genotypes in present study. Beneficial ions, such as K$^+$ and Ca$^{2+}$ are very advantageous for plant’s growth and development because they regulate the protein formation, enhance the enzymatic activities and sustain the plasma membrane and cell wall integrity (Chen et al., 2012b). Outcomes concerning the leaf ion accumulation specified that chitosan showed non-significant effect on all the above mentioned ions under normal conditions. While, significant effect was noted for leaf Na$^+$, Cl$^-$, K$^+$ and Ca$^{2+}$ under saline conditions (Fig. 4.4.5). Negative correlation exist between toxic ions concentration (Na$^+$ and Cl$^-$) in leaf tissues and plant growth, so poor growth of salt sensitive (Black Beauty) genotype can be ascribed to high accumulation of toxic ions (Na$^+$ and Cl$^-$) and low contents of K$^+$ and Ca$^{2+}$ in its leaf but tolerant-Saadia genotype exhibited the enhanced growth as it stored low ratios of these toxic ions and higher concentration of K$^+$ and Ca$^{2+}$ in its leaves. Chitosan mitigated the harsh effect of salinity by decreasing the ratios of Na$^+$ and Cl$^-$ in plant’s leaf tissues, grown under salt stress. So, it can be suggested that chitosan has slightly positive influence on ionic contents and keeps the plant safe from Na$^+$ and Cl$^-$ toxicity by declining their concentrations in leaf under saline environments. Guan et al. (2009) suggested that chitosan increased plant growth by improving water uptake and essential nutrients through cell osmotic adjustments.

Yield parameters such as numbers of fruit, average fruit weight, fruit diameter and yield per plant etc. are the vital elements in evaluating the eggplant production under saline environments. Crop yield entirely depends on fruit numbers and average fruit weight in
case of eggplant. Salinity stress reduced average weight as well as fruit diameter in both the tested eggplant genotypes but had no significance influence on number of fruits per plant (Fig. 4.4.6). Our results verify the findings of Naik et al. (2011), Kaswala et al. (2012) and Sadeghi and Rassoli (2013). But chitosan considerably reduced the salinity induced harmful effects on eggplant yield by enhancing number of fruits, average fruit weight as well as fruit diameter in this study. The enhancement in the yield of eggplant with the exposure to chitosan under stressed and non-stressed conditions might be due to the improved chlorophyll and photosynthetic rate. However exact role of chitosan in yield improvement is still unknown but it can be suggested that the progressive influence of chitosan on productivity could have been due to high production of assimilates and their translocation to the fruit. It might be due to the increase in water uptake and essential nutrient under saline regimes with the application of chitosan (Guan et al., 2009). Bittelli et al. (2001) reported that chitosan improved growth and yield in pepper under drought stress condition. Whereas, Farouk and Amany (2012) reported that foliar applied chitosan enhanced growth and yield of cowpea under drought stress as well as non-stressed conditions. Similarly, Sharifa and Abu-Muriefah (2013) suggested that foliar application of 200 mg L\(^{-1}\) chitosan solution enhanced growth, yield and quality under drought stressed and non-stressed common bean plants.

In conclusion, present study confirmed the progressive effects of chitosan in improving the eggplant growth, yield and ability of plant’s tolerance to salt stress, which were revealed by more biomass, the increase of chlorophyll content, \(Pn\), \(gs\), \(E\), \(RWC\), \(\Psi_p\), \(WUE\), \(K^+\), \(Ca^{2+}\), GB and proline content, higher antioxidant enzyme activities, and less \(\Psi_s\), \(\Psi_w\), \(Na^+\), Cl\(^-\) and MDA contents in leaves.
Chapter 6

SUMMARY

In the first part of this study, thirteen eggplant genotypes were screened out for salt tolerance and characterized as salt tolerant and sensitive on the basis of salt tolerance indices (PHSI, RLSI, SFWSI, RFWSI and PDMSI) and leaf ionic contents (Na\(^+\) and K\(^+\)). All the tested genotypes exhibited different stress tolerance potential for observed parameters. Saadia was found tolerant and Black Beauty regarded as sensitive in this investigation.

After screening experiment, one salt tolerant (Saadia) and one sensitive (Black Beauty) eggplant cultivars were subjected to various salinity stress levels (control, 3, 6, 9, 12 and 15 dS m\(^{-1}\)) in order to evaluate and compare growth, water relations, physiological, biochemical, enzymatic and ionic characteristics. Results of this investigation revealed that salinity stress imposed substantial alterations in these attributes. Stressed plants of Saadia (salt tolerant) were high in K\(^+\), Ca\(^{2+}\), Chl., Pn, E, WUE, gs, \(\Psi_w\), \(\Psi_s\) and \(\Psi_p\) whereas Black Beauty (sensitive) was at bottom in these attributes. However, proline, GB, MDA contents and activities of SOD, CAT and POD enzymes were also increased with increase in salinity stress in both tested cultivars. But, Saadia (tolerant) exhibited maximum increase in proline, GB, SOD, CAT and POD and the minimum increase in MDA contents, Na\(^+\) and Cl\(^-\).

In the second part of this study, salt tolerance was induced by foliar application of chitosan. An optimization trial was conducted with the objective of, to optimize the best dose of chitosan for foliar application. Salt tolerant and sensitive eggplant cultivars were grown in pots and after ten days of salinity i.e. 9 dS m\(^{-1}\) (optimized in second experiment) exposure to plants, specific chitosan levels (75, 100, 125, 150, 175 and 200 mg L\(^{-1}\)) were exogenously applied. Effect of chitosan was tested against the growth attributes such as plant height, root length, shoot and root fresh weight, shoot and root dry weight. Among chitosan concentrations, 150 mg L\(^{-1}\) was found to be the optimal level for vegetative growth enhancement.

After optimization experiment, another trial was conducted in which chitosan (150 mg L\(^{-1}\)) was sprayed on salt tolerant and salt sensitive cultivars. In this trial optimized chitosan level (150 mg L\(^{-1}\)) was sprayed on both the salt tolerant and sensitive eggplant cultivars under saline (9 dS m\(^{-1}\)) and non-saline (control) environments. It was noted that both the investigated eggplant cultivars responded well in term of physiological, biochemical,
enzymatic, ionic and yield attributes to an exogenous application of chitosan (150 mg L\textsuperscript{-1}) under saline as well as control conditions. Tolerant (Saadia) responded better to chitosan application than sensitive. Consequently, it was concluded that foliar application of chitosan substantially alleviated the salt induced adverse effects by improving morpho-physiological, water relation, ionic, biochemical, antioxidant enzymes and yield aspects investigated under stressed and non-stressed environment.
Recommendations and future prospects

There is a dire need to screen out the high yielding salt tolerant vegetables varieties, so that we can feed our ever growing population in an efficient way. In current investigation all the available eggplant genotypes were screened for salt tolerance and grouped into salt tolerant and non-tolerant categories. It will be of great importance for the eggplant growers, because findings of this study will give them clear information about salt sensitivity of available eggplant genotypes. The screened salt tolerant eggplant genotypes can be grown on marginal saline land for getting reasonable production from these unproductive soils and strengthening of vegetable breeding programs related to the salinity tolerance. Moreover, the development of high yielding and salt tolerant genotypes will help in poverty alleviation by utilizing the saline soils of Pakistan. In this way we might be able to get some kind of benefit from unproductive saline lands. However, available hybrids and more genotypes from various ecological sectors of the country should be screened and genotypes with high salt tolerance potential should be identified for growing in salty lands of the Pakistan. In this study various morpho-physiological, biochemical, enzymatic and ionic attributes of eggplant genotypes were investigated under saline and nonsaline regimes, so these markers will be very beneficial for the vegetables breeders in the breeding programs for abiotic stress resistance. As, the above said aspects vary from one growth stage to other, so more comprehensive investigation for salt stress at vegetative and reproductive stage is recommended before their implementation in breeding programs. From the present study, it can also be extracted that investigations for toxic effects of salinity can be extended at cellular and molecular level especially at the membranous level. This combined study of physio-morphological, biochemical, enzymatic and ionic markers under saline conditions may accelerate breeding as well as screening programs. Since, salt stress is a very serious threat to agriculture especially the vegetables. Therefore, there is a dire need to mitigate the toxic effects of salinity. In this study, foliar spray of chitosan (150 mg L\textsuperscript{-1}) significantly alleviated the drastic effects of salinity by improving the morphological, physio-biochemical, water relations and enzymatic aspects. Chitosan can be easily imported from China and can also be manufactured locally by the waste of fishing industry in Karachi. It is very economical way to boost the eggplant production in salt affected lands. Exact mechanism of chitosan stress tolerance induction is less studied and still unknown. For example, how exogenous application of chitosan influence gene expression in eggplant.
However, a comprehensive study is required to find a specific role of chitosan in regulating nutrient specific transporters, which control ions entry in stressed plants. Chitosan also accelerates the activity of other growth related plant hormones like ABA, auxins, cytokinins, and gibberellins etc. So, in future this aspect (cross talk) can also be addressed that how chitosan enhances the activity of these hormones under saline conditions. Therefore, this beneficial effect of chitosan can be studied by using advanced biotechnological approaches especially at molecular level under local environment, which may lead scientists to understand the mechanism of salt tolerance due to the exogenous application of chitosan.
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### APPENDICES

**Appendix 1** Mean squares from the analysis of variance (ANOVA) for the stress tolerance indices.

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>PHSI</th>
<th>RLSI</th>
<th>SFWSI</th>
<th>RFWSI</th>
<th>DMSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>12</td>
<td>214.9***</td>
<td>174.10***</td>
<td>309.0***</td>
<td>1398.2***</td>
<td>2726.2***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>4</td>
<td>14004.4***</td>
<td>2773.39***</td>
<td>10381.5***</td>
<td>9379.9***</td>
<td>61894.8***</td>
</tr>
<tr>
<td>G × S</td>
<td>48</td>
<td>17.0***</td>
<td>6.88NS</td>
<td>6.3***</td>
<td>378.5***</td>
<td>330.4**</td>
</tr>
<tr>
<td>Error</td>
<td>192</td>
<td>4.9</td>
<td>7.01</td>
<td>2.7</td>
<td>500.6</td>
<td>736.0</td>
</tr>
</tbody>
</table>

*aSource of variation, *bDegree of freedom *, **, *** Significant at *P* ≤0.05, *P* ≤0.01, *P* ≤0.001 respectively, NS = Non significant

**Appendix 2** Mean squares from the analysis of variance (ANOVA) for the leaf sodium (Na⁺) and potassium (K⁺) contents.

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Na⁺ Contents</th>
<th>K⁺ Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>12</td>
<td>3520***</td>
<td>189.4***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>4</td>
<td>157477***</td>
<td>10830.3***</td>
</tr>
<tr>
<td>G × S</td>
<td>48</td>
<td>323***</td>
<td>13.6NS</td>
</tr>
<tr>
<td>Error</td>
<td>192</td>
<td>60</td>
<td>17.0</td>
</tr>
</tbody>
</table>

*aSource of variation, *bDegree of freedom *, **, *** Significant at *P* ≤0.05, *P* ≤0.01, *P* ≤0.001 respectively, NS = Non significant

**Appendix 3** Mean squares from the analysis of variance (ANOVA) for the plant height, root length and shoot fresh weight.

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Plant height</th>
<th>Root length</th>
<th>Shoot fresh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>259.726***</td>
<td>27.3008***</td>
<td>57.6847***</td>
</tr>
<tr>
<td>Genotypes (G)</td>
<td>5</td>
<td>320.711***</td>
<td>2.8115***</td>
<td>81.7897***</td>
</tr>
<tr>
<td>S × G</td>
<td>5</td>
<td>10.302***</td>
<td>0.7783**</td>
<td>2.6737*</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>1.303</td>
<td>0.1599</td>
<td>0.7823</td>
</tr>
</tbody>
</table>

*aSource of variation, *bDegree of freedom *, **, *** Significant at *P* ≤0.05, *P* ≤0.01, *P* ≤0.001 respectively, NS = Non significant
### Appendix 4 Mean squares from the analysis of variance (ANOVA) for root fresh weight, root and shoot dry weight.

<table>
<thead>
<tr>
<th>SOV(^a)</th>
<th>DF(^b)</th>
<th>Root fresh weight</th>
<th>Shoot dry weight</th>
<th>Root dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>1.42141***</td>
<td>2.21880***</td>
<td>0.02525***</td>
</tr>
<tr>
<td>Genotypes (G)</td>
<td>5</td>
<td>0.62835***</td>
<td>2.89864***</td>
<td>0.01782***</td>
</tr>
<tr>
<td>S × G</td>
<td>5</td>
<td>0.05935**</td>
<td>0.09781***</td>
<td>0.00078***</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>0.01264</td>
<td>0.01814</td>
<td>0.00014</td>
</tr>
</tbody>
</table>

\(^a\)Source of variation, \(^b\)Degree of freedom, **, *** Significant at \(P \leq 0.05, P \leq 0.01, P \leq 0.001\) respectively, NS = Non significant

### Appendix 5 Mean squares from the analysis of variance (ANOVA) of for Pn, gs and E.

<table>
<thead>
<tr>
<th>SOV(^a)</th>
<th>DF(^b)</th>
<th>Photosynthetic rate (Pn)</th>
<th>Stomatal conductance (gs)</th>
<th>Transpiration rate (E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>33.5504***</td>
<td>0.01577***</td>
<td>2.03158***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>5</td>
<td>10.4815***</td>
<td>0.01234***</td>
<td>3.95494***</td>
</tr>
<tr>
<td>G × S</td>
<td>5</td>
<td>1.3432***</td>
<td>0.00038**</td>
<td>0.06543**</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>0.0254</td>
<td>0.00009</td>
<td>0.01640</td>
</tr>
</tbody>
</table>

\(^a\)Source of variation, \(^b\)Degree of freedom, **, *** Significant at \(P \leq 0.05, P \leq 0.01, P \leq 0.001\) respectively, NS = Non significant

### Appendix 6 Mean squares from the analysis of variance (ANOVA) for WUE and Chl. Index.

<table>
<thead>
<tr>
<th>SOV(^a)</th>
<th>DF(^b)</th>
<th>Water use efficiency</th>
<th>Chlorophyll index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>2.59240***</td>
<td>348.302***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>5</td>
<td>1.01489***</td>
<td>556.784***</td>
</tr>
<tr>
<td>G × S</td>
<td>5</td>
<td>0.22110***</td>
<td>23.762***</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>0.03805</td>
<td>2.640</td>
</tr>
</tbody>
</table>

\(^a\)Source of variation, \(^b\)Degree of freedom, **, *** Significant at \(P \leq 0.05, P \leq 0.01, P \leq 0.001\) respectively, NS = Non significant

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Appendix 7 Mean squares from the analysis of variance (ANOVA) for water relations.

<table>
<thead>
<tr>
<th>SOV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Water potential</th>
<th>Osmotic potential</th>
<th>Turgor potential</th>
<th>RWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>0.53619***</td>
<td>0.16305***</td>
<td>0.11256***</td>
<td>1138.04***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>5</td>
<td>0.88313***</td>
<td>0.20021***</td>
<td>0.23986***</td>
<td>1425.85***</td>
</tr>
<tr>
<td>G × S</td>
<td>5</td>
<td>0.02367***</td>
<td>0.00927***</td>
<td>0.00562**</td>
<td>40.09***</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>0.00068</td>
<td>0.00087</td>
<td>0.00106</td>
<td>7.03</td>
</tr>
</tbody>
</table>

<sup>a</sup>Source of variation, <sup>b</sup>Degree of freedom *,**,*** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, NS = Non significant

Appendix 8 Mean squares from the analysis of variance (ANOVA) for the enzymatic activities.

<table>
<thead>
<tr>
<th>SOV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SOD</th>
<th>CAT</th>
<th>POD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>2.25333***</td>
<td>0.05070***</td>
<td>7.23853***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>5</td>
<td>1.84933***</td>
<td>0.01729***</td>
<td>3.09369***</td>
</tr>
<tr>
<td>G × S</td>
<td>5</td>
<td>0.17133***</td>
<td>0.00229***</td>
<td>0.54374***</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>0.02783</td>
<td>0.00017</td>
<td>0.02165</td>
</tr>
</tbody>
</table>

<sup>a</sup>Source of variation, <sup>b</sup>Degree of freedom *,**,*** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, NS = Non significant

Appendix 9 Mean squares from the analysis of variance (ANOVA) for biochemical attributes.

<table>
<thead>
<tr>
<th>SOV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Proline</th>
<th>Glycine betaine</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>252.542***</td>
<td>17.1602***</td>
<td>3.52083***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>5</td>
<td>433.436***</td>
<td>15.9982***</td>
<td>2.68833***</td>
</tr>
<tr>
<td>G × S</td>
<td>5</td>
<td>18.233***</td>
<td>1.9492***</td>
<td>0.26583***</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>1.127</td>
<td>0.1177</td>
<td>0.05298</td>
</tr>
</tbody>
</table>

<sup>a</sup>Source of variation, <sup>b</sup>Degree of freedom *,**,*** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, NS = Non significant
Appendix 10 Mean squares from the analysis of variance (ANOVA) for ionic attributes.

<table>
<thead>
<tr>
<th>SOV(^a)</th>
<th>DF(^b)</th>
<th>Ca(^{2+}) contents</th>
<th>K(^+) contents</th>
<th>Na(^+) contents</th>
<th>Cl(^-) contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>11.213 **</td>
<td>420.083 ***</td>
<td>120.333 ***</td>
<td>129.199 ***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>5</td>
<td>107.256 ***</td>
<td>901.800 ***</td>
<td>386.150 ***</td>
<td>290.548 ***</td>
</tr>
<tr>
<td>G × S</td>
<td>5</td>
<td>4.983 **</td>
<td>50.033 **</td>
<td>12.933 ***</td>
<td>17.459 ***</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>1.052</td>
<td>9.513</td>
<td>2.346</td>
<td>2.637</td>
</tr>
</tbody>
</table>

\(^a\)Source of variation, \(^b\)Degree of freedom *,**,*** Significant at \(P\leq0.05\), \(P\leq0.01\), \(P\leq0.001\) respectively, NS = Non significant

Appendix 11 Mean squares from the analysis of variance (ANOVA) for plant height, root length and shoot fresh wt.

<table>
<thead>
<tr>
<th>SOV(^a)</th>
<th>DF(^b)</th>
<th>Plant height</th>
<th>Root length</th>
<th>Shoot fresh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan (Ch)</td>
<td>7</td>
<td>162.21 ***</td>
<td>7.1053 ***</td>
<td>47.856 ***</td>
</tr>
<tr>
<td>Genotypes (G)</td>
<td>1</td>
<td>1032.82 ***</td>
<td>41.7639 ***</td>
<td>150.277 ***</td>
</tr>
<tr>
<td>Ch × G</td>
<td>7</td>
<td>8.68 **</td>
<td>0.7318 *</td>
<td>3.117 **</td>
</tr>
<tr>
<td>Error</td>
<td>45</td>
<td>2.63</td>
<td>0.2689</td>
<td>0.767</td>
</tr>
</tbody>
</table>

\(^a\)Source of variation, \(^b\)Degree of freedom *,**,*** Significant at \(P\leq0.05\), \(P\leq0.01\), \(P\leq0.001\) respectively, NS = Non significant

Appendix 12 Mean squares from the analysis of variance (ANOVA) for root fresh wt., root and shoot dry wt.

<table>
<thead>
<tr>
<th>SOV(^a)</th>
<th>DF(^b)</th>
<th>Root fresh weight</th>
<th>Shoot dry weight</th>
<th>Root dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan (Ch)</td>
<td>7</td>
<td>0.70236 ***</td>
<td>1.33488 ***</td>
<td>0.01546 ***</td>
</tr>
<tr>
<td>Genotypes (G)</td>
<td>1</td>
<td>1.72923 ***</td>
<td>5.78102 ***</td>
<td>0.05933 ***</td>
</tr>
<tr>
<td>Ch × G</td>
<td>7</td>
<td>0.03751 *</td>
<td>0.09117 *</td>
<td>0.00111 *</td>
</tr>
<tr>
<td>Error</td>
<td>45</td>
<td>0.01558</td>
<td>0.03242</td>
<td>0.00036</td>
</tr>
</tbody>
</table>

\(^a\)Source of variation, \(^b\)Degree of freedom *,**,*** Significant at \(P\leq0.05\), \(P\leq0.01\), \(P\leq0.001\) respectively, NS = Non significant
### Appendix 13 Mean squares from the analysis of variance (ANOVA) for water relations.

<table>
<thead>
<tr>
<th>SOV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Water potential</th>
<th>Osmotic potential</th>
<th>Turgor potential</th>
<th>RWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>0.26325**</td>
<td>0.09240**</td>
<td>0.04373**</td>
<td>316.23**</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>2.34282**</td>
<td>0.70616**</td>
<td>0.47650**</td>
<td>3160.10**</td>
</tr>
<tr>
<td>Chitosan (Ch)</td>
<td>1</td>
<td>0.03437**</td>
<td>0.31080**</td>
<td>0.13845**</td>
<td>241.54**</td>
</tr>
<tr>
<td>G×S</td>
<td>1</td>
<td>0.06917**</td>
<td>0.02727**</td>
<td>0.00958*</td>
<td>74.59&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>G×Ch</td>
<td>1</td>
<td>0.00820**</td>
<td>0.02450**</td>
<td>0.00435*</td>
<td>53.12&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>S×Ch</td>
<td>1</td>
<td>0.00820**</td>
<td>0.01406**</td>
<td>0.00079&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>113.17&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>G×S×Ch</td>
<td>1</td>
<td>0.00017&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.01510**</td>
<td>0.01209*</td>
<td>33.97&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>0.00019</td>
<td>0.00057</td>
<td>0.00083</td>
<td>34.17</td>
</tr>
</tbody>
</table>

<sup>a</sup>Source of variation.  <sup>b</sup>Degree of freedom **,*** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, NS = Non significant

### Appendix 14 Mean squares from the analysis of variance (ANOVA) for Pn, E and gs.

<table>
<thead>
<tr>
<th>SOV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Photosynthetic rate</th>
<th>Stomatal conductance</th>
<th>Transpiration rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>7.1348***</td>
<td>0.00281***</td>
<td>0.2729***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>16.4021***</td>
<td>0.02645***</td>
<td>14.8444***</td>
</tr>
<tr>
<td>Chitosan (Ch)</td>
<td>1</td>
<td>13.0944***</td>
<td>0.00661***</td>
<td>0.0985***</td>
</tr>
<tr>
<td>G×S</td>
<td>1</td>
<td>0.6413&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.00080*</td>
<td>0.0775**</td>
</tr>
<tr>
<td>G×Ch</td>
<td>1</td>
<td>0.5330&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.00031&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.0126&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>S×Ch</td>
<td>1</td>
<td>0.8483</td>
<td>0.00180</td>
<td>0.0012&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>G×S×Ch</td>
<td>1</td>
<td>0.2869&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.00080</td>
<td>0.0089&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>0.1726</td>
<td>0.00008</td>
<td>0.0090</td>
</tr>
</tbody>
</table>

<sup>a</sup>Source of variation.  <sup>b</sup>Degree of freedom **,*** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, NS = Non significant

### Appendix 15 Mean squares from the analysis of variance (ANOVA) for WUE and Chl. index.

<table>
<thead>
<tr>
<th>SOV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Water use efficiency</th>
<th>Chlorophyll index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>0.2809***</td>
<td>24.85&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>11.7925***</td>
<td>1180.98***</td>
</tr>
<tr>
<td>Chitosan (Ch)</td>
<td>1</td>
<td>2.9748***</td>
<td>135.30**</td>
</tr>
<tr>
<td>G×S</td>
<td>1</td>
<td>0.0025&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>26.65</td>
</tr>
<tr>
<td>G×Ch</td>
<td>1</td>
<td>0.2287*</td>
<td>12.75&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>S×Ch</td>
<td>1</td>
<td>0.9547***</td>
<td>24.50&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>G×S×Ch</td>
<td>1</td>
<td>0.1633&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>4.50&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>0.0332</td>
<td>5.89</td>
</tr>
</tbody>
</table>

<sup>a</sup>Source of variation.  <sup>b</sup>Degree of freedom **,*** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, NS = Non significant
### Appendix 16 Mean squares from the analysis of variance (ANOVA) of the biochemical attributes.

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Proline</th>
<th>Glycine betaine</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>12.375***</td>
<td>21.945***</td>
<td>0.87781***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>940.695***</td>
<td>177.190***</td>
<td>8.10031***</td>
</tr>
<tr>
<td>Chitosan (Ch)</td>
<td>1</td>
<td>66.990**</td>
<td>83.528**</td>
<td>0.69031***</td>
</tr>
<tr>
<td>G×S</td>
<td>1</td>
<td>9.138***</td>
<td>2.940**</td>
<td>0.52531***</td>
</tr>
<tr>
<td>G×Ch</td>
<td>1</td>
<td>3.713***</td>
<td>0.945**</td>
<td>0.16531</td>
</tr>
<tr>
<td>S×Ch</td>
<td>1</td>
<td>22.950***</td>
<td>0.813***</td>
<td>0.47531***</td>
</tr>
<tr>
<td>G×S×Ch</td>
<td>1</td>
<td>2.258**</td>
<td>2.588***</td>
<td>0.11281</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>0.317</td>
<td>0.059</td>
<td>0.02317</td>
</tr>
</tbody>
</table>

*a Source of variation, b Degree of freedom *,**,*** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, NS = Non significant

### Appendix 17 Mean squares from the analysis of variance (ANOVA) for enzymatic activities.

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>SOD</th>
<th>CAT</th>
<th>POD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>0.40500***</td>
<td>0.01163***</td>
<td>1.5620***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>1.62000***</td>
<td>0.07315***</td>
<td>10.8462**</td>
</tr>
<tr>
<td>Chitosan (Ch)</td>
<td>1</td>
<td>0.45125***</td>
<td>0.01665***</td>
<td>1.3903***</td>
</tr>
<tr>
<td>G×S</td>
<td>1</td>
<td>0.08000*</td>
<td>0.00138*</td>
<td>1.2680**</td>
</tr>
<tr>
<td>G×Ch</td>
<td>1</td>
<td>0.06125*</td>
<td>0.00300*</td>
<td>0.0604</td>
</tr>
<tr>
<td>S×Ch</td>
<td>1</td>
<td>0.01125**</td>
<td>0.00633***</td>
<td>0.0587*</td>
</tr>
<tr>
<td>G×S×Ch</td>
<td>1</td>
<td>0.03125**</td>
<td>0.00165**</td>
<td>0.0428*</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>0.01214</td>
<td>0.00026</td>
<td>0.0091</td>
</tr>
</tbody>
</table>

*a Source of variation, b Degree of freedom *,**,*** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, NS = Non significant

### Appendix 18 Mean squares from the analysis of variance (ANOVA) for the ionic contents.

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Ca²⁺ contents</th>
<th>K⁺ contents</th>
<th>Na⁺ contents</th>
<th>Cl⁻ contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>19.531***</td>
<td>105.13***</td>
<td>55.125***</td>
<td>27.658***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>276.125***</td>
<td>2080.12***</td>
<td>800.000***</td>
<td>588.674***</td>
</tr>
<tr>
<td>Chitosan (Ch)</td>
<td>1</td>
<td>12.500**</td>
<td>66.13***</td>
<td>60.500***</td>
<td>31.502***</td>
</tr>
<tr>
<td>G×S</td>
<td>1</td>
<td>3.125*</td>
<td>50.00**</td>
<td>28.125**</td>
<td>14.783**</td>
</tr>
<tr>
<td>G×Ch</td>
<td>1</td>
<td>3.125*</td>
<td>4.50 NS</td>
<td>15.125**</td>
<td>12.814*</td>
</tr>
<tr>
<td>S×Ch</td>
<td>1</td>
<td>3.781*</td>
<td>24.50</td>
<td>12.500**</td>
<td>24.064**</td>
</tr>
<tr>
<td>G×S×Ch</td>
<td>1</td>
<td>0.281 NS</td>
<td>6.13 NS</td>
<td>10.125**</td>
<td>11.580*</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>0.618 NS</td>
<td>4.39</td>
<td>0.726</td>
<td>1.288</td>
</tr>
</tbody>
</table>

*a Source of variation, b Degree of freedom *,**,*** Significant at P≤0.05, P≤0.01, P≤0.001 respectively, NS = Non significant
Appendix 19 Mean squares from the analysis of variance (ANOVA) for yield attributes.

<table>
<thead>
<tr>
<th>SOV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Number of Fruits</th>
<th>Average Fruit weight</th>
<th>Fruit Diameter</th>
<th>Average Yield per Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (G)</td>
<td>1</td>
<td>8.76758&lt;sup&gt;***&lt;/sup&gt;</td>
<td>1903.4&lt;sup&gt;***&lt;/sup&gt;</td>
<td>1.620&lt;sup&gt;***&lt;/sup&gt;</td>
<td>923305&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>0.33008&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>77933.5&lt;sup&gt;***&lt;/sup&gt;</td>
<td>84.500&lt;sup&gt;***&lt;/sup&gt;</td>
<td>9356489&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chitosan (Ch)</td>
<td>1</td>
<td>5.48633&lt;sup&gt;*&lt;/sup&gt;</td>
<td>11355.2&lt;sup&gt;***&lt;/sup&gt;</td>
<td>13.005&lt;sup&gt;***&lt;/sup&gt;</td>
<td>2227422&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>G×S</td>
<td>1</td>
<td>0.04883&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>283.2&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.125&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>187&lt;sup&gt;NS&lt;/sup&gt;</td>
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<td>G×Ch</td>
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<sup>a</sup>Source of variation, <sup>b</sup>Degree of freedom *,**,*** Significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, NS = Non significant