INVESTIGATING PHYSIOLOGICAL AND BIOCHEMICAL INDICATORS OF SALT TOLERANCE IN LINSEED (Linum usitatissimum L)

A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

IN

SOIL SCIENCE

By

Muhammad Abdul Qayyum

M.Sc. (Hons) Soil Science
Declaration

I hereby declare that the contents of the thesis, “Investigating Physiological and Biochemical Indicators of Salt tolerance in linseed (Linum usitatissimum L.)” are product of my own research and no part has been copied from any published source (except the references, standard mathematical or genetic models/equations/formulate/protocols 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. (In case of any default the scholar will be proceeded against as per HEC plagiarism policy)

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(Dr. SHAHZAD M.A. BASRA)
DEDICATED TO
MY SWEET FAMILY

AND

FRIENDS

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ABSTRACT

Salinity is one of the biggest problems in soils of Pakistan due to its arid to semi-arid climate. A rapid increase in population, industrialization of arable lands along with water scarcity and poor quality ground water compels us to find new ways to tackle salinity. Salt-affected soils can be used for growing non-conventional multipurpose crops. Linseed is a medicinal and oil seed crop which can be grown on salt-affected land. To explore the genetic variation for salt tolerance and to investigate the physiological and biochemical traits of linseed (*Linum usitatissimum* L.), sixty genotypes of linseed were grown for four weeks in solution culture using three salinity levels (control, 100 and 200 mM NaCl). On the basis of biomass and K'/Na⁺ ratio, linseed genotypes (637-72, NO-303) and (S-907, C-99-3-115) were selected as salt tolerant and salt sensitive respectively. Subsequently, physiological and biochemical traits of salt tolerant were evaluated in solution culture and their yield and oil contents were determined in pot culture experiment under same set of treatments. Results revealed that germination and seedling growth were significantly affected due to salinity and linseed genotypes did not show distinct variation some in physiological traits like relative water contents (RWC), electrolyte leakage (EL), chlorophyll contents, stomatal conductance, carbonic anhydrase (CA), nitrate reductase (NR) and leaf osmotic potential but at the same time, photosynthetic rate, organic osmolytes especially glycine betain and antioxidants SOD, POD and APX were affected significantly and hence give a clue of their functional importance in conferring salt tolerance in linseed. Controlled entry of Na⁺ ion at root level, active uptake of K⁺, enhanced activity of antioxidant enzymes and reduction in lipid peroxidation seems to be the salt tolerant traits in native linseed genotypes. Furthermore, it was noted that at high salinity (200 mM NaCl), crop failed to reach maturity, however, seeds were produced at low salinity (100 mM NaCl). The results indicated that linseed genotypes are sensitive to higher level of salinity (200 mM NaCl) and express
tolerant to salinity at vegetative stage, thus can be grown on salt-affected soils for some biomass production.

Keywords: linseed, physiological, biochemical, indicators, organic osmolytes, antioxidant enzymes, yield, oil contents
CHAPTER 1

INTRODUCTION

One of the most serious and important problem in agriculture is soil salinity. Over 3% of the total land mass of the world is affected by salinity and more than half of the countries on this planet are bearing some quantity of salt-affected lands (Corbishley and Pearce, 2007). Naturally, regions of semi-arid and arid climate have evapotranspiration more than precipitation which typically cause upward movement of salts along with water and hence cause salinity. Thus, most of the countries bearing salinity are in a vast range which extends from Africa to central Asia through Middle East (Corbishley and Pearce, 2007). Within next 25 years, about 30% of arable land is predicted to be lost due to salinity while this loss may be enhanced up to 50% by the year 2025 (Mahajan and Tuteja, 2005; Wang et al., 2003). Currently, out of 1500 mha agricultural land almost 32 mha is affected by salt accumulation (FAO, 2005). On an average, salinity has affected approximately 6% of Asia–Pacific land (FAO, 2006).

Pakistan has 62,400 km long canal Indus basin irrigation system (IBIS) which irrigates 20.42 Mha area in the Indus plain. In this irrigation system, almost 16 Mha of land receives 172 billion cubic meter (BCM) of high-quality river water per year (Aslam and Prathapar, 2006). Unplanned and continuous irrigation with canal water has altered the hydrological balance in the irrigated areas of Indus basin. This situation caused a rise in water table in some areas while a decrease in water table depth cause water logging, salinity and soil erosion, in different regions of all the four provinces of Pakistan (Zaka et al., 2005; Aslam and Prathapar, 2006). The severity of problem can be judged by the fact that useful arable land is being degraded by salinity at 40,000 ha per annum (Alam et al., 2000). Obviously, the high salt concentration in soils (soil salinity) reduces the volume of agricultural raw material as well as the quality of products throughout the world (Lauchli and Grattan, 2007) and is of great anxiety for an agriculture based country like Pakistan.
In addition to salinization and degradation of arable lands, urbanization, population growth and industrialization have also contributed to the reduction in per capita arable land availability. This reduction also has a negative impact on food production which is not good news for food security of a third world or developing country like Pakistan. Reduction in arable lands has also exerted pressure on the existing forest as these are cleared to balance the loss of arable lands (WRI, 2000). Thus keeping in view the above scenario, two approaches are worth mentioning: (1) Exploring and maximizing the salt tolerant crops, i.e. saline agriculture means living with salinity (2) Using the salt-affected and marginal lands for producing non-conventional crops. Therefore, exploitation of degraded wastelands including salt-affected ones is a practical option for growing plants having economic significance. Several species of non-conventional crops are potentially good and give economic return when grown on salt-affected soils. (Dagar et al., 2004). For example, Isabgol (Plantago ovata), a potential medicinal rabi-crop can be successfully grown on soils having high pH (Dagar et al., 2006). It can also be grown on calcareous degraded land in arid regions irrigating with saline water (Tomar and Minhas, 2004b), also, as an agro-forestry crop with Acacia nilotica with no significant yield reduction (Dagar and Tomar, 2002). Similarly ajwain (Trachyspermum ammi L.) a potential medicinal plant is moderately salt tolerant (Ashraf and Orooj, 2006).

Salt stress adversely effects almost all physiological, biochemical (Munns and James, 2003; Cuartero et al., 2006; Nabati et al., 2011) and molecular processes (Mansour, 2000; Tester and Devenport, 2003) and hence reduce yield. Ultimate effect of salt stress is decrease in soil osmotic potential which, in turn, causes water stress, nutrient imbalances, specific ion toxicity or combination of all these factors (Evelin et al., 2009). Excessive salt accumulation in plants may also cause membranes disorganization, production of toxic metabolites, reduced photosynthesis and nutrient uptake and enhanced ROS production which leads to cell and/or plant death (Sun et al., 2011; Abogadallah, 2010; Chartzoulakis and Psarras, 2005).
Reactive oxygen species (ROS) exert the most devastating effect on plant growth under saline conditions and severely hamper the growth and development of plants (Szalai et al., 2009; Zhu, 2001). These are among the main causes of cell damage under all types of stress (Mittler, 2002; Gara et al., 2003; Ali et al., 2011; Bhutta, 2011). When triplet oxygen (atmospheric oxygen) gains extra energy in terms of electrons, it is converted into a number of reactive oxygen species which severely damage different living cells like proteins, lipids and nucleic acids (Abogadallah, 2011). These reactive oxygen species (also called active oxygen species, AOS) include superoxide anions (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (1$^1$O$_2$) and hydroxyl radicals (OH$^•$). ROS production occurs in plants during photosynthesis, respiration and photorespiration (Mittler, 2002; Uchida et al., 2002; Asada, 2006; Abogadallah, 2011). NADPH oxidase, amine oxidase and cell wall bound peroxidase also produce ROS (Shalata et al., 2001; Mittler, 2002). ROS production occurs also in roots due to the disruption of electron transport in root mitochondria (Fukao and Bailey-Serres, 2004) which up-regulate the antioxidative system in mitochondria and peroxisomes of roots (Mittova et al., 2004).

Two types of mechanisms operate in plant for the removal of ROS (Beak and Skinner, 2003). These include enzymatic and non-enzymatic antioxidant systems. Enzymatic oxidant system (EAS) consists of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) while non enzymatic antioxidants include vitamin A, C, E, glutathione, carotenoids and phenolics which protect cells from ROS damage and finally convert ROS into triplet oxygen and water molecule (Jaleel et al., 2009; Gara et al., 2003; Meloni et al., 2003).

Plant response under saline environment has been studied over two decades and a significant progress in understanding of plant adaptive responses to salinity has been made both at physiological and molecular levels. Plants react through adaptive responses in growth and physiology under salt stress. These responses may include; (i) intracellular K$^+$ homeostasis, (ii) ion selectivity or exclusion, (iii) ion
compartmentation at whole plant and cellular level, (iv) production of organic osmolytes, (vii) generation of antioxidants and (viii) programmed cell death (PCD) (Hasegawa et al., 2000; Zhu, 2002; Meloni et al., 2003; Shabala, 2009; Anschütz et al., 2014). Thus Na\(^+\) exclusion from the transpiration stream, sequestration of Na\(^+\) and Cl\(^-\) in the vacuoles of roots and leaf cells and K\(^+\) retention in mesophyll cells are very important and well defined mechanisms of salt tolerance in plants (Munns et al., 2006; Pandolfi et al., 2012; Hasegawa, 2013; Wu et al., 2013).

The attributes of salt tolerance vary among species and cultivars due to their complex nature (Flowers, 2004; Munns, 2007) and there are chances of increasing yield in salt-affected soils. This requires new germplasm and more efficient techniques for identifying important genes and their performance under field conditions (Shabala and Munns, 2012). For breeding salt tolerant cultivars, many approaches have been advocated, including conventional breeding, wide crossing, the use of physiological traits and, more recently, marker-assisted selection and the use of transgenic plants. None of these approaches could be said to offer a universal solution (Flowers and Flowers, 2005). Marker-based genetic transformation could be an effective tool in plant breeding if the knowledge from plant physiology must be integrated with molecular breeding techniques (Cuartero et al., 2006). Plant salt tolerance is a complex phenomenon and involves responses to cellular osmotic and ionic stresses, their secondary stresses and whole-plant co-ordination. A cascade of reaction starts involving hundreds of different genes, either directly or indirectly. Some genes are expressed at very early stages, while others become crucial at later stages of plant development (Chen et al., 2005). These variations complicate the screening for salt tolerance, and crop ranking made at one stage may differ from similar ranking made at another stage of plant growth. Thus, knowledge of physiological mechanisms and use of physiological traits is of utmost importance for efficient screening methods (Yeo, 1994; Zhu 2000).
The efforts for the selection of salt tolerant genotypes have mainly focused on screening and breeding of current and available genotypes especially of commercial value. Only partial success was achieved by these efforts because agronomic criteria like yield and survival were used mainly for selection. To develop a salt tolerant genotype, selection should be based on the criteria of physiological mechanisms of salt tolerance (Noble and Rogers, 1992; Yeo, 1994; Ashraf, 2004).

Pakistan’s domestic need for edible oil is 2.78 million tons while the local production of edible oil is 0.83 million tons. This huge gap between need and production of edible oil is filled by importing 1.9 million tons of edible oil at the cost of Rs. 111 billion. Since 1991-92, an annual increase of 6.6% in the import of edible oil has been observed in the country (GOP, 2008). In developing countries like Pakistan, demand for edible oil and hence the oilseed crops has been increased but their area of production remained same or even reduced due to the industrialization of arable lands and hence increased pressure of cereal crops on normal soils. With the emphases on increasing vegetable oil production, there has been a national effort in encouraging the cultivation of high yield potential crops on marginal salt-affected lands with proper fertilizer management (Mahmood et al., 2007).

Linseed (Linum uistatissimum L) is among the most valuable dual purpose oilseed crops and is used for the extraction of oil from seeds and fibers from plant’s stems. The prospective health benefits of linseed for cancer and cardiovascular diseases have gained great attention of nutrition works and plant scientists (Jenkins et al., 1999). In linseed, fibers, lignans and omega 3 fatty acids are major components in terms of giving health benefits (Oomah, 2001). Linseed has great adaptability and product diversity and researchers of Australia, North America, Europe and Asia are conducting research for producing its bio-products. Canada was the top producer of linseed in 2009 with 45% of the world’s linseed production while India and China were also among the top producers of linseed in the same year (FAO, 2009). In India 80% of the linseed oil is used for the industrial purpose and remaining 20% for edible purpose (Khan et al., 2007). In Pakistan, it was cultivated on an area of 3946 hectares and its production
was 2776 tons during the year 2011 (FAOSTAT, 2013). Khan et al. (2007) investigated that increasing salinity reduced almost all the growth, physiological, biochemical and yield attributes linearly in linseed genotypes. They also observed that proline offered a protection by osmotic adjustment of salt stressed plants of linseed genotypes. Similarly, Muhammad and Hussain (2010) observed that soil salinity significantly affected the agronomic parameters of linseed like survival, plant height, number of branches, shoot and root fresh and dry weights, root moisture contents, number of leaves plant\(^{-1}\) while leaf length and shoot moisture contents had no effects of salinity. They also suggested to grow linseed on saline soils because it is moderately tolerant to salinity for biomass production. Likewise, Mervat and Ebtihal (2013) found a significant decrease in yield and yield attributes like number of fruiting branches plant\(^{-1}\), number of capsules plant\(^{-1}\), capsule weight plant\(^{-1}\), number of seeds capsule\(^{-1}\), seed weight capsule\(^{-1}\) and 1000 seeds weight in linseed under saline condition. They also noted a significant decrease in oil contents in salt stressed linseed plants.

Keeping in view the medicinal, industrial and nutritional benefits of linseed, an effort has been made to evaluate physiological and biochemical variations in linseed genotypes which may contribute to salt tolerance under salt stress conditions so that it can be grown successfully on marginal salt-affected lands.

The specific objectives are:

1. To explore the genetic variations in linseed genotypes for salt tolerance and selection of tolerant and sensitive genotypes.
2. To evaluate the response of linseed at germination stage under salt stress conditions.
3. To observe the survival and ion distribution in linseed genotypes at seedling stage
4. To define the various physiological and biochemical processes having functional significance in determining salt tolerance and plant growth of linseed.
5. To investigate the effect of soil salinity on seed yield, yield attributes and seed oil contents of linseed genotypes.
CHAPTER 2
REVIEW OF LITERATURE

2.1. Agriculture: Future Challenges

In spite of reducing poverty and blistering economic growth of a country, agriculture sector has to face multiple challenges in 21st century. Some of the key issues to be addressed in future are over population, food security, urbanization of productive land, deforestation and water scarcity. In addition to these issues, the continuous increase in the extent of salt-affected soils and poor quality ground water are the ultimate challenges to agriculture sector of developing countries of arid and semi-arid regions.

2.1.1. Salt-affected Soils

Salt-affected soils exist in every part of the world but their severity and extent is high in the regions of arid and semi arid climate (Pitman and Lauchli, 2002; Qadir and Oster, 2004; Qadir et al. 2006). Almost 10% of arable land of the world is adversely affected by high salt concentration while almost half of the world’s countries are also hampered by salinity to some extent (Tabet et al., 1997; Corbishley and Pearce, 2007). Sodic and saline-sodic soils of the world are over 50% of total salt-affected land (Beltran and Manzure, 2005). At present, 20% of irrigated land of the world is salt-affected and/or irrigated with brackish water (Ghassemi et al., 1995).

Salt-affected land of Pakistan is estimated to be 6.68 mha (Khan, 1998) and 56% of it is saline-sodic in nature (Aslam et al., 1994; Mehdi et al., 2008). The province of Sindh is affected more (34.2%) due to salinity after Punjab (43.2 %). The province of Baluchistan contains 21.8 % salt-affected land while Khyber Pakhtunkhwa (KPK) province contains 0.8 % salt-affected land (MINFAL, 2000). About 25% of irrigated land is affected by salinity and almost 1.4 mha of arable land is left uncultivated and abandoned (World Bank, 2006a). Owing to this loss of productive arable land, small farmers have been getting worse year after year (Hussain et al., 2012). In Pakistan, it
is estimated that annual loss of crop due to salinity ranges between Rs. 15 and 55 billion. In addition, almost 15 billion rupees (A$340 million) has been lost in terms of land left unproductive. Taking into account the average cost of yield reduction as 35 billion rupees (A$790 million) per year, cost of salinity in 2004 was almost equal to 0.6 percent of GDP of Pakistan (World Bank, 2006a; Corbishley and Pearce, 2007). This is probably the most important reason of increasing rural poverty in Pakistan and poverty rate is 13% higher in rural areas than urban areas. This is why although southern Punjab (Multan, Bahawalpur and D.G. Khan divisions) have high per capita cultivated land with maximum irrigation (100%) but is among the poorest parts of the country having 40% of rural poverty rate (ADB, 2002).

2.1.2. Poor quality ground water

Groundwater is the principal source of drinking water, agriculture industry and households (WWF, 2007). Punjab has 80 percent fresh ground water. Sindh has less than 30 percent fresh ground water. KPK has most of the layers of saline ground water while Balochistan also has saline ground water. Best estimates give a figure of 500,000 operational tube wells in the Indus Basin (Ali et al., 2004). These tube wells are generally thought to be a reasonable source of fresh groundwater. However, total Indus basin contains fresh ground water reserves of about 55 MAF (South Asia water vision, country report 2001). Despite six times increase in diesel pumps over a period of 30 years, the ground water contributes only 48 percent of the water available. However, according to study conducted by Habib (2004) on analysis of water demand and supply, the water shortage in agriculture range from 10 MAF to 25 MAF in rainy and dry years respectively.

Pakistan is facing an acute shortage of good quality irrigation water to raise crops (Ghafoor et al., 2001). Groundwater used as a supplement source of irrigation is mostly of poor quality owing to arid to semi-arid climate, low recharge rate and over-pumping of good quality ground water. About $6.79 \times 10^{10}$ m$^3$ groundwater is pumped, of which 70-75% is hazardous for irrigation (Latif and Beg, 2004) on the basis of the criteria of the Department of Agriculture, Punjab (Muhammed and
Ghafoor, 1992). It is well known that in many areas of Sindh tube wells have been ceased due to pumping of saline water (Qureshi et al., 2011).

Extensive use of saline water by the farmers of other countries including Pakistan is merely due to the severe shortage of canal water (Qadir et al., 2007). The irrigation with saline water not only reduces the productivity but also the quality of produce is badly affected (Latif and Ahmad, 2009).

2.2. Soil Salinity and plant growth

Salt stress adversely affects the growth and development of crop plants. High salinity may cause the adverse effects on membrane integrity, enzyme activity, nutrient uptake and photosynthesis. The production of ROS during salt stress is one of the important reasons of this damage. So broadly salt stress causes four types of injuries on plants such as:

2.2.1. Osmotic or water-deficit effect

Salts affect the ability of plants to take up water and hence indirectly reduce plant growth. The plants which cannot regulate osmotic stress are unable to maintain pressure potential and ultimately close their stomata and reduce photosynthesis. This loss of pressure potential severely affects cell division and elongation and inhibits plant growth during salt stress (Qin et al., 2010). Many comprehensive reviews (Hasegawa et al., 2000; Munns, 2005; Munns and Tester, 2008) documented adverse effects of salinity in terms of water-deficit or osmotic stress at cell level.

Salinity induced water deficit reduces root and shoot cell expansion. This reduction occurs due to osmotic-induced production of abscisic acid (ABA), which promotes root growth and inhibits shoot growth (Munns and Tester, 2008). Thus root growth is affected less than shoot growth under salinity induced osmotic stress (Munns, 2011). Carbon partitioning from the shoot to the root facilitates root growth into soil regions where water is available (Saab et al., 1990; Munns and Tester, 2008; Yamazaki et al., 2012). Plants, particularly in response to moderate water deficit, can osmotically adjust and re-establish cell turgor (Greenway and Munns, 1983; Binzel et
Cells of glycophytes and most other halophytes exhibit higher cell growth yield thresholds and reduced extensibility at low water potentials that restrict cell expansion (Matthews et al., 1984; Munns and Tester, 2008; Tardieu et al., 2011; Shalaba and Mackay, 2011).

In glycophytes, leaf succulency (thickening of leaf tissues and increase in the volume of leaf sap) is a typical adaptive response under saline conditions and may be achieved by increasing the size of mesophyll cells and the relative size of their vacuoles (Gorham et al., 1985; Longstreth and Nobel, 1979). The number of spongy cell layers may also be increased under salt stress (Longstreth and Nobel, 1979). Root suberisation increases with salinity (Steudle, 2000); this may be functionally important to prevent apoplastic flow of toxic Na⁺ ions and to increase water retention in the roots of glycophytes. Some halophytes (e.g. *Mesembryanthemum crystallinum*) have made this trait constitutive, by developing an extra endodermis layer (Inan et al., 2004). Some halophytes have evolved unique adaptations such as salt glands and bladders, succulence, life cycle avoidance and salt induced facultative metabolism to cope with salinity (Flowers et al., 1986, 2010; Bohnert et al., 1995; Shabala and Mackay, 2011). In halophytes cell turgor is maintained by storage of Na⁺ and Cl⁻ in vacuoles, with the solute potential of the cytosol adjusted by accumulation of K⁺ and organic solutes (Storey and Wyn Jones, 1979; Storey, 1995; Glenn et al., 1999). According to Glenn et al. (1999), Na⁺, K⁺ and Cl⁻ contribute to maintain 80–95% of the cell sap osmotic pressure in both halophyte grasses and dicots. This results in the hyper accumulation (>10% of dry weight each) of Na⁺ and Cl⁻ in their shoots (Grattan et al., 2008), largely in vacuoles (Flowers and Colmer, 2008). At the same time, halophytes maintain cytoplasmic K⁺ concentrations similar to those of glycophytes (Flowers and Colmer, 2008) and thus have a high vacuole/cytosol Na⁺ ratio and a high cytosol/vacuole K⁺ ratio (Ye and Zhao, 2003).

In halophytes, presence of salt bladders/glands is the most remarkable feature (Shalaba and Mackay, 2011). Glands extrude salts on a regular basis, while bladders release salts only when rupture, after a prolonged period of accumulation (Tester and
Davenport, 2003). Epidermal bladder cells are also thought to be storage sites for excess Na\(^+\), Cl\(^-\) and K\(^+\) (Adams et al., 1998; Agarie et al., 2007) and may play an important role to reduce water loss and prevent UV damage (Shalaba and Mackay, 2011). In halophytes cell turgor is maintained by storage of Na\(^+\) and Cl\(^-\) in vacuoles, with the solute potential of the cytosol adjusted by accumulation of K\(^+\) and organic solutes (Storey, 1995; Glenn et al., 1999). According to Glenn et al. (1999), Na\(^+\), K\(^+\) and Cl\(^-\) contribute to maintain 80–95% of the cell sap osmotic pressure in both halophyte grasses and dicots. Thus halophytes accumulate excessive amount (>10% of dry weight each) of Na\(^+\) and Cl\(^-\) in their shoots (Grattan et al., 2008), largely in vacuoles. At the same time, halophytes maintain cytoplasmic K\(^+\) concentrations similar to those of glycophytes (Flowersand Colmer, 2008) and thus have a high vacuole/cytosol Na\(^+\) ratio and a high cytosol/vacuole K\(^+\) ratio (Ye and Zhao, 2003). Those halophytes, exhibiting enhanced fresh and dry weight gains at high NaCl concentrations, are potential genetic resources for osmotic tolerance determinants that would facilitate growth and yield stability under water deficit (Flowers et al., 1986; Greenway and Munns, 1983; Munns and Tester, 2008).

2.2.2. Ion toxicity

Under salt stress, when plants are unable to stop entry of toxic ions into their body and/or over-accumulate toxic ions like Na\(^+\), Cl\(^-\), SO\(_4\)\(^{2-}\), HCO\(_3\)-, ion toxicity may occurs. Ion toxicity reduces the productivity of plants and hence causes crop failure. However, plant response to ion toxicity differs and varies with species (Dogan et al., 2010). Not all crops are equally affected by ion toxicity and some crops and woody plant species are more sensitive than others.

In plants, ion toxicity mostly occurs in older leaves where toxic ions (Na\(^+\) and Cl\(^-\)) accumulate and cause leaf death. When salt build up in the cytoplasm surpasses the compartmentment of toxic ions in vacuoles, it reduces the activity of different enzymes and hence leads to injury and death of leaves (Munns 2002, 2005; Munns et al, 2006). This death of leaves ultimately reduces the overall photosynthetic area and hence production of photosynthates and their supply is badly affected. This leads to
the disturbance of plant carbon balance to maintain growth and physiological processes (Munns, 2002; Ulfat et al., 2007).

High Na\(^+\) interferes with K\(^+\) and Ca\(^{2+}\) nutrition and stomatal regulation, while high Cl\(^-\) concentration reduces photosynthetic capacity due to chlorophyll degradation in barley (Tavakkoli et al., 2011). Sibole et al. (1998) strongly suggested that bean is extremely sensitive to Na\(^+\). Ulfat et al. (2007) found that high Na\(^+\) concentration in leaves inhibits photosynthesis in different Brassica napus cultivars. Slabu et al. (2009) concluded that for faba bean (Vicia faba) grown at high NaCl concentration, Na\(^+\) is the primary toxic ion, because it interferes with K\(^+\) uptake and disrupts efficient stomatal regulation resulting in unproductive water loss and necrosis whereas Cl\(^-\) induces toxicity symptoms due to chlorophyll degradation. Control of Cl\(^-\) transport and Cl\(^-\) exclusion from shoots is correlated with salt tolerance in many species, particularly legumes, such as Trifolium (Winter, 1982; Rogers et al., 1997), Medicago (Sibole et al., 2003), Glycine (Luo et al., 2005), and Lotus (Teakle et al., 2007), and woody perennials, for example, Citrus and Vitis (Romero-Aranda et al., 1998; Moya et al., 2003). Severe leaf chlorosis and depression of photosynthesis were found for red kidney bean (Phaseolus vulgaris) (Hajrasuliha, 1980), and high concentrations of Cl\(^-\) led to a decrease in the growth rate. Previous investigations on soybean (Glycine max L.) clearly indicated a sensitivity of this species to high concentrations of Cl\(^-\) (Lauchli and Wieneke, 1979; Parker et al., 1983). Based on analysis of a number of field trials of wheat and chickpea crops, Dang et al. (2008) concluded that the Cl\(^-\) concentration in the soil was more important in reducing growth and yield than Na\(^+\). They found that Cl\(^-\) concentration in the youngest mature leaf of bread wheat, durum wheat, and chickpea varied much more with increasing levels of subsoil salinity than with Na\(^+\) concentration (Dang et al., 2006), suggesting that Cl\(^-\) toxicity was relatively more important to growth than Na\(^+\) toxicity. Tavakkoli et al. (2010) reported that exposure to high concentrations of Cl\(^-\) is a major cause of losses in yield due to soil salinity in faba bean.

Mechanisms for keeping cytoplasmic concentrations of Na\(^+\) and Cl\(^-\) below toxic
levels are mainly of two types: the mechanisms which minimize salt entry in the root and its transport through the plant, and other mechanisms reduce the salt buildup in the cytoplasm by sequestration in vacuoles. In fact, most of the plants exclude nearly 98% of the solutes in the soil solution and transport only about 2% solutes to the shoots via xylem. This high degree of exclusion is achieved through (i) tightly controlled uptake from the soil because the epidermis of the roots forming a virtual ‘barrier’ to the salt entry into the roots (Lauchli *et al*., 2008) and (ii) regulated movement in the xylem by controlled loading of Cl\(^-\) into the xylem (Tregeagle *et al*., 2010) or (iii) by retrieval of Na\(^+\) as it moves in the transpiration stream to the leaves (James *et al*., 2006).

The unidirectional Na\(^+\) uptake is all the same and does not differ between salt sensitive and salt tolerant genotypes in most species. But salt tolerant genotypes have the ability to more actively exclude Na\(^+\) via plasma membrane Na\(^+\)/H\(^+\) antiporters. In barley, salt tolerant varieties loaded much higher amounts of Na\(^+\) into the xylem compared with sensitive genotypes (Shabala *et al*., 2010), for more osmotic adjustment in the shoot (Shalaba and Mackay, 2011). The most obvious physiological hallmark’ distinguishing halophytes from glycophytes is their ability to select K\(^+\) from a mixture dominated by Na\(^+\) and yet accumulate sufficient Na\(^+\) for the osmotic adjustment. At the whole-plant level, the selectivity between K\(^+\) and Na\(^+\) \((S_{K/Na})\) in halophytes is within the range of 100-200, even at external salinities exceeding sea water levels. Thus, it appears that there is nothing really unique to halophytes that is not present in glycophytes; the major difference is that the halophytes control these mechanisms more efficiently than glycophytes (Shalaba and Mackay, 2011).

### 2.2.3. Nutrient imbalance

Under salinity, not only the homeostasis of Na\(^+\) but also Ca\(^{2+}\) and K\(^+\) ions are disturbed (Borsani *et al*., 2003; Xue and Liu, 2008). Na\(^+\) and/or Cl\(^-\) concentrations often exceed those of most macronutrients by one or two orders of magnitude, and by even more in the case of micronutrients. Therefore, high concentrations of Na\(^+\) and Cl\(^-\) in the soil solution may depress nutrient-ion activities and produce extreme ratios
of Na⁺/Ca²⁺, Na⁺/K⁺, Ca²⁺/Mg²⁺, and Cl⁻/NO₃⁻. As a result, the plant becomes susceptible to osmotic and specific-ion injury as well as to nutritional disorders that may result in reduced yield or quality. Nutrient imbalances can result in salt-stressed plants in various ways. Imbalances may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant’s internal requirement for that essential element (Grattan and Grieve, 1994).

Under normal conditions, cytosolic K⁺/Na⁺ ratio is fairly high, enabling normal cell metabolism. Under saline conditions, K⁺/Na⁺ ratio falls dramatically (Maathuis and Amtmann, 1999) due to excessive Na⁺ accumulation in the cytosol and increased K⁺ leakage from the cell, resulting from NaCl induced membrane depolarization under saline conditions (Zhu, 2000; Shabala, 2000; Shabala et al., 2003; Chen et al., 2005; Cuin et al., 2008). Sodium ion enters the cytosol via non-selective cation channels and depolarizes the membrane, provoking K⁺ loss via outward-rectifying K⁺ channels (KORC) and non-selective outward rectifying channels (NORC). The activity of plasma membrane H⁺-ATPase opposes NaCl induced membrane depolarization and its related K⁺ efflux. Besides, it fuels the Na⁺/H⁺ antiport, further improving the cytosolic Na⁺/K⁺ ratio (Zepeda-Jazo et al., 2008). Salt stress increases the uptake of some micronutrients like Fe, Mn, Zn, and Cu in some crop plants (Villora et al., 2000).

2.2.4. Reactive oxygen species (ROS)

ROS are produced in plants as a consequence of electron leakage onto O₂ from electron transport activity of mitochondria, chloroplast and cell membranes or from different metabolic processes occurring in various cellular organs (Dell Rio et al., 2006; Blokhina and Fagerstedt, 2010; Heyno et al., 2011). Any type of environmental stress whether biotic or abiotic stimulates the production of ROS due to disturbance of cellular homeostasis (Mittler, 2002; Hu et al., 2008; Han et al., 2009; Mishra et al., 2011; Srivastava and Dubey, 2011).

When generation of ROS surpasses the scavenging or defense mechanism, cell
bears the state of oxidative stress. Cells under oxidative stress bears lipid peroxidation, protein oxidation, DNA damage, inhibition of enzyme activities, initiation of programmed cell death due to ROS activity which ultimately leads to cell death (Verma and Dubey, 2003; Wang et al., 2003; Vinocur and Altman, 2005; Pitzschke et al., 2006; Mishra et al., 2011; Srivastava and Dubey, 2011). To quench and scavenge the ROS, plants have well defined and efficient system of non-enzymatic (tocopherols, carotenoids) and enzymatic (SOD, CAT, POD, APX) antioxidants (Kim et al., 2005; Nawaz et al., 2010; Ali et al., 2011).

Many evidences showed that high level of antioxidants cause more resistance to oxidative damage by ROS in plants (Bhutta, 2011; Nabati et al., 2011). For example, Cavalcanti et al. (2004, 2007) observed the active role of SOD, APX, CAT and GR in salt tolerance of maize and cowpea. However, Bose et al. (2014) in their recent review, argued that salt tolerant species possessing efficient mechanisms of Na⁺ exclusion from the cytosol may not require a high level of antioxidant activity, because they do not allow excessive ROS production in the first instance. They suggested that H₂O₂ signatures may operate in plant signaling networks, in addition to well-known cytosolic calcium signatures. They also suggested that intrinsically higher SOD levels in halophytes are required for rapid induction of the H₂O₂ signature and to trigger a cascade of genetic and physiological adaptive responses, while the role of other enzymatic antioxidants may be in decreasing the basal levels of H₂O₂, once the signaling has been processed.

2.3. Salt tolerance traits in plants

2.3.1. Physiological traits of salt tolerance

Laborious and time consuming efforts of conventional breeding to increase salinity tolerance in plants depend on existing variability in genetic makeup. In addition, to change a single trait which is controlled by multiple genes is very difficult. So, these constraints compel scientific community to take advantage of using advanced knowledge in plant physiological responses during salt stress tolerance.
According to Noble and Rogers (1992), using physiological responses as screening criteria can be successful in pragmatic selection for agronomic traits. When plants are exposed to salinity, both glycophytes and halophytes evolve some mechanisms through which they save them from salt damage.

2.3.1.1. Entry of ions into the roots

Roots are the only plant organs which are firstly and directly exposed to increased salt concentrations in root zone and/or in growth media. Plant and environmental factors change the ion uptake rate among plant species. These factors include plant species/genotype, plant growth stage, temperature, light intensity and relative humidity. Reduction in plant growth due to root zone salinity ultimately reduces the plant vigor and hence the plant yield.

Two types of pathways are adopted by plants to take up ions/salts from the root zone or growth media. These include symplast and apoplast. Symplast requires expenditure of energy in the form of ATP and is an active process while apoplast requires the concentration gradient and is a passive process. Osmotic potential provides the force for ion uptake and plants can regulate the uptake of Na\(^+\) and/or Cl\(^-\) ions by using this force. Different transport proteins are also involved in uptake of different ions (Na\(^+\), K\(^+\)) under normal as well as saline conditions as described by Garcia-debleas et al. (2003).

2.3.1.2. Intra-cellular accumulation/compartmentation

At cell level, different salt tolerance mechanisms operate to efficiently remove the toxic and/or lethal concentrations of ions from the plant body. In cell, different types of antiporter, symporter and carrier proteins actively participate in ion trafficking. These are termed as ion pumps and they regulate the ion homeostasis (IH). SOS (salt overly sensitive) and NHX regulatory pathways are among the best IH pathways. SOS proteins work on plasma membrane while NHX proteins work on tonoplast (vacuolar membrane). SOS family (SOS1, SOS2, SOS3) shows hypersensitivity to NaCl concentration but not to osmotic stress as these are not sensitive to mannitol. In the Arabidopsis thaliana, SOS3 activates SOS2 on cell
membrane and hence stimulates the activity of Na\(^+\)/H\(^+\) antiporter (Quintero \textit{et al.}, 2002; Guo \textit{et al.}, 2004).

After reaching the cytoplasm, Na\(^+\) is immediately pumped into the vacuole and this scavenging is regulated by NHX proteins on tonoplast (Blumwald \textit{et al.}, 2000). When Na\(^+\) is pumped into the vacuole, it is then thrown into the leaf cells before being toxic for different enzymes. The activity of Na\(^+\)/H\(^+\) antiporters is more in halophytes as compared to glycophytes and is induced by the presence of high salt concentration. Thus the over-expression of NHX proteins (vacuolar transporter) enhances the salt stress tolerance as is reported in tomato and rice (Zhang and Blumwald, 2001; Fukuda \textit{et al.}, 2004). These proteins enhance and facilitate the storage of Na\(^+\) ions by increasing uptake of Na\(^+\) to vacuoles and thus confer high salt tolerance by reducing the Na\(^+\) concentration in the cytosol.

Glycophytes as well as halophytes are not able to tolerate high Na\(^+\) concentration in their cytosol and hence both these types control Na\(^+\) entry into the cell and its accumulation in the cytoplasm for the safety and protection of metabolic machinery. A salt tolerant plant “Golden Promise” exhibited significantly low concentration of Na\(^+\) along with low ratios of Na\(^+\)/K\(^+\) and Na\(^+\)/Ca\(^{2+}\) in its younger leaf blades and sheath tissues as compared to a salt sensitive plant “Maythorpe” (Wenxue \textit{et al.}, 2003). In durum wheat, Munns and James (2003) observed that salt sensitive genotypes failed to exclude salts from the transpiration stream which ultimately hammered the new leaves and caused plant death.

In some plant species, however, controversial observations were also recorded. For instance, \textit{Lupinus luteus}, a salt tolerant species compartmented high Na contents in stem when compared with salt sensitive \textit{Lupinus angustifolius} species (Van Steveninck \textit{et al.}, 1982). Such mechanisms prevail in plant cells and even in some special plant parts showing some sort of adaptation at cell or plant level (Carden \textit{et al.}, 2003).

Keeping in view the above discussion, it can be suggested that different plant species specially glycophytes adopt both ion inclusion and exclusion as mechanisms
of salt tolerance and these mechanisms depend on ion distribution pattern among leaves and other parts of the plant body (Munns, 2002; Ashraf, 2004; Dogan et al., 2010; Nemati et al., 2011).

2.3.1.3 Na⁺/K⁺ ratio

High concentration of exchangeable Na⁺ is present in saline and/or sodic condition which increases the Na⁺/K⁺ or Na⁺/Ca²⁺ ratio in soil. Under such conditions, plants take more Na⁺ and ultimately uptake of K⁺ and Ca²⁺ is greatly reduced. Venkata et al. (2012) claimed that of the several parameters (such as biomass production, quantities of proline, glycine betaine, sulphates, etc.) tested initially, only Na⁺ and Na⁺/K⁺ ratio showed significant and consistent (over seasons) correlation with salinity tolerance. They found that in pearl millet (Pennisetum glaucum L.), hybrids involving two poor general combiners produced better salt tolerant type except for stem Na⁺/K⁺ where a combination of such parents yielded moderately tolerant hybrids. Natarajan et al. (2005) concluded that high yielding salt tolerant accessions of rice maintained a low Na⁺/K⁺ ratio and higher grain yield. Similarly, Kaya et al. (2012) noted that salt tolerant linseed genotypes had low Na⁺/K⁺ ratio and low Na⁺ concentration than salt sensitive genotypes. Rezaei et al. (2010) observed that salinity tolerance in rapeseed cultivar Kristina was associated to a restriction of Na⁺ absorption at the root level. Khan et al. (2009) investigated that wheat genotypes with higher concentration of proline, Na⁺/K⁺ ratio and chlorophyll contents were found salt tolerant under saline conditions. It was found that high K⁺ uptake over Na⁺ caused salt tolerance in maize (Akram et al., 2010), in wheat (Munns and James, 2003) and barley (Wenxue et al., 2003). Thus keeping in view the above review, Na⁺/K⁺ ratio can be suggested as an important criteria for screening genotypes under saline conditions (Wenxue et al., 2003; Natarajan et al., 2005; Khan et al., 2009; Akram et al., 2010; Rezaei et al., 2010; Kaya et al., 2012; Venkata et al., 2012). However, it is very important to separate cytosolic K⁺/Na⁺ ratio from the tissue ratio as traditional tissue analysis for Na⁺ content, based on acid tissue digestion followed by AA-spectroscopic analysis, used as a basis to determine K⁺/Na⁺ ratio in plant tissues,
cannot account for Na\(^+\) compartmentation in the vacuole. This diminishes the predictive value of the K\(^+\)/Na\(^+\) ratio in plant tissues to screen plants for salt tolerance (Chen et al., 2005).

### 2.3.1.4 Photosynthesis

Salt stress adversely affects the carbon metabolism of plant and carbon assimilation is severely affected in terms of photosynthesis. To cope with the conditions of osmotic stress, plants conserve their water and hence close stomata. This stomatal closure reduces the intracellular concentration of CO\(_2\) and hence inhibits the carboxylation process of photosynthesis. This disturbance reduces the photosynthetic rate and carbon assimilation in the plants and ultimately plant growth is sharply reduced. Some other factors are also involved in the reduction of photosynthesis in salt stressed plants. These factors include disruption of photosynthetic pigments and enzymes (Stepien and Klobus, 2006). High salt concentration causes thylakoid shrinkage and stacking of grana membranes. Ion imbalance during salt stress causes K\(^+\) deficiency in plants and hence K\(^+\) is reduced in chloroplasts which disintegrate the photo system.

One of the most notable effects of salt stress is the alteration of photosynthetic pigment biosynthesis (Maxwell and Johnson, 2000). However, contradictory reports are available in literature for increase or decrease of chlorophyll contents under salt stress. Although the reduction in chlorophyll contents during salt stress is a frequently observed phenomenon, but many studies show an increase in chlorophyll contents in salt stressed plants. For instance, increase in chlorophyll contents were observed in plants such as mustard (Jamil and Rha, 2013), cotton (Higbie et al., 2010), sugar beet and cabbage (Jamil et al., 2007b) under salt stress conditions while a decline in chlorophyll contents due to salinity was observed in *Oryza sativa* (Amirjani, 2011, Chutipaijit et al., 2011), *Vigna radiata* (Saha et al., 2010), tomato (Dogan et al., 2010), radish (Jamil et al., 2007a) and pea (Hamada and El-Enany, 1994). In different studies, the chlorophyll contents were used as a sensitive indicator of the cellular metabolic state (Chutipaijit et al., 2011).
Based on the integration and correlation between salt tolerance and photosynthetic rate, it can be suggested that photosynthetic rate is useful selection criterion only for those species in which this correlation exists under saline conditions. Thus selection, breeding or genetic engineering of the species having high rate of photosynthesis and improved performance can be fruitful on salt-affected soils.

To develop practical strategies for screening salt tolerant genotypes of any crop, it is very important to investigate that whether physiological or biochemical changes during salt stress are the part of salt tolerance mechanism or these are the consequences of detrimental effects of salt stress. Keeping in view the above discussion, it is obvious that none of the above traits or attributes can be suggested as universal criteria for the determination of plant tolerance during salt stress. So, it would be better and valuable if different attributes are specified for different individual crop species.

2.3.2. Biochemical traits of salt tolerance

2.3.2.1. Synthesis of organic osmolytes

Salinity causes water deficit or osmotic shock to the plants as a first symptom of stress condition. During this shock/stress, plants produce water potential gradient as a first reaction or strategy to cope with the new situation of stress. To create water potential gradient, plants synthesize organic compatible solutes, like proline, and glycinebetain in the cytoplasm. These solutes create sharp water potential gradient and help plants to take up water into the plant. They also protect and maintain the structure of the cell organelles and proteins (enzymes) without interfering with their activities and hence termed as osmoprotectants (Ashrafijou et al., 2010; Nabati et al., 2011). Although osmotic adjustment is essential for adapting plants to soils with low water potential, but may bring penalties in terms of carbon allocation for the rapidly growing phase of fast growing plants such as wheat and barley (Munns, 1988). Compatible solute synthesis comes with an energy cost and hence involves a potential growth penalty (Munns and Tester, 2008). Lutts et al. (1996) found that proline did not take part in osmotic adjustment in salt stressed rice and its accumulation seemed
to be a symptom of injury rather an indicator of salt tolerance. Similarly, Colmer et al. (1995) found no significant affect of proline in salt tolerance in a wheat amphiploid. On the other hand, Petrusa and Winicow (1997) noted a rapid increase in proline contents in alfalfa and similar results were reported by Madan et al. (1995) in salt tolerant lines of B. juncea. Although the synthesis of these compounds occurs at the expense of plant growth, but may allow the plant to survive and recover from the period without water (Munns, 2011).

**2.3.2.1.1. Proline**

In general, proline contents are higher in higher plants but its concentration increases markedly when induced under salt stress (Dogan et al., 2010; Nabati et al., 2011). Proline protects proteins from denaturation, cause plasma membrane stability through phospholipid interaction. Proline also serve as a quencher of hydroxyl radical and provide energy in the form of nitrogenous source. Moreover, production of proline is also linked to water stress, salinity and other abiotic plant stresses (Ashraf and Harris, 2004; Munns and Tester, 2008, Lu et al., 2009) indicating an essential role for these solutes in tolerance to these stresses. Proline accumulates under salt stress in both leaf and root tissues (Sleator and Hill, 2002) and putatively protects against the osmotic potential (Watanabe et al., 2000, Chen et al., 2007). Porgali and Yurekli (2005) observed that proline level increased in Lycopersicon esculentum showing salt sensitivity as compared to Lycopersicon pennellii which was salt tolerant. They also suggested the proline level to be a criterion in the evaluation of plants salt stress tolerance. Huang et al. (2009) observed that increasing salt concentration increases the production of proline in cucumber plant cells and also improve relative water contents and peroxidase activity in cucumber leaves.

**2.3.2.1.2. Glycine betain (GB)**

Plants initiate some defensive machinery in order to cope with stress; one of them is associated with changes in metabolites. Under saline conditions, plants synthesize another organic osmolytes of great importance i.e. glycinebetaine (GB). GB helps to eliminate the salt stress by regulating the water potential of plant cell. Even the foliar
application of GB improves plant growth under salt stress. This response was observed in *Oryza sativa* (Harinasut *et al.*, 1996) and *Zea mays* (Yang and Lu, 2005).

GB also plays a vital role in transduction of stimuli and homeostasis of certain ions (Tuteja, 2007) during plant growth in salty conditions. Cha-um *et al.* (2006) investigated that high level of glycine betain in salt-tolerant rice (*Oryza sativa* L. spp. *indica*) played a significant part by stabilizing chlorophyll pigments and through oxidation of water molecule in PSII. Meloni and Martinez, (2009) observed that in vinal (*Prosopis ruscifolia* Griesbach) increased GB also increased the activity of SOD and improved ion homeostasis in high salt concentration. Similarly, Zhang *et al.* (2009) observed over accumulation of GB as salt tolerant mechanism in transgenic cotton which also protected plasma membranes and enhanced photosynthesis in cotton.

**2.3.2.2. Antioxidative defense system**

A complex antioxidative defense system comprising of enzymatic and non-enzymatic components is present in plants to counteract the destructive oxidative damage of reactive oxygen species (Prochazkova *et al.*, 2001). Superoxide dismutases are the plant metalloenzymes responsible for the detoxification of superoxide radical and its conversion to H$_2$O$_2$ (Mittler *et al.*, 2004). Although H$_2$O$_2$ by itself is less damaging but it can form even more toxic species so its level must be monitored. In leaf peroxisomes at relatively higher concentration H$_2$O$_2$ is scavenged by catalase without any reducing power and thus provides plant with an energy efficient way. However, lower levels of H$_2$O$_2$ are eliminated form chloroplast by ascorbate peroxidase along with other peroxidases with the help of various reductants like ascorbate and glutathione (Asada, 2006). APX, DHAR and GR are the main enzymes involved in the ascorbate-glutathione cycle. In the ascorbate-glutathione cycle (Halliwell-Foyer cycle) (Noctor and Foyer, 1998) APX uses ascorbate as electron donor to reduce H$_2$O$_2$ to water. The monodehydroascorbate (MDHA) disproportionates spontaneously to ascorbate and dehydroascorbate or is regenerated to ascorbate by the NADPH dependent monodehydroascorbate reductase respectively.
The re-reduction of dehydroascorbate to ascorbate is coupled to oxidation of glutathione by dehydroascorbate reductase (DHAR), which is regenerated by the NADPH dependent glutathione reductase (GR). Enzymes of this cycle are present in the cytosol, mitochondria, peroxisomes and in the stroma and thylakoid lumen of chloroplasts (Chew et al., 2003; Shingeoka et al., 2002).

Regarding defense against singlet oxygen in the thylakoid membranes, plants have evolved two strategies. The first is the regulation of the light-harvesting apparatus to diminish triplet chlorophyll production. The second is the rapid quenching of either singlet oxygen directly or indirectly preventing its formation by quenching the triplet chlorophyll production by membrane bound quenchers like carotenoids and tocopherols (Asada, 2006). Unfortunately, cells do not possess any enzymatic mechanisms for the detoxification of highly active hydroxyl radical and only rely on mechanisms that prevent its formation. These mechanisms include the preceding elimination of superoxide radical and H₂O₂ and/or sequestering metal ions that catalyze the Haber-Weiss reaction with specific metal binding proteins (Hintze and Theil, 2006).

Therefore, salt tolerant plants, in addition to being able to regulate water and ionic relations, should also have an efficient antioxidative system for effective removal of the ROS (Rout and Shaw, 2001). Several works have provided evidence for an effective protector role of antioxidant enzymes against oxidative stress in diverse plant species (Mittler, 2002; Vaidyanathan et al., 2003; Jung, 2004). The effect of salinity (100 mM NaCl) and different nitrogen sources (NaNO₃/ (NH₄)₂SO₄) on the activity and spatial distribution of antioxidative enzymes (such as superoxide dismutase, guaiacol peroxidase, and catalase) was investigated in sunflower seedlings by Rios-Gonzalez et al. (2002). Their results indicated that salinity treated plants exhibited increased antioxidant enzyme activities. Moreover, these activities were comparatively higher in roots than in leaves as roots are the first to sense salinity and constitute the first line of adaptation reactions. Contrary to these findings the effects of 10 and 20% sea water studied in nutrient solutions in 30 day-old sunflower plants...
revealed that both APX and GR activities were significantly depressed at higher percentage of sea water. Moreover, a substantial increase in GR activity was exhibited in the leaves of plants grown in 10% sea water (Baccio et al., 2004). However, effect of long term soil salinity (ECe 5.4 and 10.6 dSm⁻¹) in salt tolerant and moderately tolerant wheat cultivars revealed that salinity stress significantly increased thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) activity in both the genotypes and at all the stages (Sairum et al., 2002). Moreover, a higher activity of SOD, CAT and GR was recorded in tolerant cultivar compared with less tolerant cultivar. Results indicated that salinity tolerance of tolerant cultivar as manifested by lower decrease in biomass and grain yield was associated with higher antioxidant activity, and lower TBARS contents. Similar findings were reported again by Sairum et al. (2005) by using salt tolerant and susceptible wheat cultivars under higher salt stress.

Later on, Mandhania et al. (2006) investigated the effect of salt stress on cell membrane damage, ion content and antioxidant enzymes in wheat seedlings of two cultivars salt tolerant and salt sensitive. 4 day old seedlings were irrigated with 0, 50 and 100 mM NaCl. Observations recorded on the 3rd and 6th day after salt treatment revealed that the activities of catalase, ascorbate peroxidase and glutathione reductase increased with increase in salt stress in both the cultivars, however, superoxide dismutase activity declined. Anyhow, MDA contents were significantly increased indicating a high degree of membrane damage by salt stress. Similarly, the effects of salt stress on the activity of SOD, APX and GR enzymes studied by Stepein and Klobus (2005) in two wheat and two maize varieties cleared the role of these enzymes in defense mechanism. In the non-saline control plants, the antioxidant enzymes activities were significantly higher for maize than for wheat indicating that C₄ plants possess stronger defense mechanism as compared to C₃ plants. Adding salt to the nutrient solution significantly increased the level of SOD, APX and GR in leaves of both maize and wheat. In addition, lipid peroxidation analyses indicated an increase in TBARS contents in both plant species grown under salinity that corresponded to the
damage that occurred in secondary oxidative stress. However, as a result of greater efficiency of antioxidant defense in maize, the TBARS quantities remained lower as compared to wheat plants.

The activity of antioxidant enzymes was also reported to increase under saline conditions in case of cotton (Meloni et al., 2003) and it was noted that salinity led to significant increase in SOD, POD and GR activities in salt tolerant cultivars but the activities remained unchanged in salt sensitive cultivars. Similarly, effect of salt stress on the activity of antioxidative enzymes and lipid peroxidation were also investigated in the leaves of two maize cultivars by Azevedo Neto et al. (2006). They reported that in the leaves of salt stressed plants, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and glutathione reductase activities was more pronounced in the salt tolerant than in the salt sensitive genotypes. Salt stress had no significant effect on catalase activity in the salt tolerant, but it was reduced significantly in the salt sensitive genotypes.

It is thus apparent from forth going discussion that a combination of characters like higher antioxidant activity leading to lower oxidative stress, higher osmotic concentration and selective uptake of useful ions and prevention of over accumulation of toxic ions contribute to salinity tolerance in diverse crop species and using physiological and biochemical attributes as salt tolerance indicators is an indirect selection criteria whose success depends on the strong integration and relationship of such indicators with each other and with plant responses to salinity stress.

### 2.3.3. Agronomic traits of salt tolerance

Although crop yield is most important criterion for the assessment of salinity tolerance of any crop, yet seed germination rate, root dry weight, shoot dry weight, number of tillers, leaf damage, leaf size, flowering, fruit size, seed size and survival rate have been suggested to be the very important agronomic traits of salt tolerance (Goudarzi and Pakniyat, 2008; Akram et al., 2011; Ghaloo et al., 2011). During salt stress, timing of crop development is severely affected and it is also worth mentioning that plant growth stages are not related to one another in terms of their salinity.
tolerance. Agronomic traits reflect the interaction of physiological mechanisms, genetic and environmental factors and affect of this interaction on crop growth and yield (Zhou et al., 2010). In different research papers, it is claimed that plant growth attributes should be observed and measured throughout the growth period of crop plants so that the salt sensitive growth stages and agronomic traits could be identified precisely (Erdem et al., 2001).

### 2.3.4 Yield and oil contents

Salt stress tolerance of oilseed crops is a complicated phenomenon as it is significantly modified by environmental conditions such as cultural, climatic and biological factors (Mahmoodzadeh, 2008). Root zone salinity affects many crops in terms of their growth and yield. The reduction in crop yield may be due to the hampered balance of plants for water and nutrients. Limited and imbalanced uptake of water and nutrients by plant roots is due to high osmotic stress and sodium and chloride toxicity (Rameeh et al., 2012).

Linseed has very high activity of antioxidants and is being considered among the most important source for oil and antioxidants. This very high activity of antioxidant is due to the presence of ascorbic acid which is the present in huge amount in linseed (Westscott and Muir, 2003; Morris, 2005). Linseed possesses high omega fatty acid ratio and now a days, it is thought to be the richest and cheapest of omega-3 fatty acid (Okuyama et al., 2007).

Many researchers claimed different results by the application of salt stress on some parameters of high value in crop plants. Both qualitative and quantitative parameters were severely affected by the application of salt stress. For example, increasing salinity reduced nearly all parameters of growth in Nigella sativa, amount of essential oil and growth of Chamomile (Razmjoo et al., 2008) and yield of lemon bark in terms of essential oil (Ozturk et al., 2004). Effect of salt stress on essential oil quality in lemon verbena showed the increased amount of geranial as salinity level was increased (Tabatabaie and Nazari, 2007). On the other hand, findings of the previous researchers are contradictory (Sarah et al., 2010). Francois (1996) observed
that hybrids of sunflower did not show any reduction in seed yield up to 4.8 dS m$^{-1}$ salinity level while oil contents of sunflower remained unaffected by increased salinity up to 10.2 dS m$^{-1}$. Rameeh et al., (2012) observed that seed yield of rapeseed genotypes was significantly affected and reduced by Cl$^{-}$ ion under saline conditions.

2.4. Oil seed Crops

Pakistan, having tremendously an agrarian economy, is facing acute shortage of edible oil production. The domestic production of edible oils only meets about 24 percent of total demand while rest of the requirement is fulfilled through import. The import during the year 2009-10 of Soybean & Palm Oil was $1,979 million that rose to $2,426 million in 2011-12, showing an average increase of 7% per annum (GOP, 2012). Edible oil is a genuine need and cannot be considered as luxury and thus its demand is inelastic and increasing with time. Lack of policy making for oilseed crops in addition to the technological deficiency and lack of awareness of farmers are among the main reasons of this acute shortage of edible oil.

The cotton seed crop has major contribution in edible oil production. Its share in domestic oil production is 51 percent. Sunflower ranks second with a contribution of 32 percent while rest of the crops (canola, rapeseed and mustard) provide 17 percent of edible oil (GOP, 2008). These few crops are the sole source of edible oil production in the country and oil production is severely affected when yield of these crops is reduced due to any reason.

Domestic need for edible oil is increasing every year due to increased population and Pakistan has to spend a huge amount of money (Rs. 45 billion per year) to import edible oil (Ali et al., 2009). To boost the domestic oilseed production, some efforts has been made but, so far, these efforts have been proved fruitless to fill the vast gap between demand and supply of edible oil.

The demand of edible oil, as well as the search for alternative crops by growers, may result in the use of salt-affected and marginal soils for the production of alternate oilseed crops (Francois, 1994; Tanveer-ul-Haq et al., 2002). Camelina (Camelina
sativa) with a low fertility requirement and a short growing season may have potential for biodiesel production (Gugel and Falk, 2006). Field pennycress (Thlaspi arvense) has high contents of erucic and linoleic acids, and grows wild in Northern states and Canada (Moser, 2009). Lesquerella (Lesquerella fendleri) is a potential moderately salt tolerant crop to produce engine lubricants (Adam et al., 2007), and unlike castor (Ricinus communis), it does not contain toxic substances (Grieve et al., 1997; Miyamoto et al., 2012). Salicornia is among the few halophytes which can be grown with highly saline or sea water (Glenn et al., 1999; Grattan, et al., 2008).

To meet the edible oil demands of the country, it is the need of the day to bring marginal lands under oil seed crops by screening and breeding the salt tolerant oil seed crops which are better able to grow on salt-affected soils (Tanveer-ul-Haq et al., 2002).

2.5. Linseed and Salinity

Linseed (Linum usitatissimum L.) is an economically important dual purpose medicinal crop. It gives fiber (flax) from stem and oil (linseed) from seed. It is grown as minor crop in a large number of countries for different types of products (Ebtihal et al., 2012). Linseed has great adaptability and product diversity and researchers of Australia, North America, Europe and Asia are conducting research for producing its bio-products.

Linseed has significant variations for salinity tolerance and its salt tolerance ability does not change with the stage of plant growth and development (Ashraf and Fatima, 1994). Biochemical markers for salt tolerance in linseed were evaluated by El-Beltagi et al. (2008) in linseed cultivars and they established that the relative salt tolerance of tolerant cultivars was linked with high activity of SOD, POD and APX enzymes and low MDA concentration. It was also noted that low Na⁺/K⁺ ratio was an indicator of salt tolerance in linseed cultivars. In another experiment, Emam and Helal (2008) investigated that flax seeds tolerated NaCl stress up to 200 mM NaCl at germination stage and germination was completely inhibited at 300 mM NaCl. In
addition, it was recorded that salt stress significantly increased soluble carbohydrates, MDA contents, reduced glutathione and proline contents but reduced the total phenols, ascorbic acid and total free amino acid contents. They also observed an increased activity of antioxidant enzymes like SOD, APX, ASO (ascorbate oxidase), phenol peroxidase, GPX and polyphenol oxidase, POX in linseed seedlings.

Linseed growth has been studied on salt-affected soils and different physiological and biochemical traits have been indicated which grant salt tolerance in linseed. But little work is done on native linseed genotypes and controversial claims regarding salt tolerance in linseed are reported. For instance, Ashraf and Fatima (1994) reported that degree of salt tolerance of linseed does not vary with growth stage and Na\(^+\) inclusion in the shoot is the physiological trait of salt tolerance in this crop. Similarly, Muhammad and Hussain (2010) studied physiological growth responses of some medicinal plants including linseed and reported that test species were tolerant to moderate salinity and can be grown on saline soils to obtain some biomass. These controversial results encouraged us to conduct comprehensive study of linseed regarding its various aspects from germination to maturity under saline conditions. Thus current study was designed to know the germination response of linseed under salt stress and its growth and yield attributes were recorded to assess the salt tolerant traits in native linseed germplasm.
CHAPTER 3

RESULTS AND DISCUSSION

Study 1

3.1: Screening of linseed genotypes (*Linum usitatissimum* L.) for salt tolerance

3.1.1. Introduction

Selection and screening of a large pool of species, varieties and/or genotypes is essential to explore and identify genes and germplasm for conventional breeding programs to produce and evolve new salt stress tolerant species, varieties and/or genotypes. This strategy involves the useful knowledge of plant responses to salt stress at various growth stages as it was studied in different crops like rice (Shannon, 1998), sorghum (Azhar and Khan, 1997), wheat (Ali *et al*., 2002; Khan *et al*., 2003b), soybean (Kamal *et al*., 2003), cotton (Azhar and Ahmad, 2000) and maize (Khatoon *et al*., 2010). These also provide clues to plant breeders looking for plants of economic importance with improved salt tolerance.

Linseed is important due to its medicinal and industrial by-products and it possess a wide range of salt tolerance and can be successfully grown on marginal salt-affected soils. Thus to assess the magnitude of variation for salt stress tolerance in linseed in quick, precise and simple way, this research was being carried out. This research focused on classification of genotypes on the basis of root and shoot dry biomass, root length, and ionic parameters, i.e., Na\(^+\), K\(^+\) and Na\(^+\)/K\(^+\) ratio.
3.1.2. Materials and method

3.1.2.1. Plant culture and experimental design

A solution culture study was conducted between October and December 2010 in the rain protected wire house of Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. Seeds of 60 linseed genotypes were collected from Ayub Agriculture Research Institute (AARI), Faisalabad. The healthy and sterilized seeds of genotypes were sown in trays containing two inches layers of sand. After 15 days, seedlings were wrapped with the foam at root shoot junction and transplanted in polystyrene sheets with holes in them floating over 200 L capacity iron tubs, lined with polythene sheet containing \( \frac{1}{2} \) strength Hoagland’s nutrients solution (Hoagland and Arnon, 1950) and aerated by the air pump continuously. The culture solution was changed on weekly basis. The design of the experiment was CRD with factorial arrangement having three replications. The pH of solution was maintained between 6.0 and 6.5 with commercial HCl or NaOH.

3.1.2.2. Salinity treatments

After one week of transplanting, salt (NaCl) was added to the nutrient solution and required levels of salinity (control, 100 and 200 mM NaCl) were developed in three increments, whereas no salt was added in control. Salinity was maintained continuously until final harvest. Electrical conductivities (ECw) of nutrient solutions were measured with an electrical conductivity meter on alternate days. Over the duration of stress, the first salt level (designated as low salinity level) of 100 mM NaCl and the second salt level (designated as high salinity level) of 200 mM NaCl were maintained during the experiment. The control, i.e., nutrient solution without added salts, had an ECw of 1.71 dS m\(^{-1}\).

3.1.2.3. Plant harvest

After four weeks of salt stress, plants were harvested and washed thoroughly and root and shoot lengths were recorded. The young fully expanded leaves were separated at harvesting time and were dried at 65±5 °C to constant weight in a forced air-driven oven (Jones and Case, 1990).
3.1.2.4. Measurements

The data regarding growth parameters like plant height, root/shoot lengths, root/shoot fresh and dry weights, number of tillers per plant and ion contents (Na$^+$ K$^+$ and Na$^+$/K$^+$ ratio) of the plant leaves were recorded for each genotype tested in the experiment. Root and shoot lengths and fresh weights were recorded just after harvesting while dry weights of roots and shoots were recorded after oven drying. For the analysis of Na$^+$ and K$^+$, 50 mg of well ground dry material of leaves were digested in 10 ml of di-acids (HNO$_3$:HClO$_4$) (Jones and Case, 1990). The filtrate was filtered with Whatman No. 1 filter paper and Na$^+$ and K$^+$ contents in the digests were determined with a flame photometer (Jenway PFP7).

3.1.2.5. Ranking of genotypes for salt tolerance

Usually in conventional screening methods, genotypes are screened on the basis of observations made for a single physiological or growth characteristics to allocate comparisons. The intervals are set on the basis of observation ranges. This method is often erroneous specifically when multiple growth and physiological characteristics of large number of genotypes are screened. In cluster group analysis, genotypes can be screened simultaneously on several physiological and ionic parameters. No score boundaries are set as genotypes are classified on the basis of cluster group rankings. Genotypes are ranked and grouped for their salinity tolerance. Ranking numbers are assigned to groups on the basis of cluster means. Genotypes are scored on the basis of ranking numbers (Khrais et al., 1998).

Salt tolerance indices were formed from the data for cluster analysis (Zeng et al., 2002). Salt tolerance index was the observation under salinity divided by the mean of control. In cluster analysis, methods of Jolliffe et al., (1989) and Khrais et al., (1998) were followed. Group ranking was obtained on the basis of Ward’s minimum variance cluster analysis for salt tolerance indices of four growth parameters at the seedling stage, i.e., root length, root dry weight, shoot dry weight and shoot/root ratio, and three ionic parameters, i.e., Na$^+$, K$^+$ and Na$^+$/K$^+$ ratio of plant leaves. The procedures are described in the SAS User’s Guide (SAS Institute, 1994). Cluster
groups were ranked from cluster means, in order from highest to lowest means. All
the numbers of group ranking at each salinity level in each genotype were added to
find the sum.

The final ranking of genotypes was done on the basis of these sums in the order
that the genotypes with lowest sums were considered most salt tolerant and those with
highest sums were considered salt sensitive when grown under salinity stress
(El-Hendawy, 2011).

3.1.2.6 Statistical Analysis

All the data presented in this study are mean of three replications. Analysis of
variance (ANOVA) was performed by using a statistical package, Statistix 8.1. Significant differences among treatments were considered at the P ≤ 0.05 levels.

3.1.3. Results

There was very significant difference among linseed genotypes in terms of growth
and ionic parameters. The interactions between salinity levels and genotypes were also
significant (P<0.05) for seedling growth [shoot dry weight (SDW), root dry weight
(RDW), root length (RL) and shoot: root ratio (S/R)] and ionic parameters (Na+, K+
and Na+/K+) measured 30 days after salinity stress, indicates that there was variable
response of genotypes to salinity from low to high levels (Table 3.1.1). The response of
linseed genotypes in terms of their growth and ionic parameters at control, 100 and 200
mM NaCl are presented and discussed here.

Table 3.1.1.F-tests and P values of main effects of salinity and genotypes and their interactions for ionic parameters and seedling growth parameters measured at 30 days from salt stress

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f</th>
<th>Ionic parameters</th>
<th>Growth parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na⁺</td>
<td>K⁺</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>2</td>
<td>876.86**</td>
<td>1703.05**</td>
</tr>
<tr>
<td>Varieties(V)</td>
<td>59</td>
<td>6.55**</td>
<td>6.41**</td>
</tr>
<tr>
<td>S×V</td>
<td>118</td>
<td>5.06**</td>
<td>6.31**</td>
</tr>
</tbody>
</table>

* Significant at α= 0.05, ** highly significant at α= 0.01
3.1.3.1. Seedling growth

Sixty genotypes of linseed were grown under hydroponic conditions under three salinity levels including control. The bulk of data regarding growth and ionic parameters was converted to salt tolerance indices (% control) to rank the sixty linseed genotypes into salt tolerant, moderate and sensitive at two salinity levels. The data regarding growth parameters indicated that vegetative growth of linseed genotypes decreased sharply with increasing levels of salt stress. It was noted that root length, shoot dry weight, root dry weight, and shoot/root ratio significantly decreased with increasing levels of salinity, however, low salinity level (100 mM NaCl) reduced these growth parameters to lesser extent than high salinity level (200 mM NaCl). The trend between reduction of root and shoot growth of linseed genotypes under salinity was rather more interesting. It was recorded that shoot growth was hampered more than root growth in salinity stress because root length of salt stressed genotypes reduced less as compared to the root length of genotypes grown under normal conditions while a sharp decrease in shoot growth of salt stressed genotypes was recorded when compared with their non-saline counterparts. However, in two genotypes 637-72 and NO-303 (ranked salt tolerant), reduction in shoot length was the minimum and these were considered the best performing genotypes under saline conditions (Fig 3.1.1). In addition, the variation among linseed genotypes in terms of relative salt tolerance (salt tolerance index) increased from low to high salinity levels.

During this study, chlorosis was observed occasionally on few plants while visual damage on plant leaves was evident at low salinity level. On the other hand, visual damage was severe at high salinity while chlorosis was observed on all plants. It was difficult to quantify the score among genotypes on the basis of visual observation and did not included in the ranking of genotypes, so data is not shown here but on the whole damage on NO-303 and 637-72 appeared less than the other genotypes. In sixty (60) linseed genotypes, half the genotypes (30) were considered salt sensitive, twenty
one (21) genotypes were moderately tolerant and nine (9) genotypes were ranked salt tolerant regarding their vegetative growth and biomass production (Table 3.1.2).

**Figure 3.1.1.** Graph showing the relative growth parameters of linseed genotypes (genotypes from best to worst performing)

**Figure 3.1.2.** Graph showing the relative ion (Na, K) contents in linseed genotypes (genotypes from best to worst performing)
Table 3.1.2. Ranking of sixty linseed genotypes for their relative salt tolerance in terms of seedling growth parameters (shoot dry weight, root dry weight, root length, shoot/root ratio) in a cluster analysis (Ward’s minimum variance analysis)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>NaCl (mM)</th>
<th>Cluster group ranking (^a)</th>
<th>Sum (^b)</th>
<th>Genotypic ranking (^c)</th>
<th>Tolerance degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-316, 637-72, 97019, NO-303</td>
<td>100</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97001, M-319, NM-14, NM-2</td>
<td>100</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NM-4, 4506-13</td>
<td>100</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L× 10 -103, L-9-20-9</td>
<td>100</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L× 10 -77</td>
<td>100</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>6601-6, NM-92-17, ASTLIA-9, NM-10</td>
<td>100</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2× 10 -7, NM-6, NM-12, 6173-8-33, 230-27-680-4, RL-70, NM-1</td>
<td>100</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-2</td>
<td>100</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>Sensitive</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA-10-127-1, L×10-7-1, 36153-538-2FR, L× 10 -165 p</td>
<td>100</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>L× 10 -10-10, DRM-311, 97007, 97017, L× 10 -3, L× 10 -207, NM-9</td>
<td>100</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>Sensitive</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NL-31, NM-3,</td>
<td>100</td>
<td>3</td>
<td>7</td>
<td>6</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>
a Cluster groups were obtained from Ward’s minimum variance cluster analysis on the means of the salt tolerance indices in seedling growth parameters (see Material and Methods). Genotypes were divided into four cluster groups at 100 mM NaCl and four clusters groups at 200 mM NaCl. The cluster group rankings were obtained from cluster means (data not shown) in the order from the highest to the lowest cluster means.

b Sums were obtained from the cluster group ranking by adding the ranking numbers at the two salt levels in each genotype.

c Genotypes were finally ranked based on the sums with the smallest sum being the relatively most tolerant

### 3.1.3.2. Ionic Tolerance

Data regarding Na\(^+\), K\(^+\) and Na\(^+\)/K\(^+\) ratio in the leaves of linseed genotypes was subjected to cluster analysis for similar ranking as done for growth parameters. But linseed genotypes showed different degree of Na\(^+\) and K\(^+\) ion accumulation in leaves and hence different ranking regarding ionic parameters. However, it was noted that most of the genotypes which were ranked salt tolerant in growth parameters, also maintained the same ranking regarding ionic parameters. Only two genotypes M-319 and Lx×10-77 which expressed tolerance regarding growth parameters, ranked moderately tolerant in ionic parameters. On the whole, most of the linseed genotypes were ranked moderately tolerant to salinity stress while few genotypes showed sensitivity to salt stress in terms of Na\(^+\), K\(^+\) and Na\(^+\)/K\(^+\) ratio in leaves at vegetative
stage of growth (Table 3.1.3). The genotypes containing very high Na⁺/K⁺ ratio were considered salt sensitive while others containing fairly low Na⁺/K⁺ ratio were ranked salt tolerant (Fig 3.1.2). Most of the genotypes which produced relatively higher biomass showed low Na⁺/K⁺ ratio in leaves under saline conditions.

Table 3.1.3. Ranking of sixty linseed genotypes for their relative salt tolerance in terms of ionic parameters (Na⁺, K⁺, Na⁺/K⁺ ratio) in a cluster analysis (Ward’s minimum variance analysis).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>NaCl (mM)</th>
<th>Cluster group ranking</th>
<th>Sum b</th>
<th>Genotypic ranking c</th>
<th>Tolerance degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO-303, E-316, 11-2, 97007,</td>
<td>100</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>Tolerant</td>
</tr>
<tr>
<td>L×10-3, NM-9, 637-72</td>
<td>200</td>
<td>1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NM-2, ASTLIA-9, 97001,</td>
<td>100</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>Tolerant</td>
</tr>
<tr>
<td>NM-10, NM-6, RL-70, 336153-538-2FR, DRM-311, L×10 -165, L×10 -10-39, 98004,</td>
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<td>2</td>
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<td>L×10 -101</td>
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<td>5</td>
<td>4</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97019, NM-14, L×10 -10,</td>
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<td>2</td>
<td>3</td>
<td>2</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2×10-7, 4506-13, NL-31, 6173-8-33, L×10-77, L×10-10-10, 166-L×10, L×10-11,</td>
<td>100</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
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<tr>
<td>M-319, NM-4, 6601-6, 230-27-680-4, L×10 -54, 97017, L×10 -207,</td>
<td>100</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>Moderate</td>
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<td></td>
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61
<table>
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<tr>
<th>Genotype</th>
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<th>4</th>
<th>3</th>
<th>200</th>
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<tbody>
<tr>
<td>E28-1, M-303, Lx10-163</td>
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<td>1</td>
</tr>
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</tr>
<tr>
<td>NM-92-17, NM-3, E-96, Lx10-29</td>
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<td>4</td>
<td>200</td>
<td>2</td>
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</tr>
<tr>
<td>Lx10-7-1, Lx10-164, S-907, Lx10-162, F-18-1,</td>
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<td>5</td>
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<tr>
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</tr>
<tr>
<td>LA-10-127-1</td>
<td>100</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>200</td>
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</tr>
<tr>
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<td>100</td>
<td>4</td>
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</tr>
<tr>
<td>GP98-3-45</td>
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<td>4</td>
<td>8</td>
<td>7</td>
<td>200</td>
<td>4</td>
</tr>
</tbody>
</table>

* Cluster groups were obtained from Ward’s minimum variance cluster analysis on the means of the salt tolerance indices in seedling ionic parameters (see Material and Methods). Genotypes were divided into four cluster groups at 100 mM NaCl and four cluster groups at 200 mM NaCl. The cluster group rankings were obtained from cluster means (data not shown) in the order from the highest to the lowest cluster means.

* Sums were obtained from the cluster group ranking by adding the ranking numbers at the two salt levels in each genotype.

* Genotypes were finally ranked based on the sums with the smallest sum being the most relatively tolerant
Fig. 3.1.3. Dendrogram showing the separation between clusters (groups)
3.1.4. Discussion

In recent years, plant breeders have made considerable achievements in enhancing salt stress tolerance in a large number of arable crops (Shannon, 1998; Ashraf, 2002; Gregorio et al., 2002). But a large number of reports in literature mainly deal with photosynthesis, water relations of plants and accumulation of inorganic ions and organic metabolites related to salt stress tolerance (Munns, 2002; Zhang et al., 2009), as metabolic sites of salinity damage and plants mechanisms to survive salt stress, are so far not well understood. In fact, there are no well defined indicators of salt stress tolerance in plants that could be used practically in increasing salt tolerance in conventional crops (Ashraf, 2002; Gregorio et al., 2002). In addition, the most direct criteria for determining responses to a large number of abiotic stresses including salinity is yield but complex genetic mechanisms for yield with significant environmental influences limit the selection of yield or dry matter production under such stresses. Thus use of physiological and ionic characteristics related to yield is a sensible option for screening of important arable crops.

In this study, salt tolerance among linseed genotypes was evaluated by using cluster analysis. As mentioned by Khrais et al. (1998) and Zeng et al. (2002), the advantages of using a multivariate analysis in the evaluation of salt tolerance are that it allows: i) a simultaneous analysis of multiple parameters to increase the accuracy of the genotype ranking; ii) the ranking of genotypes even when plants are evaluated at different salt levels and salt tolerance varies with salinity levels, especially when the salt tolerance indices are averaged across salt levels; and iii) a more convenient and accurate estimation of salt tolerance among genotypes by simply adding the numbers in cluster group ranking at different salt levels. Because there is variation of salt tolerance among the growth parameters and also among the different ionic parameters for linseed, the sensitive parameters, which can be single or multiple parameters, must be identified at different growth stages before using the cluster analysis.
This study expressed a great deal of variation in tolerance to increasing salt (NaCl) concentrations during the early growth stages of linseed. The growth and performance of some genotypes (salt tolerant) was relatively higher than others (salt sensitive). Salt tolerance is important at whole plant level to seed production but in several crops, it has been shown that tolerance at seedling stage indicates the increased salt tolerance at adult plant level. Most of the genotypes that showed salt tolerance at seedling growth parameters, also exhibited salt tolerance in ionic parameters (Table 3.1.4). For example, genotypes NO-303, 637-72, E-316, 97001, NM-14, NM-2 and 97019 showed same tolerant behavior in both growth and ionic parameters while genotypes M-319 and Lx10 -77 showed salt tolerance at seedling growth level but were ranked moderately tolerant on the basis of ionic parameters (Table 3.1.4). These findings clearly indicate that obviously there is some interaction and correlation between growth and ionic parameters which can be exploited during the screening for salt tolerance.

Four week old seedlings were used to assess variation in salt tolerance in 60 linseed genotypes and valuable information was collected at early stage of plant growth. This method has been intensively utilized to investigate salt tolerance in rice (Zeng et al., 2002), and wheat (Khan et al., 2003b; Ali et al., 2007; El-Hendawy et al., 2011). Literature review shows that seedling stage is most sensitive stage of plant growth and development and mostly the research work on different corps has been done at this stage, as in wheat (Qureshi et al., 1990; Salam et al., 1999; Khan et al., 2003b; Ali et al., 2002, 2007; El-Hendawy et al., 2011), forages (Shahriari, 2012), sorghum (Sorghum bicolor) (Azhar, 1998; Kausar et al., 2012), Lucerne (Medicago sativa) (Al-Khatib et al., 1993), pearl millet (Kebebew &McNeilly, 1996), rice (Shannon et al., 1998; Zeng et al., 2002;), maize (Zea mays) (Rao & McNeilly 1999; Khan & McNeilly, 2000), and cotton (Akhtar and Azhar, 2001).

It was concluded that linseed genotypes had a great deal of variation for salt tolerance among themselves and salt tolerance indices gave a relatively good comparison among linseed genotypes. Salt tolerant genotypes had relatively higher root and shoot dry weights, root length and shoot length, root length ratio than salt
sensitive genotypes and hence had higher salt tolerance indices than rest of the genotypes. On the other hand, salt tolerant genotypes had low Na\(^+\)/K\(^+\) ratio, high K\(^+\) and low Na\(^+\) concentration than the salt sensitive genotypes and hence had lower indices of salt tolerance. It was also observed that ranking of genotypes based on ionic parameters gave a wide range of salt tolerant genotypes (33%) while this range was narrow (15%) in case of growth parameters specially root and shoot biomass. So ionic parameters should be considered as selection criteria for the salt tolerance of genotypes but the mechanism involved in ion tolerance in linseed needs to be studied further for complete understanding of salt tolerance mechanism of linseed.
Study 2

3.2: Effect of salt stress on seed germination and ion (Na\(^+\), K\(^+\)) distribution in linseed

3.2.1. Introduction

An essential step for successful crop production is to obtain an adequate plant population, as yield is reduced by sub-optimal plant densities and uneven stands (Omami, 2005). Salinity of soil and irrigation water is a continuing threat to economic crop production especially in arid and semiarid regions of the world (Ahmad, 2009). The ability of seed to germinate under saline conditions, the cotyledons to break through a soil crust while emerging and seedlings to survive under saline conditions are crucial for crop production in salt-affected soils (Omami, 2005).

High salt concentration in growing medium significantly reduces the seed germination percentage and germination rate (Jamil et al., 2006). Several investigations of seed germination under salt stress have indicated that seeds of most species attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seedling phases of development (Ghoulam and Fares, 2001; Berrichi et al., 2010; Keshavarzi et al., 2011; El-Naim et al., 2012; Kandil et al., 2012; Moosavi et al., 2013; Sikha et al., 2013). The detrimental effect of salts occurs because of osmotic stress and specific ion toxicity (Munns et al., 2006). Seed germination is adversely affected under salt stress due to reduced availability of water, changed mobility of stored reserves and alterations in structural organization of seed proteins (Almansouri et al., 2001; Machado Neto et al., 2004). Seeds need enough water to imbibe and germinate but under the saline conditions, due to the excessive accumulation of salts around the seeds cause the osmotic stress by decreasing the availability of water for imbibitions and germination. The interaction of specific ion and osmotic effects induce a reduction in the number of germinated seeds and retardation in the rate of germination.
Germination and seedling development is very important for early establishment of plants under stress conditions. Selecting cultivars for rapid and uniform germination under saline conditions can contribute towards early seedling establishment (Omami, 2005).

Ion distribution within the plant body highlights the relative importance of different plant organs and tissues in the accumulation of toxic ions and nutrients. Variations in distribution pattern of different ions in different plant organs express various roles of those ions in plant physiology and also show relative mobility of such ions in the plant (Marschner, 1995; Dell, 1996). Ion distribution gradient also depends on tissue age. For instance, K\(^+\) transport occurs freely in xylem and phloem and hence it is quickly transported from older to younger leaves for osmotic adjustment, activation of different enzymes, photosynthetic activity, and synthesis of proteins as well as other important metabolic functions. On the other hand, Ca\(^{2+}\) has limited mobility in phloem and is strictly bound in the older tissues. Nutrient availability, uptake and distribution depend mainly on solute composition and concentration of growth medium and environmental factors. Ion competition has been reported by Grattan and Grieve (1994) and Marschner (1995) at uptake and transport sites of cell membrane between K\(^+\) and Ca\(^{2+}\), K\(^+\) and NH\(_4^+\) and Ca\(^{2+}\) and Mg\(^{2+}\).

Under salt stress conditions, Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Cl\(^-\) and SO\(_4^{2-}\) are present in high concentration in growing media and this high concentration may leads to ion competition and ultimately reduction of nutrient availability, uptake of ions and saturates the binding sites. This competition and interaction between different ions often leads to imbalance as well as deficiency and/or toxicity of ions (Grieve and Shannon, 1999). The inhibitory effects of salinity on plant growth are also attributed to specific ion toxicity, low external osmotic potential and nutrient deficiency (Parida and Das, 2005). Ion toxicity is caused by the replacement of K\(^+\) by Na\(^+\) ions in biochemical reactions and by the loss of function of proteins, as Na\(^+\) and Cl\(^-\) ions penetrate the hydration shells and interfere with the non-covalent interaction among the amino acids (Zhu, 2002).
The ability of plants to maintain a high cytosolic K+/Na+ ratio is a key feature of plant salt tolerance (Chen et al., 2005, 2007; Akram et al., 2010). The change in cytosolic K+/Na+ ratio may be crucial for triggering PCD in living cells (Shabala, 2009). A decrease in the cytosolic K+ pool stimulates caspase-like proteases and endonuclease activity leading to PCD under salt (NaCl) stress (Shabala, 2009; Demidchik et al., 2010). Hence, high cytosolic K+ (no decline in cytosolic K+ pools) is also essential to prevent salt-induced PCD (no PCD) (Shabala, 2009).

Owing to its high nutritive value and a wide adaptability to diverse environments, linseed has been considered a promising crop for marginal lands and semi-arid regions (Ebtihal et al., 2012). Salinity is one of the major limiting factors in crop production in such areas. It is necessary to understand the response of linseed to salt stress if cultivation in saline areas is considered. Little information on the effect of salt stress on linseed germination and ion (Na+, K+) distribution is available. The research objectives were to:

- Assess the response of contrasting linseed genotypes for seed germination at different levels of salinity
- Evaluate ion (Na+, K+) distribution among different parts (root, shoot, leaf) of linseed

3.2.2. Materials and method

3.2.2.1. Plant material, growth and treatment conditions

The solution culture experiment was conducted in the rain protected wire house of Saline Agriculture Research Centre (SARC), Institute of Soil and Environmental Science, University of Agriculture, Faisalabad, Pakistan. Healthy seeds of four linseed genotypes namely: S-907, C-99-3-115 (salt sensitive) and 637-72, NO-303 (salt tolerant) selected from the previous experiment, were sown in trays containing pre-washed sand. Separate trays were used for the germination of each genotype. Water was sprinkled daily over these trays to maintain optimum moisture contents for seed germination. At four leaf stage, the seedlings were wrapped with foam at root shoot junction, transplanted to thermo pore sheets with holes in them floating on 200
L capacity iron tub, lined with polythene sheet containing half strength Hoagland’s solution (Hoagland and Arnon, 1950). Plant’s roots were aerated by bubbling air through the nutrient solution for 8 hours per day by air pump and the solution was renewed twice a week. The design of the experiment was CRD with factorial arrangement using three replications. After one week of transplanting, salinity levels (control, 100 and 200 mM) were developed with NaCl salt in three increments, whereas in control no salt was added. The solution pH was adjusted to 6.5 ± 0.5 throughout the experiment with 1 M NaOH or HCl as required.

**Growth parameters**

3.3.2.2 Measurements of plant growth and biomass

Plant growth was measured in terms of measuring fresh and dry weights of roots and shoots of linseed genotypes. For the determination of dry weight, plants were separated into roots and shoots and dried at 65 ± 5°C for 48 hours.

3.3.2.3 Relative growth rate (RGR)

For the determination of relative growth rate (RGR, g g⁻¹ d⁻¹), plant dry weights of the two successive harvests were used. Three plants from each tub were harvested at two week interval. Relative growth rate was determined by the following formula:

\[
RGR = \frac{1}{W} \times \frac{dW}{dt}
\]

(Ashraf and Fatima, 1994)

Where \( W \) = dry weight of the plants at first harvest; \( dW \) = difference in dry weights of plants at two harvests and \( dt \) = time interval between the two successive harvests.

3.2.2.4 Determination of Na⁺ and K⁺ contents

After 30 days of salt stress, three plants from each treatment were harvested, washed thoroughly with distilled water and dried using blotting paper. The root, shoot and leaves were separated at harvesting time and dried at 65 ± 2°C for 2 days in a forced air-driven oven to determine Na⁺ and K⁺. For the analysis of Na⁺ and K⁺, 50 mg of well ground dry material of each of root, shoot and leaves was digested.
separately in 10 ml of di-acids (HNO₃:HClO₄) mixture. The filtrate was filtered through whatman No. 1 filter paper and Na⁺ and K⁺ contents in the filtrate were determined with a flame photometer (Jenway PFP7).

### 3.2.2.5 Seed germination

For the determination of seed germination of linseed under salt stress conditions, a separate experiment was conducted at Saline Agriculture Research Centre. Four replicates of 50 seeds were germinated in covered, sterilized, disposable petri dishes containing whatman No. 3 filter paper moistened with either distilled water (control) or 100 or 200 mM of NaCl solutions. Petri dishes were sealed with parafilm to prevent evaporation, to minimize changes in the concentration of solutions. Seeds were incubated in a growth chamber at 20 ± 1 ºC and were considered germinated with the emergence of radical. Germinated seeds were counted every day until the end of germination period of 10 days. Every three days, germinated seeds were removed from the petri dishes. To maintain adequate moisture, 5 ml of original salt solutions were added to each petri dish every three days.

### 3.2.2.6 Statistical Analysis

All the data presented in this study are mean of three replications. Analysis of variance (ANOVA) was performed by using a statistical package, Statistix 8.1. Significant differences among treatments were considered at the P ≤ 0.05 levels.

### 3.2.3 Results

#### 3.2.3.1 Effect of salt stress on seed germination

All the genotypes showed almost similar germination percentage under control conditions where distilled water was applied for seed germination while germination percentage was highly affected by the application of salt treatments (Table 3.2.1) and it was significantly reduced between control and 200 mM NaCl. The maximum germination (100%) was recorded at control treatment (distilled water) while the minimum seed germination (78%) was observed at 200 mM NaCl. Significant variations were observed among genotypes regarding seed germination at various
levels of NaCl. Linseed genotypes did not differ at control regarding their germination but seed germination declined progressively when 100 mM NaCl solution was applied and it reduced to 86-88% in salt sensitive genotypes and 93-94% in salt tolerant genotypes. At 200 mM NaCl, the maximum germination was recorded in salt tolerant genotypes (84%) while the minimum germination was recorded in S-907 (78%) followed by C-99-3-115 (80%) (Table 3.2.1).

3.2.3.2 Effect of salt stress on seedling survival

In salt stress, seedling survival of plant is one the most important determinant of salt tolerance. Application of salt stress significantly affected the survival percentage of linseed seedlings which struggled hard to survive at 200 mM NaCl and minimum survival (40%) percentage was observed at this treatment level. It was observed that there was mixed response of salt tolerant and sensitive genotypes at increasing salt levels. Obviously, maximum seedlings survived at control treatment where no salt stress was applied. At control, even 100% seedling survival was observed in all the four linseed genotypes. With increasing NaCl treatments the percent survival of all genotypes decreased progressively. For example, at 100 mM NaCl the reduction in survival was 80-90% in both sensitive and tolerant genotypes. Highest reduction in survival was recorded at 200 mM NaCl, where survival percentage of S-907 declined to 40% followed by NO-303 which expressed 50% survival percentage (Table 3.2.1). Overall, genotypes followed the order as C-99-3-115 = 637-72 > NO-303 > S-907 in their survival at higher level of 200 mM NaCl treatment.
Table 3.2.1. Effect of different levels of NaCl (mM) on germination and survival of salt sensitive (S-907 and C-99-3-115) and salt tolerant (637-72 and NO-303) genotypes of linseed.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>100±1.73</td>
<td>86±2.03</td>
<td>78±1.45</td>
<td>100±1.15</td>
<td>80±2.31</td>
<td>40±1.53</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>100±2.31</td>
<td>88±2.65</td>
<td>80±2.89</td>
<td>100±1.73</td>
<td>90±2.52</td>
<td>60±2.08</td>
</tr>
<tr>
<td>637-72</td>
<td>100±2.31</td>
<td>93±2.08</td>
<td>84±1.86</td>
<td>100±1.73</td>
<td>90±1.15</td>
<td>60±2.89</td>
</tr>
<tr>
<td>NO-303</td>
<td>100±2.89</td>
<td>94±2.65</td>
<td>84±3.06</td>
<td>100±3.21</td>
<td>80±2.31</td>
<td>50±3.46</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE
Growth parameters

3.2.3.3 Relative growth rate (RGR) of linseed genotypes

Relative growth rate has been considered very important to make more appropriate comparisons of growth among species or genotypes under salinity than absolute growth rate (Munns, 2005). The results of the study showed a progressive decrease in RGR with increasing salt concentration in the external growth medium (Table 3.2.2) and the maximum reduction was noted at 200 mM NaCl. There was significant variation in RGR of all the genotypes at all the treatments, however, salt sensitive genotype C-99-3-115 had a drastic reduction compared to other genotypes. At control, the maximum RGR was observed in salt tolerant genotype NO-303 (0.94 g g\(^{-1}\) d\(^{-1}\)) while the minimum RGR (0.80 g g\(^{-1}\) d\(^{-1}\)) was noted in salt sensitive genotype S-907. At 100 mM NaCl, RGR of NO-303 reduced to 0.80 g g\(^{-1}\) d\(^{-1}\) and in S-907, RGR reduced to 0.55 g g\(^{-1}\) d\(^{-1}\). Similarly, at 200 mM NaCl, RGR of NO-303 (0.50 g g\(^{-1}\) day\(^{-1}\)) and 637-72 was the maximum while that of C-99-3-115 (0.32 g g\(^{-1}\) day\(^{-1}\)) and S-907 (0.34 g g\(^{-1}\) day\(^{-1}\)) was the minimum (Table 3.2.2). On the basis of reduction percentage in RGR at higher salinity (200 mM NaCl) when compared to their respective control, the genotypes followed the ranking as: 637-72 (55%) > NO-303 (53%) > S-907 (42%) > C-99-3-115 (38%).

3.2.3.4 Shoot fresh and dry weight (g plant\(^{-1}\))

The shoot fresh and dry weight was recorded at the end of experiment for all the four linseed genotypes at three treatment levels i.e. control, 100 and 200 mM NaCl. Shoot fresh (SFW) and dry weight (SDW) of all the genotypes was significantly reduced with the addition of NaCl to the growth medium and this reduction was the maximum at 200 mM NaCl (Table 3.2.2). Response of genotypes was statistically different from each other at both the levels of salinity and the maximum SFW (13.30 g) and SDW (1.11 g) was measured in 637-72 while the minimum SFW (7.87 g) and dry weight (0.92 g) was noted in C-99-3-115 under non saline control. Genotypes showed sharp decrease in SFW and SDW with increasing levels of salinity and SFW decreased from 11.61 g to 10.21 g in NO-303 and from 13.30 g to 11.20 g in 637-72
genotype. Similarly SDW decreased to 1.03 g in 637-72 followed by NO303 (0.85 g) at 100 mM NaCl application. Salt sensitive genotypes attained lower fresh and dry weight at both the levels of salinity (Table 3.2.2). On the basis of SFW and SDW, genotypes were categorized as: 637-72 > NO-303 > S-907 > C-99-3-115 at all the treatment levels. On the other hand, salt tolerant genotypes exhibited different percentage of SFW and SDW at both salt levels and genotype NO-303 produced more SFW (88%) as compared to 637-72 at 100 mM NaCl.
Table 3.2.2. Relative growth rate, shoot fresh and dry weight of linseed genotypes at different salinity levels.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>0.80±0.15</td>
<td>0.55±0.13 (68)</td>
<td>0.34±0.11 (42)</td>
<td>8.27±0.32</td>
<td>5.07±0.28 (61)</td>
<td>3.43±0.24 (41)</td>
<td>0.97±0.18</td>
<td>0.54±0.12 (56)</td>
<td>0.29±0.13 (30)</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>0.84±0.14</td>
<td>0.59±0.13 (70)</td>
<td>0.32±0.09 (38)</td>
<td>7.87±0.27</td>
<td>4.80±0.42 (61)</td>
<td>2.90±0.21 (37)</td>
<td>0.92±0.17</td>
<td>0.51±0.11 (55)</td>
<td>0.27±0.07 (29)</td>
</tr>
<tr>
<td>637-72</td>
<td>0.90±0.16</td>
<td>0.73±0.13 (81)</td>
<td>0.50±0.10 (55)</td>
<td>13.30±0.34</td>
<td>11.20±0.25 (84)</td>
<td>6.55±0.28 (49)</td>
<td>1.11±0.12</td>
<td>1.03±0.13 (92)</td>
<td>0.51±0.10 (46)</td>
</tr>
<tr>
<td>NO-303</td>
<td>0.94±0.18</td>
<td>0.80±0.16 (86)</td>
<td>0.50±0.13 (53)</td>
<td>11.61±0.26</td>
<td>10.21±0.18 (88)</td>
<td>5.02±0.48 (43)</td>
<td>1.05±0.16</td>
<td>0.85±0.10 (81)</td>
<td>0.33±0.13 (32)</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE and values in parenthesis are the percent of their respective control.
3.2.3.5 Root fresh and dry weight (g plant\(^{-1}\))

Application of salt decreased the root fresh and dry weight in linseed genotypes and this reduction was more severe in salt sensitive genotypes as compared to salt tolerant genotypes (Table 3.2.3). At control, genotypes did not show great difference and the maximum root fresh weight (RFW) was recorded in NO-303 (10.51 g) at control while minimum RFW was noted in C-99-3-115 (9.03 g). Similar trend of genotypes was observed for RDW (root dry weight) at control. Salt treatments decreased RFW and RDW of all the four linseed genotypes but salt sensitive genotype C-99-3-115 produced more root biomass 4.60 g and 4.03 g than S-907 which produced 4.18 g and 3.07 g RFW at 100 and 200 mM NaCl concentrations respectively (Table 3.2.3). On the other hand, salt tolerant genotype NO-303 produced comparatively higher RFW (9.39 g) and RDW (0.71 g) than 637-72 which produced 5.54 g RFW and 0.37 g RDW at 200 mM concentration of NaCl. Overall, at both levels of salinity, the percent RFW and RDW was in the following order as: NO-303 > 637-72 > C-99-3-115 > S-907.

3.2.3.6 Effect of salt stress on Na\(^+\) contents in roots

Increasing salinity increases the concentration of Na\(^+\) in root zone as well as in the plant body. Hence maximum Na\(^+\) contents (12.63 %) were recorded at 200 mM NaCl while minimum Na\(^+\) contents were noted in control treatment (Table 3.2.4). Significant differences among genotypes were recorded in terms of Na\(^+\) accumulation in roots and relatively higher Na\(^+\) accumulation was noted in salt tolerant genotypes. At control (Hoagland’s salinity), where no additional salts were added, accumulation of Na\(^+\) in roots was not much variable among linseed genotypes but with the application of salinity, Na\(^+\) accumulation in roots of all the genotypes increased and changed sharply. For instance, at 100 mM NaCl, accumulation of Na\(^+\) in the roots of salt tolerant genotypes increased from 1.38% to 5.75% in NO-303 and from 1.36% to 7.06% in 637-72. Similarly, at 200 mM NaCl, Na\(^+\) accumulation was almost double than that at 100 mM NaCl in the roots of all linseed genotypes. The maximum Na\(^+\) accumulation was noted in the roots of 637-72 (12.63%) followed by NO-303 (10.31%) while relatively less Na\(^+\) was bound to the roots of salt sensitive genotypes S-907 (7.99%) and C-99-3-115 (10.11%) at
Table 3.2.3. Root fresh and dry weight of linseed genotypes at different salinity levels

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>9.77±0.17</td>
<td>4.18±0.13 (43)</td>
<td>3.07±0.35 (31)</td>
<td>0.83±0.19</td>
<td>0.29±0.09 (35)</td>
<td>0.19±0.08 (23)</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>9.03±0.10</td>
<td>4.60±0.29 (51)</td>
<td>4.03±0.15 (45)</td>
<td>0.67±0.13</td>
<td>0.32±0.11 (48)</td>
<td>0.23±0.12 (35)</td>
</tr>
<tr>
<td>637-72</td>
<td>9.59±0.20</td>
<td>7.42±0.10 (77)</td>
<td>5.54±0.20 (58)</td>
<td>0.74±0.15</td>
<td>0.50±0.13 (67)</td>
<td>0.37±0.10 (51)</td>
</tr>
<tr>
<td>NO-303</td>
<td>10.51±0.25</td>
<td>10.03±0.26 (95)</td>
<td>9.39±0.21 (89)</td>
<td>1.28±0.10</td>
<td>1.06±0.15 (83)</td>
<td>0.71±0.13 (55)</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE and values in parenthesis are the percent of their respective control.
200 mM NaCl (Table 3.2.4). There exists significant difference between salt tolerant (NO-303 and 637-72) and salt sensitive genotypes (C-99-3-115 and S-907) at increasing levels of salt stress while salt tolerant genotypes bound more Na\(^+\) in their roots as compared to salt sensitive genotypes.

### 3.2.3.7 Effect of salt stress on K\(^+\) contents in roots

Concentration of K\(^+\) was highly affected by the application of NaCl in the roots of linseed and K\(^+\) concentration was significantly decreased between control and 200 mM NaCl and the maximum K\(^+\) concentration was recorded at control while minimum K\(^+\) concentration was noted at 200 mM NaCl (Table 3.2.4). Genotypes also showed significant variation for K\(^+\) accumulation in roots and the maximum K\(^+\) contents were recorded in NO-303 while minimum K\(^+\) contents were noted in S-907 genotype at all the levels of treatments. In control, the maximum K\(^+\) contents were accumulated by the roots of salt tolerant NO-303 genotype (3.16\%) while the minimum K\(^+\) accumulation was noted in salt sensitive genotype S-907 (2.86\%). With increasing NaCl treatments, increasing competition between different ions decreased the K\(^+\) contents in the roots of all genotypes. The greatest reduction in K\(^+\) contents occurred at the highest salt concentrations of 200 mM NaCl. At 100 mM NaCl, K\(^+\) contents decreased from 2.86\% to 1.30\% in S-907 and from 3.16\% to 2.26\% in NO-303. Similarly, at 200 mM NaCl, K\(^+\) contents of salt tolerant genotypes NO-303 (2.46\%) and 637-72 (1.19\%) were the maximum as compared to salt sensitive genotypes C-99-3-115 (0.55\%) and S-907 (0.45\%) (Table 3.2.4). There was significant difference between salt tolerant (NO-303 and 637-72) genotypes and salt sensitive genotypes (C-99-3-115 and S-907) for their root K\(^+\) concentration but overall, salt tolerant genotypes NO-303 accumulated greater concentration of K\(^+\) ions in roots. Salt sensitive genotype C-99-3-115 accumulated higher K\(^+\) contents in roots than S-907 while similar trend was observed in salt tolerant genotype NO-303 at increasing levels of NaCl.
Table 3.2. 4. Effect of different levels of NaCl (mM) on Na⁺ and K⁺ contents in roots of salt sensitive (S-907 and C-99-3-115) and salt tolerant (637-72 and NO-303) genotypes of linseed.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>1.45±0.10</td>
<td>5.04±0.07</td>
<td>7.99±0.02</td>
<td>2.86±0.06</td>
<td>1.30±0.03</td>
<td>0.45±0.05</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>1.43±0.05</td>
<td>5.62±0.09</td>
<td>10.11±0.06</td>
<td>2.88±0.07</td>
<td>1.69±0.05</td>
<td>0.55±0.08</td>
</tr>
<tr>
<td>637-72</td>
<td>1.36±0.07</td>
<td>7.06±0.09</td>
<td>12.63±0.02</td>
<td>3.14±0.03</td>
<td>2.03±0.08</td>
<td>1.19±0.03</td>
</tr>
<tr>
<td>NO-303</td>
<td>1.38±0.05</td>
<td>5.75±0.08</td>
<td>10.31±0.06</td>
<td>3.16±0.07</td>
<td>2.26±0.08</td>
<td>2.46±0.05</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE.
3.2.3.8 Effect of salt stress on Na\textsuperscript{+} contents in shoot

Accumulation of toxic ions like Na\textsuperscript{+} and Cl\textsuperscript{-} in shoot is one of the effective mechanisms of salt tolerance in different crop plants. Accumulation of Na\textsuperscript{+} ion was highly affected by the application of salinity (NaCl) and shoot Na\textsuperscript{+} contents were significantly increased in linseed genotypes. As described earlier, increasing NaCl concentration increases Na\textsuperscript{+} concentration in growth media as well as in plant parts. Thus the maximum Na\textsuperscript{+} contents were recorded at 200 mM NaCl while the minimum Na\textsuperscript{+} contents were noted at control (Table 3.2.5). There was significant difference between genotypes for the accumulation of Na\textsuperscript{+} in their shoots but trend of Na\textsuperscript{+} accumulation was quite different than that observed in roots. Thus maximum Na\textsuperscript{+} accumulation was noted in the shoots of salt sensitive genotype S-907 and C-99-3-115 while minimum Na\textsuperscript{+} was accumulated in the shoot of salt tolerant genotypes 637-72 and NO-303. Under controlled conditions, linseed genotypes did not show much difference among themselves but increasing salinity sharply increased shoot Na\textsuperscript{+} accumulation in all the genotypes. At 100 mM NaCl, for example, it increased from 0.51\% to 3.13\% in S-907 genotype and from 0.44\% to 3.09\% in NO-303 while at 200 mM NaCl, Na\textsuperscript{+} build up in the shoots of salt sensitive genotypes S-907 (4.12\%) and C-99-3-115 (4.51\%) was more than that in salt tolerant genotypes 637-72 (3.74\%) and NO-303 (3.35\%) (Table 3.2.5). Response of salt tolerant genotypes was different compared to salt sensitive genotypes for their Na\textsuperscript{+} accumulation in shoot. Overall, salt sensitive genotypes showed high build up of Na\textsuperscript{+} in shoots as compared to salt tolerant genotypes which might have caused injurious effect on physiological and biochemical processes and overall growth and development of salt sensitive genotypes.
Table 3.2.5. Effect of different levels of NaCl (mM) on Na\(^+\) and K\(^+\) contents in shoots of salt sensitive (S-907 and C-99-3-115) and salt tolerant (637-72 and NO-303) genotypes of linseed.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>0.51±0.02</td>
<td>3.13±0.04</td>
<td>4.12±0.08</td>
<td>1.64±0.03</td>
<td>1.17±0.09</td>
<td>0.49±0.06</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>0.46±0.03</td>
<td>3.80±0.07</td>
<td>4.51±0.06</td>
<td>1.65±0.05</td>
<td>1.06±0.07</td>
<td>0.88±0.06</td>
</tr>
<tr>
<td>637-72</td>
<td>0.45±0.03</td>
<td>2.97±0.01</td>
<td>3.74±0.06</td>
<td>2.01±0.07</td>
<td>1.71±0.02</td>
<td>2.57±0.08</td>
</tr>
<tr>
<td>NO-303</td>
<td>0.44±0.02</td>
<td>3.09±0.03</td>
<td>3.35±0.03</td>
<td>2.03±0.09</td>
<td>1.53±0.05</td>
<td>2.23±0.02</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE.
3.2.3.9 Effect of salt stress on K\(^+\) contents in shoot

Highly significant effects of treatments, genotypes and their interaction were observed when K\(^+\) concentration was determined in the shoots of linseed genotypes. K\(^+\) acts as inorganic osmoticum in some plants for regulation of osmotic potential of intra cellular structure and hence plays its role in retaining water contents within the plant body. Concentration of K\(^+\) was highly affected in the shoots of linseed by the application of salinity (NaCl). The maximum K\(^+\) contents were recorded in 200 mM NaCl in salt tolerant genotypes while the minimum K\(^+\) contents were observed in salt sensitive genotypes at the same treatment (Table 3.2.5). Genotypes also showed significant difference for their K\(^+\) accumulation in shoot and the maximum K\(^+\) contents were noted in salt tolerant genotype 637-72 while the minimum K\(^+\) contents were recorded in S-907 genotype. In control, K\(^+\) concentration was highest (2.03%) in the shoot of NO-303 genotype while lowest concentration of K\(^+\) (1.64%) was noted in the shoot of S-907 genotype. Application of salt stress progressively reduced the K\(^+\) contents in the shoots of salt sensitive linseed genotypes and the maximum reduction in K\(^+\) contents occurred in same genotypes at 200 mM NaCl but . At 100 mM NaCl, decrease in shoot K\(^+\) contents ranged from 1.53% to 1.71% in NO-303 and 637-72 and from 1.06% to 1.17% in S-907 and C-99-3-115 respectively. Similarly, at 200 mM NaCl, K\(^+\) accumulation in the shoots of salt tolerant genotype 637-72 (2.57%) was the maximum followed by NO-303 (2.23%) while salt sensitive genotype S-907 had the minimum shoot accumulation (0.49%) of K\(^+\) followed by C-99-3-115 (0.88%) (Table 3.2.5). Salt tolerant (NO-303 and 637-72) and salt sensitive genotypes (C-99-3-115 and S-907) differed significantly for their K\(^+\) accumulation in their shoots. Genotype S-907 and C-99-3-115 reduced the K accumulation with increasing salinity but On the other hand, salt tolerant genotype 637-72 and NO-303 accumulated low K\(^+\) in its shoot at control but with the application of salinity its shoot K\(^+\) accumulation firstly decreased dramatically and then increased to the maximum level.

3.2.3.10 Effect of salt stress on Na\(^+\) contents in leaves

In leaves, Na\(^+\) build up is thought to be injurious and cause disruption of chlorophyll pigments as well as photosynthetic apparatus. Thus it causes adverse affects when accumulated in leaves in high concentration. Concentration of Na\(^+\) in the leaves of linseed genotypes were highly affected by the application of salinity (NaCl)
and Na$^+$ contents were significantly increased between control and 200 mM NaCl (Table 3.2.6). Significant difference was found between salt tolerant and sensitive genotypes and overall the maximum Na$^+$ contents were measured in the leaves of salt sensitive genotypes while the minimum Na$^+$ contents were recorded in the leaves of salt tolerant genotypes. With increased NaCl concentration, the Na$^+$ contents in the leaves of all linseed genotypes increased progressively. The maximum increase in Na$^+$ contents of leaves was noted at the highest NaCl concentration of 200 mM. Concentration of Na$^+$ in the leaves of S-907 and C-99-3-115 increased from 0.14% to 3.55% and 0.18% to 3.42% respectively while in 637-72 and NO-303, Na$^+$ contents increased from 0.22% to 3.37% and from 0.14% to 3.44% respectively at 100 mM NaCl. At 200 mM NaCl, Na$^+$ concentration in the leaves of salt sensitive genotypes S-907 and C-99-3-115 (4.51%) were almost equal to that in the salt tolerant genotypes 637-72 and NO-303 (3.34-4.51%) (Table 3.2.6). There was no significant difference between salt tolerant (NO-303 and 637-72) genotypes and salt sensitive genotypes (C-99-3-115 and S-907) at control but linseed genotypes remained different in their leaf Na$^+$ concentration at increasing levels of salinity. Overall, Na$^+$ build up was more in the leaves of salt sensitive genotypes which might impose adverse effects on the growth of these genotypes.

3.2.3.11 Effect of salt stress on K$^+$ contents in leaves

K$^+$ plays direct role in stomatal conductance and photosynthesis by regulating the opening and closing of stomata and hence its presence in leaves is a beneficial character in terms of growth and development of crop plant especially under stress condition. Concentration of K$^+$ was highly affected in the leaves of linseed by the application of salinity (NaCl) and K$^+$ contents were significantly decreased between control and 200 mM NaCl (Table 3.2.6). Linseed genotypes also differed significantly and the maximum K$^+$ contents were measured in the leaves of salt tolerant genotype NO-303 while the minimum K$^+$ contents were noted in C-99-3-115 genotype. In control, K$^+$ contents were more in the leaves of C-99-3-115 than that of S-907 while genotype NO-303 had more K$^+$ accumulation than 637-72 genotype. With increasing NaCl treatments, the K$^+$ contents in the leaves of all linseed genotypes declined sharply.
Table 3.2.6. Effect of different levels of NaCl (mM) on Na\(^+\) and K\(^+\) contents in leaves of salt sensitive (S-907 and C-99-3-115) and salt tolerant (637-72 and NO-303) genotypes of linseed.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control Na(^+) (%)</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
<th>Control K(^+) (%)</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>0.14 ± 0.03</td>
<td>3.55 ± 0.05</td>
<td>4.51 ± 0.03</td>
<td>1.80 ± 0.06</td>
<td>0.76 ± 0.03</td>
<td>0.40 ± 0.05</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>0.18 ± 0.03</td>
<td>3.42 ± 0.03</td>
<td>4.51 ± 0.02</td>
<td>1.81 ± 0.05</td>
<td>0.68 ± 0.02</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>637-72</td>
<td>0.22 ± 0.09</td>
<td>3.37 ± 0.04</td>
<td>4.51 ± 0.01</td>
<td>1.87 ± 0.02</td>
<td>1.67 ± 0.04</td>
<td>1.89 ± 0.06</td>
</tr>
<tr>
<td>NO-303</td>
<td>0.14 ± 0.06</td>
<td>3.44 ± 0.05</td>
<td>3.34 ± 0.02</td>
<td>1.88 ± 0.03</td>
<td>1.68 ± 0.01</td>
<td>2.00 ± 0.03</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE.
However, at 200 mM NaCl, different trend in leaf K\(^+\) contents was recorded and salt K\(^+\) contents increased in the leaves of salt tolerant genotype and found the maximum in NO-303 (2.0%) followed by 637-72 (1.89%) while the leaf K\(^+\) contents of salt sensitive genotypes C-99-3-115 (0.21%) and S-907 (0.40%) were the minimum (Table 3.2.6). There was significant difference between salt tolerant (NO-303 and 637-72) and salt sensitive genotypes (C-99-3-115 and S-907) at increasing levels of salinity i.e. 100 mM NaCl and 200 mM NaCl.

### 3.2.3.12 Effect of salt stress on K\(^+\)/Na\(^+\) ratio in roots

K\(^+\)/Na\(^+\) ratio is considered one of the most important selection criteria of salt tolerance in crop plants. Thus determination of K\(^+\)/Na\(^+\) ratio in different plant parts was of utmost necessity. K\(^+\)/Na\(^+\) was highly affected in the roots of linseed by the application of salinity (NaCl) as K\(^+\)/Na\(^+\) ratio was significantly decreased between control and 200 mM NaCl. The maximum K\(^+\)/Na\(^+\) ratio was recorded at control while the minimum K\(^+\)/Na\(^+\) ratio was measured at 200 mM NaCl (Table 3.2.7). There was significant difference between genotypes regarding their root K\(^+\)/Na\(^+\) ratio and the maximum K\(^+\)/Na\(^+\) ratio was noted in salt tolerant genotypes while the minimum K\(^+\)/Na\(^+\) ratio was noted in salt sensitive genotypes. At 100 mM NaCl, the root K\(^+\)/Na\(^+\) ratio reduced from 1.98 to 0.26 in S-907 genotype and from 2.32 to 0.29 in 637-72 genotype. Similarly, at 200 mM NaCl, K\(^+\)/Na\(^+\) ratio of salt tolerant genotypes 637-72 (0.10) and NO-303 (0.24) remained higher than that of salt sensitive genotypes C-99-3-115 (0.05) and S-907 (0.06). Linseed genotypes exhibited variation in their root K\(^+\)/Na\(^+\) ratio at all the treatments and genotype 637-72 showed more reduction in root K\(^+\)/Na\(^+\) ratio as compared to NO-303 at 100 mM NaCl, however, salt sensitive genotype C-99-3-115 showed almost equal root K\(^+\)/Na\(^+\) ratio to salt tolerant genotype 637-72 at low salinity levels.

### 3.2.3.13 Effect of salt stress on K\(^+\)/Na\(^+\) ratio in shoot

K\(^+\)/Na\(^+\) ratio was highly affected in the shoots of linseed genotypes by the application of salinity (NaCl) and K\(^+\)/Na\(^+\) ratio decreased significantly between control and 200 mM NaCl (Table 3.2.7). Linseed genotypes differed significantly regarding K\(^+\)/Na\(^+\) ratio in their shoots and the maximum K\(^+\)/Na\(^+\) ratio was recorded in the shoots of salt tolerant genotypes 637-72 and NO-303 while the minimum K\(^+\)/Na\(^+\) ratio was noted in the shoots of salt sensitive genotypes S-907 and C-99-3-115. At
control, shoot K⁺/Na⁺ ratio did not differ to great extent between salt tolerant and sensitive genotypes but increasing NaCl salinity sharply declined the K⁺/Na⁺ ratio in the shoots of all linseed genotypes. An abrupt decrease in shoot K⁺/Na⁺ ratio was measured at 100 mM NaCl and it decreased from 4.51-4.62 to 0.57-0.49 in 637-72 and NO-303 genotypes and from 0.38-0.28 to 0.12-0.19 in S-907 and C-99-3-115 genotypes respectively. At 200 mM NaCl, shoot K⁺/Na⁺ ratio of salt tolerant genotypes 637-72 (0.69) and NO-303 (0.67) was more as compared to salt sensitive genotypes C-99-3-115 (0.19) and S-907 (0.12). Compared to root K⁺/Na⁺ ratio, linseed genotypes showed significant variations for their shoot K⁺/Na⁺ ratio at all the treatment levels (Table 3.2.7).

3.2.3.14 Effect of salt stress on K⁺: Na⁺ ratio in leaves

Ratio of K⁺/Na⁺ was highly affected by the application of NaCl salinity in the leaves of linseed and a significant reduction in leaf K⁺/Na⁺ ratio was noted among treatments. The maximum K⁺/Na⁺ ratio was recorded in the leaves of plant grown in non saline control while the minimum K⁺/Na⁺ ratio was recorded at highest NaCl level of 200 mM (Table 3.2.7). At control, leaf K⁺/Na⁺ ratio did not differ to high extent but increasing NaCl concentration significantly decreased leaf K⁺/Na⁺ ratio of all genotypes. The maximum reduction in leaf K⁺/Na⁺ ratio occurred at the highest NaCl concentration of 200 mM. For example, leaf K⁺/Na⁺ ratio reduced from 15.07-10.72 to 0.21-0.20 in S-907 and C-99-3-115 genotypes with increasing salinity of 100 mM NaCl and from 18.55-11.51 to 0.50-0.49 in salt tolerant genotypes NO-303 and 637-72 at the same stress level. At 200 mM NaCl, leaf K⁺/Na⁺ ratio of 637-72 (0.42) and NO-303 (0.60) was higher than that of C-99-3-115 (0.05) and S-907 (0.09) (Table 3.2.7).
Table 3.2.7. Effect of different levels of NaCl (mM) on K⁺: Na⁺ ratios in roots, shoots and leaves of salt sensitive (S-907 and C-99-3-115) and salt tolerant (637-72 and NO-303) genotypes of linseed.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>K⁺:Na⁺ ratio in roots</th>
<th>K⁺:Na⁺ ratio in shoots</th>
<th>K⁺:Na⁺ ratio in leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (100 mM)</td>
<td>NaCl (200 mM)</td>
<td>Control (100 mM)</td>
</tr>
<tr>
<td>S-907</td>
<td>1.98± 0.11</td>
<td>0.26±0.04</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>2.02± 0.12</td>
<td>0.30±0.05</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>637-72</td>
<td>2.32± 0.09</td>
<td>0.29±0.02</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>NO-303</td>
<td>2.30± 0.08</td>
<td>0.39±0.02</td>
<td>0.24±0.01</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE
3.2.3.15 Partitioning of Na⁺ ion in linseed genotypes at different salinity levels

Na⁺ partitioning in linseed genotypes was examined after the application salt stress. It was noted that salt tolerant genotypes (637-72 and NO-303) bound more Na⁺ in the root section after the application of salt treatments. At control, salt sensitive and tolerant genotypes bound almost equal percentage of Na⁺ in roots but with the application of salt stress, ability of salt tolerant genotypes to accumulate Na⁺ in roots increased rapidly and genotypes 637-72 accumulated more Na⁺ (53% and 60%) followed by NO-303 (47% and 61%) as compared to S-907 (43% and 48%) and C-99-3-115 (44% and 53%) at 100 and 200 mM NaCl concentration respectively (Fig. 3.2.1). It was also noted that Na⁺ accumulation was more in shoots and leaves of salt sensitive genotypes which perhaps damaged the growth and development of these plants. At 100 mM NaCl, leaf Na⁺ concentration was 27% and 30% in S-907 and C-99-3-115 which was higher than 22% and 25% in 637-72 and NO-303 genotypes respectively. Similarly, shoot Na⁺ concentration remained higher 24-25% in salt sensitive genotypes as compared to salt tolerant genotypes which accumulated 18-20% Na⁺ in their shoots at 200 mM NaCl. As leaves are the most sensitive part of plants, so Na⁺ accumulation was less in the leaves of all linseed genotypes at 200 mM NaCl when compared at 100 mM NaCl (Fig. 3.2.1).

![Fig. 3.2.1. Distribution of Na⁺ ion in different parts of linseed genotypes at different salinity levels](image-url)
3.2.3.16 Partitioning of K\(^+\) ion in linseed genotypes at different salinity levels

Application of salinity decreased the K\(^+\) accumulation in roots but increased it in leaves (Fig. 3.2.2). Almost all the genotypes reduced K\(^+\) accumulation in roots and the minimum K\(^+\) accumulation was observed in salt tolerant genotypes at 100 mM NaCl. In leaves of linseed genotypes, a different behavior of K\(^+\) accumulation was recorded with the increasing levels of salinity. It was found that salt tolerant genotypes accumulated less K\(^+\) in shoots than salt sensitive genotypes. For example, salt sensitive genotype S-907 accumulated 36% K\(^+\) in shoot while salt tolerant genotype 637-72 accumulated 31% K\(^+\) in its shoot. Similarly, C-99-3-115 accumulated more K\(^+\) (32%) than NO-303 (31%) at 100 mM NaCl. At 200 mM, genotypes followed the order as NO-303 > C-99-3-115 > S-907 > 637-72 in K\(^+\) accumulation of shoot part. In leaf K\(^+\) accumulation, all the genotypes of linseed showed different pattern among themselves. At control, K\(^+\) accumulation was almost equal in both salt tolerant and sensitive genotypes, but with the application of 100 mM NaCl, accumulation of K\(^+\) in leaves increased dramatically in tolerant genotypes 637-72. At 200 mM NaCl, genotypes expressed a mixed behavior and followed the different order of 637-72 > S-907 > C-99-3-115 > NO-303 for their leaf K\(^+\) accumulation (Fig. 3.2. 2).

![Fig 3.2.2. Distribution of K\(^+\) ion in different parts of linseed genotypes at different salinity levels](image-url)
3.2.4 Discussion

Lab experiment regarding germination of linseed genotypes revealed that an increase in NaCl concentration adversely affected seed germination and seedling survival percentage of four linseed genotypes. It was noticed that seed germination was 100% under normal conditions (control) but 200 mM NaCl solution reduced seed germination to 84% in salt tolerant genotypes while in salt sensitive genotypes, this percentage was even less (78-80%). These finding clearly indicate that seed germination of linseed is inhibited by the presence of salts in the growth medium. The results demonstrated genotypic variation in seed germination responses of linseed to salinity stress. The genotype NO-303 attained the highest final germination percentages at all the salinity levels but the reduction in germination due to increases in salt level was much lower than in the other genotypes. The reduction in seed germination under salt stress might be either due to the decreased rate of water uptake by the seed coat (osmotic shock) and/or alterations of enzymatic or hormonal activities by certain toxic ion accumulation as suggested by Kaya et al. (2006) and Atak et al. (2006). Salt sensitive genotypes were affected more by the application of salt stress in terms of reduction in seed germination as compared to salt tolerant genotypes. Reduction in linseed germination under salt stress has also been reported by Guo et al. (2012).

Salt tolerance during early seedling growth was assessed on the basis of absolute growth at the given salt concentration relative to control. The results demonstrated genotypic variations in seedling growth responses of linseed to salinity stress. Seedling survival of all the genotypes was significantly affected by NaCl and reduced to 50% in salt tolerant genotypes and 40% in salt sensitive genotypes. This reduction in survival of linseed seedling was highly correlated to germination percentage of seeds. It was observed with decreasing germination, survival of seedlings also decreased. Croser et al. (2001) found that there was a little effect of salinity on the emergence of picea glauca, P. mariana and Pinus banksiana, however, later seedling growth was reduced. We observed the same trend that the genotypes sensitive to salinity at germination stage may also show sensitivity at seedling stage.
The variation in seedling growth and survival might also be due to seed reserves as higher seed weight resulted in vigorous seedling growth as speculated by Soltani et al. (2002) in chickpea, Kaya et al. (2008) in chickpea and Kaya and Day (2008) in sunflower.

RGR and shoot fresh weight (SFW) of plant reflect its vigour and are considered a good index to its exposure to stresses of all sorts (Lutts, 2004; Yang et al., 2007). The results of present work clearly revealed the response of linseed genotypes exposed to increasing NaCl concentrations as RGR and shoot fresh weight decreased significantly. This reduction in RGR and root and shoot biomass might be due to ion toxicity or decreased osmotic potential as well as low cell wall extensibility (Grieve et al., 2001; Halperin and Lynch, 2003). There are several reports on osmotic stress and ion toxicity resulted from salt stress in linseed (El-Beltagi et al., 2008; Muhammad and Hussain, 2010; Kaya et al., 2012). Relative growth rate (RGR) of salt sensitive genotypes was severely reduced by the application of salinity and it reduced to 38-40% of control as compared to 53-55% of control in salt tolerant genotypes at 200 mM NaCl. This reduction in RGR might reduce the root and shoot biomass production of all linseed genotypes at higher levels of salinity and severe reduction in root biomass was noted as compared to shoot biomass at higher level of salinity especially in salt sensitive genotypes. The two tolerant linseed genotypes produced relatively greater fresh and dry biomass of both shoots and roots than the sensitive genotypes at different concentrations of NaCl of the growth medium. These results are in agreement with the findings of Ashraf and Fatima (1994) and Khan et al., (2007) who reported the same results in linseed. Similarly, Parida and Das, (2005); Hajer et al. (2006); Turan et al. (2009) and Kaya et al. (2012) observed reduction in RGR and root and shoot biomass of plants under saline conditions.

Salt tolerant genotypes had high ability to restrict the entry of Na⁺ ion to the upper parts of plant and bound more Na⁺ in their roots as compared to salt sensitive genotypes. It is also possible that salt tolerant genotypes had more carriers required for the fast rate of ion uptake than the salt sensitive genotypes (Greenway and Munns, 1983; Flowers and Yeo, 1986). Thus salt tolerant genotypes had low Na⁺ accumulation in shoots and leaves. On the other hand, salt tolerant genotypes proved themselves as the high accumulator of K⁺ in shoots. Root Na⁺ and K⁺ contents did not show significant interaction with dry matter production. Interaction of shoot and leaf
Na\(^+\) contents with shoot dry weight was less than that of shoot and leaf K\(^+\) contents. K\(^+\)/Na\(^+\) of root, shoot and leaves showed even better interaction with shoot dry weight of linseed genotypes and proved important criteria for salt stress tolerance.

Increased salinity reduced K\(^+\)/Na\(^+\) ratio in salt sensitive genotypes while salt tolerant genotypes possessed higher K\(^+\)/Na\(^+\) ratio in roots, shoots and leaves. Shoot K\(^+\)/Na\(^+\) ratio of linseed genotypes was more than that of roots and leaves. Natarajan et al. (2005) reported the same results in rice while Kaya et al. (2012) also observed that salt tolerant genotypes of linseed possessed lower Na\(^+\)/ K\(^+\) ratio as compared to salt sensitive genotypes. Our results are in contradiction with that of Ashraf and Fatima (1994) who found that high K\(^+\)/Na\(^+\) ratio was the characteristics of salt sensitive accessions of linseed. It is possible that the vigorous growth of the two salt tolerant genotypes may have provided enough energy for the active uptake of K\(^+\) and for active removal of Na\(^+\) at root level across the plasma membrane and tonoplast.

In conclusion, the salt tolerant genotypes of linseed showed a relatively good seed germination and seedling survival under salt stress. It is obvious that salt sensitive genotypes of linseed decreased in germination and survival percentage under salinity stress. In addition, it was noted that salt tolerant genotypes showed higher K\(^+\)/Na\(^+\) in their roots, shoots and leaves as compared to salt sensitive genotypes. Ion selectivity especially of Na\(^+\) provides an important tool for salt tolerance in these genotypes and high interaction of ionic parameters especially K\(^+\)/Na\(^+\) ratio in shoots and leaves indicates its importance in biomass production under salt stress and hence can be used as useful criteria of salt tolerance in linseed genotypes. It was also observed that the ability of genotypes to accumulate Na\(^+\) at root levels and to restrict the entry of this ion to upper parts of the plant seems to be one of the distinct features of salt tolerant genotypes.
Study 3

3.3: Physiological and biochemical characterization of linseed genotypes in response to NaCl stress

3.3.1. Introduction

The nature of the damage due to high salt concentrations on plants is not entirely clear. The integrity of cellular membranes, the activities of various enzymes, nutrient acquisition and function of photosynthetic apparatus are all known to be prone to the toxic effects of high salt stress. Salinity stress is known to affect various growth processes including photosynthesis, stomatal conductance, water relations, synthesis and transport of organic compounds (Ashraf, 2004) and ultimately growth of the plant is reduced. An important cause of damage might be reactive oxygen species (ROS) generated by salt stress. Plants subjected to salt stress display complex molecular responses including the production of stress proteins and compatible osmolytes (Zhu et al., 1997). Many of the osmolytes and stress proteins with unknown functions probably detoxify plants by scavenging ROS or prevent them from damaging cellular structures (Zhu, 2001).

The inhibitory effects of salinity on plant growth are also attributed to specific ion toxicity, low external osmotic potential and nutrients deficiencies (Parida and Das, 2005). Ion toxicity is caused by the replacement of K⁺ by Na⁺ in biochemical reactions and by the loss of function of proteins, as Na⁺ and Cl⁻ ions penetrate the hydration shells and interfere with the non-covalent interaction among the amino acids (Zhu, 2002). Sodium translocation from the leaves and lower leaf accumulation of Na⁺ could result in the maintenance of higher K⁺/Na⁺ ratios, which would be suitable for the metabolic processes occurring within the plants (Ashraf and Khanum, 1997). Hence, the ability of plants to maintain a high cytosolic K⁺/Na⁺ ratio is considered to be one of the important physiological mechanisms contributing to salt tolerance in many oat species (Chen et al., 2005; Akram et al., 2010).

Salinity poses several undesirable effects on several plant processes, leading to membrane disorganization, increase in reactive oxygen species (ROS) levels and metabolic toxicity (Hasegawa et al., 2000). High concentration of salts disturbs several biochemical processes and enzyme activities, particularly of CO₂ and nitrate assimilation. The enzyme carbonic anhydrase (CA) is found in abundance in the photosynthesizing tissues of both C₃ and C₄ plants and regulates the availability of
CO₂ to ribulose bisphosphate carboxylase (rubisco) by catalyzing the reversible hydration of CO₂ (Badger and Price, 1994). Whereas nitrate reductase (NR) is the enzyme that catalyses the first step of nitrate assimilation, which appears to be a rate-limiting process in the acquisition of nitrogen (Flores et al., 2002). Limited uptake of CO₂ results in decreased carbon reduction by Calvin cycle, which in turn leads to non-availability of oxidized NADP⁺ for acceptance of electrons during photosynthesis, stimulating the formation of ROS such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radicals (Peltzer et al., 2002). The toxic effects of O₂⁻ and H₂O₂ generate hydroxyl radicals and other destructive species such as lipid peroxides (Vaidyanathan et al., 2003).

For ameliorating salt stress, plants have evolved complex mechanisms that contribute to the adaptation to both osmotic and oxidative stresses caused by salinity. The mechanisms that include osmotic adjustment is usually accomplished by either uptake of organic ions from external solution or by de novo synthesis of some compatible solutes (osmoprotectants) such as amino acids and soluble sugars which act as osmolytes (Shabala et al., 2000; Rontein et al., 2002; Ghoulam et al., 2002; Sakamoto and Murata, 2002; Ashraf and Harris, 2004). Osmoprotectants are neutral molecules that stabilize proteins and membranes against denaturation effect of high concentration of salts (Munns, 2002). Moreover, plant cell must adjust osmotic potential to prevent water losses, maintaining cell turgor under salt stress (Naidoo and Naidoo, 2001).

To minimize the effect of oxidative stress, plant cell have evolved a complex antioxidant system, which is composed of antioxidant compounds (glutathione, ascorbate, β-carotene and α-tocopherol) as well as ROS scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD) (Apel and Hirt, 2004). When ROS production suppresses the antioxidant system capacity, oxidative stress occurs, resulting in protein, DNA, damage and lipid peroxidation (Shalata and Neumann, 2001). These enzymes play significant roles in detoxifying ROS. SOD dismutases superoxide radical to H₂O₂. H₂O₂ reacts with various targets inducing damage to proteins and DNA and also cause lipid peroxidation. Thus CAT and POD are involved in converting H₂O₂ into water and oxygen (Hussain et al., 2007). Ascorbate peroxidase (APX) is the most important
peroxidase, catalyzing the reduction of H₂O₂ to water using the reducing power of ascorbate. Glutathione reductase (GR) plays a crucial role in catalyzing the last and rate-limiting step of the Halliwell-Asada enzymatic pathway (Bray et al., 2000). Malondialdehyde (MDA) contents are considered as the general indicator of lipid peroxidation (Meloni et al., 2003; Wang and Zhou, 2006). Thus antioxidants and compatible solutes may provide a strategy to enhance salt tolerance in plants.

The present study was designed to:

1. Investigate the effect of salt stress on some specific processes having functional significance in C-assimilation and nitrogen status of linseed under stress conditions.
2. Elucidate the effects of salt stress on the activity of anti-oxidative enzymes and lipid peroxidation in leaves.
3. Assess the mechanism of osmotic adjustment in linseed under salt stress.

3.3.2. Materials and methods

3.3.2.1 Seedling growth and treatments

The experiments were conducted in rain protected wire house of Zhejiang University, Hangzhou, China and plant analyses were completed in College of Agriculture and Biotechnology, Zhejiang University. Seeds of two salt tolerant genotypes 637-72 and NO-303 and two salt sensitive genotypes S-907 and C-99-3-115 (based on previous screening experiments, conducted in wire house of Saline Agriculture Research Centre, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan) were germinated in moist quartz sand in greenhouse. Fifteen days old seedlings with uniform growth were selected and transplanted into 20 L containers covered with a foamed plastic plate containing evenly spaced holes. The containers contained half strength Hoagland’s nutrient solution (Hoagland and Arnon, 1950). The design of the experiment was CRD with factorial arrangement using three replicates. After one week of transplanting, salinity levels (control, 100 and 200 mM) were developed with NaCl salt in three increments, whereas in control no salt was added. The solution pH was adjusted to 6.5±0.5 throughout the experiment with 1 M NaOH or HCl, as required. The nutrient media in the pots was continuously aerated using aquatic pumps and media was changed after every five days. Measurements were made after 30 days of salt stress.
Physiological parameters

3.3.2.2 Photosynthetic rate ($P_n$) and Stomatal conductance ($g_s$)

$P_n$ and $g_s$ were measured in cloudless clear days at light-saturating intensity between 11.00 and 12.00 hours on the uppermost, fully expanded leaves, using Infrared Gas Analyser (IRGA), LICOR-6200 portable photosynthesis system (LI-COR Lincoln, NE, USA).

3.3.2.3 Chlorophyll a,b

During the experiment, before dealing amount of proline, chlorophyll ‘a’ and chlorophyll ‘b’ contents were measured in the laboratory. Photosynthetic pigments (chlorophyll a and b) were measured in fresh leaf samples, a week before the harvest. One plant per replicate was used for chlorophyll determination. Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Leaf samples (0.5 g) were homogenized with acetone (80% v/v), filtered and make up to a final volume of 5 mL. Then the solution was centrifuged at 3000 rpm for 10 minutes. Pigment concentrations were calculated from the absorbance of extract at 663 and 645 nm using the formula (Ashraf et al., 1994; Arnon, 1975) given below:

Chlorophyll a (mg/g FW) = \[12.7 \times (A_{663}) - 2.69 \times (A_{645})\] \times 0.5

Chlorophyll b (mg/g FW) = \[22.9 \times (A_{645}) - 4.69 \times (A_{663})\] \times 0.5

Chlorophyll a+b (mg/g FW) = \[20.2 \times (A_{645}) - 8.02 \times (A_{663})\] \times 0.5

3.3.2.4 Relative water contents (RWC)

Relative water contents were measured as described by Yamasaki and Dillenburg (1999). Leaves were collected from mid section of plant in order to minimize age effect. For each treatment, 10 leaves were taken. To obtain their fresh weight (fr wt), the leaves were weighed just after removal from the stem. In order to determine turgid weight (t wt), the leaves were kept in Double Distilled water (DDW) inside a closed perti dish for four hours. After gentle wiping the water from the leaf surface with tissue paper, the leaves were weighed. To determine dry weight (dry wt), the leaf samples were dried at 80 °C for 48 h. Values for fr wt, t wt and dry wt were used to calculate leaf RWC using the equation below:

$$\text{RWC (\%)} = \frac{(\text{fr wt} - \text{dry wt})}{(\text{t wt} - \text{dry wt})} \times 100$$
3.3.2.5 Electrolyte leakage (EL)

EL is used to assess membrane permeability as described by Lutts et al. (1995). Samples were washed three times with DDW to remove surface contamination. Young leaf discs from each sample were taken. Leaf discs were placed in a closed vial containing 10 mL of DDW and incubated on rotator shaker for 24 h subsequently the electrical conductivity of solution (EC1) was determined. Samples were then autoclaved at 120 °C for 20 min and last electrical conductivity (EC2) was noted after cooling the solution at room temperature. The electrolyte leakage was calculated as:

\[ EL (\%) = \left( \frac{EC1}{EC2} \right) \times 100 \]

3.3.2.6 Leaf osmotic potential

A fully expanded youngest leaf was excised from each plant and was immediately frozen. After two weeks, the frozen sap was extracted by crushing the leaf material and sap was used directly for osmotic potential determination in an osmometer (Wescor, 5500 Vapour pressure osmometer) (Scholander et al., 1965).

Biochemical parameters

3.3.2.7 Carbonic anhydrase (CA) activity

The activity of CA enzyme was determined by the method of Dwivedi and Randhava (1974). The leaf samples were cut into small pieces and suspended in cystein hydrochloride solution. The samples were incubated at 4 °C for 20 min. the pieces were blotted and transferred to the test tubes containing phosphate buffer (pH 6.8) followed by the addition of alkaline bicarbonate solution and bromothymol blue indicator. The test tubes were incubated 5 °C for 20 min. After addition of 0.2 mL of methyl red indicator, the reaction mixture was titrated against 0.05 N HCl. The results were expressed as μ mol CO₂ kg⁻¹ leaf fr wt s⁻¹.

3.3.2.8 Nitrate reductase (NR) activity

NR activity was estimated by the method of Jaworski (1971). Fresh leaf samples were weighed and transferred to plastic vials. To each vial, 2.5 mL phosphate buffer (pH 7.5), 0.2 M potassium nitrate and 5% isopropanol solutions were added. Each vial was then incubated for 2 h in the dark at 30 °C. To the incubated mixture, 1% sulphanilamide and 0.2% NED-HCl (N-1-nephthylethylene-diamine dihydrochloride) were added. The reaction mixture was kept for 20 min for color development. The
absorbance was read at 540 nm and was compared with that of the calibration curve. The activity of NR was expressed as \( \mu \text{mol NO}_2 \text{ h}^{-1} \text{ g}^{-1} \text{ leaf fr wt.} \)

### 3.3.2.9 Determination of Proline

Proline contents were determined spectrophotometrically adopting the ninhydrin method of Bates *et al.* (1973). As much as 300 mg of fresh leaf samples were homogenized in sulphosalicylic acid. To the extract, 2 mL each of acid ninhydrin and glacial acetic acid were added. The samples were then heated to 100 °C. The mixture was extracted with toluene and the free toluene was quantified spectrophotometrically at 528 nm using L-proline as a standard.

### 3.3.2.10 Determination of Glycine betaine (GB)

Glycine betaine contents were estimated by the method of Grieve and Grattan (1983). Leaves were weighed and oven dried at 80 °C and the dried leaves were finely ground with deionized water at 100 °C for 60 min. GB concentration was determined at 365 nm, using aqueous extracts of dry ground leaf material after reaction with potassium tri-iodide solution.

### 3.3.2.11 Determination of total soluble sugars (TSS)

Total soluble sugars were extracted following the method adopted by Homme *et al.* (1992). Sugar free residues were extracted with 1.5 N H\(_2\)SO\(_4\) following the method by Naguib (1963). Soluble sugars and those resulting after polysaccharides hydrolysis were estimated by anthrone reagent (Fairbairn, 1953).

### 3.3.2.12 Determination of soluble proteins

Soluble proteins were extracted according the method described by Hassanein (1977). Water insoluble residues remaining after extraction of soluble proteins were extracted with 1N NaOH. Soluble proteins and those resulting after insoluble residue hydrolysis were measured by using BIO-RAD protein assay dye reagent according to the method adopted by Bradford (1976).

### 3.3.2.13 Assay of antioxidant enzymes

Fully expanded leaves of plant samples were collected for enzymatic analysis after 30 days of salt stress. The 0.5 g fresh weight of leaves was homogenized in a pre-chilled mortar under ice-cold conditions in 5.0 ml 50 mM cold sodium phosphate buffer (pH 7.8). After centrifuging at 13,000 \( \times \) g for 30 min, the supernatants were stored at 4 °C and used for measurements of various antioxidant enzymes.
Superoxide dismutase (SOD) activity was determined by the nitro blue tetrazolium (NBT) method (Giannopolitis and Ries, 1977) by measuring the photoreduction of NBT at 560 nm. The reaction mixture (3 ml) contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 10 µM EDTA, 2 mM riboflavin and enzyme extract (100µl). Reaction was started by placing tubes below two 15 W fluorescent lamps for 10 min, then stopped by switching off the light. Non-illuminated and illuminated reactions without supernatant served as calibration standards. The absorbance was measured at 560 nm. One unit of SOD was defined as the quantity of enzyme that produced 50% inhibition of NBT reduction under the experimental conditions.

Catalase (CAT) activity was determined according to Aebi (1974). The assay mixture (3 ml) consisted of 100µl enzyme extract, 100µl H₂O₂ (300 mM) and 2.8 ml 50mM phosphate buffer with 2 mM EDTA (pH 7.0). The CAT activity was assayed by monitoring the decrease in the absorbance at 240 nm as a consequence of H₂O₂ disappearance (molar extinction coefficient (ε) = 39.4 mM cm⁻¹).

Peroxidase (POD) activity was measured according to the method of Amako et al. (1994) with some modification. The reaction mixture (3 ml) consisted of 100µl enzyme extract, 100µl guaiacol, 100µl H₂O₂ (300 mM) and 2.7 ml 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). Increase in the absorbance due to oxidation of guaiacol was measured spectrophotometrically at 470 nm (ε = 26.6 mM cm⁻¹).

Ascorbate peroxidase (APX) activity was assayed according to the method of Nakano and Asada (1981). The reaction mixture consisted of 100µl enzyme extract, 100µl ascorbate (7.5 mM), 100µl H₂O₂ (300 mM) and 2.7 ml 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). The oxidation of ascorbate was determined by the change in absorbance at 290 nm (ε = 2.8 mM cm⁻¹)

3.3.2.14 Lipid peroxidation (MDA)

The level of lipid peroxidation in plant tissues, expressed as malondialdehyde (MDA), was determined according to Hodges et al. (1999). Fresh samples (0.5 g) were homogenized in 4.0 ml of 1% trichloro acetic acid (TCA) solution and centrifuged at 10,000 × g for 10 min. The supernatant was added to 1 ml 0.5% (w/v) TBA made in 20% TCA. The mixture was heated in boiling water for 30 min and the
reaction was stopped by placing the tubes in an ice bath. The samples were centrifuged at 10,000×g for 10 min, and the absorbance of the supernatant was recorded at 532 nm. Correction of non-specific turbidity was made by subtracting the absorbance value read at 600 nm. The level of lipid peroxidation was expressed as nmol g⁻¹ fresh weight by using its molar extinction coefficient of 0.155 mM cm⁻¹.

3.3.2.15 Statistical analysis

All the data presented in this study are mean of three replications and standard errors (SE). Analysis of variance (ANOVA) was performed by using a statistical package, Statistix 8.1. Significant differences among treatments were considered at the P ≤ 0.05 level.

3.3.3. Results

Physiological parameters

3.3.3.1 Relative water contents (RWC) and electrolyte leakage (EL)

To assess the plant water status, relative water content (RWC) is easy to measure and widely used method for assessing plant water status while electrolyte leakage (EL) shows degree of membrane damage under stress conditions. Addition of NaCl in the growth medium caused significant reduction in relative water contents (RWC) of all four linseed genotypes (Table 3.3.1). On the other hand, salt application significantly increased the EL of all the genotypes under stress. There was no significant variation among linseed genotypes for their RWC and EL, however, salt tolerant genotypes maintained relatively higher RWC than salt sensitive genotypes. Contrary to that, these genotypes showed less EL as compared to salt sensitive genotypes at all the treatment levels. The maximum RWC was noted in salt tolerant genotype 637-72 (21.50%, 15.77%) followed by NO-303 (21.47%, 15.73%) at 100 mM and 200 mM NaCl while at 100 mM NaCl, the minimum RWC was noted in salt sensitive S-907 (21.10%) genotype while at 200 mM the minimum RWC 15.57%. Unlike RWC, EL was the maximum in salt sensitive genotypes and the minimum in salt tolerant genotypes. The increase in EL of all the genotypes was much pronounced at higher salinity of 200 mM NaCl and it was 26.27% and 26.10% in 637-72 and NO-303
Table 3.3.1. Relative water contents (RWC) and electrolyte leakage (EL) of linseed genotypes at different salinity levels

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Relative water contents (%)</th>
<th>Electrolyte leakage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>NaCl (100 mM)</td>
</tr>
<tr>
<td>S-907</td>
<td>31.40±0.31</td>
<td>21.10±0.38</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>31.53±0.24</td>
<td>21.13±0.49</td>
</tr>
<tr>
<td>637-72</td>
<td>31.80±0.46</td>
<td>21.50±0.29</td>
</tr>
<tr>
<td>NO-303</td>
<td>31.76±0.44</td>
<td>21.47±0.15</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE
respectively (Table 3.3.1). On the other hand, EL was relatively higher i.e. 26.90% and 26.83% in salt sensitive genotypes S-907 and C-99-3-115 respectively at 200 mM NaCl.

3.3.3.2 Effect of salt stress on photosynthetic pigments

Effect of NaCl application on chlorophyll ‘a’, ‘b’ contents and chlorophyll a+b in four linseed genotypes are depicted in Table 2. Salinity treatments caused significant decrease in chlorophyll ‘b’ in all the four genotypes while there was no significant decrease in chlorophyll ‘a’ contents in linseed genotypes at all the treatment levels. On the other hand, a significant decrease in chlorophyll a+b was observed in all the four genotypes with increasing levels of salinity (Table 3.3.2). Maximum chlorophyll ‘a’ (0.25 mg g⁻¹ FW) and chlorophyll ‘b’ (0.23 mg g⁻¹ FW) was recorded in control treatment while minimum concentration of chlorophyll ‘a’ (0.21 mg g⁻¹ FW) and ‘b’ (0.10 mg g⁻¹ FW) was observed at 200 mM NaCl. Similarly, the maximum chlorophyll a+b was recorded at control and the minimum quantity of chlorophyll a+b was recorded at 200 mM NaCl. There was no significant difference in linseed genotypes for chlorophyll ‘a’ at all the treatments but chlorophyll ‘b’ contents were significantly varied among genotypes. At 100 mM NaCl, chlorophyll ‘b’ decreased progressively and its reduction ranged from 0.23 to 0.19 mg g⁻¹ FW in NO-303 genotype and from 0.20 to 0.15 mg g⁻¹ FW in S-907 genotype. While at 200 mM NaCl, chlorophyll ‘b’ further reduced to 0.16 mg g⁻¹ FW in salt tolerant genotypes while it was 0.10 mg g⁻¹ FW in salt sensitive genotypes. At 200 mM NaCl, the maximum chlorophyll a+b contents were found in salt tolerant genotypes while the minimum were measured in S-907 (Table 3.3.2). On % control basis, genotype 637-72 performed better followed by NO-303 at both 100 and 200 mM NaCl concentrations.
Table 3.3.2. Chlorophyll contents (a, b) and chlorophyll a+b in leaves of salt sensitive (S-907 and C-99-3-115) and salt tolerant (637-72 and NO-303) genotypes of linseed.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Chlorophyll ‘a’ (mg g⁻¹ FW)</th>
<th>Chlorophyll ‘b’ (mg g⁻¹ FW)</th>
<th>Chlorophyll a+b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (100 mM)</td>
<td>NaCl (200 mM)</td>
<td>Control (100 mM)</td>
</tr>
<tr>
<td>S-907</td>
<td>0.24±0.12</td>
<td>0.24±0.10</td>
<td>0.21±0.14</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>0.24±0.09</td>
<td>0.24±0.08</td>
<td>0.22±0.10</td>
</tr>
<tr>
<td>637-72</td>
<td>0.24±0.11</td>
<td>0.24±0.14</td>
<td>0.23±0.09</td>
</tr>
<tr>
<td>NO-303</td>
<td>0.25±0.10</td>
<td>0.24±0.09</td>
<td>0.23±0.06</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE
3.3.3.3 Gas exchange

The data regarding plant stomatal conductance ($g_s$) and photosynthetic rate ($P_n$) in young leaves of linseed genotypes is shown in Table 3.3.3. Genotypes showed significant variations for their stomatal conductance and photosynthetic rate at all the levels of salt stress. The maximum stomatal conductance as well as photosynthetic rate was recorded in salt tolerant genotypes while the minimum stomatal conductance and photosynthetic rate was noted in salt sensitive genotypes. Stomatal conductance and photosynthetic rates of salt tolerant genotypes (637-72 and NO-303) was statistical with respect to salt sensitive genotypes C-99-3-115 and S-907 under control treatment. With the application of salts, a clear decline in $P_n$ and $g_s$ was found in stressed plants of all the four linseed genotypes and this reduction was more prominent in salt sensitive genotypes than that in salt tolerant genotypes. At 100 mM NaCl, stomatal conductance changed from 1.93 to 1.57 mmol m$^{-2}$ s$^{-1}$ and photosynthetic rate reduced from 16.83 to 12.70 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ in genotype 637-72 while these parameters reduced from 1.77 to 1.33 mmol m$^{-2}$ s$^{-1}$ and 16.50 to 11.93 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ respectively in C-99-3-115 genotype. At 200 mM NaCl, stomatal conductance and photosynthetic rates of salt sensitive genotypes S-907 (0.50 mmol m$^{-2}$ s$^{-1}$; 9.33 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) and C-99-3-115 (0.57 mmol m$^{-2}$ s$^{-1}$; 9.83 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) were less than that of salt tolerant genotypes 637-72 (0.93 mmol m$^{-2}$ s$^{-1}$; 10.93 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) and NO-303 (0.83 mmol m$^{-2}$ s$^{-1}$; 10.87 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) (Table 3.3.3). Compared to control treatment, salt sensitive genotypes showed 74-75% stomatal conductance at 100 mM NaCl as compared to salt tolerant genotypes which showed 81.88% stomatal conductance at the same treatment. However, at 200 mM NaCl, stomatal conductance reduced more in salt sensitive genotypes (30-32% of control) rather than in salt tolerant genotypes (45-48%). Similarly, photosynthetic rate of salt sensitive genotypes reduced to 71-72% of respective control at 100 mM NaCl while in salt tolerant genotypes it was 75% of respective control at same salinity level while at 200 mM NaCl, photosynthetic rate was reduced to 57-60% in salt sensitive genotypes when compared with control but salt tolerant genotypes showed 65% photosynthetic rate of their respective control.
Table 3.3.3. Stomatal conductance ($g_s$) and photosynthetic rate ($P_n$) of linseed genotypes at different salinity levels.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>NaCl</th>
<th>NaCl</th>
<th>Control</th>
<th>NaCl</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(100 mM)</td>
<td>(200 mM)</td>
<td>(100 mM)</td>
<td>(200 mM)</td>
<td>(100 mM)</td>
<td>(200 mM)</td>
</tr>
<tr>
<td><strong>S-907</strong></td>
<td>1.67±0.18</td>
<td>1.24±0.19</td>
<td>0.50±0.15</td>
<td>16.33±0.20</td>
<td>11.67±0.35</td>
<td>9.33±0.24</td>
</tr>
<tr>
<td><strong>C-99-3-115</strong></td>
<td>1.77±0.15</td>
<td>1.33±0.12</td>
<td>0.57±0.09</td>
<td>16.50±0.32</td>
<td>11.93±0.26</td>
<td>9.83±0.29</td>
</tr>
<tr>
<td><strong>637-72</strong></td>
<td>1.93±0.18</td>
<td>1.57±0.12</td>
<td>0.93±0.15</td>
<td>16.83±0.22</td>
<td>12.70±0.21</td>
<td>10.93±0.32</td>
</tr>
<tr>
<td><strong>NO-303</strong></td>
<td>1.85±0.13</td>
<td>1.63±0.09</td>
<td>0.83±0.12</td>
<td>16.70±0.26</td>
<td>12.50±0.29</td>
<td>10.87±0.43</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE
3.3.3.4 Carbonic anhydrase (CA) and nitrate reductase (NR)

To sustain the carboxylation reaction of photosynthesis, carbonic anhydrase (CA) enzyme rapidly converts atmospheric CO$_2$ to HCO$_3^-$ and is considered the first step in photosynthesis while nitrate reductase (NR) is one of the most important enzymes in the assimilation of exogenous nitrate, the predominant form of nitrogen available to green plants growing in soil. Activity of this enzyme in plants gives a good estimate of the nitrogen status of the plant and is very often correlated with growth and yield. CA and NR activity of all the genotypes significantly decreased by the increased levels of salinity (NaCl). At control, activity of CA had no difference and genotypes showed almost equal activity of CA but significant difference was observed among genotypes in their NR activity (Table 3.3.4). A progressive decrease in the activity of CA and NR was observed in genotypes and CA activity of salt sensitive genotypes decreased from 341.07 to 265.20 µmol CO$_2$ kg$^{-1}$ LFW s$^{-1}$ in S-907 and from 341.60 to 265.43 µmol CO$_2$ kg$^{-1}$ LFW s$^{-1}$ in NO-303 genotype at 100 mM NaCl. Similarly, NR activity decreased in salt tolerant genotype NO-303 from 0.58 to 0.48 µmol NO$_2$ h$^{-1}$ g$^{-1}$LFW s$^{-1}$ and from 0.41 to 0.33 µmol NO$_2$ h$^{-1}$ g$^{-1}$LFW s$^{-1}$ in salt sensitive genotype C-993-115 at same level of salt stress. At 200 mM NaCl, the maximum activity of CA was measured in NO-303 (191.50 µmol CO$_2$ kg$^{-1}$ LFW s$^{-1}$) followed by 637-72 (191.47 µmol CO$_2$ kg$^{-1}$ LFW s$^{-1}$) while the minimum activity was noted in S-907 (191.25 µmol CO$_2$ kg$^{-1}$ LFW s$^{-1}$) and C-993-3-115 (191.27 µmol CO$_2$ kg$^{-1}$ LFW s$^{-1}$) (Table 4). Activity of NR differed greatly among genotypes and the maximum activity occurred in genotype NO-303 (0.48 µmol NO$_2$ h$^{-1}$ g$^{-1}$LFW s$^{-1}$) and 637-72 (0.44 µmol NO$_2$ h$^{-1}$ g$^{-1}$LFW s$^{-1}$) when compared with C-993-3-115 (0.33 µmol NO$_2$ h$^{-1}$ g$^{-1}$LFW s$^{-1}$) and S-907 (0.35 µmol NO$_2$ h$^{-1}$ g$^{-1}$LFW s$^{-1}$) genotypes at 100 mM NaCl. When compared with percent of control, genotypes were in the order as 637-72 > NO-303 > C-993-3-115 = S-907 regarding their CA concentration at 100 mM NaCl levels while at 200 mM NaCl, 56% activity of CA was observed in all genotypes of their respective control (Table 3.3.4). A different type of order was observed in genotypes regarding the activity of NR at 100 mM NaCl while at 200 mM, the order of genotypes was as 637-72 (69%) = NO-303 (69%) > S-907 (64%) > C-993-3-115 (63%).
Table 3.3.4. Activities of carbonic anhydrase and nitrate reductase enzymes in the leaves of linseed genotypes at different salinity levels

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Carbonic anhydrase activity (µmol CO₂ kg⁻¹ LFW s⁻¹)</th>
<th>Nitrate reductase enzyme (µmol NO₂ h⁻¹ g⁻¹LFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (100 mM)</td>
<td>NaCl (200 mM)</td>
</tr>
<tr>
<td>S-907</td>
<td>341.07±0.52</td>
<td>265.20±0.31</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>341.13±0.13</td>
<td>265.27±0.23</td>
</tr>
<tr>
<td>637-72</td>
<td>341.53±0.29</td>
<td>275.33±0.33</td>
</tr>
<tr>
<td>NO-303</td>
<td>341.60±0.23</td>
<td>265.43±0.26</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE
3.3.3.5 Leaf osmotic potential

The results showed high effect of salinity on leaf osmotic potential among linseed genotypes (Table 3.3.5). The leaf osmotic potential decreased to 109% of respective control in salt tolerant genotypes NO-303 and 637-72 respectively at 200 mM NaCl while the magnitude of leaf osmotic potential was 107% of respective control in salt sensitive genotypes S-907 and C-99-3-115 respectively at the same level of salt stress. Genotypes showed progressive decrease in their leaf osmotic potential with increasing salinity and it varied from -1.07 to -1.11 MPa in S-907 to from 1.10 to -1.13 MPa in NO-303 genotype at 100 mM NaCl while at control almost similar leaf osmotic potential was restored by all genotypes. At 200mM NaCl, leaf osmotic potential of NO-303 (-1.20 MPa) and 637-72 (-1.19 MPa) genotypes was the maximum while that of C-99-3-115 (-1.15 MPa) and S-907 (-1.14 MPa) was the minimum (Table 3.3.5). On percent of control basis, genotypes showed the order as 637-72 = NO-303 > S-907 = C-99-3-115.

Table 3.3.5. Leaf osmotic potential (-MPa) of linseed genotypes at different salinity levels

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>1.07±0.19</td>
<td>1.11±0.10</td>
<td>1.14±0.09</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>1.08±0.19</td>
<td>1.12±0.12</td>
<td>1.15±0.14</td>
</tr>
<tr>
<td>637-72</td>
<td>1.09±0.09</td>
<td>1.12±0.10</td>
<td>1.19±0.14</td>
</tr>
<tr>
<td>NO-303</td>
<td>1.10±0.10</td>
<td>1.13±0.13</td>
<td>1.20±0.15</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE

3.3.3.6 Proline and Glycinebetaine (GB)

During salt stress, plants accumulate organic osmolytes, also called compatible solute such as proline and glycinebetaine to protect themselves from osmotic stress (Sakamoto and Murata, 2002). Proline (Pro) and glycinebetaine (GB) contents of all the genotypes was significantly affected by the increased levels of salinity (NaCl) and their concentration significantly increased with increasing salinity. Proline and
Table 3.3.6. Effect of different levels of NaCl (mM) on proline and glycine betaine concentration of salt tolerant and salt sensitive genotypes of linseed

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Proline (mg g⁻¹ FW)</th>
<th>Glycine betaine (µmol g⁻¹ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (100 mM)</td>
<td>NaCl (200 mM)</td>
</tr>
<tr>
<td>S-907</td>
<td>11.33±0.36</td>
<td>12.20±0.31</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>11.42±0.32</td>
<td>11.88±0.34</td>
</tr>
<tr>
<td>637-72</td>
<td>11.73±0.50</td>
<td>13.47±0.33</td>
</tr>
<tr>
<td>NO-303</td>
<td>11.70±0.31</td>
<td>13.10±0.42</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE
glycinebetaine concentrations in salt tolerant genotypes NO-303 and 637-72 were not significantly different from salt sensitive genotypes (S-907 and C-99-3-115) in control treatment. However, genotypes showed progressive increase in their proline and GB concentration with increasing salinity and proline concentration varied from 11.33 to 12.20 mg g\(^{-1}\) FW while GB contents increased from 10.53 to 12.03 µmol g\(^{-1}\) DW in S-907 genotypes. Similarly, proline contents increased from 11.73 to 13.47 mg g\(^{-1}\) FW and GB increased from 10.83 to 13.58 µmol g\(^{-1}\) DW in 637-72 genotype at 100 mM NaCl. At 200 mM NaCl, proline concentration of salt tolerant genotypes NO-303 (15.17 mg g\(^{-1}\) FW) and 637-72 (14.93 mg g\(^{-1}\) FW) was higher than C-99-3-115 (14.67 mg g\(^{-1}\) FW) and S-907 (13.57 mg g\(^{-1}\) FW) (Table 3.3.6). It was observed that genotype 637-72 had more proline concentration at 100 mM NaCl but at 200 mM NaCl, NO-303 produced more proline than 637-72. Similar trend was observed in case of GB concentration and relatively high concentration of GB was noted in salt tolerant genotype 637-72 (13.58 µmol g\(^{-1}\) DW) at 100 mM NaCl but at 200 mM NaCl, NO-303 produced more GB (15.03 µmol g\(^{-1}\) DW) as compared to 637-72 (14.97 µmol g\(^{-1}\) DW) while salt sensitive genotypes had lower concentrations of proline and GB at both levels of salt stress.

### 3.3.3.7 Total soluble sugars (TSS) and total proteins

Among different organic osmotica, soluble sugars contribute up to 50% of total osmotic potential in glycophytes under salt stress conditions (Cram, 1976). Proteins which accumulate in plants under salinity stress may provide a storage form of nitrogen that is re-utilized when stress is over (Singh et al., 1987) and are helpful in organic adjustment. Total soluble sugar and total proteins of all the genotypes was significantly affected by the application of salts and their concentration was significantly increased under saline conditions (Table 3.3.7). Response of linseed genotypes also differed significantly for their total soluble sugars and protein contents and the maximum TSS (8.70 and 8.73 mg g\(^{-1}\) FW) and total proteins (10.90 and 10.83 mg g\(^{-1}\) FW) were recorded in NO-303 and 637-72 genotypes under control conditions as compared to S-907 in which minimum concentration of TSS (8.30 mg g\(^{-1}\) FW) and total protein (10.67 mg g\(^{-1}\) FW) was noted under control treatment. It was also observed that linseed genotypes did not show great difference in TSS and TP under non saline conditions but showed sharp increase in their TSS and total proteins with increasing NaCl salinity. At 100 mM NaCl, TSS concentration increased to 9.63 mg
Table 3.3.7. Effect of salinity on total soluble sugar and total protein concentration of salt sensitive and salt tolerant genotypes of linseed

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Total soluble sugars (mg g⁻¹ FW)</th>
<th>Total protein (mg g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>NaCl (100 mM)</td>
</tr>
<tr>
<td>S-907</td>
<td>8.30±0.40</td>
<td>8.60±0.32</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>8.37±0.29</td>
<td>8.79±0.36</td>
</tr>
<tr>
<td>637-72</td>
<td>8.73±0.47</td>
<td>9.63±0.32</td>
</tr>
<tr>
<td>NO-303</td>
<td>8.70±0.25</td>
<td>9.43±0.23</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE
g\(^{-1}\) FW in 637-72 followed by NO-303 (9.43 mg g\(^{-1}\) FW) while TP contents were higher in NO-303 (12.67 mg g\(^{-1}\) FW) followed by 637-72 (12.53 mg g\(^{-1}\) FW) as compared to S-907 (11.80 mg g\(^{-1}\) FW) and C-99-3-115 (11.73 mg g\(^{-1}\) FW) genotypes. Similarly, at 200 mM NaCl, TSS concentration of NO-303 (13.97 mg g\(^{-1}\) FW) and 637-72 (13.77 mg g\(^{-1}\) FW) genotypes was the maximum while that of C-99-3-115 (12.60 mg g\(^{-1}\) FW) and S-907 (12.50 mg g\(^{-1}\) FW) was the minimum (Table 3.3.7). At the same treatment, total protein concentration of salt tolerant genotypes ranged 26.87 to 27.17 mg g\(^{-1}\) FW while in salt sensitive genotypes it ranged from 25.40 to 25.70 mg g\(^{-1}\) FW. On the basis of percent of control, NO-303 produced 161% total soluble sugars of its respective control followed by 637-72 which produced 158% higher total soluble sugars of its respective control at 200 mM NaCl. On the other hand, 637-72 genotype produced more total proteins (248% of respective control) followed by NO-303 (247% of respective control). Salt sensitive genotypes S-907 and C-99-3-115 showed same trend in total soluble sugars and total protein contents and their percent of respective control was 151% (for TSS) and 240% and 241% for total proteins for C-99-3-115 and S-907 respectively at 200 mM NaCl.

### 3.3.3.8 Superoxide dismutase (SOD) and catalase (CAT) enzymes

The activity of SOD and CAT enzymes in salt sensitive and tolerant genotypes are presented in Table 3.3.8. The results of this study showed that activity of SOD increased while that of CAT decreased to a significant level under salt stress conditions in all the genotypes of linseed and the maximum SOD (371.67 unit mg\(^{-1}\) protein) and the minimum CAT activity (5.17 unit mg\(^{-1}\) protein) was recorded at 200 mM NaCl. Significant change was observed among genotypes for their SOD and CAT activities by the application salt treatments. Salt tolerant genotypes had well efficient SOD system and its activity increased from 198.0 to 303.3 unit mg\(^{-1}\) protein in 637-72 genotype followed by NO-303 (204.0 to 283.3 unit mg\(^{-1}\) protein) while in salt sensitive genotype C-99-3-115, the concentration ranged from 186.85 to 222.00 unit mg\(^{-1}\) protein for SOD at 100 mM NaCl. On the other hand, salt tolerant genotype NO-303 showed higher reduction in CAT activity (5.62 unit mg\(^{-1}\) protein) than that of 637-72 (4.80 unit mg\(^{-1}\) protein) at 100 mM NaCl. Similarly, salt sensitive genotype C-99-3-115 expressed high activity CAT (6.06 unit mg\(^{-1}\) protein) as compared to S-907 (5.76 unit mg\(^{-1}\) protein) at NaCl level of 100 mM. At 200 mM NaCl, SOD activity of NO-303 (371.67 unit mg\(^{-1}\) protein) and 637-72 (343.33 unit mg\(^{-1}\) protein)
Table 3.3.8. Effect of salinity on superoxide dismutase (SOD) and catalase (CAT) enzyme concentration of linseed genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>SOD (Unit mg⁻¹ protein)</th>
<th>CAT (Unit mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(100 mM)</td>
<td>(200 mM)</td>
</tr>
<tr>
<td><strong>S-907</strong></td>
<td>187.33±0.48</td>
<td>197.67±0.33</td>
<td>231.67±0.44</td>
</tr>
<tr>
<td><strong>C-99-3-115</strong></td>
<td>186.85±0.28</td>
<td>222.00±0.40</td>
<td>246.67±0.48</td>
</tr>
<tr>
<td><strong>637-72</strong></td>
<td>198.00±0.23</td>
<td>303.33±0.33</td>
<td>343.33±0.44</td>
</tr>
<tr>
<td><strong>NO-303</strong></td>
<td>204.00±0.35</td>
<td>283.33±0.33</td>
<td>371.67±0.49</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE
genotypes was the maximum while that of C-99-3-115 (246.67 unit mg⁻¹ protein) and S-907 (231.67 unit mg⁻¹ protein) was the minimum (Table 3.3.8). Catalase activity showed inverse relation with that of SOD activity and CAT activity decreased with increasing levels of salinity and it was also noted that salt sensitive genotypes showed relatively high activity of CAT as compared to salt tolerant genotypes at different levels of salinity. Thus CAT activity remained lower in salt tolerant genotypes and it ranged from 4.60 to 5.32 unit mg⁻¹ protein at 200 mM NaCl as compared to salt sensitive genotypes in which CAT activity ranged from 5.17 to 5.72 unit mg⁻¹ protein. Genotypes had a specific order for CAT activity at all treatment levels which was as C-99-3-115 > S-907 > NO-303 > 637-72 while for SOD activity, order of genotypes was different for different salinity level.

3.3.3.9 Peroxidase (POD) and ascorbate peroxidase (APX) enzyme

The activities of peroxidase (POD) and ascorbate peroxidase (APX) were significantly affected by applying different levels of NaCl concentration in all the four linseed genotypes (Table 3.3.9). POD activity was significantly decreased in salt sensitive genotypes but increased in salt tolerant genotypes by the application of salinity. On the other hand, salt application significantly increased the APX level in all four genotypes of linseed. At control, there was no significant difference among linseed genotypes for POD but salt stress increased the gap between salt tolerant and sensitive genotypes regarding POD activity. For example, at 100 mM NaCl salt sensitive genotypes showed decline in POD activity and POD decreased from 5.80 to 5.59 unit mg⁻¹ protein in S-907 followed by C-99-3-115 (5.84 to 5.62 unit mg⁻¹ protein) while on the other hand POD activity increased from 6.24 to 6.84 unit mg⁻¹ protein in NO-303 followed by 6.21 to 6.75 unit mg⁻¹ protein. At 200 mM NaCl, POD activity of NO-303 (7.16 unit mg⁻¹ protein) and 637-72 (7.11 unit mg⁻¹ protein) genotypes was found higher than the activity of POD in C-99-3-115 (5.49 unit mg⁻¹ protein) and S-907 (5.42 unit mg⁻¹ protein) (Table 3.3.9). APX expressed different trend in all the linseed genotypes and at control, APX activity was much variable in all the genotypes, however, salt sensitive genotypes had lower activity of APX as compared to salt tolerant linseed genotypes. But activity of APX more in salt tolerant genotypes at all salinity levels. At 100 mM NaCl, genotype 637-72 regulated highest APX activity (8.61 unit mg⁻¹ protein) followed by NO-303 (6.67 unit mg⁻¹ protein).
Table 3.3.9. Concentration of peroxidase (POD) and ascorbate peroxidase (APX) in salt sensitive and tolerant linseed genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>5.80±0.13</td>
<td>5.59±0.24</td>
<td>5.42±0.25</td>
<td>1.78±0.16</td>
<td>2.50±0.29</td>
<td>2.57±0.26</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>5.84±0.15</td>
<td>5.62±0.12</td>
<td>5.49±0.28</td>
<td>3.12±0.34</td>
<td>3.96±0.36</td>
<td>4.10±0.37</td>
</tr>
<tr>
<td>637-72</td>
<td>6.21±0.15</td>
<td>6.75±0.19</td>
<td>17.11±0.18</td>
<td>5.22±0.17</td>
<td>8.61±0.22</td>
<td>10.54±0.18</td>
</tr>
<tr>
<td>NO-303</td>
<td>6.24±0.33</td>
<td>6.84±0.11</td>
<td>7.16±0.20</td>
<td>4.31±0.25</td>
<td>6.67±0.32</td>
<td>8.14±0.17</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE
Similarly, APX was higher in salt tolerant genotypes (8.14-10.54 unit mg\(^{-1}\) protein) as compared to salt sensitive genotypes (2.57-4.10) at the salt level of 200 mM NaCl. On the basis of percent of control, POD activity in genotypes was in the order as NO-303 > 637-72 > C-99-3-115 > S-907 while for APX activity this order was as 637-72 > NO-303 > C-99-3-115 > S-907.

3.3.3.10 Lipid peroxidation (MDA) contents

Significant increase in lipid peroxidation (MDA) was noted in linseed genotypes by the application of salt stress. A wide range of difference was noticed between salt sensitive and salt tolerant genotypes regarding their MDA contents and lipid peroxidation levels. In salt sensitive genotypes S-907 and C-99-3-115, lipid peroxidation and MDA contents greatly increased with increasing salt concentration when compared with salt tolerant genotypes 637-72 and NO-303. At control, higher MDA contents were found in C-99-3-115 (5.45 nmol g\(^{-1}\) FW) and S-907 (5.11 nmol g\(^{-1}\) FW) while lowest MDA contents were found in NO-303 (4.58 nmol g\(^{-1}\) FW) followed by 637-72 (4.82 nmol g\(^{-1}\) FW). Salt sensitive genotypes showed progressive increase in their MDA concentration and it was 8.50 nmol g\(^{-1}\) FW in S-907 genotype and 8.40 nmol g\(^{-1}\) FW in C-99-3-115 genotype at 100 mM NaCl. On the other hand, salt tolerant genotypes had lower MDA contents (6.37-6.99 nmol g\(^{-1}\) FW) at 100 mM NaCl concentration. Similarly, at 200 mM NaCl, MDA concentrations of NO-303 (8.30 nmol g\(^{-1}\) FW) and 637-72 (8.39 nmol g\(^{-1}\) FW) genotypes were the minimum while that of C-99-3-115 (11.23 nmol g\(^{-1}\) FW) and S-907 (11.70 nmol g\(^{-1}\) FW) was the maximum (Table 3.3.10). Linseed genotypes possessed similar order for MDA contents for both salinity levels and it was S-907 > C-99-3-115 > NO-303 > 637-72 at 200 mM NaCl.
Table 3.3.10. Lipid peroxidation (MDA) contents of linseed as affected by salt stress

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>NaCl (100 mM)</th>
<th>NaCl (200 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>5.11±0.16</td>
<td>8.40±0.21</td>
<td>11.70±0.15</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>5.45±0.12</td>
<td>8.50±0.17</td>
<td>11.23±0.15</td>
</tr>
<tr>
<td>637-72</td>
<td>4.82±0.13</td>
<td>6.99±0.26</td>
<td>8.39±0.21</td>
</tr>
<tr>
<td>NO-303</td>
<td>4.58±0.13</td>
<td>6.37±0.19</td>
<td>8.30±0.17</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE

3.3.4. Discussion

The genetic variations between crop plants provide a precious tool in the selection of genotypes with desirable characters (Misra and Dwivedi, 2004). The present study clearly demonstrates some physiological and biochemical traits of salt tolerance in linseed genotypes subjected to different levels of salinity. It is obvious that salt stress reduced plant growth by affecting almost all the physiological processes, like photosynthetic activities, stomatal conductance, carbon and nitrogen assimilation, and ROS scavenging antioxidant enzymes.

In general, an appropriate growth strategy is key to fitness in a competitive situation, so too in linseed genotypes, their growth strategy is critical to survive (Paz and Marinez-Ramos, 2003; Du and Huang, 2008). Salt induced effects on relative water contents (RWC) has been used as one of the imperative water relation attributes for assessing the degree of salt tolerance in different crops like pea (Noreen and Ashraf, 2009), Safflower (Siddiqui and Ashraf, 2008) and hot pepper (Zaif et al., 2009). In the present study, salinity caused significant reduction in RWC of all the four linseed genotypes, however, both salt tolerant and sensitive genotypes did not differ significantly in their RWC. Decrease in RWC under increasing levels of salinity
in linseed genotypes was due to the decreased water potential of growth medium due to the high salt concentration as speculated by Sairam et al. (2002); Khan et al. (2007); and Siddiqui and Ashraf (2008).

Salt stress resulted in increased concentration of toxic ions (Na\textsuperscript{+} and Cl\textsuperscript{-}) which caused injury to cell membrane and hence reduced the membrane permeability. In addition, production of ROS under salt stress also caused significant reduction in membrane permeability and hence increases electrolyte leakage (EL). Thus EL is used as a criterion for salt tolerance to assess the membrane permeability of plants under stress conditions. Salt stress significantly increased the EL in linseed genotypes but there was no significant difference between salt tolerant and sensitive genotypes in this aspect. Thus in linseed EL may not be used as salt tolerant trait.

One of the most notable effects of salt stress is the alteration of photosynthetic pigment biosynthesis (Maxwell and Johnson, 2000). The decrease in chlorophyll contents under salt stress is a commonly reported phenomenon. In various studies, the chlorophyll contents were used as a sensitive indicator of the cellular metabolic state (Chutipaijit et al., 2011). It is obvious from the results that salinity stress lead to the reduction in chlorophyll contents (Chlorophyll ‘a’ and ‘b’) in linseed genotypes. In linseed genotypes, chlorophyll ‘a’ was affected less (89-97% of respective control) than chlorophyll ‘b’ (48-71% of respective control) under salt stress. Similar results were found in Oryza sativa where reduction of chlorophyll ‘a’, and ‘b’ contents was observed after NaCl treatment (200 mM NaCl for 14 days) where reduction of chlorophyll ‘b’ contents (41%) was more than the chlorophyll ‘a’ contents (33%) (Amirjani, 2011). In another study, Saha et al. (2010) observed a linear decrease in the levels of total chlorophyll, chlorophyll ‘a’, chlorophyll ‘b’, carotenoids and xanthophylls as well as the intensity of chlorophyll fluorescence in Vigna radiata under increasing concentrations of NaCl treatments. The results revealed that chlorophyll ‘b’ was affected more than chlorophyll ‘a’ in all the genotypes but this reduction was more in salt sensitive genotypes than salt tolerant genotypes. The decrease in chlorophyll ‘a’ and ‘b’ in linseed genotypes might occur due to salt
induced acceleration of chlorophyll enzymes degradation (Hernandez and Almansa, 2002) and/or disorder of chloroplast structure and associated proteins (Cha-um and kirdmanee, 2009). The decrease in chlorophyll contents in plants grown under NaCl stress may be the consequence of the activation of chlorophyllase (Reddy and Vora, 1986), the enzyme that degrades chlorophyll, which is activated by various stresses.

It is well established that photosynthetic capacity in crop plants is vital for final biological yield. The photosynthetic capacity of plants is reduced by the harmful effects of salinity on different photosynthesis related traits especially photosynthetic rate and stomatal conductance (Fisarakis et al., 2001; Sudhir and Murthy, 2004). In the present study, photosynthetic rate (57-65% of respective control) and stomatal conductance (30-48% of respective control) were significantly reduced due to increased concentration of NaCl. The reduction in photosynthetic rates in linseed under salt stress might be due to the reduction in water potential and high concentrations of Na\(^+\) and/or Cl\(^-\) which are accumulated in chloroplasts and hence affects carbon metabolism or photophosphorylation as reported by Sudhir and Murthy (2004). Some other factors that reduce photosynthetic rates under salt stress are; enhanced senescence, changes in enzyme activity, induced modifications in cytoplasmic structure and negative feedback by reduced sink activity (Iyengar and Reddy, 1996).

The reduction in stomatal conductance which results in restricting the availability of CO\(_2\) for carboxylation reactions is also a factor that reduces photosynthesis under salt stress (Brugnoli and Bjorkman, 1992). In addition, stomatal closure minimizes loss of water through transpiration and this affects light-harvesting and energy-conversion systems thus leading to alteration in chloroplast activity (Iyengar and Reddy, 1996). Rubisco, the key enzyme that determines the Pn in plants, is regulated by a number of factors, including CO\(_2\) concentration (Hopkins, 1999). Salinity stress may have decreased CO\(_2\) availability by inducing stomatal closure (Bethke and Drew, 1992); therefore, partly inhibiting rubisco activity (Soussi et al.,
1999) and, consequently, the Pn. Moreover, the decrease in CA activity and lowered quantity of chlorophyll pigment may be the other reasons that the Pn decreased.

To sustain the carboxylation reaction of photosynthesis, carbonic anhydrase (CA) enzyme rapidly converts atmospheric CO$_2$ to HCO$_3^-$ and is considered the first step in photosynthesis. In the current study, activity of CA enzyme was significantly reduced (56% of respective control) in linseed genotypes under the increased levels of salinity. CA catalyzes the reversible inter-conversion of CO$_2$ and HCO$_3^-$ in plants, whose level is regulated by photon flux density, CO$_2$ concentration, and availability of zinc (Tiwari et al., 2005). Salinity stress is reported to cause stomatal closure, thereby decreasing CO$_2$ partial pressure (Bethke and Drew, 1992). The fall in CO$_2$ levels in NaCl grown plants seems to be the cause of the decrease in CA activity.

NR (nitrate reductase) activity is determined by several external and internal factors. Campbell (1999) has highlighted at least 4, which include (a) the availability of substrate (NO$_3^-$) at the level of cytoplasm; (b) the level of functional NR; (c) the activity level of functional NR; (d) the overall metabolic state of the plant. Salinity was found to affect nitrate uptake in at least 2 ways: by direct competition of chloride with nitrate, and at the membrane level and/or membrane proteins by changing plasmalemma integrity (Cramer et al., 1985). This may have led to restricted nitrate influx, thus decreasing substrate availability. Since nitrate (substrate) is a key regulator of NR (Solomonson and Barber, 1990), the activity of NR decreased in response to saline stress. Moreover, the degradation/inactivation, and reduction in gene expression and NR-protein synthesis in response to NaCl stress (Ferrario et al., 1998) may be another cause of lower NR activity. Thus, nitrate reductase (NR) enzyme was significantly decreased (63-69% of respective control) with the increasing salinity in linseed genotypes.

Water potential, solute potential and turgor potential are inter-related in plant cells and are markedly affected when plants are exposed to salt stress. As a general principle, when plants experience high osmotic stress because of a low external water potential, a lowering of the solute potential (more negative) is stimulated, a process
referred to as osmotic adjustment. Osmotic adjustment under salt stress is due to uptake of ions from the external medium and/or accumulation of organic osmotica (Hernandez & Almansa, 2002; Taiz & Zeiger, 2002; Chaparzadeh et al., 2003). We did not find significant interaction between saltinity and genotypes regarding leaf osmotic potential, however, genotypes differed significantly in their leaf osmotic potential.

Proline accumulation in salt-stressed plants is a key defense response to sustain the osmotic pressure in a cell, which is reported in salt tolerant and salt sensitive genotypes of many crops (Kumar et al., 2003; Misra and Gupta, 2006; Koca et al., 2007). Similarly, glycine betaine (GB) accumulates in response to stress in many crops, including spinach, barley, tomato, potato, rice, carrot and sorghum (Mohanty et al., 2002; Yang et al., 2003). This organic compound is mainly localized in chloroplasts and plays a vital role in chloroplast adjustment and protection of thylakoid membranes, thereby maintaining photosynthetic efficiency (Genard et al., 1991). Murata et al. (1992) reported that GB protects the photosystem II (PSII) complex by stabilizing the association of the extrinsic PSII complex proteins under salt stress. Salinity stress increased (120-130% of respective control) proline contents in linseed but the concentration of glycine betaine increased (140% of respective control) markedly under same conditions. In some plant species, proline plays a major role in osmotic adjustment such as in potato (Claussen, 2005). But in linseed, GB played significant role compared to proline in osmotic adjustment in leaves. Cha-um et al. (2006) investigated that high level of glycine betain in salt-tolerant lines of rice (Oryza sativa L. spp. indica) played a significant role as a salt defensive response mechanism in terms of chlorophyll pigment stabilization and water oxidation in PSII, resulting in high net photosynthetic rate (NPR) and growth efficiency. A positive correlation between GB and SOD activity was observed in linseed genotypes which might indicate its role in up-regulating the SOD activity. These antioxidant enzymes activity depends on osmolyte concentration in cell, as these enzyme need availability of in vivo melieu for maximal catalytic activity (Burg and Ferraris, 2008; Sahu et al.,
2010). Previously, it was reported by Meloni and Martinez, (2009) in vinal (*Prosopis ruscifolia* Griesbach) that GB enhanced salinity tolerance by an antioxidant mechanism involving enhanced SOD activity and improving ion homeostasis under conditions of high salinity. Similarly, Zhang *et al.* (2009) observed that transgenic cotton over-expressing choline monooxygenase gene (Ah-CMO) was more tolerant to salt stress due to elevated accumulation of glycinebetaine, which provided greater protection of the cell membrane and photosynthetic capacity than in non-transgenic cotton.

In most of the plants grown under salt stress conditions, soluble sugars play an important role in osmotic adjustment. Many reports indicate that sucrose is produced or accumulated in plants tolerating drought or salt stress (Nabati *et al.*, 2011). Total soluble sugars of linseed genotypes increased significantly under salt stress conditions and high accumulation of sugars was observed in salt tolerant genotypes (161% of respective control) as compared to salt sensitive genotypes (151% of respective control) of linseed.

Several salt-induced proteins have been identified in plant species and have been classified into two distinct groups (Pareek *et al.*, 1997; Ali *et al.*, 1999; Mansour, 2000); i) salt stress proteins, which accumulate only due to salt stress, and ii) stress associated proteins, which also accumulate in response to heat, cold, drought, water logging, and high and low mineral nutrients. Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is re-utilized when stress is over (Singh *et al.*, 1987) and may play a role in osmotic adjustment. Proteins may be synthesized *de novo* in response to salt stress or may be present constitutively at low concentration and increase when plants are exposed to salt stress (Pareek *et al.*, 1997; Tamas *et al.*, 2001). In linseed genotypes, concentration of total proteins increased significantly with increasing salinity but physiologically salt sensitive and tolerant genotypes did not differ significantly with respect to protein contents. However, total proteins showed positive correlation with leaf osmotic
potential and hence may play its role in osmotic adjustment in linseed genotypes under salt stress conditions.

An unavoidable consequence of aerobic metabolism is production of reactive oxygen species (ROS). ROS include free radicals such as superoxide anion ($O_2^-$), hydroxyl radical (OH) as well as non radical molecules like hydrogen peroxide ($H_2O_2$) and singlet oxygen ($^1O_2$). Environmental stresses such as drought, salinity, chilling, metal toxicity, and UV-B radiation as well as pathogens attack lead to enhanced generation of ROS in plants due to disruption of cellular homeostasis (Mittler, 2002; Sharma and Dubey, 2005, 2007; Mishra et al., 2011; Srivastava and Dubey, 2011). When the level of ROS exceeds the defense mechanisms, a cell is said to be in a state of “oxidative stress.” The enhanced production of ROS during environmental stresses can pose a threat to cells by causing phytotoxic reactions such as peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway and ultimately leading to death of the cells (Wang et al., 2003; Meriga et al., 2004; Vinocur and Altman, 2005; Pitzschke et al., 2006; Srivastava and Dubey, 2011).

To minimize the effect of oxidative stress, plant cell have evolved a complex antioxidant system, which is composed of antioxidant compounds (glutathione, ascorbate, β-carotene and α-tocopherol) as well as ROS scavenging enzymes such as: Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), and glutathione reductase (GR) (Apel and Hirt, 2004). Of these, SOD, CAT, APX and POD play significant roles in detoxifying ROS. SOD dismutates superoxide radicals to $H_2O_2$, where CAT and POD are involved in converting $H_2O_2$ into water and oxygen. Antioxidant enzymes are known to protect the cell structures against ROS generated by stress conditions (Reddy et al., 2004). Our results indicated that under salt stress conditions, a significant increase in the activities of SOD (173-182% of respective control), APX (189-202% of respective control) and POD (114-115% of respective control) were noted in salt tolerant genotypes of linseed while salt sensitive genotypes had low SOD (124-132% of
respective control), CAT (90-96% of respective control), APX (132-145% of respective control) and POD activity (93-94% of respective control). SOD is a key enzyme in the active oxygen scavenger system and is considered to be the first line of defense against ROS (Hamilton and Heckathorn, 2001) which dismutates superoxide anion to H$_2$O$_2$ (Costa et al., 2005). The CAT and POD destroy the H$_2$O$_2$ produced by SOD and other reactions (Badawi et al., 2004a). In plants, a number of enzymes regulate H$_2$O$_2$ at intracellular levels, but POD, CAT and APX are considered the most important. Relatively high activities of ROS scavenging enzymes have been observed in salt tolerant genotypes in linseed as compared to salt sensitive genotypes, suggesting that the antioxidant system played an important role in conferring tolerance against salt stress. In the present study, the responses of SOD, CAT, POD, APX enzymes activities and MDA contents suggest that oxidative stress is an important component of stress conditions in linseed. Our data showed that CAT activity in leaves of both salt tolerant and sensitive genotypes was decreased under the application of increased NaCl concentration. Thus our results suggest that POD and APX activities coordinated with SOD activity play a central protective role in the superoxide and H$_2$O$_2$ scavenging process under salt stress (Liang et al., 2003; Badawi et al., 2004). The possibility of using antioxidant enzymes as biochemical indicators for assessing salt tolerance were reported by Wang and Huang (2004). Many authors argued that an increase in the SOD, POD and APX enzymes may be due to the increase of mRNA levels in the plant tissues (Sairam et al., 2002).

Salinity caused lipid peroxidation, which has often been used as indicator of salt induced oxidative damage in membranes (Hernandez and Almansa, 2002). In the present study, it was observed that MDA contents were significantly increased in the leaves of linseed genotypes. The lower level of lipid peroxidation in salt tolerant genotypes (174-181% of respective control) and higher level of lipid peroxidation in salt sensitive genotypes (206-229% of respective control) of linseed suggests that tolerant plants are better protected from oxidative damage under salinity stress. Similar results correlating lipid peroxidation to antioxidative system activity was also
reported by other researchers (El-Beltagi et al., 2008; Emam and Helal, 2008; Khan et al., 2010). The reduction of MDA contents was due to increased antioxidative enzyme activities, which reduced H$_2$O$_2$ levels and membrane damage (Lin and Kao, 2000; Hernandez and Almansa, 2002).

Results of the present study revealed that under salt stress, there was no significant difference between salt tolerant and sensitive genotypes of linseed in terms of leaf RWC (responsible for turgidity), CA and NR activities, P$_n$ and chlorophyll contents, proline, total soluble sugars, total proteins and CAT activity. The apparent reduction in these traits could be due to the reduction in water potential of growth medium as the addition of NaCl results in a decrease in water potential which directly or indirectly affects stomatal conductance, and hence all the other processes are disturbed and reduced. Moreover, salinity may retard the uptake of NO$_3^-$ which is a substrate and an inducer of NR (Katerji et al., 1997). All these factors lead to the reduction in growth among linseed genotypes. Salt tolerant linseed genotypes showed positive correlation of GB with the activity of SOD as compared to the other antioxidant enzymes and hence help in the induction of antioxidative enzyme system and cause salt tolerance. An increased activities of antioxidant enzymes specially SOD, POD and APX significant played important role in reducing lipid peroxidation (MDA contents) in salt tolerant genotypes of linseed while salt sensitive genotypes possessed least effective antioxidant system. Thus the antioxidant system along with lipid peroxidation may be considered as salt tolerant traits in linseed genotypes.
Study 4

3.4: Effect of salt stress on yield and oil contents of linseed

3.4.1. Introduction

Pakistan, the sixth most populous country in the world (U.S. 2008), has predominantly an agrarian economy. But despite this fact, it has been chronically deficient in edible oil production.

Linseed (Linum usitatissimum L) is one of the most important oilseed crops for the extraction of oil (from seeds) and fibers (from plant’s stems). In India 80% of the linseed oil goes for the industrial purpose and remaining 20% is used for edible purpose (Khan et al., 2007). To meet the edible oil demands of the country, it is the need of the day to bring marginal lands under oil seed crops by screening and breeding the salt tolerant oil seed crops which are better able to grow on salt-affected soils than currently available (Tanveer-ul-Haq et al., 2002). Linseed, while a minor crop, is grown in a wide range of countries, climates and for many different products (Ebtihal et al., 2012). Because of its adaptability and product diversity, it is being considered as a platform for the development of novel bio-products. Research on use of linseed for bio-product production is being conducted in Australia, North America, Europe and Asia. In 2009, the top producers of linseed were Canada, India and China, with 45% of world production being in Canada (FAO, 2009). In Pakistan, linseed is grown on marginal and sub-marginal lands under irrigated as well as rain-fed conditions of Punjab and Sindh provinces.

Although direct screening based on grain yield takes more time, is laborious and expensive, the evaluation of salt tolerance of genotypes based on final grain yield is necessary before any recommendation can be made with regard to the authenticity of reliable different traits as screening criteria or recommendation of selected genotypes for using a good donor to increase the salt tolerance for linseed genotypes in breeding programs. The agronomic and physiological traits may be important not only to be used as quick and easy screening criteria if they would be closely associated with
grain yield of genotypes (Noble and Rogers, 1992; Munns and James, 2003), but also to improve the salt tolerance that needs a better understanding of salt tolerance mechanisms of linseed genotypes. In this study, therefore, genotypic differences for salt tolerance were identified among genotypes on the grain yield and agronomic parameters at maturity stage; grain yield was used as a reference to determine validity of physiological traits as screening criteria for salt tolerance of linseed genotypes.

Keeping in view the above scenario, a pot experiment was conducted to achieve the following objectives:

1. To assess the effects of salt stress on yield attributes of linseed
2. To evaluate the effects of salt stress on oil contents of linseed

3.4.2. Materials and methods

3.4.2.1 Plant material, growth and treatment conditions

A pot experiment was conducted in the rain protected wire house of Institute of Soil and Environmental Science, University of Agriculture, Faisalabad during October-November 2011. The healthy and uniform seeds of four linseed genotypes (*Linum usitatissimum* L) identified as salt sensitive (S-907 and C-9-3-115) and salt tolerant (637-72 and NO-303) in previous experiment of screening were sown at the depth of 2 cm in glazed earthen pots having well pulverized 12 kg soil. The recommended doses of fertilizers (90 kg N + 30 kg P + 30 kg K ha\(^{-1}\)) were applied. The sources for nitrogen, phosphorus and potassium were urea, DAP and SOP respectively. Full dose of phosphorus and potassium and half dose of nitrogen were applied at the time of sowing while remaining half nitrogen was applied at vegetative stage. The required salinity levels (control, 100 mM NaCl, 200 mM NaCl) in pots were developed by mixing required amount of NaCl in the soil before filling the pots. The pots were irrigated with tap water (EC = 0.88 dS m\(^{-1}\)) when required.

3.4.2.2 Plant harvest

Plants were harvested at maturity and subjected to sun drying after harvesting. Pods of linseed were threshed before shattering and/or seed dispersal.
3.4.2.3 Measurements of plant yield attributes

The effect of salinity on yield attributes was studied in terms of number of branches plant⁻¹, number of pods plant⁻¹, number of seeds plant⁻¹, 1000 seed weight and seed yield plant⁻¹. Linseed genotypes at highest salinity level (200 mM NaCl) were unable to produce seeds, that’s why its data for yield attributes and oil contents are not presented here, however, data regarding number of branches plant⁻¹ and number of pods plant⁻¹ was recorded. At drying, 3 plants from each replicate were randomly separated from others and were used for computing yield parameters. After counting the number of branches plant⁻¹ and number of pods plant⁻¹, pods were crushed and cleaned to assess number of seeds pod⁻¹. For 1000 seed weight, seeds were counted through seed counter and then weighed on weighing balance.

3.4.2.4 Measurements of oil contents

To obtain oil contents by solvent extraction, method of Popa et al. (2012) was used. The crushed seeds of linseed were extracted with petroleum ether (Merck, 40-60°C) in a Soxhlet apparatus and the remaining solvent was removed by distillation. After extraction, the oil samples were filtered and stored. Extracted oil samples had a light yellow color.

3.4.2.5 Statistical analysis

All the data presented in this study are mean of three replicates. Analysis of variance (ANOVA) was performed by using a statistical package, Statistix 8.1. Significant differences among treatments were considered at the P ≤ 0.05.

3.4.3. Results

3.4.3.1 Effect of salt stress on number of branches in linseed

Number of branches plant⁻¹ of all the genotypes were significantly affected by the application of salinity and number of branches were significantly reduced between control and 100 mM NaCl (Table 3.4.1). The maximum number of branches plant⁻¹ (9.00) were recorded at control while the minimum number of branches plant⁻¹ (2.67) were observed at 200 mM NaCl. There was no significant difference among linseed
genotypes regarding number of branches plant\(^{-1}\) at control but salt stress adversely affected the genotypes in the production of branches. Genotypes showed sharp decrease in number of branches plant\(^{-1}\) with increasing levels of salinity. The number of branches of salt tolerant genotypes were more in salt tolerant genotypes when compared with salt sensitive genotypes and it varied with salt treatment from 8.67 and 7.67 in 637-72 and NO-303 genotypes respectively. Similarly, salt sensitive genotypes S-907 and C-99-3-115 possessed 6.33 and 6.00 number of branches plant\(^{-1}\) at 100 mM NaCl (Table 3.4.1). Linseed genotypes did not show greater differences in number of branches but effect of salinity was more pronounced. At 200 mM NaCl, the maximum number of branches were recorded in NO-303 (3.33) followed by 637-72 and S-907 (3.00) while the minimum number of branches were noted in C-99-3-115 (2.67).

3.4.3.2 Effect of salt stress on number of pods plant\(^{-1}\)

Salinity stress drastically affected the number of pods plant\(^{-1}\) of all the genotypes and number of pods plant\(^{-1}\) were significantly reduced between non saline and saline soil (200 mM NaCl). Linseed genotypes expressed considerable variations regarding number of pods plant\(^{-1}\) at all the treatment levels and the maximum number of pods plant\(^{-1}\) were calculated in NO-303 (67.0) followed by 637-72 (65.0), C-99-3-115 (59.0) and S-907 (53.0) at control. The variations among genotypes became more significant when grown on salt-affected soils and genotypes showed sharp decrease in number of pods plant\(^{-1}\) with increasing levels of salinity and drastic decrease in salt sensitive
Table 3.4.1. Effect of different levels of salinity on number of branches plant$^{-1}$ and number of pods plant$^{-1}$ of linseed genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number of branches plant$^{-1}$</th>
<th>Number of pods plant$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>100 mM NaCl</td>
</tr>
<tr>
<td>S-907</td>
<td>9.00±1.15</td>
<td>6.33±0.88 (70)</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>9.00±1.15</td>
<td>6.00±1.00 (67)</td>
</tr>
<tr>
<td>637-72</td>
<td>9.00±1.15</td>
<td>8.67±0.88 (96)</td>
</tr>
<tr>
<td>NO-303</td>
<td>9.00±0.58</td>
<td>7.67±1.45 (85)</td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE and values in parenthesis are the percent of their respective control.
genotypes S-907 (36.67) and C-99-3-115 (39.67) was recorded as compared to salt tolerant genotypes 637-72 (50.0) and NO-303 (54.0) at 100 mM NaCl (Table 3.4.1). Similarly, salinity level of 200 mM NaCl severely reduced the number of pods plant\(^{-1}\) and genotype S-907 (salt sensitive genotype) exhibited severe reduction (15.0) in its number of pods plant\(^{-1}\). On the basis of percent of control, genotypes expressed the order as NO-303 > 637-72 > S-907 > C-99-3-115 regarding number of pods plant\(^{-1}\).

3.4.3.3 Effect of salt stress on number of seeds pod\(^{-1}\)

In linseed genotypes, seed production was badly affected by salt stress and number of seeds pod\(^{-1}\) of all the genotypes were considerably affected by the application of increased levels of salinity. Thus number of seeds pod\(^{-1}\) were significantly reduced between control and 100 mM NaCl (Table 3.4.2) while at 200 mM NaCl, genotypes were not able to produce seed in pods. There was no considerable variations among linseed genotypes regarding number of seeds pod\(^{-1}\) and the maximum number of seeds pod\(^{-1}\) were counted in NO-303 (9.67) followed by 637-72 (8.67), however, S-907 produced least number of seeds pod\(^{-1}\) (7.67) as compared to other salt sensitive genotype C-99-3-115 at normal (non saline) soil. Number of seeds pod\(^{-1}\) of salt tolerant genotypes NO-303 (7.33) and 637-72 (6.33) was statistically significant from salt sensitive genotypes C-99-3-115 and S-907 (4.67) at 100 mM NaCl. Although, salt sensitive genotypes produced equal number of seeds pod\(^{-1}\) but on the basis of percent of control genotype S-907 produced more seeds (61\%) as compared to C-99-3-115 (56\%) at 10 10dS m\(^{-1}\). On the other hand, salt tolerant genotype NO-303 produced even higher number of seed pod\(^{-1}\) (76\% of respective control) than salt sensitive genotypes followed by 637-72 (73\% of respective control) at same salinity level.

3.4.3.4 Effect of salt stress on seed yield plant\(^{-1}\)

Soil salinity affects seed yield of many crops and this affect was very drastic in linseed genotypes, although salt tolerant genotypes showed relatively higher seed yield plant\(^{-1}\) as compared to salt sensitive genotypes. Statistically, the interaction between
Table 3.4.2. Effect of different levels of salinity on number of seeds pod\(^{-1}\) and seed yield plant\(^{-1}\) of linseed genotypes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>100 mM NaCl</th>
<th>200 mM NaCl</th>
<th>Control</th>
<th>100 mM NaCl</th>
<th>200 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>7.67±0.33</td>
<td>4.67±0.88 (61)</td>
<td>Genotypes did not produce seeds in pods</td>
<td>0.26±0.08</td>
<td>0.13±0.07 (51)</td>
<td>Genotypes did not produce seeds in pods</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>8.33±0.33</td>
<td>4.67±0.88 (56)</td>
<td>0.35±0.06</td>
<td>0.16±0.09 (47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>637-72</td>
<td>8.67±0.88</td>
<td>6.33±0.33 (73)</td>
<td>0.40±0.10</td>
<td>0.26±0.09 (66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO-303</td>
<td>9.67±0.33</td>
<td>7.33±0.67 (76)</td>
<td>0.49±0.08</td>
<td>0.34±0.07 (71)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE and values in parenthesis are the percent of their respective control.
genotypes and treatments was also significant regarding seed yield plant$^{-1}$. It was also noted that linseed genotypes showed greater variations for their seed yield plant$^{-1}$ even when grown on non saline (control) soil. The maximum reduction in seed yield was recorded in S-907 (0.13 g) followed by C-99-3-115 (0.16 g) at 100 mM NaCl (Table 3.4.2). Among treatments, the maximum seed yield plant$^{-1}$ was recorded at non saline (control) and the minimum seed yield plant$^{-1}$ was measured at 100 mM NaCl while at 200 mM NaCl, plants could not survive till maturity. Salt tolerant genotypes NO-303 and 637-72 produced relatively more seed yield plant$^{-1}$ and the maximum seed yield plant$^{-1}$ was recorded in NO-303 (0.34 g) followed by 637-72 (0.26 g). On the basis of seed yield (percent of control), genotypes expressed the order as NO-303 > 637-72 > S-907 > C-99-3-115.

3.4.3.5 Effect of salt stress on 1000 seed weight

Seed weight of all the genotypes was significantly affected by the application of salinity and 1000 seed weight was significantly reduced between control and 100 mM NaCl (Table 3.4.3). The maximum 1000 seed weight was recorded in control treatment while the minimum 1000 seed weight was noted at 100 mM NaCl. There was considerable variation among linseed genotypes regarding 1000 seed weight and the maximum 1000 seed weight was measured in NO-303 (0.22 g) while the minimum 1000 seed weight was noted in S-907 (0.11 g). At control, linseed genotypes did not show greater difference in their 1000 seed weight, however, 1000 seed weight of salt tolerant genotypes NO-303 (0.26 g) and 637-72 (0.25 g) was statistically significant from salt sensitive genotypes C-99-3-115 (0.24 g) and S-907 (0.20 g). Genotypes showed sharp decrease in 1000 seed weight with increasing levels of salinity and it decreased considerably with salt treatment. For example, 1000 seed weight decreased from 0.26 g to 0.22 g in NO-303 and from 0.25 g to 0.19 g in 637-72 genotypes at 100 mM NaCl. Similarly, salt sensitive genotypes showed a drastic reduction in 1000 seed weight at 100 mM NaCl and it reduced to 0.14 g and 0.11 g in C-99-3-115 and S-907 respectively (Table 3.4.3). On percent of control basis, genotypes were in the order as NO-303 > 637-72 > C-99-3-115 > S-907.

3.4.3.6 Effect of salt stress on oil contents of linseed

Application of salt stress on linseed genotypes drastically affected the oil contents of all the four genotypes and percent oil contents of linseed was significantly
Table 3.4.3. Effect of different levels of salinity on 1000 seed weight and oil contents of linseed genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>100 mM NaCl</th>
<th>200 mM NaCl</th>
<th>Control</th>
<th>100 mM NaCl</th>
<th>200 mM NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-907</td>
<td>0.20±0.06</td>
<td>0.11±0.05</td>
<td>0.11±0.03</td>
<td>26.60±0.35</td>
<td>20.68±0.33</td>
<td>Genotypes did not produce seeds in pods</td>
</tr>
<tr>
<td>C-99-3-115</td>
<td>0.24±0.08</td>
<td>0.14±0.07</td>
<td>0.14±0.07</td>
<td>27.80±0.52</td>
<td>21.15±0.43</td>
<td>Genotypes did not produce seeds in pods</td>
</tr>
<tr>
<td>637-72</td>
<td>0.25±0.09</td>
<td>0.19±0.06</td>
<td>0.19±0.08</td>
<td>34.90±0.35</td>
<td>24.46±0.32</td>
<td></td>
</tr>
<tr>
<td>NO-303</td>
<td>0.26±0.06</td>
<td>0.22±0.09</td>
<td>0.22±0.08</td>
<td>35.80±0.45</td>
<td>27.58±0.33</td>
<td></td>
</tr>
</tbody>
</table>

Each value is an average of 3 replications ± SE and values in parenthesis are the percent of their respective control.
reduced between control and 100 mM NaCl (Table 3.4.3). Data regarding oil contents clearly indicate the wider differentiation between salt sensitive and tolerant genotypes in linseed. Salt tolerant genotypes expressed clear difference in their percent oil contents even at control where no salt (NaCl) was added in soil. The maximum seed oil contents was measured in NO-303 (35.80%) followed by 637-72 (34.90%) while the minimum seed oil contents were noted in S-907 (26.60%) and C-99-3-115 (27.80%). Seed oil contents of salt tolerant genotypes NO-303 (35.80%) and 637-72 (34.90%) were statistically significant from that of salt sensitive genotypes C-99-3-115 (27.80%) and S-907 (26.60%) in control treatment. Genotypes showed sharp decrease in seed oil contents with increasing levels of salinity, but salt tolerant genotypes contained relatively higher percent oil contents than salt sensitive genotypes of linseed. For example, it was noted that salt tolerant genotypes NO-303 contained 27-58% oil contents and 637-72 contained 24.46% oil contents in their seeds which was higher than that contained in salt sensitive genotypes C-99-3-115 (21.15%) and S-907 (20.68%) (Table 3.4.3).

3.4.4. Discussion

Salt tolerance at vegetative stage is crucial for yielding vigorous plants for tolerating salt stress at later stages of growth. That is why, crop failed to reach at maturity at highest salinity level of 200 mM NaCl. The advantage of the utilization of branching and number of pods in the evaluation for salt tolerance is that both parameters can be determined at early growth stage. Therefore, screening salt tolerance of genotypes at early growth stage based on agronomic parameters can shorten the period for experiments to screen salt tolerance of linseed genotypes. However, it is only true if the changes in salt tolerance exhibit the same pattern at all growth stages (Allen et al., 1985; Ashraf and Waheed, 1993). Because evaluating salt tolerance among genotypes based on grain yield needs a long period for the experiment, the work in the literature suggests that evaluating salt tolerance of genotypes on the basis of agronomic parameters, especially for parameters that initiate at early growth stage and significantly correlate with grain yield, can be used as more quick and feasible traits to screen large
number of genotypes rather than grain yield. Therefore, it is necessary to identify salinity-sensitive agronomic parameters that initiate at early growth stage. In our study, branching and pod number of linseed initiate during vegetative stages and are sensitive parameters to salinity in other crops such as rice (Zeng et al., 2002). The advantage of the utilization of both parameters in the evaluation for salt tolerance is that both parameters can represent the evaluation of genotypic differences for salt tolerances in terms of total grain yield. Thus, both parameters can be used as more quick and feasible traits to evaluate large number of linseed genotypes in breeding programs rather than grain yield.

In the present study, the observed reduction in branching (30-37% of respective control) and number of pods plant⁻¹ (26-32% of respective control) due to salt stress directly affects the productivity, biomass and seed yield of linseed genotypes of Pakistan origin. Decreased branching due to salt stress in different oil seed crops (Mensah et al., 2006; Sadat-Noori et al., 2008) has been reported and might be due to the reduction in photosynthesis. The deleterious effect of salinity on yield attributes namely number of pods plant⁻¹, number of seed pod⁻¹was not surprising. The limited synthesis of photosynthates would adversely affect their partitioning to developing sink and thus resulting in lower values of these attributes would naturally be responsible for decreasing seed yield plant⁻¹. Khan et al. (2007) and Muhammad and Hussain (2010) also reported that productivity of linseed was decreased under saline conditions.

It was interesting to note that different yield attributes played a positive role in increasing oil contents in linseed genotypes and a comparison among different yield attributes revealed that 1000 seed weight played a very important role and exhibited a positive correlation with percent oil contents. However, contribution of seed yield plant⁻¹ towards percent oil contents of linseed is far better than 1000 seed weight.

The decrease in seed oil content of all the four genotypes with the increase in salt concentration of soil parallels the situation in some salt tolerant and salt sensitive lines of *Brassica juncea* (Ashraf and Naqvi, 1992). Previously, Semiz et al. (2012) observed that with the increasing salinity levels reduction in water potential, plant height, fresh yield, biomass production, seed yield, and 1000-grain (seed) weight of fennel
(Foeniculum vulgare Mill.). Similar results were reported in peppermint (Khorasaninejad et al., 2010), Brassica juncea (Shanker et al., 2011), and in soybean (Taher-Soula and Mohammadi, 2013).

Results of present study revealed that all the four genotypes of linseed did not express tolerance in terms of grain yield at salt level of 200 mM NaCl, which proved their sensitivity at this salinity level. However, these genotypes produced grains at lower level of salinity, i.e. 100 mM NaCl. This shows that native linseed genotypes can be grown on soils having higher than 100 mM NaCl for biomass production and not for grain yield. Moreover, salt stress severely reduced the branching and pods production in linseed and both of them have significant role in improving seed yield in linseed genotypes. It was also noted that dry land (soil) salinity affected growth and development of linseed and crop failed to reach maturity at highest level of salinity (200 mM NaCl). Salt stress also drastically affected number of seeds in pods and hence reduced the overall seed yield. Salt stress not only reduced the number of seeds in pods but also severely reduced the seed weight and 1000 seed weight was drastically reduced under salinity stress. Reduction in seed yield and seed weight played a significant role in the reduction of oil contents in linseed. Hence, salt stress indirectly affects seed yield and oil contents by affecting the yield attributes of linseed.
CHAPTER 4

GENERAL DISCUSSION

Salinity is one of the major abiotic stresses which affect crop productivity in one quarter to one third of all agricultural lands. The problem becomes more severe due to the irrigation with saline water and uses of uncultivable soils to fulfill the demand of the increasing population all over the world (Munns, 2002). Salt stress causes a number of changes in plant metabolism. Of them, ion toxicity, osmotic stress, disturbing the uptake and translocation of nutritional ions, disturb protein synthesis and energy production, reduction in plant growth and photosynthesis are most prominent (Mittler, 2002; Misra and Dwivedi, 2004; Parida and Das, 2005). Salinity stress also caused the oxidative damage through the production of reactive oxygen species which are the by-products of hyper osmotic and ionic stresses and responsible for oxidative damage in plants (Sun et al., 2011). These effects can disturb the physiological and biochemical functions of the plant cell, leading to cell death (Xiong et al., 2002; Zhu, 2002).

Advance research in plant physiology, genetic makeup and plant molecular biology make it easy to understand plant responses to salinity stress (Flowers, 2004; Munns, 2007). The complex mechanism of salinity tolerance and high extent of variation at intra-specific and inter-specific levels in plant contributing many difficulties to recognize a single indicator which could be used as an effective selection criteria. But, currently quick and economical viable short gun approach has been extensively used to ameliorate the injurious effects of salinity on plant growth (Cuartero et al., 2006; Ashraf and Foolad, 2007). In the past few decades, various types of organic and inorganic chemicals have been used to ameliorate the harmful effects of salinity on different crops. However, the extent of their ameliorative effect depends on a number of factors such as type of crop, the mode of their application, the type of chemical and its interaction with different types of salts in the growth medium of plants and different growth stages at which they are applied.
Screening of germplasm is very crucial to identify salt tolerant genotypes for breeding program to sort out salt tolerant and high yielding varieties. A lot of work has been done on screening of many field crops at vegetative stage such as wheat (Qureshi et al., 1990; Salam et al., 1999; Ali et al., 2002, 2007; El-Hendawy et al., 2011), rice (Shannon et al., 1998; Zeng et al., 2002), maize (Rao and McNeilly, 1999; Khan and McNeilly, 2000; Zeng et al., 2002) and sorghum (Azhar and Khan, 1997; Kausar et al., 2012) but little information is available regarding screening of linseed. Thus sixty linseed genotypes were grown in solution culture experiment at 0, 100 and 200 mM NaCl. The data regarding growth parameters like plant height, root/ shoot lengths, root/shoot fresh and dry weights, number of tillers per plant and ion contents (Na	extsuperscript{+}, K	extsuperscript{+} and Na	extsuperscript{+}/K	extsuperscript{+} ratio) of the plant leaves were recorded for each genotype tested in the experiment. In cluster group analysis, genotypes were screened simultaneously on several physiological and ionic parameters. Genotypes were ranked and grouped for their salinity tolerance. The differences among linseed genotypes in terms of growth and ionic parameters and interactions between salinity levels and genotypes were also significant (P<0.05) for seedling growth and ionic parameters measured 30 days after salinity stress, which indicated variable response of genotypes to salinity from low to high levels. This study revealed a great deal of variation in tolerance to increasing salt (NaCl) concentrations during the early growth stages of linseed. The linseed genotypes which produced relatively high biomass compared to others were ranked salt tolerant and vice versa were ranked salt sensitive genotypes.

After ensuring the great deal of variation among linseed genotypes, it was thought to investigate the affect salt stress on the germination of linseed genotypes. In addition, distribution pattern or accumulation of Na	extsuperscript{+} and K	extsuperscript{+} in different parts (root, shoot, leaf) of linseed could be a useful tool to understand the salinity control at whole plant level. Overall motive of this study was to know the most salt sensitive stage of growth in linseed. Keeping in view the importance of seed germination and ion distribution in plants under salt stress, second study was conducted which planned to investigate the effect of salt stress on germination, survival and ion distribution in linseed.
The results of study 2 revealed that salinity caused significant reduction in seed germination (78-84% of respective control) and survival percentage (40-60% of respective control). These findings clearly expressed the sensitivity of linseed germination against salinity stress. Ion (K⁺, Na⁺) distribution among root, shoot and leaves of linseed revealed that the main difference between salt tolerant and sensitive genotypes was hyper accumulation of Na⁺ ions in roots which seemed to be the most distinct feature of salt tolerant genotypes. It is possible that the vigorous growth of salt tolerant genotypes may have provided enough energy to restrict the entry of Na⁺ at root level and enhance the K⁺/Na⁺ ratio in shoot.

Salt tolerance is a complex phenomenon and plants showing salt tolerance possess some specialities in terms of physiological and biochemical traits which play a dominant role in their adoptability under saline environment. It was very important to know these traits in linseed. Hence study 3 was conducted in hydroponics and different traits having functional importance in growth under salinity were recorded. Results of study 3 revealed that the exposure of linseed genotypes to increasing NaCl concentrations significantly reduced RWC (relative water contents). This reduction was only due to the decrease in water potential of saline medium. The reduction in RGR and root and shoot biomass might be due to ion toxicity or decreased osmotic potential as well as low cell wall extensibility (Grieve et al., 2001; Haplerin and Lynch, 2003). Effect of salinity on relative water contents (RWC) has been used as one of the very important water relation parameters for assessing degree of salinity tolerance in linseed (Khan et al., 2007). Salinity caused a significant reduction in relative water contents for all linseed genotypes but genotypes did not differ significantly in their RWC. Salt stress significantly increased the electrolyte leakage (EL) in linseed genotypes. Although the salt tolerant genotypes showed physiologically non significant electrolyte leakage than salt sensitive genotypes but injury to membranes was obvious under salt stress conditions. Salt stress resulted in increased concentration of toxic ions (Na⁺ and Cl⁻) which caused injury to cell membrane and hence reduced the membrane permeability. In addition, production of ROS under salt stress also caused significant reduction in membrane permeability and hence increases EL (Kaya et al., 2001a, 2002a). Thus in
linseed RWC and EL may not be useful traits regarding salinity tolerance. The imposition of salinity stress significantly inhibited the photosynthetic pigments [chlorophyll ‘a’ (89-97% respective control) and chlorophyll ‘b’ (49-71% of respective control)] and gas exchange parameters [photosynthetic rate (57-65% of respective control) and stomatal conductance (30-48% of respective control)] in all genotypes of linseed. The decrease in chlorophyll ‘a’ and ‘b’ in linseed genotypes might occurred due to salt induced acceleration of chlorophyll enzymes degradation (Hernandez and Almansa, 2002) and/or disorder in chloroplast structure and associated proteins (Cha-um and Kirdmanee, 2009). Stomatal regulation is a major factor in controlling photosynthetic rate as well as water balance of plants growing under salinity stress (Dubey, 2005; Sun et al., 2011). In our study, photosynthetic rate and stomatal conductance were significantly decreased due to increased concentration of NaCl. The reduction in photosynthetic rates in linseed under salt stress might be due to the reduction in water potential and high concentrations of Na+ and/or Cl− which are accumulated in chloroplasts and hence affect carbon metabolism or photophosphorylation as reported by Sudhir and Murthy (2004). The activity of carbonic anhydrase (CA) enzyme was significantly reduced (56% of respective control) in linseed under the increased levels of salinity. Carbonic anhydrase catalyzes the reversible inter-conversion of CO$_2$ and HCO$_3^−$ in plants, whose level is regulated by photon flux density, CO$_2$ concentration, and availability of zinc (Tiwari et al., 2005). Salinity stress cause stomatal closure, thereby decreasing CO$_2$ partial pressure (Bethke and Drew, 1992). The fall in CO$_2$ levels in NaCl grown plants seems to be the cause of the decrease in CA activity. Salinity also affects the nitrate reductase (NR) activity (63-69% of respective control) as it reduces nitrate uptake by direct competition of chloride with nitrate which led to restricted nitrate influx, thus decreasing substrate availability. Since nitrate (substrate) is a key regulator of NR (Solomonson and Barber, 1990), the activity of NR decreased in response to saline stress.

Salinity causes water deficit or osmotic shock to the plants as a first symptom of stress condition. During this shock/stress, plants produce water potential gradient as a first reaction or strategy to cope with the new situation of stress. To create water
potential gradient, plants synthesize organic osmolytes, also called osmoprotectants and compatible solutes, such as proline, glycine betain, sugars, free amino acids and quarternary compounds in the cytoplasm. These solutes create sharp water potential gradient and help plants to take up water into the plant. They also protect and maintain the structure of the cell organelles and proteins (enzymes) without interfering with their activities and hence termed as osmoprotectants (Ashrafijou et al., 2010; Nabati et al., 2011).

Significant improvement in GB, total soluble sugars and total protein contents occurred in linseed genotypes under saline conditions. The accumulation of GB (133-140% of respective control) played a considerable role in up regulation of antioxidant enzymes like SOD and hence helped in salt tolerance in linseed. Cha-um et al. (2006) investigated that high level of glycine betain in salt-tolerant lines of rice (Oryza sativa L. spp. indica) played a significant role as a salt defensive response mechanism in terms of chlorophyll pigment stabilization and water oxidation in PSII, resulting in high net photosynthetic rate (NPR) and growth efficiency. Soluble sugars play a very significant role in salt stress tolerance by playing major role in osmotic adjustment in linseed genotypes.

Our results indicate that under salt stressed conditions, a significant increase in the activities of SOD, APX and POD were noted in salt tolerant genotypes of linseed while salt sensitive genotypes had low POD activity under salt stress. SOD is a key enzyme in the active oxygen scavenger system and is considered to be the first line of defense against ROS (Hamilton and Heckathorn, 2001) which dismutates superoxide anion to H$_2$O$_2$ (Costa et al., 2005). The CAT and POD destroy the H$_2$O$_2$ produced by SOD and other reactions (Badawi et al., 2004a). Relatively high activities of ROS scavenging enzymes (SOD, POD, APX) have been observed in salt tolerant genotypes in linseed as compared to salt sensitive genotypes, suggesting that the antioxidant system played an important role in plant tolerance against salt stress. Thus, linseed genotypes respond differently to salinity stress as a result of variations in their antioxidant systems (Emam and Helal, 2008; El-Beltagi et al., 2008; Khan et al., 2010)
Continuously increasing human population is exerting great pressure on normal lands for arable cultivation of food and fiber crops. Therefore, exploitation of degraded wastelands including salt-affected ones is a practical option for growing plants having medicinal and aromatic significance. Several non-conventional plant species have good growth potential and economic production under high saline conditions. Some medicinal and aromatic plants resist soil salinity and alkalinity to a considerably higher level than do traditionally grown agricultural crops (Dagar et al., 2004).

Pot study was conducted to investigate the effect of salt stress on yield and oil contents of linseed genotypes. In this study, it was found that linseed genotypes could not survive to maturity and failed to grow at highest salinity level of 200 mM NaCl while linseed genotypes were grown successfully till maturity in control (normal soil) and 100 mM NaCl salinity levels.

Results of pot study revealed that sole stress of salinity reduced yield attributes (number of branches plant\(^{-1}\), number of pods plant\(^{-1}\), number of seed pod\(^{-1}\), seed yield plant\(^{-1}\) and 1000 seed weight) and percent oil contents of linseed. However, number of branches plant\(^{-1}\), number of pods plant\(^{-1}\) were severely affected and thus indirectly reduced the seed yield of linseed. Seeds pod\(^{-1}\), 1000 seed weight and seed yield showed a positive correlation with oil contents and thus their reduction under salt stress indirectly decreased the oil contents in linseed genotypes.

It can be concluded that under salinity stress, increased concentration of Na\(^+\) ion in leaves and shoots significantly affected the photosynthetic rates and hence reduced the biomass production in terms of plant height, root and shoot fresh and dry weights. It was noted that photosynthetic rate is the most sensitive parameters to salinity stress while stomatal conductance, relative water contents and enzyme (CA, NR) activities were indirectly affected by salinity stress. Salt tolerant genotypes NO-303 and 637-72 had high ability to restrict Na\(^+\) at root level. Organic osmolytes (particularly GB) played significant role in regulating leaf osmotic potential and hence helped in osmotic adjustment of linseed under salt stress conditions. Moreover, GB played a significant role in enhancing the activity of SOD enzyme which works as the first line of defense in oxidative stress tolerance. The activities of SOD along with POD and APX helped in
reducing lipid peroxidation in salt tolerant genotypes. Salt stress indirectly affected the yield and oil contents of linseed by affecting the yield attributes and seed weight of linseed. Thus most crucial trait in native linseed genotypes to salinity tolerance is their ability to restrict Na\(^+\) in roots as well as K\(^+\) accumulation in shoot and leaves. In addition, reduction in lipid peroxidation (MDA contents) due to the antioxidant enzymenactivity (speciappy POD) is also a worth mentioning trait regarding salt tolerance in linseed. Moreover, glycine betain contributed more than proline in conferring salinity tolerance to linseed genotypes. Thus, ability of linseed to restrict the Na\(^+\) entry into roots by promoting K\(^+\) uptake in addition to enhanced activity of POD is the trait that can be targeted in the breeding program of producing salt tolerant genotypes. Linseed genotypes can be grown successfully on salt-affected soils till 100 mM NaCl. The growing of linseed on high salinity soils can give some biomass but crop is unable to produce economic yield on such soils. Linseed has wide range of adaptation on salt-affected soils and exploitation of its genetic potential for salt stress tolerance may also give better and more salt tolerant crop.
CHAPTER 5

SUMMARY

Salinity is the big threat throughout the world that drastically reduced the crop productivity. Increase in salinity tolerance to fulfill the increasing the increasing demand of food production and maintain food security of mankind, are important challenges to the world agriculture as the world population is increasing very rapidly than the area of agricultural land to support it. In addition, urbanization and industrialization on arable lands, shortage of good quality irrigation water and ever increasing salt-affected soils are among the worst problems in developing countries like Pakistan and compels to utilize every part of land for the production of crops. There are many crops which possess more salt tolerance and low water requirements than the conventional crops. Thus utilizing salt-affected soils and growing multipurpose crops on marginal lands to get benefits in terms of oil, medicine or other by-products of economic importance is the need of time especially in developing world. Keeping in view the above scenario, the present research work was planned on linseed for achieving the following objectives:

- To explore the genetic variations in linseed genotypes for salt tolerance and selection of tolerant and sensitive genotypes.
- To assess the effect of salt stress on germination and ion distribution in linseed
- To define the various physiological and biochemical traits having functional significance in determining salt tolerance and plant growth of linseed.
- To evaluate the effect of salt stress on yield and oil contents of linseed

To achieve these objectives, solution culture and pot experiments were conducted at wire house of Saline Agriculture Research Centre (SARC), University of Agriculture, Faisalabad. Sixty linseed genotypes were grown for four weeks in hydroponics using
three levels of salinity (control, 100 mM NaCl and 200 mM NaCl) to screen out salt tolerant and salt sensitive linseed genotypes. On the basis of some growth and ionic parameters, genotypes were identified as salt tolerant and salt sensitive genotypes.

In 2nd study, seeds of four linseed genotypes 637-72 and NO-303 identified as salt tolerant and S-907 and C-99-3-115 recognized as salt sensitive genotypes were grown in petri dishes and solution culture with three salinity levels (control, 100 mM NaCl and 200 mM NaCl). An increase in concentration of NaCl significantly affected seed germination of the four linseed genotypes under investigation. The genotype NO-303 attained the highest final germination percentages at all the salinity levels but the reduction in germination due to increase in salt level was much lower than in the other genotypes. Seedling survival of all the genotypes was significantly affected by NaCl. The results revealed that the genotypes sensitive to salinity at germination stage may also show sensitivity at seedling stage. Na\(^+\) contents increased with increasing salinity level and peaked at 200 mM NaCl. This means that Na\(^+\) accumulation was harmful because the genotypes with low Na\(^+\) contents like 637-72 and NO-303 possessed more vigorous seedling growth than the other genotypes. Both salt tolerant genotypes (637-72 and NO-303) accumulated considerably less concentrations of Na\(^+\) in their shoots and leaves and more in the roots as compared to the two salt sensitive genotypes (S-907 and C-99-3-115).

In study 3, solution culture experiment was conducted to assess the effect of salt stress on physiological and biochemical processes of salt tolerance in linseed. Salt tolerant and salt sensitive genotypes were used in this study with the same levels of salinity as in study 2. This study was carried out in College of Agriculture and Biotechnology, Zhejiang University, China. It is obvious that salinity stress reduced plant growth by affecting plant physiological processes, relative water contents, electrolyte leakage, decreasing stomatal conductance, activity of CA and NR and reducing photosynthetic rate, altering accumulation of organic osmolytes and antioxidant activities of all the four linseed genotypes. Salt tolerant linseed genotypes 637-72 and NO-303 performed better in salinity stress as compared to salt sensitive genotypes. Proline, GB, total soluble sugars and total proteins are accumulated more
and hence provide a good source to decrease osmotic potential and protect cellular organelles from the adverse effects of increased salt concentration. Activity of antioxidant enzymes, especially SOD, POD and APX may help them from protecting oxidative damage. Membrane damage in terms of lipid peroxidation was more severe in salt sensitive genotypes S-907 and C-99-3-115 which may be another important biochemical trait for better salinity tolerance in linseed genotypes.

In order to evaluate the effect of salt stress on yield, yield attributes and oil contents of linseed, a pot experiment was conducted in wire house of SARC, UAF. Same linseed genotypes (salt sensitive S-907, C-99-3-115 and salt tolerant 637-72 and NO-303) were grown in pots with following treatments (1) control; (2) 100 mM NaCl; (3) 200 mM NaCl salinity. In pot culture, crops failed to reach maturity at highest level of salinity (200 mM NaCl) but at other treatment level, yield attributes and oil contents of linseed were measured and analysed. In this study, it was revealed that soil salinity affects significantly on linseed yield attributes like number of branches plant$^{-1}$, number of pods plant$^{-1}$, number of seed pod$^{-1}$, seed yield plant$^{-1}$ and 1000 seed weight primarily by affecting the production of photosynthates and their distribution between sinks. Oil contents of linseed were also affected by the application of salt stress but salt tolerant genotypes produced more oil contents than salt sensitive genotypes.

By summarizing all these results, it can be concluded that salinity stress caused significant reduction on plant growth by affecting plant physiological characteristics especially photosynthetic rate and electrolyte leakage while the other physiological traits like relative water contents, stomatal conductance and enzyme activities were indirectly affected. Salt tolerant genotypes of linseed accumulate more Na$^+$ in roots than shoots and leaves while germination stage is not much sensitive to salt stress. Hence restricted entry of toxic ions at root level, increased uptake of K$^+$ ion and reduction in lipid peroxidation due to enhanced activity of antioxidant enzymes seems to be the salt tolerance traits in linseed genotypes of Pakistan.
LITERATURE CITED


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Appendix:

Figure 3.1.1: Graph showing relative shoot dry weight (g) of sixty linseed genotypes under salinity stress

Figure 3.1.2: Graph showing relative root dry weight (g) of sixty linseed genotypes under salinity stress
Figure 3.1.3: Graph showing relative root length (cm) of sixty linseed genotypes under salinity stress

Figure 3.1.4: Graph showing relative shoot: root ratio of sixty linseed genotypes under salinity stress
Figure 3.1.5: Graph showing relative leaf Na contents (mmol/g dw) in sixty linseed genotypes under salinity stress

Figure 3.1.6: Graph showing relative leaf K contents (mmol/g dw) in sixty linseed genotypes under salinity stress
Figure 3.1.7: Graph showing relative leaf Na: K ratio in sixty linseed genotypes under salinity stress