Bismillah hir rehman nir raheem | Translation: Shuru karta hun Allah ke naam se jo bada meherbaan aur nehayat rehem karne wala hai.

Selection and characterization of different wheat genotypes against salinity and low calcium

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

Doctor of philosophy

By

Muhammad Arshad

M.Sc. (Hons.) Agriculture

INSTITUTE OF SOIL AND ENVIRONMENTAL SCIENCES

FACULTY OF AGRICULTURE

UNIVERSITY OF AGRICULTURE

FAISALABAD, PAKISTAN

2014

ACKNOWLEDGEMENT

All the praises and thanks are for Almighty ALLAH, Who bestowed me with the opportunity and ability to contribute a little material to the existing knowledge. Trembling lips and wet eyes praise for Holy Prophet, Muhammad (Peace be upon him) who forever remains a torch of guidance and knowledge for humanity as a whole.

The work reported in this thesis was funded by Higher Education Commission (HEC) of Pakistan. I acknowledge their support in enabling me to pursue this multifaceted research.

The completion of this thesis owes a lot to many individuals who have been instrumental in providing me with much needed assistance and support during the course of my studies. First and foremost, with deep sense of honor, I wish to thank my supervisor Dr. Muhammad Saqib for his help, guidance and suggestions for my dissertation work and write up of the manuscript. His critical reading of this manuscript and valuable suggestions are highly appreciable. Special thanks are also extended to Prof. Dr. Javaid Akhter and Dr. Muhammad Asghar for their suggestions and guidance.

I am thankful to all of my colleagues(Atiq-u-Rehman, Gulam Abbas) and friends (Mohson shehzad, Imran Mustaq, Naveed Ahmed, Zia)for providing me company, help, and members of the Institute particularly to my Saline Agriculture Research Centre fellows.

I am grateful to my spiritual father(Mufti Muhammad Amin sahib) lovely father and mother and elder brother(Prof. Dr Muhammad Sarwar) for their love , moral ,financial and guidance support through out my life. I also wish to thank my other brothers and sisters for their love and affection. I am so much obliged to all of my family members for their whole hearted support and untiring company during all phases of my life.

I reserve a special place for my dear wife (and children) and she has my heartfelt gratitude for cheering me up during some of those dull moments of Ph.D duration and also for your coping so well in my absence.

Muhammad Arshad

88-ag-1056

The Controller of Examinations,

University of Agriculture,

Faisalabad.

We, the supervisory committee, certify that the contents and form of the thesis submitted by **Muhammad Arshad Regd. 88-ag-1056** have been found satisfactory and recommend that it be processed for evaluation, by External Examiner(s) for the award of Ph.D degree.

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ABSTRACT

Salt affected soils are those soils which have higher concentration of soluble salts and/or exchangeable sodium affecting normal growth of most of the crops. Under salinity and particularly when associated with sodicity, the availability and uptake of Ca^{2+} is reduced that results in the loss of membrane integrity and other disorders associated with Ca^{2+} deficiency in plants. A wheat genotype efficient in uptake and utilization of calcium under saline conditions may be better able to withstand saline and sodic conditions in the field. Very little information is available on wheat response to salinity and low Ca^{2+} as screening of wheat genotypes has usually been done against salinity alone. The studies reported in this thesis evaluate and characterize different wheat genotypes against salinity and low calcium. All of the physical growth parameters including shoot length, root length, shoot and root fresh and dry weights were decreased significantly due to salinity and low calcium alone as well as under their combined presence. Reduction was more pronounced under the combined stress of salinity and low calcium and different genotypes differed significantly in different stress treatments. In saline treatment, the genotypes 25-SAWSN-39 and 25-SAWSN-31 produced more shoot fresh and dry weights, showed less accumulation of Na^{+} and Cl^{-} and higher K^{+} and Ca^{2+} where as the genotypes 25-SAWSN-35 and 25-SAWSN-47 produced less shoot fresh and dry weights and had less accumulation of K^{+} and Ca^{2+} and high accumulation of Na^{+} and Cl^{-}. In salinity + low calcium treatment the genotype 25-SAWSN-39 behaved as a tolerant genotype whereas 25-SAWSN-31 behaved similar to the sensitive genotype and these differences were due to high accumulation of Ca^{2+} and low Ca^{2+}: Na^{+} ratio in 25-SAWSN-39 and vice versa. Different physiological and biochemical parameters of the genotypes 25-SAWSN-39 and 25-SAWSN-31 supported their growth under saline and saline + low calcium treatments. The behaviour of the selected genotypes was further tested in the salt affected field conditions where salinity and low availability of calcium co-exist. The genotype 25-SAWSN-39 was promising under salt-affected field conditions too, and can be recommended to the farmers and may also be used by the breeders for the development of more salinity tolerant wheat genotypes.

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CHAPTER-1

INTRODUCTION

Salinity is a major agricultural problem that decreases plant growth and yield all over the world. About 7% of the total world land area is affected by salinity (Flowers *et al*., 1997). The world's total irrigated area is 230 mha out of which 45 mha (about 20%) is salt affected (FAO, 2007). According to another report soil erosion and salinity have damaged about 15% of the total land area of the world (Rengasamy, 2006). Most of the world's arid and semi arid areas are subjected to salinity problem due to high temperature, limited rainfall and high evapo-transpiration (Azevedo Neto *et al*., 2006). The salt affected lands have poor structure and drainage, and are mostly poorly managed.

Pakistan's total agricultural land is 22 mha. About 6.67 mha of this is affected by salinity (Khan, 1998). This makes salinity a serious issue in Pakistani agriculture as salinity degrades the productive soil and converts it into useless land and deserts. The management and reclamation of these lands is technically possible but difficult due to large area and unavailability of good quality irrigation water (Qureshi *et al*., 1990). Therefore, reclamation does not seem to be feasible for large tracts of salt-affected lands of Pakistan. However, by selection of salt tolerant crop genotypes and plant species and by improvement of salt tolerance of such species and genotypes, good economical results can be obtained from such soils (Qureshi and Barrett-Lennard, 1998).

The world food production is needed to be increased by 38% by the year 2025 to fulfil the food demand of the growing world population at the current level (Rengasamy, 2006). This food production may not be increased by expansion in cultivated area because most of the suitable land has already been brought under cultivation. The increasing land degradation due to soil salinity, sodicity and allied problems poses a serious threat to the sustainability of world food production. The situation requires efforts to utilize salt-affected soils by developing crops that are tolerant to salinity and sodicity.

Salinity reduces the uptake of water and different nutrients by the plants and causes ion toxicity in the plants (Saqib *et al*., 2005). Ashraf and Foolad (2007) have listed different effects of salinity as osmotic effect, nutrient and hormonal imbalances, ionic effects and production of reactive oxygen species (ROS). Salinity inhibits photosynthesis by stomatal and non-stomatal

factors (Seemann and Critchley, 1985). As salinity decreases photosynthesis, it therefore decreases plant growth (Heuer and Plaut, 1989). In most crops, as the sodium concentration increases in leaves, the photosynthetic activity significantly reduces and shows a negative correlation (Yeo, 1998). In a study on wheat (James *et al*., 2002), it has been found that at a sodium concentration of 350 mM in leaves, the photosynthetic rate was decreased by 50%. Photosynthesis, leaf water relations and transpiration rate have been used as screening criteria in breeding for salinity tolerance in many crops (James *et al*., 2002; Rivelli *et al*., 2002). James *et al*. (2002) found a positive relationship between photosynthetic rate and growth of wheat and the dry matter production variation was due to the differences in photosynthetic rates. Rivelli *et al*. (2002) revealed that stomatal conductance of wheat crop was decreased with high sodium concentration of 150 mM NaCl, and increased with decrease in sodium concentration.

The stress conditions caused by salinity lead to formation of different reactive oxygen species (ROS) (Ali and Alqurainy, 2006) like hydroxyl radical (Temple *et al*. 2005), superoxide, hydrogen peroxide (H_2O_2) (Tolbert, 1982) and singlet oxygen (Elstner, 1987). Lipids are degraded by these reactive oxygen species (Fridovich, 1986; Wise and Naylor, 1987) and nucleic acids and proteins are also denatured (Fridovich, 1986; Imlay and Linn, 1988). Plants produce different enzymatic and non enzymatic antioxidants which can detoxify the reactive oxygen species resulting from salinity stress. The super oxide dismutase, peroxidase and catalase are enzymatic in nature and can defend the plant cells from reactive oxygen species (Chen and Asada, 1989; Asada, 1992). The superoxide dismutase converts oxygen to hydrogen peroxide. Hydrogen peroxide is broken down by catalase and peroxidases (Chang *et al*., 1984). When plants were subjected to salt stress the antioxidative enzymes such as catalase (CAT), peroxidase (POD), glutathione reductase (GR), and superoxide dismutase rose to a high level and a relationship between enzymes and salinity tolerance has been observed (Lee *et al*., 2001; Mittova *et al*., 2004).

Ca^{2+} is an essential nutrient for plants (Marschner, 2003). Its ability to form linkages between molecules makes it a key element in maintaining the integrity and structure of membranes and cell walls (Hanson, 1984). Ca^{2+} also plays an important role of a secondary messenger in many of the signal transduction pathways in the cell (Bush, 1995). The defects associated with low Ca^{2+} include poor root development, leaf necrosis and curling, blossom end rot, bitter pit, fruit cracking, poor fruit storage and water soaking (White and Broadley, 2003).

The causes of these defects are not clear. However, two sites in the cell are recognized as the important targets. First is the cell wall, where Ca^{2+} plays a key role in cross linking of acidic pectin residues and second is the cellular membrane system where low Ca^{2+} increases the permeability of the plasma membrane

The higher concentration of sodium decreases root and shoot growth and calcium concentration in plants (Maas and Grieve, 1987). It is also observed that crop species sensitive to salinity required more calcium than the species which were tolerant to salinity (Greenway and Munns, 1980). Saline and saline sodic soils are usually poor in calcium availability and sodium induced calcium deficiency is very common in plants grown on such soils. Salinity reduces influx of Ca^{2+} in the tissues which results in deficiency of Ca^{+2} (Lynch and Läuchli, 1985; Davenport *et al.*, 1997; Lazof and Bernstein, 1999). Sodium greatly displaces Ca^{+2} from the membranes, if there is low Ca^{+2} in the external solution (Cramer *et al*., 1985; Lynch *et al*., 1987). High Na^{+}: Ca^{+2} ratios cause the deficiency of Ca^{+2} in different species (Maas and Grieve, 1987). Different concentrations of sodium have different effects on displacement of Ca^{+2} from the membranes. It was observed that less displacement of Ca^{+2} occur by NaCl salinity from membranes in salt tolerant barley (Bittisnich *et al*., 1989) and melon genotypes (Yermiyahu *et al*., 1997) than in the respective salt sensitive genotypes. The effect of low Ca^{+2} is not as much studied as the Na^{+} toxicity.

Wheat is a moderately salt-tolerant crop (Maas and Hoffman, 1977) and it has significant genotypic differences for salinity tolerance (Saqib *et al*., 2005). The wheat genotypes said to be tolerant to salinity have a capacity to keep away the sodium ions from the shoot (Schachtman *et al*., 1989). Wheat is one of the most important crops contributing to the daily protein and calorie requirements of people. Wheat is the staple food of Pakistan and most popular cereal crop throughout the country. The total area under wheat every year in Pakistan is about 8.8 mha with most of it in Punjab (Anonymous, 2011). It is very important to increase its production to feed the growing population. A viable option is to screen, develop and grow wheat genotypes with higher tolerance to salinity and sodicity as the non-saline soil may not be available to increase wheat acreage in future. Large tracts of the salt-affected soils are available and the utilization of these salt affected lands and waters by growing salt-tolerant plant species is more economical for the poor farmers of the marginal lands in the developing countries than the reclamation of these lands (Qureshi and Barrett-Lennard, 1998).

The genotypes are checked and selected against salinity by observing their fresh and dry mass production, growth and yield after growing under saline and non saline soil conditions. To check salt tolerance of genotypes most experiments have been conducted in hydroponic conditions (Schachtman *et al*., 1992; Munns and James, 2003; Genc *et al*., 2010) and extrapolated to soil conditions. Ca^{2+} is usually low in saline and sodic soils with a considerable range of Na^{+}: Ca^{2+} ratio in the soil solutions (Naidu *et al*., 1995; Genc *et al*. 2010). Therefore the plants usually face calcium deficiency in addition to sodium toxicity under saline and sodic soil conditions in the field. However, the hydroponic studies usually do not take into account the low calcium concentration available in the salt-affected fields which may be a reason of low success rate of solution culture selected wheat genotypes when grown on salt-affected fields.

There exists genetic variation among crop species and genotypes for tolerance to ion toxicities and deficiencies. Genetic variation also exists among the wheat genotypes for acquisition and utilization of Ca^{2+} under non-saline conditions (Genc *et al*. 2010) and this genetic variation may also prevail under salt-affected conditions. A wheat genotype efficient in uptake and utilization of calcium under saline conditions may be better able to withstand saline and sodic field conditions as these soils usually have low Ca^{+2}. Little information is available on wheat response to salinity and low Ca^{+2} as screening of wheat genotypes has usually been done against salinity alone. Keeping in view the field conditions and the lack of information of the response of wheat to salinity and low calcium the present studies have been planned and conducted with the hypothesis that the wheat genotypes with better calcium uptake and utilization efficiency will perform better under saline sodic field conditions.

CHAPTER – II

REVIEW OF LITERATURE

Salt affected soils are those soils which have elevated concentrations of soluble salts or exchangeable sodium affecting normal growth of most of the crops. Salt-affected soils include saline, sodic and saline sodic soils. These types of soils are predominantly present in arid and semi arid regions of the globe. In these areas annual rainfall is less than evopo-transpirational losses of water. Under humid and sub humid conditions salt affected soils do occur but at a smaller scale. Both soils and plants face different types of problems due to the presence of salts.

2.1 Extent of salinity problem

Salinity is a worldwide problem. Total land surface of the world is about 13.2×10^9 ha, out of which 7×10^9 ha are arable and 1.5×10^9 ha are cultivated (Massoud, 1981). More than 100 countries of the world are facing the problem of soil salinity. More than 4,000,000 square kilo meter of the world is affected to some extent by salinity (FAO, 2006). About 23% of the world cultivated land is saline and 37% is sodic in nature. On average, almost 6% of all land in the Asia-Pacific region is affected by salinity. Pakistan, Australia, and Thailand together have 6.8% of the world's land area but are having more than 10% of the world's land affected by salinization. With respect to absolute size affected, Australia ranks third with 254,000 square kilometers, Pakistan eighth and Thailand forty-fifth (FAO, 2006).

According to Saboora *et al*. (2006) salinity is increasing at a rate of 10% per year throughout the world. Salinity and sodicity are most serious soil issues in Pakistan. Total cultivated area of Pakistan is about 22.94 mha and irrigated agriculture is practiced on about 18.78 mha. Salt affected area of Pakistan is about 6.67 mha (Khan, 1998). About 75-80% of the pumped ground water in Punjab province is unfit for irrigation owing to high EC, SAR and RSC which is adversely affecting the yield of crops (Ghafoor *et al*., 2001).

2.2 Effects of salinity on plants

According to Greenway and Munns (1980) salinity depresses plant growth because plants may undergo three main types of stresses under salinity: (a) reduction in water potential (b) nutritional imbalance and (c) specific ion toxicity. Production of reactive oxygen species under under salinity is also harmful. These harmful effects of salinity can be seen on cell as well as whole plant levels. Inhibition in growth and metabolism occurs in all types of plants in response to salinity but the tolerance potential of different species varies widely.

2.2.1 Osmotic stress

Under salinity stress the water potential of the medium becomes more negative as compared to the plant itself. This reduction in water potential restricts water uptake by plants and probably the movement of water outside the plant tissues. So under highly saline conditions although water is present in the soil, the plants are not able to take it up. This is called physiological drought. This is the osmotic effect of salts and is the primary effect of salinity on plants resulting in a marked reduction in growth. Osmotic stresses caused by salinity and drought have similar cellular and metabolic effects on plants. The production of the new leaves depends mainly on the water potential of the medium. The fast growing cells can accommodate salts inside their vacuoles. In this way the growth of young leaves is not affected (Munns, 2005). Growth reduction of leaves and roots is a result of osmotic stress and not due to specific effects of different salts (Munns, 2002). For instance, in wheat growing in 120 mM NaCl, Na^+ concentration in the growing tissues of leaves was merely 20 mM, and only 10 mM in the fast growing zones, and Cl^- was just 50 mM (Hu *et al*., 2005). This Na^+ concentration was not harmful for the plant growth but was beneficial as it might be used for osmotic adjustment in the expanding vacuole. The fast growth of the new cells might be helpful in keeping the concentrations of the salts at lower levels. Hormonal signals from roots might be responsible for controlling the rate of cell enlargement under water deficit conditions (Munns *et al*., 2000). Reduction in the growth of the plants due to salinity depends on the intensity of the stress. Moderate osmotic stress results in rapid inhibition of growth of leaves and stems. On the other hand root growth may be increased (Hsiao and Xu, 2000).The major factors controlling the

harmful effects of osmotic stress include type of plant species, type of tissue under consideration and the way in which stress is imposed (Ashraf, 1994; Munns *et al*., 2000).

2.2.2 Specific ion toxicity

This is due to accumulation of certain toxic ions from the saline medium in the plant body. The main toxic ions are sodium, chloride and sulphate. Plants tend to accumulate salts in their older leaves, and if this accumulation is continued over a long period of time in transpiring leaves, eventually cell death may occur. The excessive build up of salts may cause injury in the cell when the salt concentration is so much that it cannot be compartmentalized into the vacuole. In such a situation salts rapidly inhibit enzymatic activity in the cytoplasm. On the other hand they might accumulate in the cell walls and result in the dehydration of the cell (Munns, 2005). Cytosolic Na^+ concentrations of the root cells are in the order of 10 - 30 mM (Tester and Davenport, 2003) whereas leaf cytosolic Na^+ levels are not exactly recognized, but mostly are found below 100 mM (Wyn Jones and Gorham, 2002). The toxic levels of Cl^- are least recognized. Roots try to eliminate excessive Na^+ and Cl^- in order to sustain the growth of the plants. According to Husain *et al*. (2003) wheat genotypes having Na^+ exclusion trait produced more grain yield and showed less leaf injury. This Na^+ exclusion trait is important only at moderate salinity levels because at higher salinity levels the osmotic effect outweighs the salt-specific effect on growth and yield of the plants. Higher concentrations of the sodium have inhibitory effects on seed germination, because it affects plant water relations and also by displacing Ca^{2+} from important cell wall binding sites, interrupting cell wall formation and in this way inhibiting plant growth (Xue *et al*., 2004). Plant morphology is changed when Cl^- concentration exceeds 80 mM in total tissue water. Chloride is very mobile in the soil and after being taken up by roots its movement to the leaves is very rapid. The higher concentration of the chloride causes leaf burn which occurs initially at the margins of older leaves and later on expands to the whole leaf and ultimately the plant becomes defoliated (Loreto and Bongi, 1987).

2.2.3 Nutritional imbalance

The excessive uptake of salts may result in reduced concentration of essential nutrients and their deficiencies (McCue and Hanson, 1990). Competition of Na^+ with K^+, Ca^{2+}, and Mn^{2+} and of chloride and sulphate with nitrate and phosphate leads to upset ionic balance in the cell.

High sodium to potassium ratio in the cell inhibits enzyme activity and different metabolic processes (Booth and Beardall, 1991). The excess Na^+ inhibits K^+ uptake and results in K^+ deficiency which firstly results in yellowing of leaves followed by their death (Gopa and Dube, 2003). Potassium is required for osmoregulation, synthesis of protein, cell turgidity and for carrying out normal photosynthesis (Freitas *et al*., 2001; Ashraf, 2004). On the other hand $Ca^{2+}$is necessary for maintaining the integrity and proper working of cell membranes (Wenxue *et al*., 2003). Under salinity stress, decreased uptake of Ca^{2+} by the roots results in loss of membrane integrity and dissolution of membranes (Kinraide, 1998). Reduced uptake of K^+ affects activities of different enzymes and ultimately the growth of the plants. Exogenous application of Ca^{2+} has ameliorative effects on plants against salinity by facilitating higher K^+/Na^+ selectivity, osmotic adjustment, accumulation of compatible solutes and reducing oxidative damage (Liu and Zhu, 1997).

2.2.4 Oxidative stress

The reactive oxygen species (ROS) are produced under stress conditions which can disturb many important functions in the cell (Asada, 1999). The reactive oxygen species include superoxide, hydrogen peroxide and the hydroxyl radicals. The detrimental effects of these ROS include lipid peroxidation, protein degradation and DNA mutation (Mittler, 2002). These are also responsible for membrane injury, damages to D1 protein of photo system II causing photo-inhibition. Under stress, stomata are closed, which results in low levels of CO_2 in the chloroplasts and causes a reduction in $NADP^+$ level with the simultaneous production of ROS (Foyer and Noctor, 2003). Although these reactive oxygen species are produced in low concentrations under normal growth conditions (Polle, 2001), they are over produced under environmental stresses (Laloi *et al*., 2004). For example due to osmotic stress, when stomata are closed the supply of CO_2 for photosynthesis is reduced and results in excessive amounts of superoxide in the chloroplast leading to photo-inhibition and photo-oxidation damage in the cell (Ashraf, 2009).

Salinity causes impairment of the cellular electron transport within different subcellular compartments resulting in the generation of ROS (Ali and Alqurainy, 2006). Salinity induced photorespiration and NADPH activity are also responsible for the increased production of H_2O_2 leading to inactivation of enzymes by oxidizing their thiol groups. Plants have specific

mechanisms to detoxify these ROS which include activation of antioxidant enzymes (Smirnoff, 2005) and accumulation of non enzymatic antioxidants (Johnson *et al*., 2003).

2.2.5 Other physiological disorders

Different physiological processes like photosynthesis and respiration are also affected by salinity. It is well documented that salinity results in the reduction of photosynthesis by injuring photosynthetic apparatus and altering the process of gas exchange and light reactions. It has often been stated that elevated levels of salinity increase the rate of respiration. Salt sensitive species showed a greater increase in respiration than salt tolerant ones (Semikhatova *et al*., 1993; Fidalgo *et al*., 2004). High salt concentration decreases the rate of photosynthesis by affecting photosynthetic enzymes, gas exchange and light reactions. Salt stress limits photosynthesis by stomatal and non-stomatal factors. Non-stomatal factors which reduce photosynthesis involve higher concentration of toxic ions like Na^+ and Cl^- contents in leaves. It is observed that chlorophyll content of salt-tolerant species is less affected as compared to salt-sensitive species. Robinson *et al*. (1983) reported a 65% inhibition in the rate of photosynthesis and stomatal conductance under saline conditions. However, chlorophyll concentrations remained unchanged. An antagonistic relationship was found between rate of photosynthesis and leaf Na^+ content in many crop species such as rice and wheat (Yeo, 1998; Saqib *et al*., 2006) and leaf Cl^- content in woody perennials such as citrus (Lu *et al*., 2010). Leaf total chlorophyll contents significantly reduced under saline conditions and the extent of reduction depends upon salt concentrations and salt tolerance of plant species. There is an increase in chlorophyll content of salt-tolerant species, whereas it was decreased in salt-sensitive species (Ashraf and McNeilly, 1988; Bai *et al*., 2006). In salt-sensitive species chlorophyll content was significantly reduced due to Cl^- accumulation (Velegaleti *et al*., 1990; Lu *et al*., 2010). Salinity directly affects the functioning of respiratory enzymes (Moradi and Ismail, 2007) and the rate of respiration is found to be increased due to salinity.

2.3 Mechanism of salinity tolerance

There are different physiological, biochemical and molecular mechanisms adopted by plants to cope with salinity stresses which are discussed below.

2.3.1 Ion regulation and compartmentalization

Ionic homeostasis is disturbed due to salinity. Plants do not have the ability to accommodate higher concentration of salts in their cytoplasm. To carry out normal metabolism in the cell, plants either limit the surplus salts in the vacuolar cells or compartmentalize them in various tissues (Zhu, 2003). Salt sensitive plants restrict sodium uptake in their cells or they partition it in older tissues. On the death of the older tissues plant get rid of these toxic ions. Exclusion of Na^+ out of cytoplasm or compartmentalization in the vacuoles is made possible by a Na^+/H^+ antiporter (Apse *et al*., 1999). Under salinity stress, plants try to keep more K^+ and less Na^+ in the cytosol by regulating the activities of K^+ and Na^+ transporters and of H^+ pumps.

Exogenous application of Ca^{2+} reduces the toxicity of salinity by keeping higher K^+/Na^+ selectivity (Liu and Zhu, 1997). Salt secretion and exclusion are two other mechanisms of tolerance. Secretion of salts is made possible by specialized structures known as salt glands. These glands secrete salts (particularly NaCl) from their leaves and help in maintaining its low concentration in the cell (Hogarth, 1999). Exclusion of salt occurs at the root level to normalize the leaf salt concentration in many plants (Levitt, 1980).

2.3.2 Compatible solutes

Compatible solutes are low-molecular weight compounds synthesised in the cytoplasm to balance the higher ionic concentrations in the vacuoles of the cell. They are called so because they do not interfere with normal metabolic processes (Zhifang and Loescher, 2003). Their main functions in the cell are replacement of water in biochemical reaction, protection of important cellular structures, osmotic balance which make the influx of water possible and scavenging of ROS (Hasegawa *et al*., 2000). They include simple sugars, sugar alcohols, complex sugars, quaternary amino acid derivatives, tertiary amines, and sulfonium compounds. These osmolytes are produced under stress conditions due to metabolic diversion into unique biochemical reactions.

2.3.3 Antioxidants

Antioxidants are those compounds which detoxify the free radicals produced under any type of stress. Their activities are increased many folds under stress conditions. There is a strong

relation between the levels of these compounds and salt tolerance of plants (Mittova *et al*., 2003). These may be enzymes or non enzymes. Enzymes include superoxide dismutase (SOD), catalase and various peroxidases. Non enzymes are tocopherol (vitamin E) carotene (vitaminA) and ascorbic acid (vitamin C). Superoxide dismutase (SOD) converts superoxide radical to H_2O_2 whereas catalase and a variety of peroxidase catalyze the breakdown of H_2O_2 (Chang *et al*., 1984).

2.3.4 Plant hormones

Salinity stress also results in increased contents of plant hormones like abscisic acid (ABA), cytokinins and jasmonates. Salinity-induced genes are changed due to ABA. Abscisic acid induced genes were found to be involved in salinity tolerance of rice (Gupta *et al*., 1998). Positive effects of ABA on photosynthesis, growth and translocation of assimilates are well documented under salinity stress (Popova *et al*., 1995). A higher level of ABA under salt stress results in more Ca^{2+} uptake and in this way increase the integrity of membranes, enabling the plants to regulate ion fluxes. According to Gomez Cadenas *et al*. (2002) ABA reduces ethylene production and shedding of leaves under salinity in citrus by reducing the uptake of Cl^- in the leaves. Jasmonates are also considered important in salinity tolerance of plants. It is observed that salt-tolerant varieties of tomato have a higher jasmonates level than salt-sensitive ones (Hilda *et al*., 2003). Jasmonates are thought to intervene signalling in defence, flowering, and senescence.

2.4 Genetic variability in wheat in response to salinity

Zheng *et al*. (2008) compared salinity tolerance of two genotypes of wheat under salinity. They found that photosynthesis, stomatal conductance, ion contents, and dry matter production were reduced in both genotypes due to salinity. However, the salt-tolerant genotype showed less reduction in these parameters compared to the sensitive one. Dulai *et al*. (2011) found that 200 mmol L^{-1} NaCl concentration substantially reduced net CO_2 assimilation rate and resulted in the closure of stomata. Changes of these parameters were less significant for barley as compared to wheat.

Response of contrasting wheat genotypes to salinity (100 and 200 mM NaCl) were studied by Sairam *et al*. (2005) and they found that a tolerant genotype (Kharchia 65) showed less reduction in relative water content, chlorophyll content, membrane stability index as compared to a sensitive genotype (HD 2009). On the other hand the activities of different antioxidants were more in the tolerant genotype as compared to the sensitive one. Mandhania *et al*. (2006) compared the response of two wheat genotypes i.e. KRL-19 (tolerant) and WH-542 (sensitive) to various levels of salinity (0, 50 and 100 mM NaCl). Relative water content decrease was more in WH-542 than KRL-19 whereas K^+/Na^+ ratio was more in KRL-19 than WH-542. There was more damage to membranes in WH-542 due to lipid peroxidation. In both genotypes, the activities of antioxidants except superoxide dismutase increased with increasing salinity. Chen *et al*. (2011) observed that under NaCl stress, oxidative damage was more severe and the potassium (K), calcium, zinc, and iron accumulations were lower in Shi 4185 (sensitive cultivar) than in Cang 6001 (tolerant one). Exogenous application of ascorbic acid and N-acetyl-L-cysteine improved Zn and Fe contents in the plants. More activity of the superoxide dismutase was found in Cang 6001 than in Shi 4185. Effect of seawater salinity (10% and 25% of original strength) on different growth parameters, water relations, protein and nucleic acids in flag leaves of two wheat genotypes Gemmieza-9 (sensitive) and Sids-1 (tolerant) was investigated by Aldesuquy *et al*. (2012) during grain-filling.

Esfandiari *et al*. (2011) grew two durum wheat genotypes, 'Egypt - 449' (salt-tolerant) and 'Syria - 371' (salt-sensitive), under control and salt stress (200 mM NaCl) conditions. The activities of antioxidant like ascorbate peroxidase and guaiacol peroxidase were increased in 'Egypt 449' in response to salinity where as of SOD and CAT were not changed. On the other hand activities of superoxide dismutase, catalase and GPX in cultivar 'Syria 371' were lower than control as compared to Egypt 449. Membrane stability index and K^+/Na^+ of both genotypes were reduced due to salt stress. Similarly, Hameed *et al*. (2008) applied various levels (5, 10 and 15 dS m^{-1} NaCl) of salinity to threedaysold wheat seedlings for 6 days. They found that salinity reduced the growth and protein content, mainly at 15 dS m^{-1} NaCl. Catalase activity was decreased at all salinity levels in both genotypes indicating that high salinity generally reduced the catalase activity. These findings show that Lu-26, exhibits a better protection mechanism against salt stress by lower salt induced proteolysis, higher biomass accumulation and protein content as compared to sensitive genotype Pak-81.

2.5 Calcium and its importance for plants

Calcium (Ca^{2+}) is derived from the Latin word *calx*, which means lime. Its mass is 40.08 and a valence of 2 (Ca^{2+}). It ranks fifth with respect to quantity in the soil. Mostly Ca^{2+} salts are very soluble, but some like calcium phosphates are very insoluble. The ionic radius of Ca^{2+} is 0.099 nm and is very close to that of Na^{+}. Because of being divalent calcium has more charge density than sodium (Cramer, 2002). Ionic behavior is not ideal in saline solutions. Due to ion pairing and precipitation the activities of the ions are mostly lower than their actual concentrations (Cramer and Lauchli, 1986) which is more distinct for calcium as compared to sodium. As a result the Na^{+}: Ca^{2+} ratio on a concentration basis is much different from that of activity basis. Ca is a vital nutrient for all plants (Marschner, 1995). Its capacity to form linkages between molecules gives it a key role in keeping the membranes and cell walls in proper structure and working (Hanson, 1984). Calcium also plays the role of secondary messenger in the cell (Bush, 1995).

Root hairs take up calcium from the soil and transport it via the vascular system to the sink organs by means of the dynamic force produced by evapotranspiration (Hirschi, 2004). The calcium concentration in the soil is sufficient to avoid its deficiency in plant cells. Calcium is very vital for the firmness and extension of cell walls and applies useful effects on plant life cycle. Calcium is known as a signal molecule (Hetherington and Brownlee, 2004). Calcium as a secondary messenger encodes changes in biotic and abiotic factors. Decoding of information carried by calcium should let the cell produce a biological response (Xiong *et al*., 2006). NADP from NAD^{+} for photosynthetic reduction to NADPH, is triggered by Ca^{+2} (Kreimer *et al*., 1985). It is suggested that H^{+}/Ca^{+2} antiport process powered by ATP is responsible for Ca^{+2} uptake (Muto *et al*., 1982) but the experimental data state that the driving force is mostly due to alterations in membrane potentials (Kreimer *et al*., 1985).

2.6 Interaction of salinity with calcium

2.6.1 Effects on plant growth

Many ions present in saline-sodic soils of which sodium is most important and decides the availability of water to root medium and its concentration decides the function of growth of

root. Salinity is also dependent on the source of salinity and sodium. Sodium chloride is more toxic than sodium sulphate (Zaman, *et al*., 2002a). Potassium concentration in plants was controlled by calcium (Lauchli, 1990). In saline or saline-sodic soils availability of macronutrients is necessary for plant growth and yield. Zaman, *et al*. (2002b) found that with increased sodium the dry mass was decreased.

Growth of plant is affected by salt toxicity because it leads to lower water potential and toxicity of specific ions which causes nutrient imbalances (Greenway and Munns, 1980). Uptake and transport of calcium is decreased by saline stress (Halperin *et al*., 1997), and therefore sodium harmful effects are attenuated by a sufficient supply of calcium in many plants helping in growth and development (Cramer, 2002). Cell wall and plasma membrane structure needed electrostatically bound calcium for proper functioning and to protect the structure. In sodium chloride salinity, the main harmful effect is that Na^+ replaced the Ca^{2+} which results in transport of nutrients through disturbed membranes (Cramer *et al*., 1985; Rengel, 1992). Sodium increased in leaves could be tackled by the sufficient supply of calcium which prevent the uptake and translocation of sodium (Halperin *et al*., 1997). The major competitor of sodium which enters into the root is potassium, and external calcium help and enhance potassium uptake by the non selective channels (Maathuis and Sanders, 2001). Genotype is a major criterion and plants from different genotypes respond differently to external calcium. Cramer (2002) investigated different species with respect to their response to calcium. Photosynthesis in maize was reduced by the antagonism effect of Mg^{2+} and Ca^{2+} that was grown under high Ca^{2+}: Na^+ ratios. Photosynthesis was decreased in such situation due to the Mg^{2+} deficiency instead of Na^+ toxicity induced by calcium (Plaut and Grieve, 1988).

The interaction of salinity and incremental calcium for growth was investigated for leaves of maize by Cramer (1992). After one day of salinity application, cell wall expansion was reduced. The plants which were grown under high Na^+: Ca^{2+} ratio, showed a reduction in hydraulic conductance. However supplemental calcium improved growth by restoring hydraulic conductivity (Cramer, 1992). In leaves of sorghum, the growth zone length was reduced due to 100 mM NaCl. When solution Ca^{2+} level increased from 1 - 10 mM, the reduction in growth zone was minimized (Bernstein *et al.*, 1993). On the other hand, supplemental calcium had no influence on the growing zone length in salinized roots of cotton (Zhong and Läuchli, 1993b).

The impact of supplemental calcium on salanized roots growth is abrupt (Cramer *et al.*, 1988). When 80 mM NaCl was added prior to addition of additional Ca^{2+}, the growth of roots was highly repressed. Though, when the concentration of Ca in the nutrient solution culture was excessive, the growth of roots was not affected (Cramer *et al.*, 1988). The variation in the growth of maize leaf (Cramer, 1992) and bean (Ortiz *et al.*, 1994) were highly affected by varying Na^{+}: Ca^{2+} ratios.

2.6.2 Effects on cell shape and formation of new cells

The ratio of Na^{+}: Ca^{2+} in soil solution has a significant impact on the cell shape and formation of new cells. In cotton roots, a high ratio of Na^{+}: Ca^{2+} resulted in formation of isodiametric cortical cells compared to controls (Kurth *et al.*, 1986). On the other hand, a low Na^{+}: Ca^{2+} ratio caused the root cells to become much longer and thinner. Supplemental Ca^{2+} resulted in the formation of 20% more roots in cotton. In roots treated by 0.4 mM Ca^{2+}, cell division was repressed at 50 mM NaCl. Where as under 10mM Ca^{2+}, this happened at 200 mM NaCl. In maize, 100 mM NaCl decreased root cortex growth by 50% compared to control (Azaizeh *et al.*, 1992. Salinity without supplemental Ca reduced cell volume, however not in treatments with additional Ca (Azaizeh *et al.*, 1992).

2.6.3 Effects on photosynthetic parameters

In *Citrus sinensis*, development is susceptible to Cl^{-} salinity (Banuls and Primo-Millo, 1992). Saline treatments significantly decreased photosynthesis. When concentrations of Ca^{2+} were amplified to 30 mM , the photosynthetic rates significantly improved. This was due to reduction of chloride concentrations in the leaf cells. In *Vaccinium ashei*, another species tremendously delicate to chloride, additional Ca^{2+} showed similar results (Wright *et al.*, 1993). In both species, growth was directly related to photosynthesis . Calcium in the company of 50 mM sodium chloride, limit the decrease of stomatal conductance in *Aster tripolium* (Perera *et al.*, 1995). Such reactions are due to interaction of Na^{+}-Ca^{2+} in the plasma membrane of the guard cells and its impact on K^{+}, Na^{+} and Ca^{2+} fluxes (Perera *et al.*, 1995). Ashraf and O'Leary (1997) reported that transpiration and stomatal conductance remained unaffected in one line of sunflower, by changing the ratios of Na^{+}: Ca^{2+}, whereas in the second line a significant reduction was observed

as the ratio of Na^+: Ca^{2+} amplified. Transpiration rates were reduced due to salinity in barley, but additional Ca^{2+} did not change these levels (Cramer *et al.*, 1989).

2.6.4 Effects on cell wall composition

Almost 50% Ca^{2+} of the cell is attached to carboxyl groups in the cell wall, mainly in pectins (Hanson, 1984). Ion exchange takes place in the cell walls (Grignon and Sentenac, 1991) and that is accompanied by clear Na-Ca interactions (Zid and Grignon, 1985). In normal environments, ratios of Na:Ca are associated to segregation of the secondary walls (Ripoll *et al.*, 1993). Under salt stressed *Citrus aurantium*, sodium competing ion of Ca^{2+} for negative sites in the cell walls of the leaves which are very specific for calcium (Zid and Grignon, 1985). Higher concentrations of sodium may decrease cellular turgor (Flowers *et al.*, 1991). In maize leaves, the restrictions of cell development by salt stress are linked to hardening of cell wall and not link to turgor effects (Neumann, 1995). Because of the fact that roots are less sensitive to salt stress (Munns and Sharp, 1993) there was found more cell wall loosening (Wu *et al.*, 1996b).

In barley, salinity increased the ratios of Na^+: Ca^{2+} in leaf tissues of growing leaves and the growth of leaves was decreased significantly (Lynch *et al.*, 1988). Na^+: Ca^{2+} ratios have a marked effect on cell wall biosynthesis (Zhong and Lauchli, 1993a). A high ratio of Na^+: Ca^{2+} restricts cellulose and non cellulosic polysaccharide biosynthesis in cotton roots cell walls (Zhong and Lauchli, 1988). On the other hand, at lower ratios of Na^+: Ca^{2+}, only non cellulosic polysaccharide biosynthesis is repressed. High ratios of Na^+: Ca^{2+} amplified the uronic acid level with a resultant decrease in the cellulose content of cell walls (Zhong and Läuchli, 1993a). Additional Ca reduced these variations in cellulose content and uronic acid. It has been proposed that these variations in cell wall configuration by salt stress may result from calcium deficiency induced by salinity (Kafkafi and Bernstein, 1996).

2.6.5 Effects on membranes

In salinized roots of cotton, dislocation of membrane-bounded calcium by sodium, from plasma membrane was found (Cramer *et al.*, 1985). In a separate study, salinity stress also exiled membrane-associated calcium on maize and barley protoplasts (Lynch and Lauchli, 1988). These findings show that fractional mitigation of NaCl reticence of cell expansion with the help of calcium may in fragment be associated with the amount of membrane-associated calcium.

Kafkafi (1991) found that the salinity tolerance of four melon genotypes was related to membranes of their roots to fix more calcium. It was discerned that calcium was moved from two diverse locations, one is high-affinity fixing site, which was related to proteins, and other was low-affinity site, which was related to phospholipids (Cramer and Lauchli, 1986). Other findings give proof for even more binding sites for calcium at cell membranes (Murata *et al.*, 1998a). Dislocation of calcium from diverse spots may cause different impacts. In the case of cotton, dislocation of membrane bound calcium from root hairs was found to be comparable to Na, whereas this dislocation was not found in case of Barium or mannitol. Consequently, it is presumed that this effect is explicit for sodium. In support of this assumption, Na-specific impacts were renowned in tobacco, but nonspecific impacts were found in barley (Murata *et al.*, 1998a). Therefore, different genotypes appear to have intrinsically diverse responses.

2.6.6 Effects on ion transport and salinity tolerance

Alteration of the ion transportation and their amounts in plants by salinity is quite obvious (Cramer, 1997). As the amount of sodium in the solution increases, Na uptake increases resulting in the reduction of calcium concentrations (Lazof and Bernstein, 1999). One outcome of this Na^+: Ca^{2+} interaction was a decrease in K concentration, which might be prohibited with more calcium supply. A higher Na^+: Ca^{2+} ratio can cause calcium deficiency in different species (Bernstein *et al.*, 1995). Total tissue calcium contents and calcium influx are limited due to salinity (Cramer *et al.*, 1994b). Calcium concentration of the expanding tissue is reduced because of salinity, and is recovered by increasing the amount of calcium (Lazof and Bernstein, 1999).

Salinity reduced the activity of calcium in the cytoplasm of the maize roots (Cramer, 1997) and *Arabidopsis* (Cramer and Jones, 1996). Likewise, mannitol treatments also resulted in the reduction of calcium activity of the cytoplasm in the roots of *Arabidopsis* (Cramer and Jones, 1996) and tobacco (Jones *et al.*, 1998). There exists a variety of mechanisms by which sodium can enter the cells and among these one mechanism at least, is insensate to calcium (Tyerman and Skerrett, 1999). Ca^{2+} enters cells via ion channels (Pineros and Tester, 1997) which are permeable to sodium as well (White, 1998a). Potassium also moves via a calcium channel interfering with calcium movement (Pineros and Tester, 1997); it is quite possible that sodium play the same role. Calcium may operate on transportation via a calcium signaling passageway. The appearance calcinuerin derived from yeast in engineered tobacco improved the plants salt

tolerance (Pardo *et al.*, 1998). Much data is available to support the contribution of Ca^{2+} signaling in salinity tolerance. Messenger RNA quantity of a Ca-ATPase in tomato (Wimmers *et al.*, 1992) and a Ca-dependent protein kinase in mungbean (Botella *et al.*, 1996) become higher after salinity treatment. Crassulacean acid metabolism (CAM) induction as a result of in the halophyte, *Mesembryanthemum crystallinum*, is reliant upon Ca-signaling mechanisms (Taybi and Cushman, 1999). Salinity causes an increase in pH of the vacuole and extra supply of calcium lowers it. It is assumed that lower levels of sodium in cytoplasm by supplemental Ca^{2+}, result in reduction of Na^+/H^+ antiport activity at the tonoplast and thus lower pH of the vacuole. Proton exclusion is enhanced in salinized mungbean roots and extra supply of calcium reduced it (Nakamura *et al.*, 1992).

2.7 Salinity calcium interaction in wheat

Hawkinst and Lewis (1993) studied the growth, ionic composition and gas exchange characteristics of wheat grown on ammonium and nitrate nutrition at different calcium concentrations in the nutrient solution. The reduction in the biomass was greater in nitrate supplied plants as compared to ammonium supplied plants. Supplemental calcium decreased Na^+ content of salt stressed nitrate supplied plants whereas it had no effects on all of the parameters of ammonium supplied plants. Kinraide (1999) observed that except osmotic stress, higher concentrations of Na^+ in the rooting medium and in the tissues of the plants are not toxic unless Ca^{2+} is also deficient. If such situation exists, it will lead to insufficient compartmentation and excessive buildup of salts in the cytoplasm. Genc *et al*. (2010) examined Ca^{2+} requirements of wheat under saline and normal environment by using five wheat genotypes were grown at 100 mM NaCl with different amounts of Ca^{2+}. This resulted in various Na^+: Ca^{2+} ratios. They observed that salinity tolerance was the maximum when the Na^+: Ca^{2+} ratio was from 5 to 15 andnutrient imbalances/ osmotic stress occurred at lower or higher than this ratio with resulting in a reduction in crop growth. Davenport *et al*. (1997) compared interactions between sodium and calcium in two wheat genotypes having different sodium uptake rates under salt stress. The rate of sodium uptake was similar for the salt-sensitive (Modoc) and the tolerant (Kharehia) genotypes except at a small amount of calcium, when Kharehia accumulated more sodium. Calcium movement was more repressed due to sodium in the sensitive genotype (Modoc) and it was due to the greater salt sensitivity of this genotype. Niazi *et al*. (2007) determined the role of

added calcium in the form of $CaSO_4$ on the growth of wheat (cv. LU-26) under salinity stress. The plants were grown in solution culture at two salinity levels of 0 and 50 mM NaCl. Calcium was applied at 3 and 6 mM to plants. They found that fresh weight of shoot and root increased by 44 and 41 % respectively at 50 mM NaCl, with increased application of $CaSO_4$ from 3 to 6 mM. Dry mass was increased by 46% at 50 mM of NaCl with the application of 6 mM of $CaSO_4$. Ehret *et al*. (1990) studied salinity-calcium interactions in two species of barley and wheat. They found that wheat exhibited more decline in growth and more Ca^{2+} insufficiency symptoms as compared to barley when grown in saline conditions. Modification of the solution with calcium lessened the effects of salinity more in wheat as compared to barley. Calcium supply enhanced tissue $Ca^{2+}/(Na^{+}+Mg^{2+})$ ratios in case of both species however wheat showed more calcium content, as compared to barley.

Salah *et al*. (2005) compared 13 wheat genotypes for their salinity tolerance. Salinity caused a marked reduction in RGR, water relations, photosynthetic parameters and K^{+} and Ca^{2+} concentrations in different plant parts, whereas it increased, Na^{+} and Cl^{-} contents. Salinity affected the growth of tolerant varieties mainly because of reduction in photosynthesis rather than decline in leaf area. They reported that exclusion of Na^{+} and Cl^{-} was not the only parameter reflecting the salt tolerance and K^{+} and Ca^{2+} concentrations were closely related to genotypic differences in salt tolerance. Pervaiz (2003) studied the effect of calcium on two wheat genotypes (Bao-119, sensitive) and (FSD-85, tolerant) under salinity. They found that salinity levels of 200 mol m^{-3} NaCl significantly decreased, dry matter yield, K^{+} contents, leaf area and dehydrogenase activity of roots whereas, Na^{+} concentration and membrane. Permeability was found to be increased. The addition of 8 mol m^{-3} calcium to nutrient solution significantly ameliorated the harmful effects of salinity. The sensitive genotype was more responsive to calcium as compared to tolerant one.

2.8 Need for the present project

Salt-affected soils are usually saline sodic in nature with a low availability of calcium under these conditions. Therefore the crops naturally face the problem of high salt concentration as well as low calcium under salt-affected field conditions. Screening of crop genotypes for salt tolerance is usually carried out against salinity alone in the solution culture.

However, the success rate of the selected genotypes in the field is very low (Saqib, 2002). A possible reason may be the lack of consideration of the allied problems like low calcium under field conditions during the process of screening of genotypes. Keeping in view the little information available on salinity and low calcium interaction in wheat, the present study has been designed with the following specific objectives:

1. To evaluate the performance of different wheat genotypes against salinity and low calcium.
2. To study the physiological and biochemical characteristics of tolerant and sensitive wheat genotypes in response to salinity and low calcium.
3. To study the growth and yield performance of the selected wheat genotypes under salt affected field conditions.

CHAPTER-3

MATERIALS AND METHODS

The research work reported in this thesis has been conducted at the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. This work has been conducted to evaluate the performance of different wheat genotypes against salinity and low calcium. Four different experiments as given below were conducted to achieve the specific objectives of this research project:

Experiment-1 Screening of different wheat genotypes against salinity and low calcium

Experiment-2 Effect of salinity and low calcium on water relations and photosynthetic parameters of different wheat genotypes

Experiment-3 Comparative oxidative stress tolerance of different wheat genotypes against salinity and low calcium

Experiment-4 Comparative performance of selected wheat genotypes under normal and saline sodic field conditions

3.1 Experimental techniques

3.1.1 Experiment-1: Screening of different wheat genotypes against salinity and low calcium

This study has been conducted with the object to evaluate the salt-tolerance of different wheat genotypes under saline conditions with and without the provision of adequate calcium. It is hypothesized that the wheat genotypes having better ability for growth under low calcium conditions can better perform under low calcium saline conditions.

3.1.1.1 Genotypes used in this experiment: The following genotypes were used in this first study:

a. 25-SAWSN-8	b. 25-SAWSN-35	c. SARC-7
d. 25-SAWSN-12	e. 25-SAWSN-39	f. SARC-1
g. 25-SAWSN-25	h. 25-SAWSN-42	
i. 25-SAWSN-31	j. 25-SAWSN-47	

Except SARC-1 and SARC-7, the other genotypes are newly developed genotypes with no information available on their salt tolerance. SARC-1 and SARC-7 genotypes have been used as a check and are known as salt-tolerant and salt-sensitive, respectively.

3.1.1.2 Seed source

All of the mentioned wheat genotypes were collected from Dept. of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan and the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.

3.1.1.3 Experimental details:

This study was conducted in the wire house at the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. The average weather conditions during the experiment were: (11.4 ± 2.1)°C minimum temperature, (26.3 ± 2.2)°C maximum temperature, (45.5 ± 5.1)% minimum relative humidity, (78.3 ± 4.9)% maximum relative humidity, and sunshine hours: 7 h and (32 ± 31) min (no other light source except sun was used). Healthy seeds of selected wheat genotypes were sown in trays each having 5 cm layer of washed sand. The sand was kept moistened with water and nutrient solution after seedling emergence. At the two leaf stage, seedlings of all genotypes under study were transplanted to foam plugged holes in polystyrene sheets floating on nutrient solution contained in 100 liter tubs (1m x 0.5m x 0.25m) (see Table-3.1 for nutrient solution details). There were four treatments (control; 9.0 mM Ca^{2+}, low Ca^{2+} ($1/4^{th}$ of the Ca^{2+} conc. in control; 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline. In the low Ca^{+2} treatment tubs the level of calcium was kept at $1/4^{th}$ of the normal level (9.0 mM Ca^{2+}). After two days of transplantation, salinity (125 mM NaCl) was developed in three increments (one per day) in saline treatment tubs of adequate and low calcium levels. No salt was added in control and low calcium alone treatments. The pH of the solution was adjusted to 6.0 ± 0.5 with diluted NaOH and/or HCl daily and the treatment solutions were changed weekly. The experiment was arranged following a split plot arrangement with four replications and two plants per replication. Salinity and low calcium treatments were kept in the main plot where as the genotypes were kept in the split plot. The plants were harvested after four weeks of growth (early jointing stage) in the solutions having above the mentioned treatments. Genotypic resistance to salinity and low Ca^{2+} was determined based on relative shoot weight.

Table 3.1: The composition of the nutrient solution used in the solution culture studies

Reagent	Concentration (mM)	Reagent	Concentration (µM)
$Ca(NO_3)_2 .4H_2O$	4	H_3BO_3	1
KH_2PO_4	0.2	$MnSO_4.H_2O$	0.5
K_2SO_4	1	$ZnSO_4.7H_2O$	0.5
$MgSO_4.7H_2O$	0.6	$CuSO_4.5H_2O$	0.3
$CaCl_2. 2H_2O$	5	$(NH_4)Mo.O_{24}$	0.01
Fe-EDTA	0.2		

Measurements taken in this experiment:

The following parameters (detailed methodology given in section 3.3) were recorded/ determined in this study:

1. Root length
2. Shoot length
3. Root fresh and dry weight
4. Shoot fresh and dry weight
5. Shoot Na^+ concentration
6. Shoot K^+ concentration
7. Shoot Ca^{2+} concentration
8. Shoot Cl^- concentration

3.1.2 Experiment-2: Effect of salinity and low calcium on water relations and photosynthetic parameters of different wheat genotypes

3.1.2.1 Genotypes used in this experiment:

The genotypes used in this study are listed below

1. 25-SAWSN-31
2. 25-SAWSN-35
3. 25-SAWSN-39
4. 25-SAWSN-47

3.1.2.2 Experimental details:

This study was carried out in the wire house at the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. In this experiment, 2 salt sensitive and 2 salt resistant wheat genotypes identified from Experiment No. 1 were used. The experimental procedures were same as used in the Experiment No. 1. The experiment was arranged following split plot design with four replications and two plants per replication. Photosynthetic attributes and the water relations were determined before harvest in the fourth week of treatment. Photosynthetic attributes were recorded on the second fully expanded leaf from top with a portable gas exchange system (details are given in section 3.3.3) and SPAD absorbance was determined using a hand-held SPAD-502 meter from the centre of the third leaf from top. Leaf water (Ψ_w) and osmotic (Ψ_π) potentials were taken from the centre of the subsequent youngest mature leaf, and leaf turgor pressure (T_p) was determined as a difference of Ψ_π and Ψ_w (details are given in section 3.3.3-5). After harvest, fresh and dry weights of roots and shoots were taken along with the lengths of roots and shoots. Leaf area meter was used (Δ MK2, England) to measure leaf area. Flame Photometer (Sherwood- 410, Japan) was used to determine Na^+ and K^+ concentrations in the leaves and Cl^- was determined by chloride analyzer (Sherwood-926, Japan. Calcium was determined with atomic absorption spectrophotometer (PerkinElmer, 100 AAnalyst, Waltham, USA).

3.1.2.3 Measurements for this study:

To avoid the high cost and heterogeneity in the salt-affected field, the researchers have tried a number of parameters under controlled conditions to get good correlation with grain yield performance of a genotype in the salt-affected field. Following parameters (detailed methodologies given in section 3.3) were recorded/ determined in this study:

1. Stomatal conductance
2. Shoot fresh weight
3. Net photosynthetic rate
4. Shoot dry weight
5. Transpiration rate
6. Root length
7. Leaf SPAD absorbance
8. Shoot length
9. Leaf water potential (Ψ_w)
10. Number of tillers plant^{-1}
11. Leaf osmotic potential (Ψ_π)
12. Shoot Na^+ concentration
13. Leaf turgor pressure (T_p)
14. Shoot K^+ concentration

15. Root fresh weight
16. Shoot Cl^- concentration
17. Root dry weight
18. Shoot Ca^{2+} concentration
19. Shoot K^+: Na^+
20. Shoot Na^+: Ca^{2+} ratio

3.1.3 Experiment 3: Comparative oxidative stress tolerance of different wheat genotypes against salinity and low calcium

3.1.3.1 Experimental details:

The experimental details regarding the genotypes used, treatment application and duration are the same as for Experiment No. 2. The experiment was arranged following split plot design (salinity and calcium treatments in main plot and genotypes in split plot) with four replications and two plants per replication. The plants were harvested after four week growth in the treatment solutions and different parameters as detailed below were recorded. The activities of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were determined in this study. The action of superoxide dismutase was considered on the basis of its capability to stop the photoreduction of nitroblue tetrazolium (NBT) as reported by Giannopolitis and Ries (1977). Chance and Maehly (1955) method was used to determine the performance of catalase (CAT) and peroxidase (POD).

3.1.3.2 Measurements of this study:

The following parameters were recorded/ determined in this study:

1. Root fresh and dry weight
2. Shoot fresh and dry weight
3. Root length
4. Shoot length
5. Protein contents
6. Superoxide dismutase activity (SOD)
7. Peroxidase activity (POD)
8. Catalase activity (CAT)

3.1.4 Experiment 4: Comparative performance of selected wheat genotypes under normal and saline sodic field conditions

3.1.4.1 Experimental details:

In this final study, four wheat genotypes, two sensitive and two resistant (as mentioned in Experiment No. 2) were used. This study was carried out simultaneously at two sites, one having non-saline and non-sodic soil (ISES Farm, UAF; the experimental plot size was 3m x 1.5m) and the other having a saline sodic soil (Proka Farm, UAF; the experimental plot size was 3m x 1.5m) (Table 3.2). The two sites are situated about 15 kilometres apart and fall under semi-arid climatic conditions. Each genotype had four replications at each site and the experiment was arranged following a randomized complete block design (RCBD). Plants were grown in the field up to maturity using recommended doses of fertilizers (NPK at the rate of 120:60:60 kg ha^{-1}) and following the recommended agronomic practices (field preparation, weeding and irrigation). The experiment was planted on 05.11.11 and 06.11.11 at Proka Farm and ISES Farm, respectively and continued until maturity (for about 165 days). Different parameters regarding soil and irrigation water quality were recorded before and after the crop harvest. The crop was harvested at maturity and data regarding grain and straw yields, and different yield components were recorded. At booting stage one leaf next to the flag leaf was detached, rinsed in distilled water and used for ionic analysis.

3.1.4.2 Measurements recorded in this experiment:

Following parameters were recorded/ determined manually at maturity in this study:

1. Grain and straw yields
2. Plant height
3. Spike length
4. Tillers per meter strip

The plants were harvested manually and their height was recorded with a meter rod. The number of tillers per meter strip was also recorded manually. These plants were separated into spikes and straw. Straw weight was recorded using a weighing balance. Spike length was measured with a measuring scale. Spikes were threshed manually and grains were collected and weighed using a weighing balance.

Table 3.2.1 The characteristics of soil and water used for field study

Soil characteristics

Soil Characteristics	**Normal Soil**	**Salt-affected Soil**
Texture	Sandy clay loam	Sandy clay loam
Particle size analysis	Sand:54%; Silt:23%; Clay:23%	Sand:57%; Silt:22%; Clay:21%
Saturation percentage (%)	30	29
pH	7.8	8.68
EC (dS m^{-1})	2.6	14.7
Ca + Mg (mmol L^{-1})	4.74	14.97
Na (mmol L^{-1})	17.85	137.39
SAR (mmol $L^{-1})^{1/2}$	8.2	35.5
Organic matter (%)	0.5	0.65
Nitrogen (mg kg^{-1})	397	363
Phosphorous (mg kg^{-1})	7.1	6.5
Potassium (mg kg^{-1})	117	103

3.2.2 Irrigation water characteristics

Characteristics	**Used on normal Soil**	**Used on salt-affected Soil**
EC (dS m^{-1})	1.34	2.55
TSS (mmol L^{-1})	13.4	25.5
SAR (mmol $L^{-1})^{1/2}$	7.43	11.2
RSC (mmol L^{-1})	Nil	Nil

3.2 SOIL ANALYSIS

3.2.1 Mechanical analysis

Mechanical analysis was carried out by Bouyoucos hydrometer method and international textural triangle was used for designating the textural class.

3.2.2 pH of saturated soil paste

Saturated paste of soil was prepared and its pH value was measured with the pH (HI 9811-5, HANNA) meter following USDA Hand Book 60.

3.2.3 Electrical conductivity (EC) of saturation extract

Saturated paste of soil was prepared and its extract was taken following USDA Hand Book 60. Electrical conductivity of saturated soil extract was measured on EC meter using 0.01N KCl for standardization.

3.2.4 Sodium and potassium

Na^+ and K^+ in the saturated soil extract were determined using Sherwood 410 Flame Photometer with the help of self prepared standard solutions using reagent grade salts of NaCl and KCl. These standards were used to develop a standard curve. The soil extract samples were diluted as required and run on the instrument. The ionic concentrations in these samples were determined using the standard curve.

3.3 PLANT ANALYSIS

3.3.1 Plant growth measurements

At harvesting the plants were removed from the thermopole sheets, washed with distilled water and separated into roots and shoots. Shoot and root lengths were determined using a meter rod. Root length refers to the length of the longest root. The fresh weight of shoots and roots was recorded after blotting where as their dry weights were recorded after drying at 70°C to a constant weight in an oven. The details of ionic analysis is given in section 3.3.1

3.3.2 Sodium, potassium and calcium

The shoot/ leaf samples were oven dried at 65°C for 72 hours and the dry weight was recorded. The ashing of dried samples was done in muffle furnace at 550°C. The ashed samples were digested in 2.5 ml, 5 M HNO_3. After digestion volume was topped up to 50 ml with distilled water and used for ionic analysis. Na^+ and K^+ in plant samples were determined by Sherwood 410 Flame Photometer with the help of self prepared standard solutions using reagent grade salts of NaCl and KCl. Calcium in the plant samples was determined by Atomic Absorption Spectrophotometer with the help of self prepared standard solutions using reagent grade salt of $CaCl_2$.

3.3.3 Gas exchange characteristics

Photosynthetic attributes were determined before harvest in the fourth week of treatment application. Data of net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate were recorded on second fully expanded leaf from top of each plant using an open system LCA-4 ADC Portable Infrared Gas Analyzer (Analytical Development Company, Hoddesdon, England). The leaf was placed in the leaf chamber of the instrument for few minutes and data were recorded. The data were recorded from 9 to 11 am with the following specification adjustments; molar flow of air per unit leaf area: 403.3 mmol m^{-2} S^{-1}, water vapor pressure in chamber ranged from 6.0 to 8.9 m bar, atmospheric pressure: 99.9 kPa, photosynthetically active radiation at leaf surface was up to 1711 umol m^{-2} S^{-1}, temperature of leaf ranged from 28.4 to 32.7 °C, ambient temperature ranged from 22.7 to 29.9 °C, ambient CO_2 concentration was 352 µmol mol^{-1}.

3.3.4 Leaf water potential

The third leaf from top (fully expanded youngest leaf) was excised to determine the leaf water potential with a Scholander Type Pressure Chamber (Arimad-2-2, Japan).

3.3.5 Leaf osmotic potential

The same leaf used for water potential was frozen at -10 °C thawed and cell sap was extracted with the help of a disposable syringe. The sap so extracted was directly used for the determination of osmotic potential using an osmometer (Wescor-5500).

3.3.6 Turgor potential

Turgor potential was calculated as the difference between osmotic potential and water potential values as given below:

$$\Psi p = \Psi w - \Psi s$$

3.3.7 Superoxide dismutase (SOD) activity

SOD activity was determined by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) using the method as described by Giannopolitis and Ries (1977). The reaction mixture (3 ml) contained 50 µM NBT, 13 mM methionine, 1.3 µM riboflavin, 50 mM phosphate buffer (pH 7.8), 75 nM EDTA and 20 to 50 µl of enzyme extract. The test tubes containing the reaction solution were irradiated under light (15 fluorescent lamps) at 78 µmol m^{-2} s^{-1} for 15 min. The absorbance of the irradiated solution was recorded on UV-VIS-spectrophotometer at 560 nm. One unit of SOD activity was defined as the amount of enzyme required for 50% inhibition of nitroblue tetrazolium (NBT) reduction.

3.3.8 Catalase (CAT) and Peroxidase (POD) activities

For the determination of activities of peroxidase and catalase the method of Chance and Maehly (1955) was used with some modification. The reaction mixture (3 ml) contained 5.9 mM H_2O_2, 50 mM phosphate buffer (pH 7.0) and 0.1 ml enzyme extract. The reaction was initiated by addition of the enzyme extract. The changes in absorbance of the reaction mixture were recorded after every 20 seconds at 240 nm. One unit enzyme activity was defined as change in absorbance of 0.01 units per minute. The POD reaction mixture contained (3 ml), 20 mM guaiacol, 50 mM phosphate buffer (pH 5.0), 40 mM H_2O_2 and 0.1 ml enzyme extract. The changes in absorbance of the reaction mixture were recorded every 20 s at 470 nm. One unit of POD activity was defined as an absorbance change of 0.01 units per min. Activity of each enzyme was expressed on protein basis and protein content of the extract was determined by the method of Bradford (1976) using bovine serum albumin as standard.

3.4 Statistical analysis

The collected data in different experiments were subjected to analysis of variance (ANOVA) following the methods given by Steel *et al*. (1997). The significance of the differences among the genotypes and treatments was analyzed using the least significant difference (LSD) test.

CHAPTER-IV

RESULTS AND DISCUSSION

4.1 Experiment-1: Screening of different wheat genotypes against salinity and low calcium

4.1.1 BRIEF INTRODUCTION TO THIS STUDY

Salinity is an important agricultural problem affecting plant growth through osmotic effect, ion toxicity and ion imbalance (Munns and Tester, 2008). In saline sodic soil conditions Ca^{2+} concentration and availability is low (Fowler and Hamm, 1980; Naidu *et al*., 1995) therefore the plants usually face calcium deficiency in addition to sodium toxicity under these conditions. The Ca^{2+} deficiency induced by sodium has been reported in many plants including cereals (Adcock *et al*. 2001; Cramer 2002). The sodium present in high concentrations under saline conditions displaces membrane-associated calcium (Ca^{2+}) (Cramer *et al*., 1985; Kinraide, 1999) leading to Ca^{2+} deficiency in the plants.

Reclamation of saline sodic soils is technically possible but not feasible in many cases because of decreasing availability of good quality water. This necessitates the exploitation of genetic variation among the crop species and genotypes for tolerance to salt-affected soil conditions. Screening against salinity is very common however, with little success in the field that may be due to lack of consideration of low calcium under saline sodic soil conditions. There may be genetic variation among the wheat genotypes for acquisition and utilization of Ca^{2+} under non-saline and saline conditions. A wheat genotype efficient for Ca^{2+} uptake and utilization under saline conditions may be better able to withstand saline and sodic conditions in the field. Very little information is available on wheat response to salinity and low Ca^{2+} as screening of wheat genotypes has usually been done against salinity alone. The present study has been designed to evaluate the performance of different wheat genotypes against salinity and low calcium.

4.1.2 BRIEF METHODOLOGY OF THIS STUDY

Ten genotypes of wheat (*Triticum aestivum* L.) were collected from Dept. of Plant Breeding and Genetics, and the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. The genotypes included 25-SAWSN-8, 25-SAWSN-12, 25-SAWSN-25, 25-SAWSN-31, 25-SAWSN-35, 25-SAWSN-39, 25-SAWSN-42, 25-SAWSN-47, SARC-7 and SARC-1. The experiment was carried out in a green-house at the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. The healthy seeds of ten wheat genotypes were sown in trays each containing 5 cm layer of washed sand. These sown seeds were kept moistened with water and with nutrient solution after seedling emergence. At the two leaf stage, seedlings were transplanted in foam plugged holes in polystyrene sheets floating over nutrient solution in 100 litre tubs (1m x 0.5 m x 0.25m). In the low Ca^{2+} treatment tubs the level of calcium was kept at $1/4^{th}$ of the normal level. After two days of transplanting salinity (125 mM NaCl) was developed in three increments (one per day) in the salinity treatment tubs of different calcium levels whereas no salt was added in control. The solution pH was adjusted at 5.5±1 with diluted NaOH or HCl and the solution was changed weekly during the period of study. Plants were harvested after 4 week growth in the treatment solutions and the data regarding root and shoot lengths, root and shoot fresh weights were recorded. The ion concentrations (Na^+, K^+, Ca^{2+} and Cl^-) of the shoot were determined. Data were analyzed statistically and genotypic resistance to salinity and low Ca^{2+} was determined based on relative shoot weight. A detailed methodology of the procedures used has been given in Chapter-3.

4.1.3 RESULTS

4.1.3.1 Shoot fresh weight

The analysis of variance indicated significant differences among treatments and genotypes and there was a significant genotype x treatment interaction (Table 4.1.1). On overall mean basis the shoot fresh weight (SFW) was decreased significantly by the stress treatments

Table 4.1.1 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on shoot fresh weight (g plant^{-1}) of different wheat genotypes. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl). Calcium has been added as $Ca(NO_3)_2$ and $CaCl_2$.

Genotypes	Treatments			
	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	10.4	7.50	5.55	2.75
25-SAWSN-31	10.7	7.85	6.53	4.05
25-SAWSN-25	10.0	7.0	5.43	2.95
25-SAWSN-12	10.3	7.25	6.48	2.92
25-SAWSN-35	10.2	6.22	4.68	2.18
25-SAWSN-39	10.6	8.78	6.78	4.28
25-SAWSN-42	10.5	8.53	6.5	4.03
25-SAWSN-47	10.0	6.44	4.44	1.94
SARC-7	10.5	7.32	5.32	2.99
SARC-1	10.2	7.98	5.73	3.52
Mean	10.4	7.49	5.74	3.16

Values are mean of four replications. LSD: 0.63; $P \leq 0.05$.

in the following trend i.e. low calcium < saline < low calcium + saline. In low calcium treatment (Ca^{2+} conc. 1/4th of the control treatment) percent decrease in SFW as compared to control was 28%, in saline treatment (125 mM NaCl) it was 45 % and in combined treatment (125 mM NaCl + low calcium) it was 69%. The genotypes also differed significantly in different stress treatments. The comparison of genotypes in different treatments showed that in low calcium treatment the maximum SFW was produced by 25-SAWSN-39 and it did not differ significantly with 25-SAWSN-12, 25-SAWSN-42 and SARC-1. The minimum SFW was observed in 25-SAWSN-35 which was statistically at par with the genotype 25-SAWSN-25, 25-SAWSN-31. In saline treatment (125 mM NaCl), 25-SAWSN-39, 25-SAWSN-12, 25-SAWSN-31, 25-SAWSN-42 and SARC-1 were statistically similar and performed better than 25-SAWSN-35 and 25-SAWSN-47. The minimum SFW in the salinity treatment was found in 25-SAWSN-47. In the combined treatment (125 mM NaCl + low calcium) the maximum SFW was observed in 25-SAWSN-39 and it was statistically similar to 25-SAWSN-12, 25-SAWSN-42 and SARC-1 whereas the minimum SFW was found in 25-SAWSN-47 and it did not differ significantly with 25-SAWSN-31 which was among the salt-resistant genotypes under salinity alone treatment.

4.1.3.2 Shoot dry weight

The analysis of variance indicated significant differences among treatments and genotypes for shoot dry weight (SDW) production (Table 4.1.2). There was also a significant genotype x treatment interaction for this parameter. On overall mean basis the shoot dry weight was the maximum in control followed by low calcium (Ca^{2+} conc. 1/4th of the control treatment), salinity (125 mM NaCl) and salinity + low calcium treatments, respectively. All of the stress treatments significantly decreased the shoot dry weight with the minimum SDW observed in the low calcium + salinity treatment. The maximum SDW in the low calcium treatment was observed for the genotype 25-SAWSN-39 which did not differ significantly from the genotypes 25-SAWSN-25, 25-SAWSN-42 and SARC-1. The minimum SDW in this treatment was found in 25-SAWSN-35 and 25-SAWSN-47 and these genotypes did not differ significantly. In saline treatment (125 mM NaCl), 25-SAWSN-39 produced the maximum SDW which was statistically similar to the SDW of 25-SAWSN-12, 25-SAWSN-31, 25-SAWSN-42 and SARC-1. In the interactive treatment (125 mM NaCl + calcium deficient) the maximum SDW was observed in

Table 4.1.2 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on shoot dry weight (g plant^{-1}) of different wheat genotypes. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

Genotypes	Treatments			
	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	1.15	0.95	0.68	0.41
25-SAWSN-31	1.17	0.99	0.82	0.54
25-SAWSN-25	1.10	1.00	0.70	0.47
25-SAWSN-12	1.15	0.95	0.81	0.45
25-SAWSN-35	1.16	0.79	0.62	0.35
25-SAWSN-39	1.19	1.08	0.84	0.58
25-SAWSN-42	1.14	1.04	0.80	0.53
25-SAWSN-47	1.11	0.81	0.59	0.32
SARC-7	1.16	0.91	0.67	0.41
SARC-1	1.14	1.01	0.77	0.51
Mean	1.15	0.95	0.73	0.46

Values are mean of four replications. LSD: 0.044; $P \leq 0.05$.

25-SAWSN-39 and it did not differ significantly with 25-SAWSN-12, 25-SAWSN-42 and SARC-1. The minimum SDW in saline and saline + low calcium treatments was found in 25-SAWSN-35 and 25-SAWSN-47 and these genotypes did not differ significantly.

4.1.3.3 Root fresh weight

The analysis of variance indicated significant differences among treatments and genotypes for root fresh weight (RFW) production (Table 4.1.3). The interaction between genotypes and treatments was also significant. On overall mean basis RFW was the maximum in control. In all of the other treatments it was significantly decreased in the following trend: low calcium < saline < saline + low calcium. In low calcium treatment the average percent decrease in RFW as compared to control was 25%, in saline treatment it was 45% whereas in interactive treatment (low calcium + 125 mM NaCl) it was 71%. The comparison of genotypes in each treatment showed that in low calcium treatment the maximum RFW was produced by 25-SAWSN-39 and it did not differ significantly with 25-SAWSN-12, 25-SAWSN-25, 25-SAWSN-42 and SARC-1. The minimum RFW in this treatment was found in 25-SAWSN-35 followed by 25-SAWSN-47. In saline treatment (125 mM NaCl) the minimum RFW was produced by 25-SAWSN-35 and it was statistically at par with 25-SAWSN-47 and SARC-7. The genotype 25-SAWSN-39 produced the maximum RFW in saline treatment and differed significantly only from the genotypes producing the minimum RFW. In the treatment 125 mM NaCl + low calcium 25-SAWSN-39, 25-SAWSN-12, 25-SAWSN-42 and SARC-1 were statistically similar and produced better RFW than the rest of the genotypes.

4.1.3.4 Root dry weight

The treatments and genotypes have significant effect on the root dry weight (RDW) production with a significant interaction between these two factors (Table 4.1.4). The mean RDW was the maximum in control. In all of the stress treatments it was decreased significantly with the maximum reduction in the case of low calcium + saline treatment followed by saline and low calcium treatments, respectively. In the low calcium treatment percent decrease in RDW as compared to control was 25%, in saline treatment it was 45% and in interactive treatment (125

Table 4.1.3 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on root fresh weight (g plant^{-1}) of different wheat genotypes. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	5.17	3.43	2.65	1.07
25-SAWSN-31	5.25	3.65	3.05	1.67
25-SAWSN-25	4.94	3.69	2.72	1.14
25-SAWSN-12	5.16	3.30	2.90	1.05
25-SAWSN-35	5.32	3.10	2.20	1.03
25-SAWSN-39	5.13	3.97	3.03	1.72
25-SAWSN-42	4.92	3.89	2.83	1.58
25-SAWSN-47	4.90	3.31	2.04	1.04
SARC-7	4.99	3.41	2.31	1.05
SARC-1	4.86	3.71	2.67	1.43
Mean	5.30	3.55	2.64	1.28

Values are mean of four replications. LSD value at $P \leq 0.05$ is 0.21.

Table 4.1.4 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on root dry weight (g plant^{-1}) of different wheat genotypes.
(Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	0.37	0.25	0.18	0.08
25-SAWSN-31	0.38	0.28	0.22	0.12
25-SAWSN-25	0.36	0.27	0.20	0.10
25-SAWSN-12	0.35	0.23	0.21	0.08
25-SAWSN-35	0.34	0.22	0.16	0.07
25-SAWSN-39	0.37	0.28	0.22	0.14
25-SAWSN-42	0.33	0.27	0.20	0.13
25-SAWSN-47	0.35	0.21	0.15	0.08
SARC-7	0.36	0.22	0.15	0.09
SARC-1	0.33	0.25	0.19	0.10
Mean	0.35	0.25	0.19	0.10

Values are mean of four replications. LSD value at $P \leq 0.05$ is 0.02.

mM NaCl + low calcium) it was 71%. The comparison of genotypes in each treatment showed that in low calcium treatment the maximum RDW was produced by 25-SAWSN-39 and it was statistically at par with 25-SAWSN-8, 25-SAWSN-12, 25-SAWSN-25, 25-SAWSN-42 and SARC-1. The minimum RDW in this treatment was found in 25-SAWSN-35. In saline treatment (125 mM NaCl), 25-SAWSN-39 performed better than 25-SAWSN-8, 25-SAWSN-35, 25-SAWSN-47, and SARC-7 but was statistically at par with the rest of the genotypes. In the interactive treatment (125 mM NaCl + calcium deficient), the maximum RDW was observed in 25-SAWSN-39 and it was statistically at par with 25-SAWSN-12, 25-SAWSN-25, 25-SAWSN-42 and SARC-1. In this treatment the rest of the genotypes were statistically at par with25-SAWSN-35 producing the minimum root dry weight.

4.1.3.5 Shoot length (cm)

There was a significant treatment effect on shoot length (SL). However, there were no genotype effect and there was no interaction between the treatments and genotypes for this growth parameter (Table 4.1.5). On overall mean basis SL was the maximum in control. In all other treatments it decreased in the order low calcium < saline < low calcium + saline. In low calcium treatment percent decrease in SL as compared to control was 17 %, in saline treatment (125 mM NaCl) it was 43% whereas in interactive treatment (125 mM NaCl + low calcium) it was 59%.

4.1.3.6 Root length (cm)

The analysis of variance indicated significant differences among the treatments for root length but the effect of genotype as well as interaction between genotypes and treatments was non-significant (Table 4.1.6). On overall mean basis the maximum root length was observed in control. In all of the other treatments it was significantly decreased with the highest reduction in low calcium + saline treatment followed by salinity and low calcium treatments, respectively. In low calcium treatment, the percent decrease in root length as compared to control was 18%, in saline treatment it was 58% whereas in interactive treatment (125 mM NaCl + low calcium) it was 83%.

Table 4.1.5 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on shoot length (cm) of different wheat genotypes. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	47.2	39.4	26.5	19.6
25-SAWSN-31	47.9	41.4	29.6	21.8
25-SAWSN-25	46.8	38.9	26.9	18.9
25-SAWSN-12	46.3	39.6	26.6	19.0
25-SAWSN-35	44.8	36.5	24.8	17.1
25-SAWSN-39	47.0	40.0	27.6	21.0
25-SAWSN-42	46.5	38.7	25.8	18.7
25-SAWSN-47	45.5	35.9	24.6	16.6
SARC-7	46.4	37.1	25.9	17.5
SARC-1	46.6	37.6	27.2	20.0
Mean	46.5	38.5	26.5	19.0

Values are mean of four replications. LSD value at $P \leq 0.05$ is 2.05.

Table 4.1.6 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on root length (cm) of different wheat genotypes. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	31.1	25.7	14.4	7.31
25-SAWSN-31	32.3	26.9	16.8	9.32
25-SAWSN-25	29.3	24.9	14.8	7.56
25-SAWSN-12	30.4	25.6	15.2	7.21
25-SAWSN-35	31.3	23.9	13.3	5.54
25-SAWSN-39	32.5	26.8	16.6	9.69
25-SAWSN-42	30.0	25.5	15.1	7.78
25-SAWSN-47	28.8	23.0	13.1	5.34
SARC-7	30.7	25.1	14.4	6.78
SARC-1	28.9	24.3	15.6	6.44
Mean	30.5	25.2	14.9	7.30

Values are mean of four replications. LSD value at $P \leq 0.05$ is 2.1.

4.1.3.7 Leaf sodium concentration

Significant differences were observed among treatments as well as genotypes regarding leaf sodium concentration (Table 4.1.7). Similarly, the interaction between genotypes and treatments was also significant. Salinity significantly increased the leaf Na^+ concentration. On overall mean basis, in saline treatment (125 mM NaCl) Na^+ concentration was 1.08 mmol g^{-1} dw whereas in interactive treatment (125 mM NaCl + low calcium) it was 1.59 mmol g^{-1} dw which was significantly higher than the leaf Na^+ concentration in the saline treatment. The low calcium treatment under non-saline conditions did not affect the leaf Na^+ concentration significantly. The comparison of genotypes in saline treatment showed that the maximum leaf Na^+ concentration was found in 25-SAWSN-35 (1.29 mmol g^{-1} dw) and SAWSN-47 and they did not differ significantly. In saline treatment, 25-SAWSN-39 and 25-SAWSN-12 accumulated statistically similar and significantly lower leaf Na^+ concentration than the other genotypes. In the interactive treatment (125 mM NaCl + low calcium) the maximum leaf Na^+ concentration was found in 25-SAWSN-47 followed by 25-SAWSN-35 whereas the minimum leaf Na^+ concentration was found in 25-SAWSN-12 followed by 25-SAWSN-39 and 25-SAWSN-42.

4.1.3.8 Leaf potassium concentration

Leaf potassium concentration has been significantly affected by different treatments and genotypes (Table 4.1.8). There was also a significant interaction between genotypes and treatments. On overall mean basis, the minimum leaf K^+ concentration was found in combined treatment (125 mM NaCl + low calcium) followed by salinity alone (125 mM NaCl). The low calcium treatment did not affect the leaf K^+ concentration significantly and the genotypes did not differ significantly in control or low calcium treatments. In saline treatment, 25-SAWSN-35 and 25-SAWSN-47 accumulated statistically similar leaf K^+ concentration which was lower than the other genotypes. On the other hand the maximum leaf K^+ concentration in this treatment was found in 25-SAWSN-39 followed by 25-SAWSN-12. In the interactive treatment (125 mM NaCl + low calcium) the maximum leaf K^+ concentration was found in 25-SAWSN-12 followed by

Table 4.1.7 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on leaf sodium concentration (mmol g^{-1} dry wt.) of different wheat genotypes. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	0.18	0.26	1.15	1.57
25-SAWSN-31	0.17	0.23	0.82	1.39
25-SAWSN-25	0.18	0.26	1.13	1.65
25-SAWSN-12	0.18	0.26	1.01	1.69
25-SAWSN-35	0.18	0.28	1.29	1.80
25-SAWSN-39	0.16	0.23	0.86	1.44
25-SAWSN-42	0.17	0.24	1.03	1.52
25-SAWSN-47	0.17	0.28	1.25	1.83
SARC-7	0.18	0.26	1.15	1.56
SARC-1	0.18	0.26	1.07	1.48
Mean	0.18	0.26	1.08	1.59

Values are mean of four replications. LSD value at $P \leq 0.05$ is 0.053.

Table 4.1.8 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on leaf potassium concentration (mmol g^{-1} dry wt.) of different wheat genotypes. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	1.85	1.93	1.13	0.78
25-SAWSN-31	1.82	1.94	1.29	0.99
25-SAWSN-25	1.89	1.88	1.05	0.76
25-SAWSN-12	1.91	1.90	1.08	0.69
25-SAWSN-35	1.84	1.85	0.82	0.55
25-SAWSN-39	1.78	1.86	1.35	0.96
25-SAWSN-42	1.81	1.90	1.27	0.86
25-SAWSN-47	1.79	1.87	0.92	0.58
SARC-7	1.89	1.94	1.07	0.67
SARC-1	1.92	1.98	1.14	0.81
Mean	1.85	1.91	1.11	0.76

Values are mean of four replications. LSD value at $P \leq 0.05$ is 0.057.

25-SAWSN-39 whereas the minimum leaf K^+ concentration was found in 25-SAWSN-35 followed by 25-SAWSN-47.

4.1.3.9 Leaf calcium concentration

Shoot calcium concentration was decreased significantly with the application of low calcium and salinity. Significant differences were observed among the treatments as well as the genotypes and there was a significant genotype x treatment interaction (Table 4.1.9). On overall mean basis, in interactive treatment the minimum calcium concentration (0.14 mmol g^{-1} dw) was found as compared to the other treatments. In low calcium treatment, it was 0.29 mmol g^{-1} dw whereas in saline treatment it was 0.26 mmol g^{-1} dw. The comparison of genotypes in each treatment showed that in low calcium treatment the maximum Ca^{2+} concentration was found in 25-SAWSN-12 which was statistically at par with 25-SAWSN-8, 25-SAWSN-39, 25-SAWSN-42 and SARC-1. The minimum Ca^{2+} concentration was found in 25-SAWSN-47 and it differed significantly from the genotypes mentioned in the previous sentence. In saline treatment, 25-SAWSN-47 accumulated the minimum Ca^{2+} in its leaves and did not differ significantly from all of the other genotypes except 25-SAWSN-12, 25-SAWSN-39, 25-SAWSN-42 and SARC-1 which accumulated higher leaf Ca^{2+} concentration. A similar genotypic trend was observed in the interactive treatment where low calcium was combined with 100 mM NaCl salinity.

4.1.3.10 Leaf chloride concentration

Leaf chloride concentration has been significantly affected by the treatments and genotypes with a significant interaction between the treatments and genotypes (Table 4.1.10). Salinity (125 mM NaCl) alone and in combination with low calcium significantly increased leaf Cl^- concentration. The average leaf Cl^- concentration was 1.42 mmol g^{-1} dw in the saline treatment (125 mM NaCl) and 1.95 mmol g^{-1} dw in combined treatment (125 mM NaCl + low calcium). The genotypes did not differ significantly in the control or low calcium treatment. In saline treatment the genotype SARC-1 accumulated the minimum leaf Cl^- concentration followed by 25-SAWSN-39 where as 25-SAWSN-35 accumulated the highest leaf Cl- concentration and was statistically at par with 25-SAWSN-47.

Table 4.1.9 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on leaf calcium concentration (mmol g^{-1} dry wt.) of different wheat genotypes. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	0.48	0.30	0.25	0.14
25-SAWSN-31	0.47	0.34	0.33	0.20
25-SAWSN-25	0.49	0.29	0.26	0.12
25-SAWSN-12	0.50	0.27	0.24	0.12
25-SAWSN-35	0.48	0.26	0.22	0.10
25-SAWSN-39	0.47	0.33	0.32	0.18
25-SAWSN-42	0.47	0.34	0.30	0.20
25-SAWSN-47	0.49	0.25	0.21	0.11
SARC-7	0.50	0.27	0.24	0.12
SARC-1	0.51	0.31	0.28	0.17
Mean	0.48	0.29	0.26	0.14

Values are mean of four replications. LSD value at $P \leq 0.05$ is 0.047.

Table 4.1.10 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on leaf chloride concentration (mmol g^{-1} dry wt.) of different wheat genotypes. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	0.39	0.30	1.63	1.99
25-SAWSN-31	0.40	0.27	1.29	1.81
25-SAWSN-25	0.41	0.31	1.53	1.88
25-SAWSN-12	0.45	0.33	1.26	2.19
25-SAWSN-35	0.50	0.38	1.87	2.30
25-SAWSN-39	0.42	0.26	1.09	1.64
25-SAWSN-42	0.44	0.36	1.25	1.83
25-SAWSN-47	0.52	0.39	1.75	2.20
SARC-7	0.45	0.32	1.48	1.99
SARC-1	0.45	0.29	1.04	1.69
Mean	0.44	0.32	1.42	1.95

Values are mean of four replications. LSD value at $P \leq 0.05$ is 0.095.

In the interactive treatment (125 mM NaCl + low calcium) the maximum leaf Cl^- concentration was found in 25-SAWSN-35 that did not differ significantly from 25-SAWSN-31 and 25-SAWSN-47. The minimum leaf Cl^- concentration in this treatment was found in 25-SAWSN-39 which was statistically similar to 25-SAWSN-42 and SARC-1.

4.1.4 DISCUSSION

All of the physical growth parameters like shoot length, root length, shoot and root fresh and dry weights were decreased significantly due to salinity and low calcium alone as well as under the combined stress. Calcium is required for cell wall and membrane integrity and a decrease in calcium availability reduces plant growth. A decrease in Ca^{2+} concentration of saline solution may further reduce plant growth over salinity alone. It might be due to removal of Ca^{2+} ions from the cell plasma lemma and internal pool (Cramer *et al*., 1987; Epstein and Lauchli, 1991). All of these parameters were decreased more by a combined stress of low calcium and salinity followed by salinity alone and low calcium alone, respectively.

The concentration, of Na^+ and Cl^- were increased with the application of salinity and this increase was the maximum in the combined treatment of low calcium and salinity. On the other hand the concentrations of Ca^{2+} and K^+ were decreased with salinity and low calcium. High sodium concentration in saline solution competes with Ca^{2+} and K^+ for uptake at root level and reduces the uptake of these ions where by the uptake and accumulation of Na^+ is increased. External calcium supply alters the selectivity of cation channels facilitating the uptake of potassium, which is one of the main competitors of sodium entrance into the roots (Maathuis and Sanders, 2001). However, if there is a deficiency of calcium, then the reverse seems true i.e. non selective cation channels are used for Na^+ hyper accumulation in the cell. At higher concentrations, sodium also displaces calcium associated with membranes (Cramer *et al*. 1985). Calcium deficiency caused by sodium has been observed in many plant species, including cereals (Ehret *et al*. 1990; Cramer 2002). This situation becomes worse with low solution calcium concentration under saline conditions. Under sodic and saline sodic field conditions, calcium is usually very low in soils with a wide range of Na^+: Ca^{2+} ratio in the soil solution (Naidu *et al*. 1995). Therefore these conditions lead to more growth reduction than under salinity alone due to high sodium uptake and reduced calcium and potassium uptake and accumulation.

Genc *et al*. (2007) and Saqib *et al*. 2005) reported that there is a great variability among wheat genotypes for sodium exclusion and tolerance to elevated levels of sodium in the plant cells. The wheat genotypes used in the present study also performed differently in different treatments. In the saline + low calcium stress the genotype 25-SAWSN-39 performed better as compared to the other genotypes where as the performance of 25-SAWSN-35 and 25-SAWSN-47 was the poorest of all of the genotypes. This process has occurred more in the case of 25-SAWSN-35 and 25-SAWSN-47 which accumulated more Na^+ as compared to the other genotypes. As a result of excessive Na^+ and Cl^- uptake their growth was very low as compared to 25-SAWSN-39 which proved to be relatively more tolerant to both salinity and low calcium supply in the surrounding medium. The better growth performance of 25-SAWSN-39 might be due to its better adaptation to both salinity and low calcium. The performance of genotype 25-SAWSN-31 has been very interesting as it performed better than the salt-sensitive genotypes under saline conditions but did not differ significantly from the salt-sensitive genotypes under salinity + low calcium. This shows that it cannot extract calcium efficiently under low calcium conditions which is evident from its calcium concentration in low calcium and low calcium + salinity treatments.

Salinity resistance is the ability of a genotype to grow and/or produce yield in a saline environment. It is generally determined as the relative biomass production or relative yield in saline conditions compared to non-saline conditions. Selection of a salinity resistant wheat genotype has always been very tricky and difficult. Grain yield production is the basis of salinity resistance of a genotype under salt affected field conditions however; it is very difficult and costly to do it for a large number of genotypes. After a detailed study on screening of wheat for salinity Qureshi *et al*. (1990) proposed that the relative shoot fresh weight of a wheat genotype under salinity as compared with the non-saline conditions gives a good correlation with grain yield performance of that genotype under salt-affected conditions. Therefore, following Qureshi *et al*. (1990) the relative shoot fresh weight (Table 4.1.11) of a genotype under stress conditions has been used as a selection criterion in this study. Based on this selection criterion wheat genotypes 25-SAWSN-39 has been selected as wheat genotype resistant to salinity and salinity + low calcium and 25-SAWSN-35 and 25-SAWSN-47 has been selected as sensitive genotypes to both the conditions. The genotype 25-SAWSN-31 has also been selected as it has been a very

interesting genotype. It performed well under saline conditions in the presence of high calcium but did not perform well under saline conditions in the presence of low calcium.

Table 4.1.11 Relative shoot fresh weight of different wheat genotypes in different treatments. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} ($1/4^{th}$ of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline ($1/4^{th}$ of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

Genotypes	Control	Low Calcium	Salinity	Low Calcium + Salinity
25-SAWSN-8	100	72	53	26
25-SAWSN-12	100	73	61	38
25-SAWSN-25	100	70	54	30
25-SAWSN-31	100	70	63	28
25-SAWSN-35	100	61	46	21
25-SAWSN-39	100	83	64	40
25-SAWSN-42	100	81	62	38
25-SAWSN-47	100	64	44	19
SARC-7	100	70	51	29
SARC-1	100	78	56	34

4.2 Experiment-II: Effect of salinity and low calcium on water relations and photosynthetic parameters of different wheat genotypes

4.2.1 BRIEF INTRODUCTION TO THIS STUDY

This study has been carried out using two salt-sensitive and two salt-resistant wheat genotypes identified from the Experiment No.1. It has been carried out to investigate the water relations and photosynthetic attributes of the selected wheat genotypes in response to salinity and low calcium, alone and in combination.

4.2.2 BRIEF METHODOLOGY OF THIS STUDY

The nursery was grown and at two leaf stage the plants were transplanted to thermopole sheets floating on nutrient solution. Four treatments including control, low calcium, salinity (125 mM NaCl) and salinity + low calcium were applied as detailed in Chapter-3. The experiment was continued for 28 days after the stress imposition. Before the harvest, net photosynthesis rate, stomatal conductance and transpiration rate were determined on the second fully expanded leaf with a portable gas exchange system and SPAD absorbance of leaf were determined using a hand-held SPAD-502 meter. Leaf water potential (Ψ) and osmotic potential ($\Psi\pi$) were measured from the center of the subsequent youngest mature leaf. Turgor pressure (Tp) was determined from $\Psi\pi$ and Ψw. At harvest, fresh and dry weights of roots and shoots were taken along with the lengths of roots and shoots. Flame photometer was used to determine Na^+ and K^+ concentration in leaves and Cl^- was determined by chloride analyzer. Calcium was determined with atomic absorption spectrophotometer.

4.2.3 RESULTS

4.2.3.1 Shoot fresh weight

The shoot fresh weight (SFW) production was significantly affected by different treatments and genotypes. The interaction between genotypes and treatments was also significant (Fig. 4.2.1). On overall treatment mean basis shoot fresh weight (SFW) was the maximum in

control. In all of the other treatments it was decreased in the given trend: low calcium < saline < low calcium + saline. In the low calcium treatment the percent decrease in SFW as compared to control was 41%, in saline treatment it was 55% and in low calcium + saline treatment, it was 81%. The comparison of genotypes in each treatment showed that in low calcium treatment the maximum SFW was produced by 25-SAWN-39 and it was significantly higher than the other genotypes, whereas the minimum SFW was found in 25-SAWN-35 and it did not differ significantly from 25-SAWN-47 and 25-SAWN-31. In saline treatment, 25-SAWN-39 and 25-SAWN-31 produced significantly higher shoot fresh weight than the other two genotypes, 25-SAWN-47 and 25-SAWN-35. In combined treatment (saline + low calcium), the maximum SFW was observed in 25-SAWN-39 and it differed significantly from the other genotypes, whereas the minimum SFW was found in 25-SAWN-47 and it was statistically at par with 25-SAWN-31 and 25-SAWN-35.

4.2.3.2 Shoot dry weight

The treatments and genotypes significantly affected the shoot dry weight (SDW) production of wheat (Fig. 4.2.2). The interaction between genotypes and treatments was also significant as the performance of genotypes differed in different treatments. On overall mean basis SDW was the maximum in control and significantly decreased in the stress treatments with the minimum decrease in the case of low calcium followed by saline and low calcium + saline treatments, respectively. In low calcium treatment percent decrease in SDW as compared to control was 40%, in saline treatment it was 56% whereas in saline + low calcium, it was 72%. The comparison of genotypes in each treatment showed that in low calcium treatment the maximum SDW was produced by 25-SAWN-39 and it was statistically different from the other genotypes where as the minimum SDW was found in 25-SAWN-47 and it was statistically at par with 25-SAWN-31 and 25-SAWN-35. In saline treatment 25-SAWN-39 performed better than the other genotypes except 25-SAWN-31 whereas the minimum SDW was produced by 25-SAWN-47 and it was statistically at par with 25-SAWN-35. In the saline + low calcium treatment, the maximum SDW was observed in 25-SAWN-39 which differed significantly from all of the other genotypes. The minimum SDW in this combined treatment was produced by 25-SAWN-47 and it did not differ significantly from 25-SAWN-31 and 25-SAWN-35.

Fig. 4.2.1 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on shoot fresh weight (g plant^{-1}) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

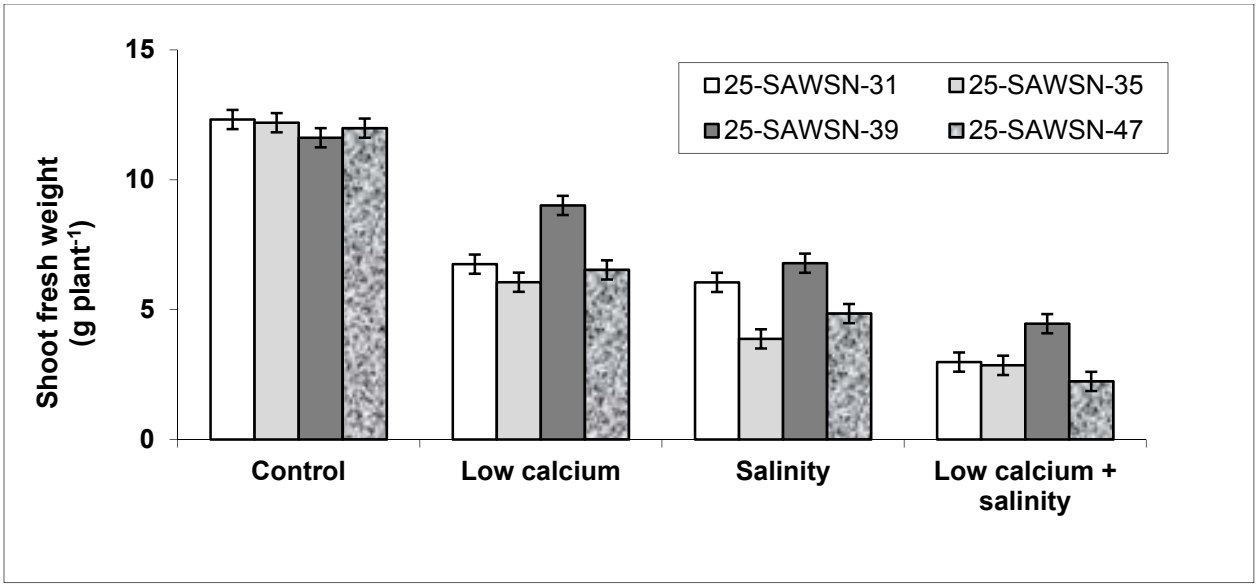
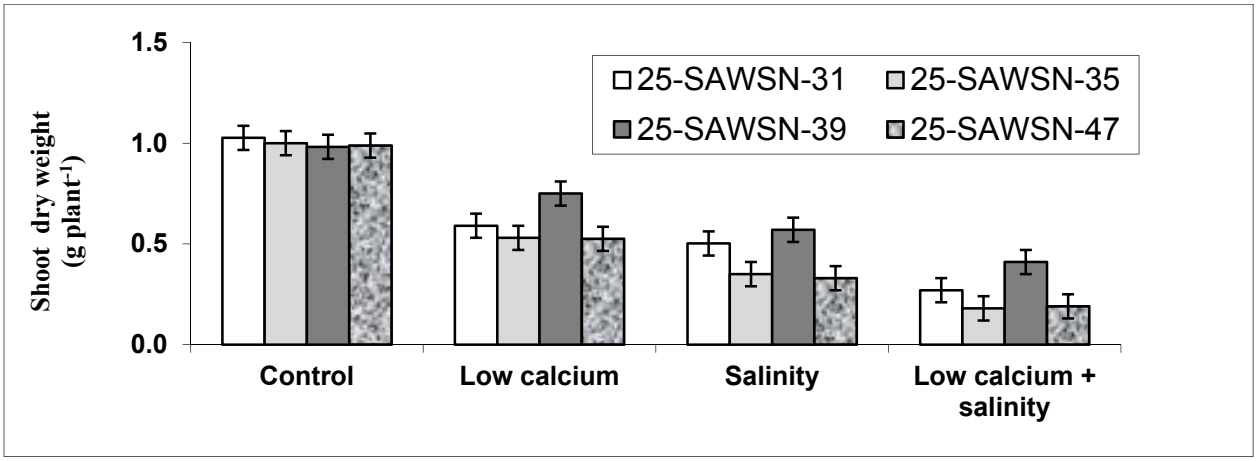


Fig. 4.2.2 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on shoot dry weight (g plant^{-1}) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.



4.2.3.3 Root fresh weight

The treatments and genotypes differed significantly regarding RFW (Fig. 4.2.3). There was also a significant genotype x treatment interaction as the performance of genotypes different in different treatments. On overall mean basis RFW was the highest in control and the application of stress resulted in significant reduction in the RFW of different wheat genotypes. The maximum reduction was found in the case of combined stress treatment (salinity + low calcium). In low calcium treatment percent decrease in RFW as compared to control was 34%, in saline treatment it was 53% where as in saline + low calcium treatment it was 70%. In low calcium treatment the maximum RFW was produced by 25-SAWN-39 and it was significantly higher than the other genotypes, whereas the minimum RFW was found in 25-SAWN-35 and it was at par with 25-SAWN-31 and 25-SAWN-47. In saline treatment, 25-SAWN-39 and 25-SAWN-31 produced more RFW as compared to the other genotypes and did not differ significantly from one another. On the other hand, the performance of 25-SAWN-47 was the poorest and at par with 25-SAWN-35 in salinity alone treatment. In the saline + low calcium treatment, the maximum RFW was observed in 25-SAWN-39 and the minimum RFW was found in 25-SAWN-35 which was statistically at par with 25-SAWN-31 and 25-SAWN-47.

4.2.3.4 Root dry weight

The treatments and genotypes significantly affected the RDW of wheat as shown in Fig. 4.2.4. The interaction between genotypes and treatments was also found to be significant. The maximum average RDW was obtained in control. The application of stress significantly reduced the root dry weight. The maximum reduction was found in the case of combined stress treatment (saline + low calcium) with a percent decrease of 75% as compared to control. In low calcium treatment percent decrease in RDW as compared to control was 40% and in saline treatment it was 53%. In low calcium treatment, maximum RDW was produced by 25-SAWN-39 and it was significantly different from the other genotypes which produced the minimum RDW and did not differ statistically from one another. In saline treatment, 25-SAWN-39 and 25-SAWN-31 did not differ significantly and performed better as compared to the other genotypes. On the other hand, the minimum RDW was found in 25-SAWN-47 and it was statistically at par with 25-SAWN-35. In saline + low calcium treatment, the maximum RDW

Fig. 4.2.3 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on root fresh weight (g plant^{-1}) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.

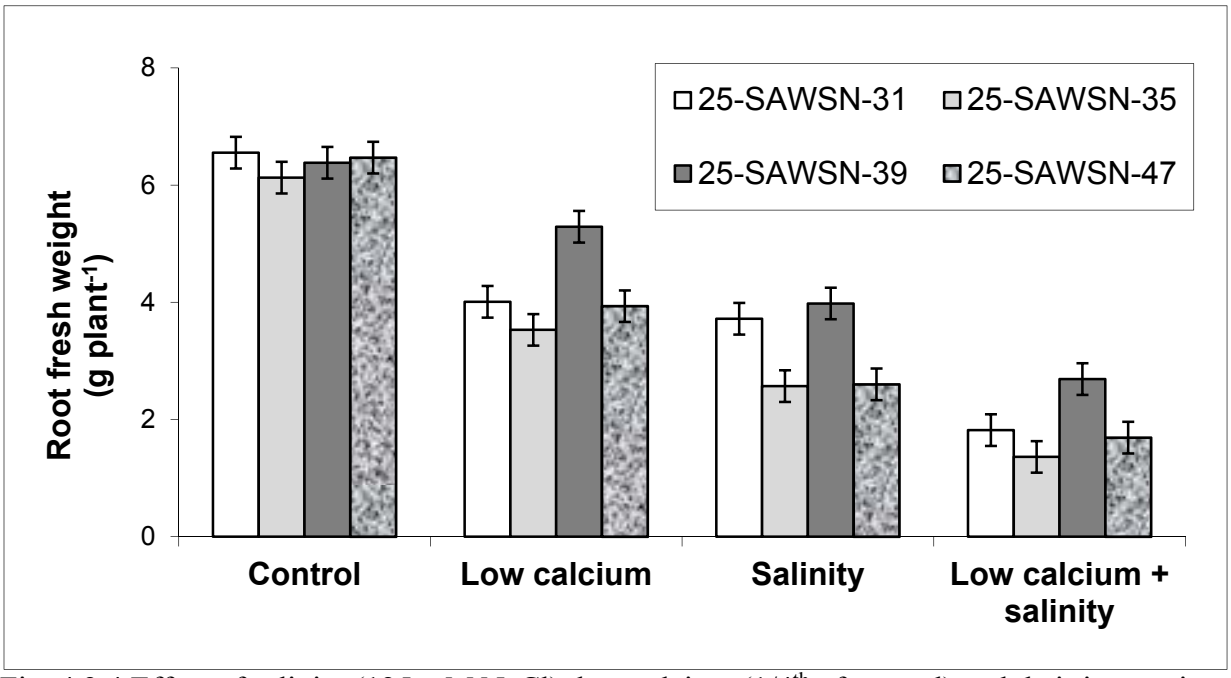
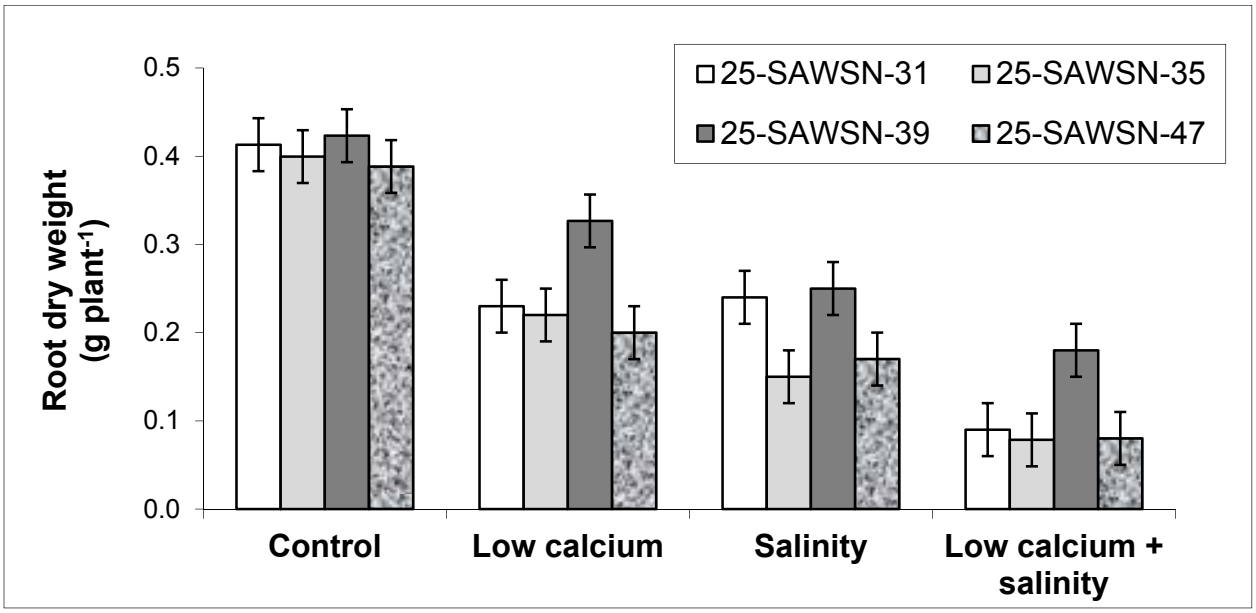


Fig. 4.2.4 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on root dry weight (g plant^{-1}) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.



was observed in 25-SAWN-39 and it was significantly higher than the RFW of 25-SAWN-47 and 25-SAWN-35 and 25-SAWN-31 which were statistically similar.

4.2.3.5 Shoot length

The differences among treatments and genotypes regarding shoot length were significant (Fig. 4.2.5). The interaction between genotypes and treatments was also found significant. On overall treatment mean basis shoot length (SL) was the maximum in control with a significant reduction in all of the other treatments in the following trend: low calcium < saline < low calcium + saline. In low calcium treatment percent decrease in SL as compared to control was 32%, in saline treatment it was 56% whereas in low calcium + saline it was 80%. The comparison of genotypes in each treatment showed that in low calcium treatment the maximum SL was produced by 25-SAWN-39 while the other three genotypes produced significantly lower SL than 25-SAWN-39 and did not differ from one another. In saline treatment, 25-SAWN-39 performed better as compared to other genotypes but was statistically at par with 25-SAWN-31, whereas the minimum SL was found in 25-SAWN-35 that was statistically similar to 25-SAWN-47. In the combined treatment (saline + low calcium), the maximum SL was observed in 25-SAWN-39 which was statistically better than the other genotypes whereas the minimum SL was found in 25-SAWN-47 which did not differ significantly from 25-SAWN-35 and 25-SAWN-31.

4.2.3.6 Root length

The differences among the treatments and genotypes regarding root length (RL) were significant (Fig. 4.2.6). The interaction between genotypes and treatments was also significant. On overall mean basis RL was the maximum in control and was decreased significantly by different stress treatments with the minimum reduction in the case of low calcium alone treatment followed by saline alone and saline + low calcium treatments, respectively. In low calcium treatment percent decrease in RL as compared to control was 32%, in saline treatment it was 67% whereas in saline + low calcium, it was 81%. The comparison of genotypes in each treatment showed that in low calcium treatment, the maximum RL was produced by 25-SAWN-39 whereas the minimum root length was produced by 25-SAWN-35 which was statistically at par with the other genotypes. In saline treatment, 25-SAWN-39 performed better than all of the other genotypes followed by 25-SAWN-31. On the other hand minimum RL was found in 25-SAWN-47 which differed significantly from the other genotypes except 25-SAWN-35. In the

saline + low calcium treatment, the maximum RL was observed in 25-SAWN-39 which was statistically different from other genotypes whereas the minimum RL was produced by 25-SAWN-47 and it differed significantly only from 25-SAWN-39.

4.2.3.7 Number of tillers per plant

The number of tillers per plant decreased significantly with the treatment of low calcium and salinity. Significant differences were also observed among treatments as well as genotypes (Fig. 4.2.7). The interaction between genotypes and treatments was also significant. On overall treatment mean basis, the saline + low calcium treatment, had the lowest number of tillers per plant, compared to other treatments. In low calcium treatment percent decrease in number of tillers per plant as compared to control was 32%, in saline treatment it was 52% whereas in low calcium + saline treatment, it was 77%. In low calcium treatment, maximum number of tillers per plant was found in 25-SAWN-39 where as it was minimum in 25-SAWN-35. In saline treatment, 25-SAWN-39 produced more tillers per plant as compared to the other genotypes but was statistically at par with 25-SAWN-31. On the other hand the lowest number of tillers per plant was found in 25-SAWN-47 and 25-SAWN-35 and both of these were statistically similar. In the saline + low calcium treatment, the maximum number of tillers per plant was found in 25-SAWN-39 whereas rest of the genotypes produced significantly lower number of tillers and did not differ significantly from one another.

4.2.3.8 Leaf SPAD absorbance

SPAD absorbance decreased significantly in the presence of low calcium and salinity. Significant differences were observed among treatments as well as genotypes and there was a significant interaction between genotypes and treatments (Fig. 4.2.8). On overall treatment mean basis, the lowest SPAD absorbance was found in the saline + low calcium as compared to the other treatments. In low calcium treatment the percent decrease in SPAD absorbance as compared to control was 29%, in saline treatment it was 43% whereas in saline + low calcium it was 67%. The comparison of genotypes in each treatment showed that in low calcium treatment the maximum SPAD absorbance was found in 25-SAWN-39 and the minimum was found in 25-SAWN-35 which was statistically similar to 25-SAWN-47 and 25-SAWN-31. In saline treatment, 25-SAWN-47 and 25-SAWN-35 had significantly lower SPAD absorbance than 25-SAWN-31 and 25-SAWN-39. In the saline + low calcium treatment, the maximum

Fig. 4.2.5 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on shoot length (cm) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.

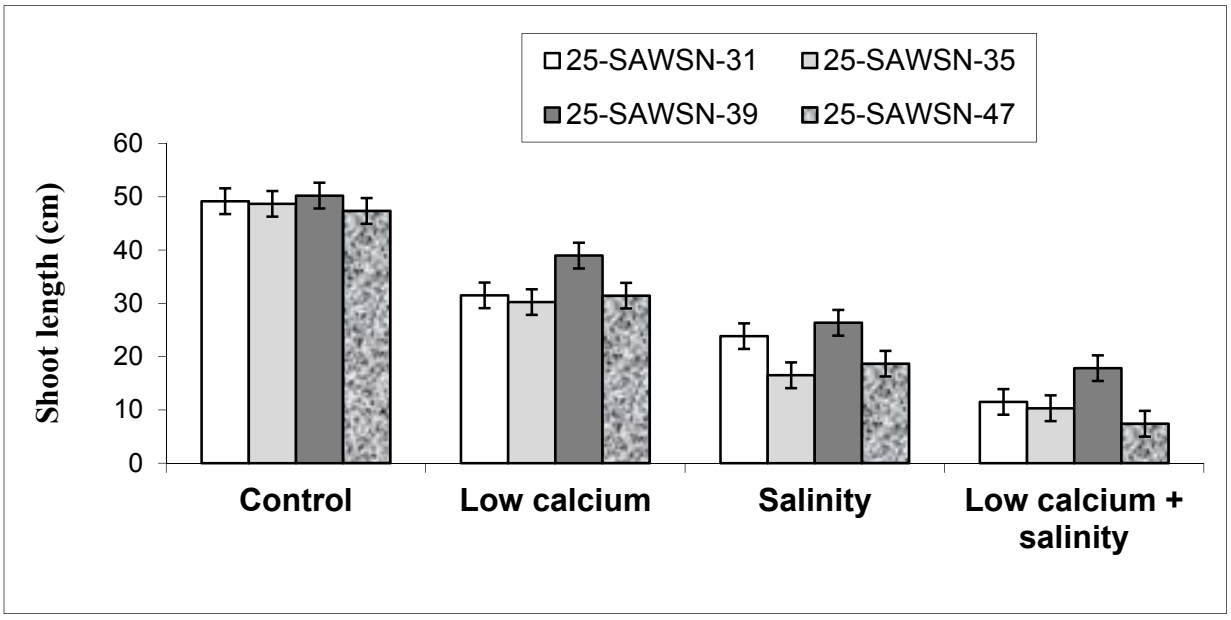
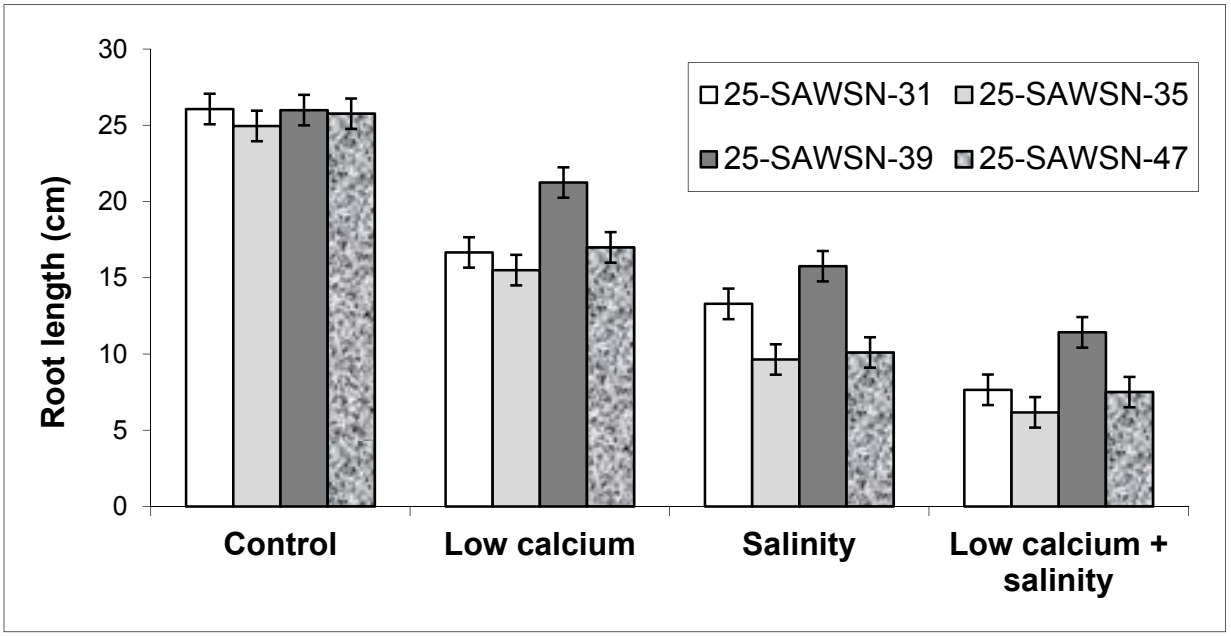


Fig. 4.2.6 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on root length (cm) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.



SPAD absorbance was found in and 25-SAWN-39 where as it was the minimum in 25-SAWN-35 which was statistical at par with 25-SAWN-47 and 25-SAWN-31.

4.2.3.9 Net photosynthetic rate

There were significant differences among treatments and genotypes regarding photosynthetic rate (Fig. 4.2.9). There was also a significant interaction between genotypes and treatments. On overall treatment mean basis, photosynthetic rate was the maximum in control and it was decreased significantly in all of the other treatments with the following trend, low calcium < saline < saline + low calcium. In all stress treatments, genotype 25-SAWN-39 significantly outperformed all the other genotypes, while genotype 25-SAWN-47 had the poorest performance. The relative difference between this genotype and the others was most pronounced in treatment low calcium + salinity. As for the control treatment, the different genotypes did not differ significantly.

4.2.3.10 Transpiration rate

The transpiration rate (E) was affected significantly by different treatments and it also varied among the genotypes (Fig. 4.2.10). The interaction between genotypes and treatments was also significant. On overall treatment mean basis, transpiration rate was the maximum in control and was decreased significantly by different stress treatments in the given trend (low calcium < saline < saline + low calcium). In the low calcium treatment percent decrease in transpiration rate as compared to control was 30%, in saline treatment it was 53% whereas in saline + low calcium it was 72%. The genotypic comparison within individual treatments showed that in low calcium treatment maximum transpiration rate was found in 25-SAWN-39 and differed significantly from 25-SAWN-35, 25-SAWN-31 and 25-SAWN-47 which were statistically at par with one another. In the saline treatment, the transpiration rate was the maximum in 25-SAWN-39 and 25-SAWN-31. The genotypes 25-SAWN-35 and 25-SAWN-47 showed the lowest transpiration rate in the saline treatment. In the saline + low calcium treatment, the maximum transpiration rate was found in 25-SAWN-39 and it was significantly higher than the other genotypes which were statistically similar to one another.

Fig. 4.2.7 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on number of tiller per plant of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.

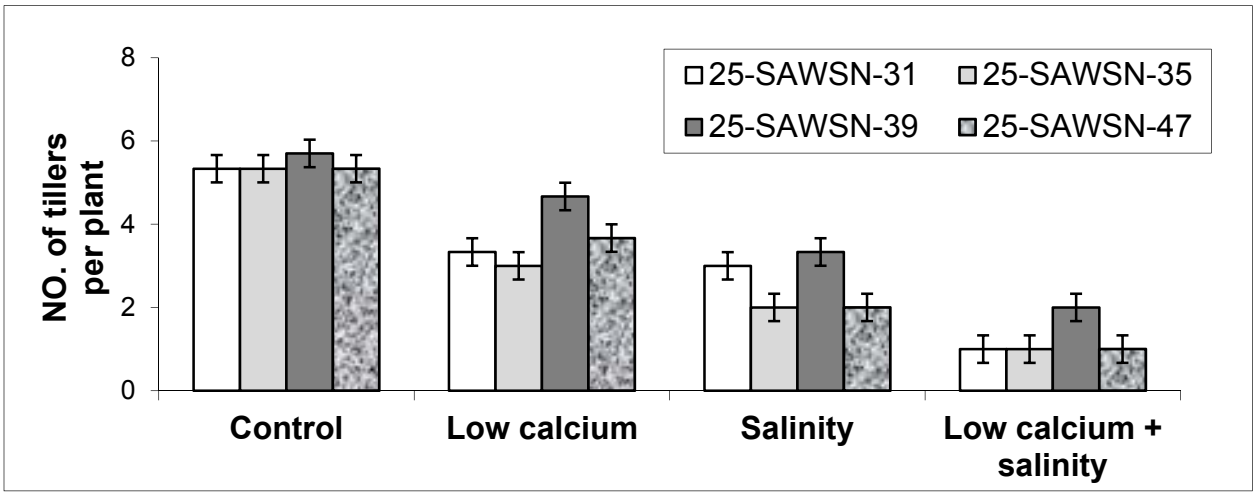
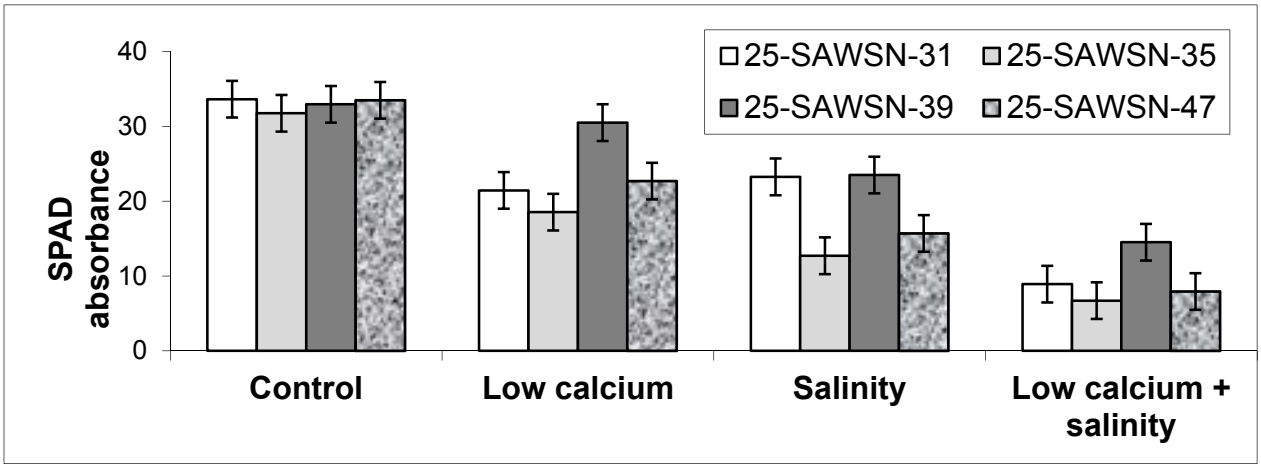


Fig. 4.2.8 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on SPAD absorbance of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.



4.2.3.11 Stomatal conductance

There were significant differences among treatments and genotypes regarding stomatal conductance (Fig. 4.2.11). The interaction between genotypes and treatments was also significant. On overall treatment mean basis, stomatal conductance was the maximum in control. In all of the other treatments it was decreased significantly in the given trend (low calcium < saline < saline + low calcium). In the low calcium treatment, percent decrease in stomatal conductance as compared to control was 28%, in saline treatment it was 50% and in saline + low calcium it was 71%. The comparison of genotypes in each treatment showed that in low calcium treatment the maximum stomatal conductance was found in 25-SAWN-39 whereas it was minimum in 25-SAWN-47 which was at par with 25-SAWN-35. In saline treatment, 25-SAWN-35 was at par with 25-SAWN-47 but showed less stomatal conductance as compared to the other genotypes. On the other hand, higher stomatal conductance was found in 25-SAWN-39 which was at par with 25-SAWN-31. In saline + low calcium treatment, the maximum stomatal conductance was found in 25-SAWN-39 and it differed significantly from the rest three genotypes viz. 25-SAWN-47, 25-SAWN-31 and 25-SAWN-35.

4.2.3.12 Water potential

Water potential data as shown in Fig. 4.2.12 revealed that water potential decreased significantly with low calcium and salinity and further decreased under the combined stress of salinity and low calcium. Significant differences were observed among treatments as well as genotypes regarding water potential and the interaction between genotypes and treatments was also significant. On over all treatment mean basis, the control treatment had the greatest value of water potential (-0.62 MPa). In the low calcium treatment water potential was -0.99 MPa, in saline treatment it was -1.18 MPa and in saline + low calcium, it was -1.19 MPa. In the low calcium treatment water potential was found more negative in 25-SAWN-31 which was statistically at par with 25-SAWN-35. The lowest water potential in this treatment was found in 25-SAWN-39. In saline treatment, 25-SAWN-47 and 25-SAWN-35 showed more negative values of water potential as compared to 25-SAWN-39 and 25-SAWN-31. In saline + low calcium treatment, lower water potential was found in 25-SAWN-35 and it did not differ significantly from 25-SAWN-31 and 25-SAWN-47, whereas it was the highest in the case of 25-SAWN-39.

Fig. 4.2.9 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on photosynthetic rate (µmol m^{-2} s^{-1}) of four wheat genotypes. Error bars show the values of LSD at P ≤ 0.05. n=4. The treatment details are same as in Fig. 4.2.1.

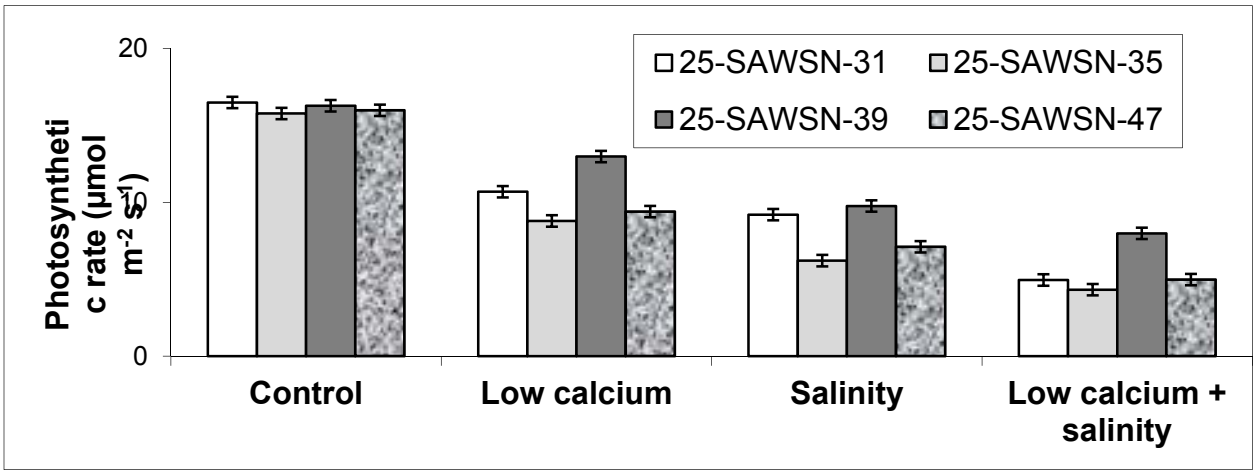
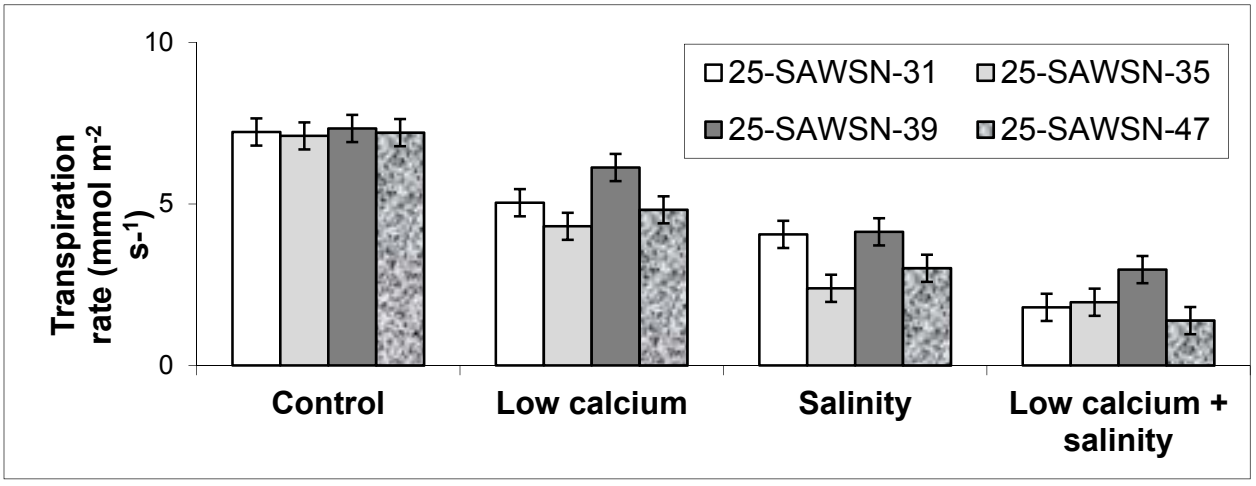


Fig. 4.2.10 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on transpiration rate (mmol m^{-2} s^{-1}) of four wheat genotypes. Error bars show the values of LSD at P ≤ 0.05. n=4. The treatment details are same as in Fig. 4.2.1.



4.2.3.13 Osmotic potential

Osmotic potential became more negative with the application of low calcium and salinity and this value became even more negative under the combined effect of salt stress and low calcium (Fig. 4.2.13). Significant differences were observed among treatments however, there was not a significant interaction between genotypes and treatments. On over all treatment mean basis, the maximum value of osmotic potential (-0.75 MPa) was found in control, in low calcium it was -1.37 MPa whereas in the saline treatment it was -2.26 MPa and in saline + low calcium it was -2.29 MPa. The genotypes did not differ significantly when compared within a particular treatment.

4.2.3.14 Turgor potential

The turgor potential decreased significantly under salinity and salinity + low calcium treatments however, low calcium alone did not affect the turgor potential significantly (Fig. 4.2.14). The interaction between genotypes and treatments was also significant. On over all treatment mean basis, in control the value of turgor potential was 0.13 MPa, in low calcium treatment it was 0.38 MPa, in saline treatment it was 1.08 MPa and in saline + low calcium treatment it was 1.10 MPa. In low calcium treatment, the genotypes did not differ significantly. In saline treatment, 25-SAWN-31 showed the maximum turgor potential followed by 25-SAWN-39 where as the minimum water potential was observed in 25-SAWN-35. In the saline + low calcium treatment, the highest turgor potential was found in 25-SAWN-39 followed by 25-SAWN-31, whereas it was the lowest in the case of 25-SAWN-35 followed by 25-SAWN-47.

4.2.3.15 Leaf sodium concentration

Significant differences were observed among the treatments as well as genotypes regarding leaf sodium concentration and there was a significant treatment x genotype interaction (Fig. 4.2.15). The leaf Na^+ concentration was increased significantly by salinity and salinity + low calcium treatments.

Fig. 4.2.11 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on stomatal conductance (mmol m^{-2} s^{-1}) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.

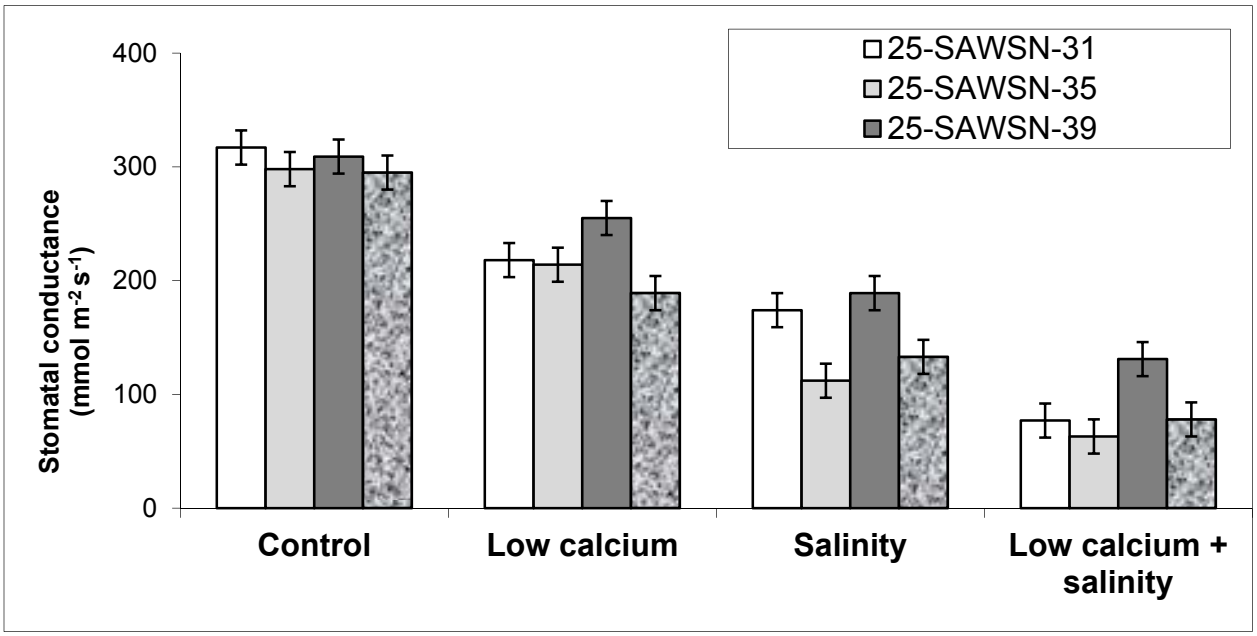
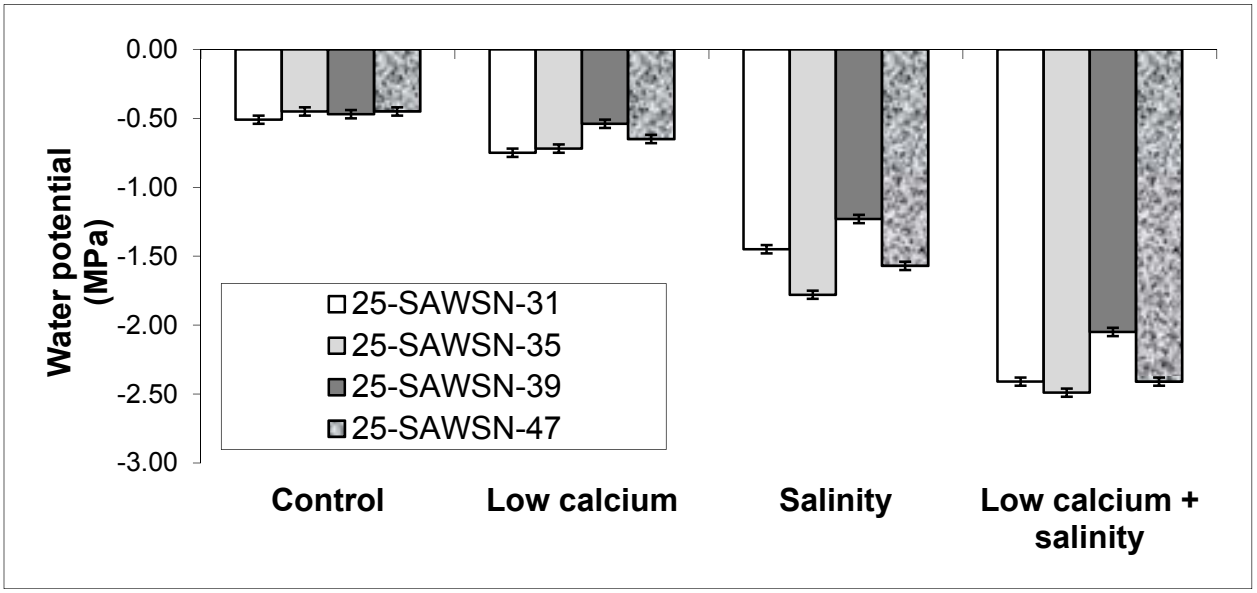


Fig. 4.2.12 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on water potential (MPa) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.



On overall mean basis, in low calcium treatment, leaf Na^+ concentration was the lowest followed by saline treatment and saline + low calcium treatment in an ascending order. In saline treatment, the maximum leaf Na^+ concentration was found in 25-SAWN-47 and 25-SAWN-35 which did not differ significantly where as the minimum leaf Na^+ concentration was found in 25-SAWN-39 and 25-SAWN-31 and both were statistically at par with each other. In the saline + low treatment, the maximum shoot Na^+ was found in 25-SAWN-47 which differed non significantly from and 25-SAWN-35 and 25-SAWN-31. On the other hand, the minimum Na^+ was found in 25-SAWN-39 which was significantly different from the other genotypes.

4.2.3.16 Leaf potassium concentration

Significant differences were observed among treatments as well as genotypes regarding leaf potassium concentration (Fig. 4.2.16). The interaction between genotypes and treatments was also significant. On overall treatment mean basis, in low calcium treatment, K^+ concentration was the highest followed by saline treatment and saline + low calcium treatment in a descending order. The comparison of genotypes in each treatment showed that in saline treatment, the maximum K^+ concentration was found in 25-SAWN-39 and it was statistically at par with 25-SAWN-31. The minimum leaf K^+ concentration was found in the leaves of 25-SAWN-47 which was statistically at par with 25-SAWN-35. In the saline + low calcium treatment, the maximum leaf K^+ concentration was found in 25-SAWN-39 whereas the minimum was found in 25-SAWN-35 which was statistically at par with 25-SAWN-31 and 25-SAWN-47.

4.2.3.17 Leaf calcium concentration

Leaf calcium concentration was decreased significantly due to low calcium and salinity. Significant differences were observed among the treatments as well as genotypes and there was a significant interaction between genotypes and treatments (Fig. 4.2.17). On overall treatment mean basis the lowest calcium concentration was found in saline + low calcium treatment followed by saline treatment and low calcium treatment in an ascending order. The comparison of genotypes in each treatment showed that in low calcium treatment the maximum Ca^{2+} concentration was found in 25-SAWN-39 which was significantly different from the other genotypes, whereas the minimum Ca^{2+} concentration was found in 25-SAWN-47 which

Fig. 4.2.13 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on osmotic potential (MPa) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.

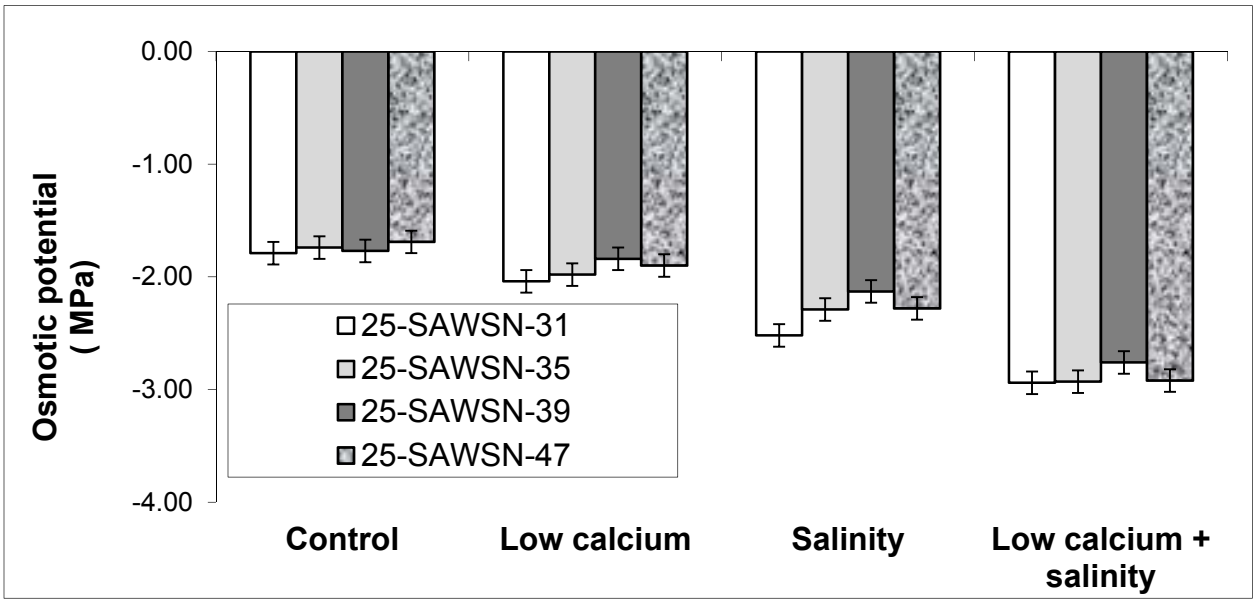
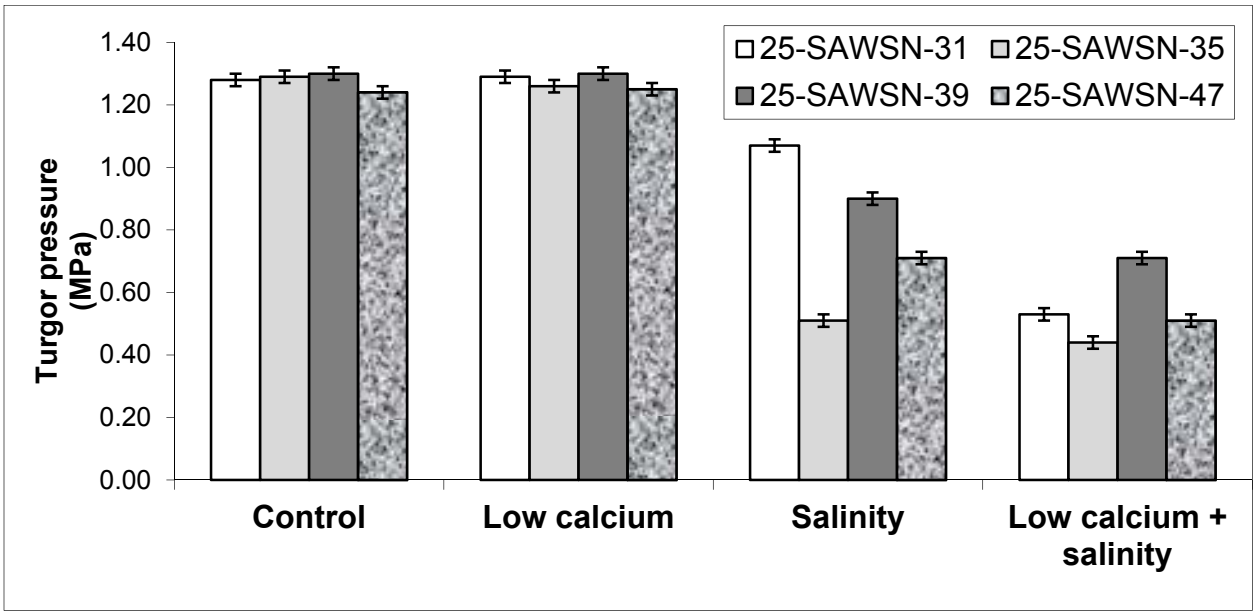


Fig. 4.2.14 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on turgor pressure (MPa) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.



was statistically at par with 25-SAWN-35 and 25-SAWN-31. In saline treatment, the maximum leaf Ca^{2+} concentration was found in 25-SAWN-39 followed by 25-SAWN-31. On the other hand, 25-SAWN-47 and 25-SAWN-35 accumulated the minimum leaf Ca^{2+} concentration and were statistically similar. In the saline + low calcium treatment, the maximum shoot Ca^{2+} concentration was found in 25-SAWN-39 and it was significantly different from all of the other genotypes. The minimum leaf Ca^{2+} concentration was found in 25-SAWN-35 and it did not differ significantly from 25-SAWN-31 and 25-SAWN-47.

4.2.3.18 Leaf chloride concentration

Significant differences were observed among the treatments as well as genotypes regarding leaf chloride concentration (Fig. 4.2.18). Similarly the interaction between genotypes and treatments was also significant. On overall mean basis, in low calcium treatment Cl^- concentration was the lowest followed by saline treatment and saline + low calcium treatment in an ascending order. In saline treatment, the maximum leaf Cl^- concentration was found in 25-SAWN-47 and it was statistically at par with 25-SAWN-35. On the other hand, 25-SAWN-39 accumulated less Cl^- concentration in its leaves and was followed by 25-SAWN-31. In the saline + low calcium treatment, the maximum leaf Cl^- concentration was found in 25-SAWN-47 which was statistically similar to leaf Cl^- concentration of 25-SAWN-35 and 25-SAWN-31. The genotype 25-SAWN-39 showed significantly lower leaf Cl^- as compared to the other genotypes.

4.2.3.19 Leaf K^+: Na^+

The leaf K^+: Na^+ ratio has been significantly affected by different treatments and genotypes with a significant interaction between genotypes and treatments (Fig. 4.2.19). On overall treatment mean basis leaf K^+: Na^+ was the maximum (6.43) in control. In low calcium treatment it was 3.20, in saline treatment it was 0.64 and in low calcium + saline treatment, it was 0.33. The comparison of genotypes in each treatment showed that in low calcium treatment this ratio was the maximum in 25-SAWN-31 and it was statistically at par with 25-SAWN-39, whereas the minimum leaf K^+: Na^+ ratio was found in 25-SAWN-47. In saline treatment, 25-SAWN-39 and 25-SAWN-31 showed higher leaf K^+: Na^+ ratio as compared to the other genotypes and these two genotypes differed significantly from 25-SAWN-47 and 25-SAWN-35

Fig. 4.2.15 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on leaf sodium concentration (mmol g^{-1} dry wt.) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.

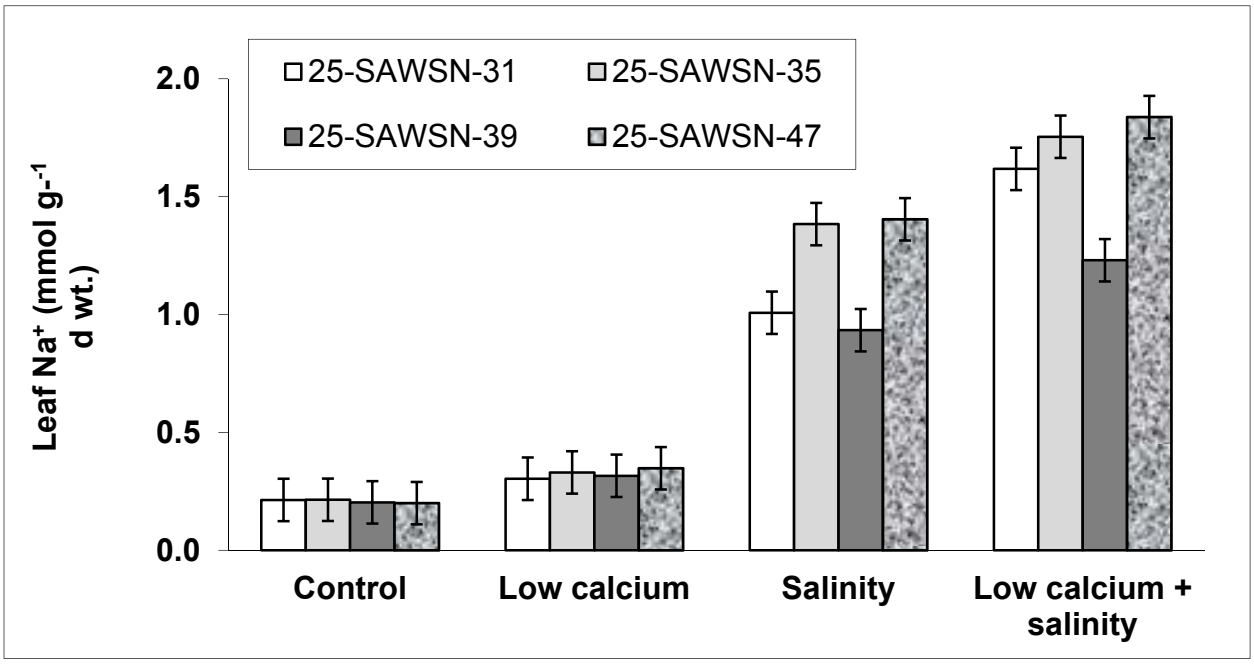
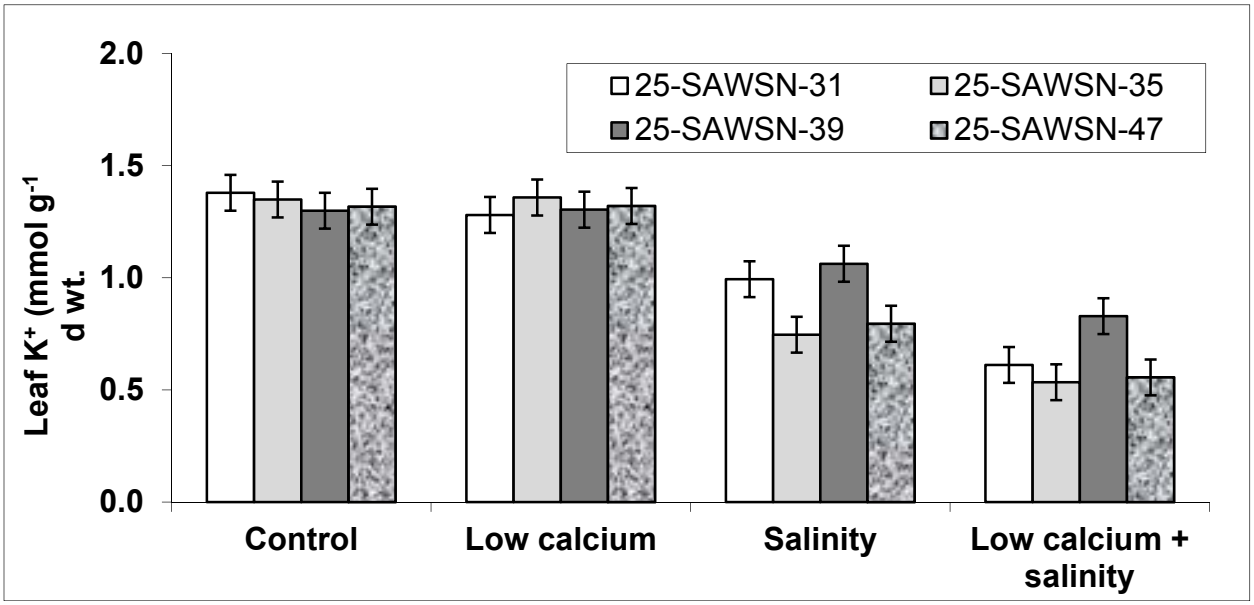


Fig. 4.2.16 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on leaf potassium concentration (mmol g^{-1} dry wt.) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.



in which lower leaf K^+: Na^+ ratio was recorded. In combined treatment (saline + low calcium), the maximum leaf K^+: Na^+ ratio was observed in 25-SAWN-39 and it differed significantly from the other genotypes, whereas the minimum leaf K^+: Na^+ ratio was found in 25-SAWN-35 and it was statistically at par with 25-SAWN-31 and 25-SAWN-47.

4.2.3.20 Leaf Ca^{2+}: Na^+ ratio

Ca^{2+}: Na^+ ratio has been significantly affected by different treatments and genotypes and there was a significant interaction between genotypes and treatments (Fig. 4.2.20). On overall treatment mean basis, Ca^{2+}: Na^+ was the maximum (4.78) in control followed by low calcium treatment it was: 1.40, saline treatment: 0.37 and low calcium + saline treatment: 0.17. The comparison of genotypes in each treatment showed that in low calcium treatment this ratio was the maximum in 25-SAWN-39 and it was statistically different from all of the other genotypes, whereas the minimum Ca^{2+}: Na^+ ratio was found in 25-SAWN-47. In saline treatment also, 25-SAWN-39 showed the highest Ca^{2+}: Na^+ ratio as compared to the other genotypes followed by 25-SAWN-31 whereas 25-SAWN-47 and 25-SAWN-35 showed the minimum leaf Ca^{2+}: Na^+ ratio and did not differ significanlty. In combined treatment (saline + low calcium), the maximum Ca^{2+}: Na^+ ratio was observed in 25-SAWN-39 and it differed significantly from all of the other genotypes whereas the minimum Ca^{2+}: Na^+ ratio was found in 25-SAWN-35 and it was statistically at par with 25-SAWN-31 and 25-SAWN-47.

Fig. 4.2.17 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on leaf calcium concentration (mmol g^{-1} dry wt.) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.

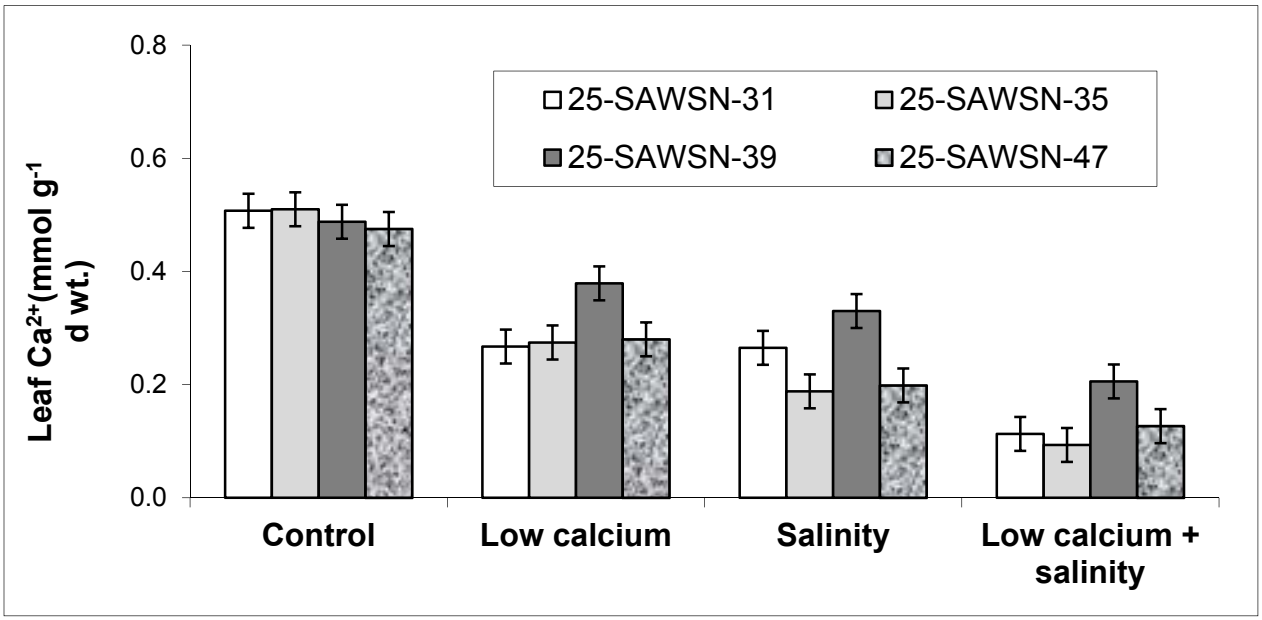


Fig. 4.2.18 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on leaf chloride concentration (mmol g^{-1} dry wt.) of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.

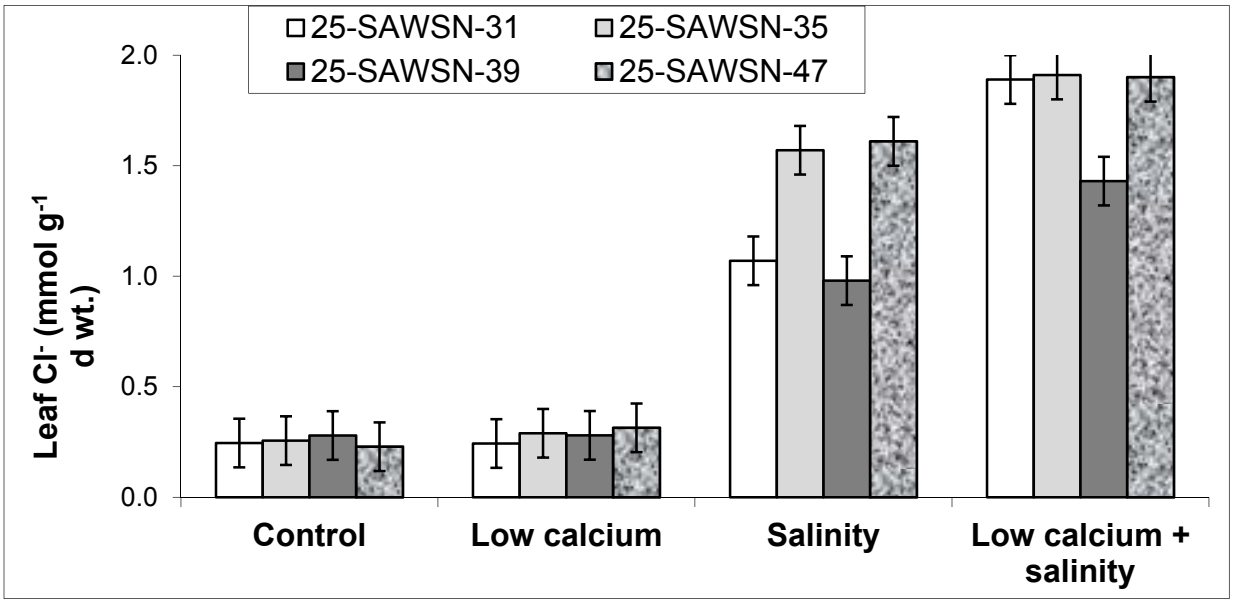


Fig. 4.2.19 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on leaf K^+: Na^+ ratio of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.

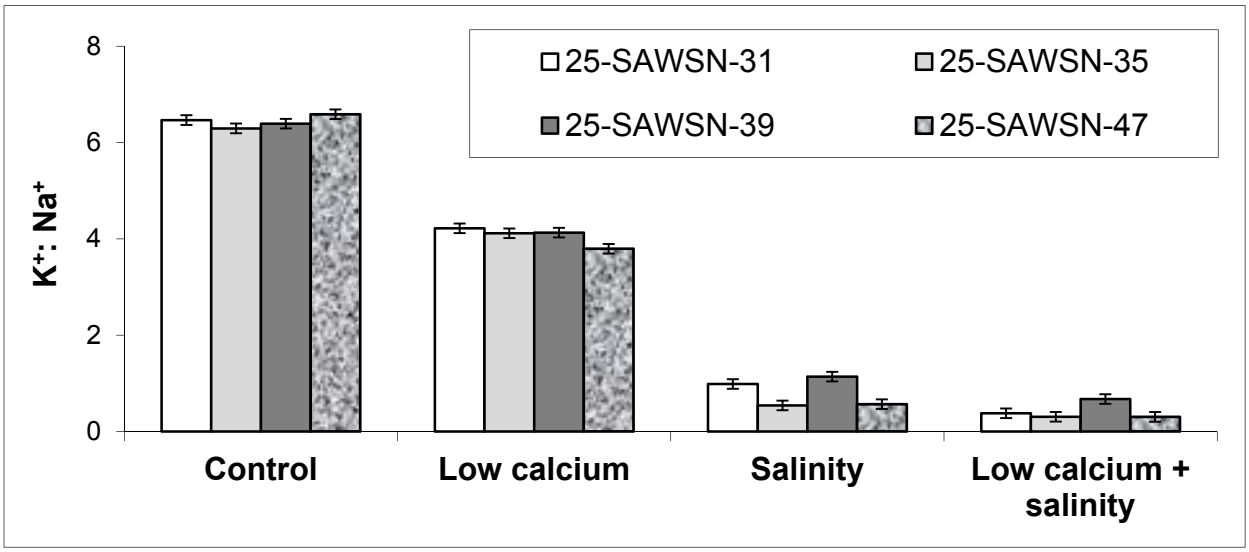
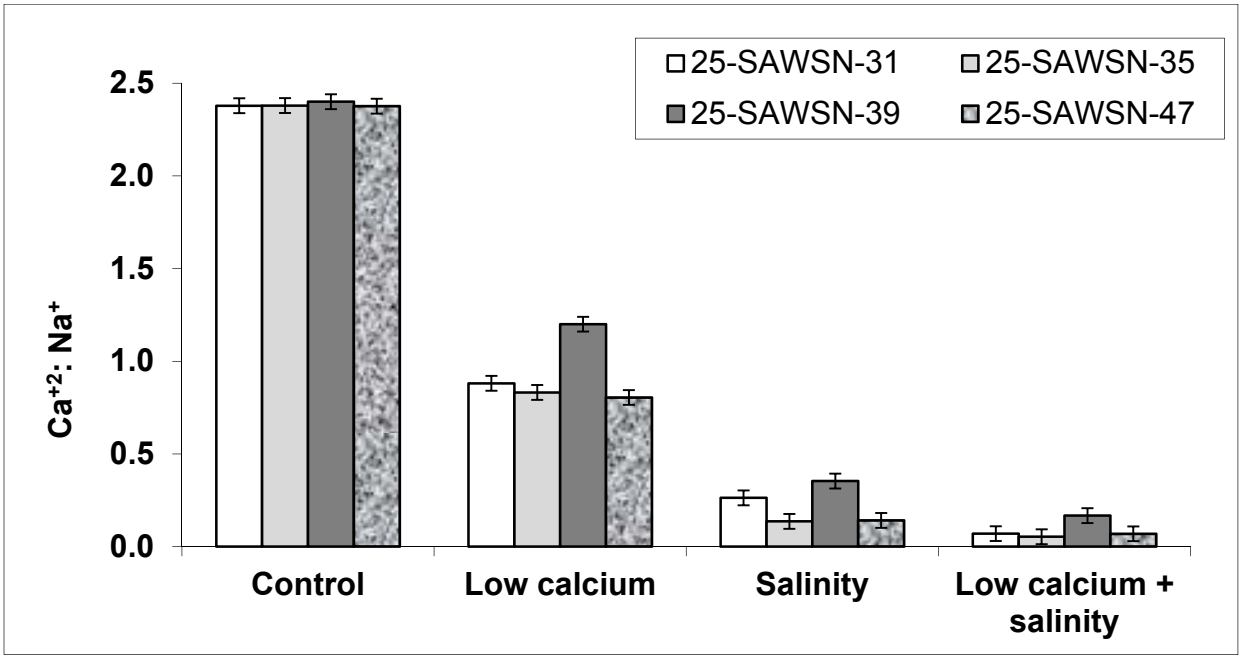


Fig. 4.2.20 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on leaf Ca^{2+}: Na^+ ratio of four wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. n=4. The treatment details are same as in Fig. 4.2.1.



4.2.4 DISCUSSION

This study explores the physiological basis of differences of growth performance of salt-resistant and sensitive wheat genotypes in response to salinity and low calcium. A significant reduction in the growth parameters of the wheat genotype was observed due to low calcium and salinity. Salinity + low calcium caused a higher growth reduction than their individual effects. Moreover, the toxic effect of low calcium + salt stress was more pronounced in the case of genotypes 25-SAWN-31, 25-SAWN-35 and 25-SAWN-47 as compared to 25-SAWN-39. High salt concentration disturbs several physiological processes of plants which results in the reduction of plant growth and development (Taffouo *et al*., 2004). Reduction in plant growth due to salinity has been reported by many workers including Turan *et al*. (2009). Because of the fact that the genotypes 25-SAWN-39 accumulated more Ca^{2+} and less Na^{+}, their growth performance was better as compared to the other genotypes.

There was a significant reduction in chlorophyll content in terms of SPAD absorbance due to salinity alone and in combination with low calcium. Chlorophyll content and salinity have a very strong negative correlation with each other (Parida and Das, 2005). Salinity decreased chlorophyll content in rice (Sultana *et al*., 1999), cotton (Meloni *et al*., 2003) and wheat (Sairam *et al*., 2002). The stability and functioning of the PSII is reduced due to accumulation of excessive sodium (Watson *et al*., 2001). So the tolerant genotypes like 25-SAWN-39 and 25-SAWN-31 avoided these destructive effects by maintenance of lower leaf sodium and higher potassium content possibly by selective ion transport at root level under saline conditions. However, under salinity + low calcium only the wheat genotype 25-SAWN-39 could maintain better photosynthesis and ionic composition.

An interactive effect of salt stress and low calcium decreased the stomatal conductance and photosynthetic rate more as compared to the individual stresses. This reduction was more pronounced in the case of 25-SAWN-35, 25-SAWN-47 and 25-SAWN-31 than 25-SAWN-39. Photosynthesis is one of the most important processes inhibited under salt stress (Munns, 2002; Ashraf and Shahbaz, 2003) and reduction in photosynthetic rate due to salinity has also been reported earlier in wheat (Zheng *et al*., 2008; Sharma *et al*., 2005).. Reduction in growth and yield is directly related to the reduction in photosynthetic activity (Meloni *et al.*, 2003). The decreased photosynthesis may be because of stomatal closure that reduces the availability of CO_2 or due to direct salt effects on the photosynthetic system (Brugnoli and Lauteri, 1991; Pessarakli,

1994). As the water uptake from soil is reduced, a decrease in stomatal conductance occurs because of closure of the stomata. An increase in leaf Na^+ concentration reduces leaf gas exchange under saline conditions (Walker *et al*., 1993). Similarly, high leaf Cl^- concentrations have also been held responsible for reduced photosynthetic capacity and stomatal conductance (Banuls *et al*., 1997). In the present study both the Na^+ and Cl^- seem to have played their role in reducing the stomatal conductance, transpiration rate and photosynthesis. Calcium is involved in the regulation of guard-cell turgor and stomatal aperture (Webb *et al*., 1996). Therefore, photosynthetic attributes were also affected negatively due to low calcium availability in the root medium and decrease in photosynthetic parameters due to salinity was further accelerated with low calcium supply.

On the other hand, extra supply of calcium has ameliorative effects on water relations (Cavajal *et al*., 1999; Kaya and Higgs, 2002). The results of water relations from this study match the findings of Aranda *et al*. (2001) and Khan (2010). Decreased availability of water to plants, due to low external water potential is considered to be the first cause of growth restriction under saline conditions (Munns and James, 2003). For proper growth under salinity a decrease in intracellular water potential is needed (Greenway and Munns, 1980) by increasing the amount of such solutes in the tissues which are osmotically active (Gorham *et al*., 1985). The decrease of tissue solute potential would compensate the salt-induced lowering of root zone water potential. This would help the plant maintain turgor pressure and functioning of cells under adverse water conditions. The genotypes 25-SAWN-39 and 25-SAWN-31 responded to salinity by decreasing leaf solute and water potential more than 25-SAWN-35 and 25-SAWN-47, so they can be regarded as more adapted to saline conditions. Due to better osmotic adjustment both these genotypes have better leaf turgor under salinity however, under salinity + low calcium only the genotype 25-SAWN-39 could maintain better photosynthetic attributes and water relations because of efficient management of ionic uptake and accumulation. The wheat genotype 25-SAWN-31 could manage its ion uptake under good calcium availability but could not under low calcium availability. Therefore, for a salt-resistant wheat genotype to be successful under saline sodic field conditions it needs to be an efficient calcium user.

4.3 Experiment-III: Comparative oxidative stress tolerance of different wheat genotypes against salinity and low calcium

4.3.1 BRIEF INTRODUCTION TO THIS STUDY

This study has been carried out using two salt-sensitive and two salt-resistant wheat genotypes identified from Experiment No.1. It has been carried out to investigate the comparative oxidative stress tolerance of the selected wheat genotypes in response to salinity and low calcium, alone and in combination.

4.3.2 BRIEF METHODOLOGY OF THIS STUDY

This study has been carried out following the procedures used for experiment No. 1. The experiment was continued for 28 days after the treatment applications as detailed in Chapter No. 3. At the time of harvest physical growth parameters were recorded and leaf ionic composition was determined. Flame photometer was used to determine Na^+ and K^+ concentration in leaves and Cl^- was determined by chloride analyzer. Calcium was determined with atomic absorption spectrophotometer. The leaf samples were collected and processed for the determination of different antioxidant enzymes. The performance of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were determined as detailed in Chapter No. 3. The action of superoxide dismutase was considered on the basis of its capability to stop the photoreduction of nitroblue tetrazolium (NBT) as reported by Giannopolitis and Ries (1977). Chance and Maehly (1955) method was used to determine the performance of catalase (CAT) and peroxidase (POD).

4.3.3 RESULTS

4.3.3.1 Shoot fresh weight

There were significant differences among treatments and genotypes regarding shoot fresh weight production. The interaction between genotypes and treatments was also significant (Fig.

4.3.1). On overall treatment mean basis shoot fresh weight was the maximum in control. In all other treatments it decreased significantly in the given trend: low calcium < saline < low calcium + saline. In low calcium treatment percent reduction in SFW as compared to control was 35%, in saline treatment it was 58% whereas in low calcium + saline treatment, it was 81%. The comparison of genotypes in each treatment showed that in low calcium treatment the maximum SFW was produced by 25-SAWSN-39 and it was statistically different from all of the other genotypes, whereas the minimum SFW was found in 25-SAWSN-47. In saline treatment, 25-SAWSN-39 and 25-SAWSN-31 performed better as compared to the other two genotypes 25-SAWSN-47 and 25-SAWSN-35 which produced significantly lower shoot fresh weight. In the combined treatment (saline + low calcium), the maximum SFW was observed in 25-SAWSN-39 and it differed significantly from all of the other genotypes whereas minimum SFW was found in 25-SAWSN-35 and it was statistically at par with 25-SAWSN-31 and 25-SAWSN-47.

4.3.3.2 Shoot dry weight

The shoot dry weight (SDW) was significantly affected by different treatments and genotypes with a significant interaction between genotypes and treatments (Fig. 4.3.2). On overall mean basis SDW was the maximum in control. In all of the other treatments it was decreased in the given trend: low calcium < saline < low calcium + saline. In the low calcium treatment, percent decrease in SDW as compared to control was 42%, in saline treatment it was 76% whereas in saline + low calcium, it was 90%. The comparison of genotypes in each treatment showed that in low calcium treatment maximum SDW was produced by 25-SAWSN-39 and it was statistically different from the other genotypes whereas the minimum SDW was found in 25-SAWSN-47. In saline treatment 25-SAWSN-39 performed better as compared to the other genotypes followed by 25-SAWSN-31 whereas the minimum SDW was produced by 25-SAWSN-35. In the saline + low calcium treatment, the maximum SDW was observed in 25-SAWSN-39 which differed significantly from the other genotypes. On the other hand, the minimum SDW was produced by 25-SAWSN-47 which was statistically at par with 25-SAWSN-35 and 25-SAWSN-31.

Fig. 4.3.1 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on shoot fresh weight (g plant^{-1}) of different wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. (Control; 9.0 mM Ca^{2+}, low Ca^{2+} (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca), saline (125 mM NaCl) and low Ca^{2+} + saline (1/4th of the Ca^{2+} conc. in control i.e. 2.25 mM Ca+125 mM NaCl).

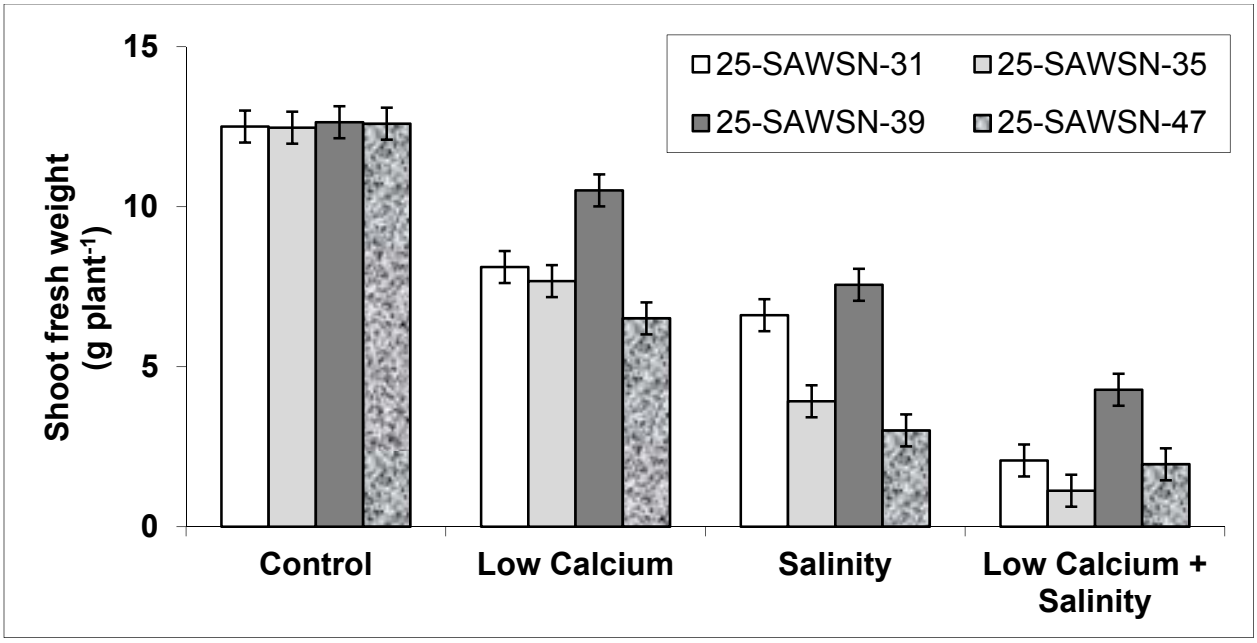
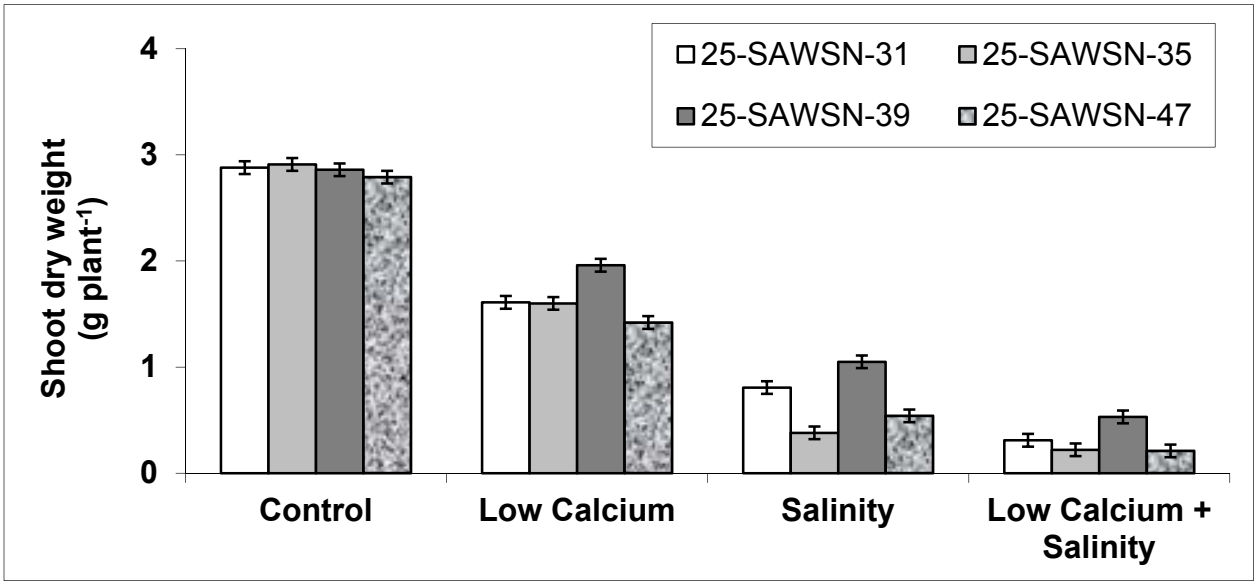


Fig. 4.3.2 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on shoot dry weight (g plant^{-1}) of different wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. The treatment details are same as in Fig. 4.3.1.



4.3.3.3 Root fresh weight

The treatments and genotypes differed significantly for their effect on root fresh weight (RFW) production (Fig. 4.3.3). The interaction between genotypes and treatments was also significant. On overall mean basis RFW was the maximum in control and the application of stress resulted in a significant reduction of RFW. The maximum reduction was found in the case of combined stress treatment (saline + low calcium). In low calcium treatment percent decrease in RFW as compared to control was 35%, in saline treatment it was 60% whereas in saline + low calcium it was 79%. In low calcium treatment the maximum RFW was produced by 25-SAWSN-39 and it was statistically different from 25-SAWSN-31 and 25-SAWSN-35, whereas the minimum RFW was found in 25-SAWSN-47. In the saline treatment, 25-SAWSN-39 produced the maximum RFW followed by 25-SAWSN-31. On the other hand, 25-SAWSN-47 produced the minimum RFW and did not differ significantly with 25-SAWSN-35. In the saline + low calcium treatment, the maximum RFW was observed in 25-SAWSN-39 and the minimum RFW was found in 25-SAWSN-47 which was statistically at par with 25-SAWSN-31 and 25-SAWSN-35.

4.3.3.4 Root dry weight

Both treatments and genotypes differed significantly regarding root dry weight (RDW) as shown in Fig. 4.3.4. The interaction between genotypes and treatments was also found significant. On overall mean basis RDW was the maximum in control and the application of salinity and low calcium stresses resulted in significant reduction in the RFW. The maximum reduction was found in the case of combined stress treatment (saline + low calcium). In low calcium treatment percent decrease in RDW as compared to control was 35%, in saline treatment it was 66% whereas in saline + low calcium it was 83%. In low calcium treatment, the maximum RDW was produced by 25-SAWSN-39 and it differed significantly from all of the other genotypes viz. 25-SAWSN-35, 25-SAWSN-31and 25-SAWSN-47 which were statistically similar to one another. In saline treatment, 25-SAWSN-39 performed better as compared to other genotypes and was followed by 25-SAWSN-31. On the other hand the minimum RDW in this treatment

Fig. 4.3.3 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on root fresh weight (g plant^{-1}) of different wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. The treatment details are same as in Fig. 4.3.1.

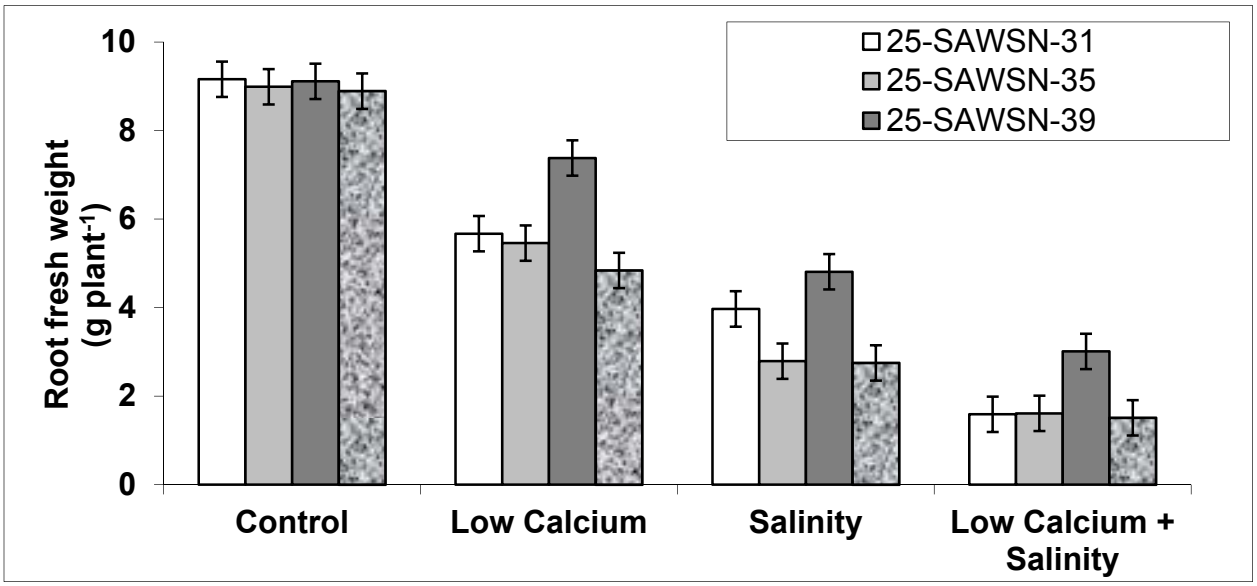
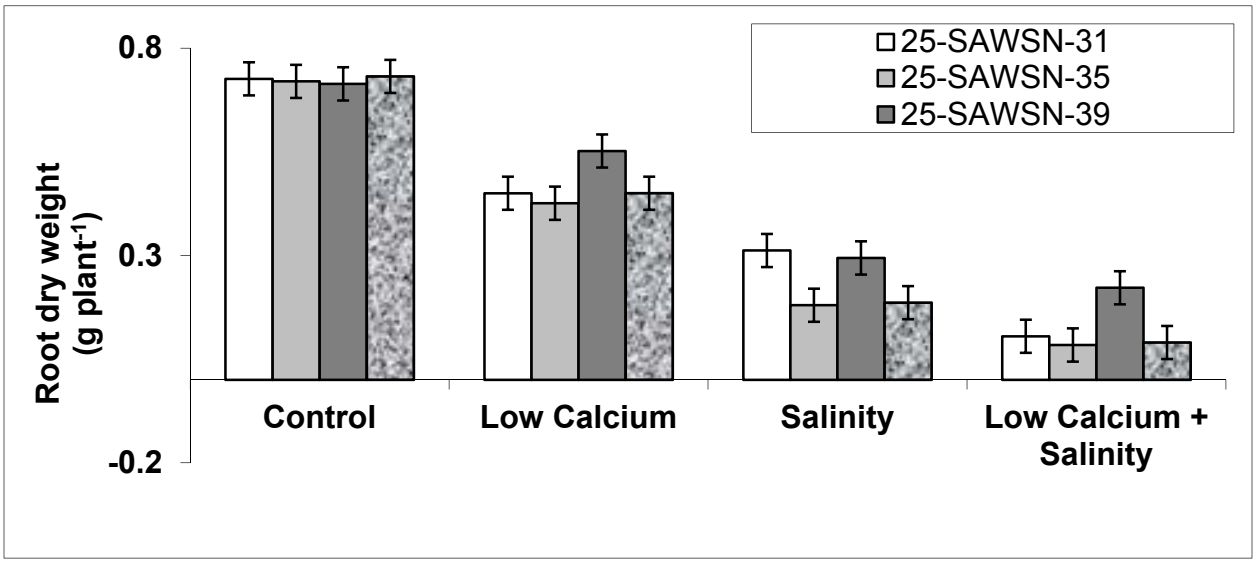


Fig. 4.3.4 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on root dry weight (g plant^{-1}) of different wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. The treatment details are same as in Fig. 4.3.1.



was found in 25-SAWSN-35 and it was statistically at par with 25-SAWSN-47. In the saline + low calcium treatment, the maximum RDW was observed in 25-SAWSN-39 and was significantly different from 25-SAWSN-47 and 25-SAWSN-35 and 25-SAWSN-31. The genotypes other than 25-SAWSN-39 were also statistically similar to one another in this treatment.

4.3.3.5 Protein content

Low calcium and salinity treatments significantly decreased protein content and this decrease was the maximum under the combined stress of low calcium and salinity. Significant differences were observed among treatments as well as genotypes with a significant interaction between genotypes and treatments. In low calcium treatment protein content was the maximum in 25-SAWSN-39 which was significantly different from the other genotypes. On the other hand it was the minimum in 25-SAWSN-47. In saline treatment, 25-SAWSN-39 showed more protein content followed by 25-SAWSN-31, whereas the minimum protein content was found in 25-SAWSN-47 which was statistically similar to 25-SAWSN-35. In the saline + low calcium, the maximum protein content was found in 25-SAWSN-39 and it was significantly different from the other genotypes which did not differ significantly from one another.

4.3.3.6 Peroxidase activity

The peroxidase (POD) activity was increased due to low calcium and salinity and this increase was the maximum under the combined effect of salt stress and low calcium (Fig. 4.3.6). Significant differences were observed among treatments as well as genotypes regarding POD activity and the interaction between genotypes and treatments was also significant. In low calcium treatment POD activity was the maximum in 25-SAWSN-39 which was significantly different from the other genotypes. In saline treatment, 25-SAWSN-31 and 25-SAWSN-39 were statistically similar and showed significantly more POD activity as compared to the other two genotypes, 25-SAWSN-47 and 25-SAWSN-35. In saline + low calcium, the maximum POD activity was found in 25-SAWSN-39, whereas it was the minimum in 25-SAWSN-35 which was statistically at par with 25-SAWSN-47 and 25-SAWSN-31.

Fig. 4.3.5 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on protein contents (mg g^{-1} fresh wt.) of different wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. The treatment details are same as in Fig. 4.3.1.

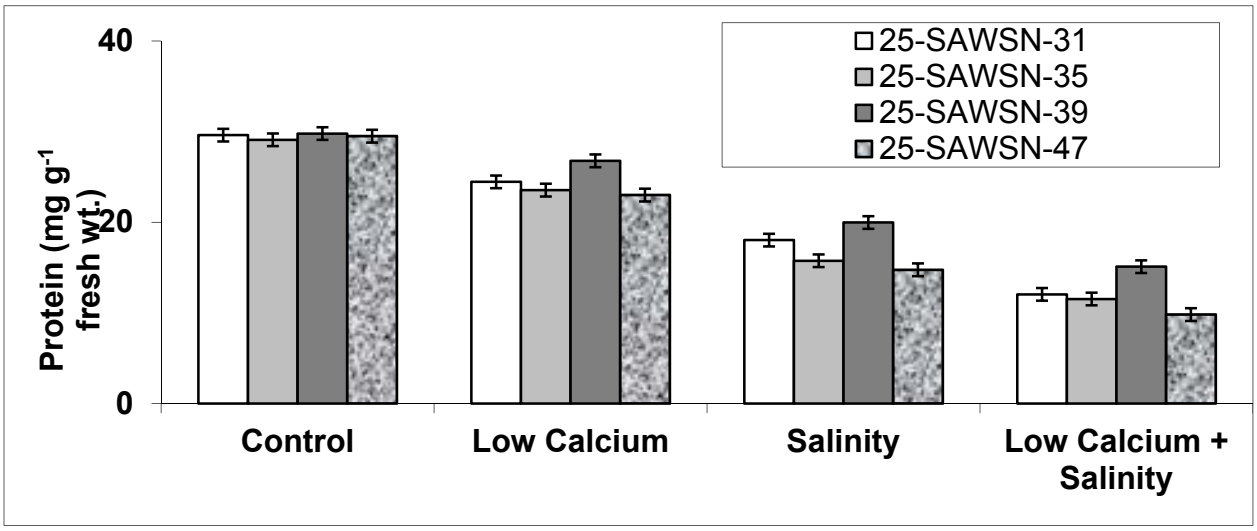
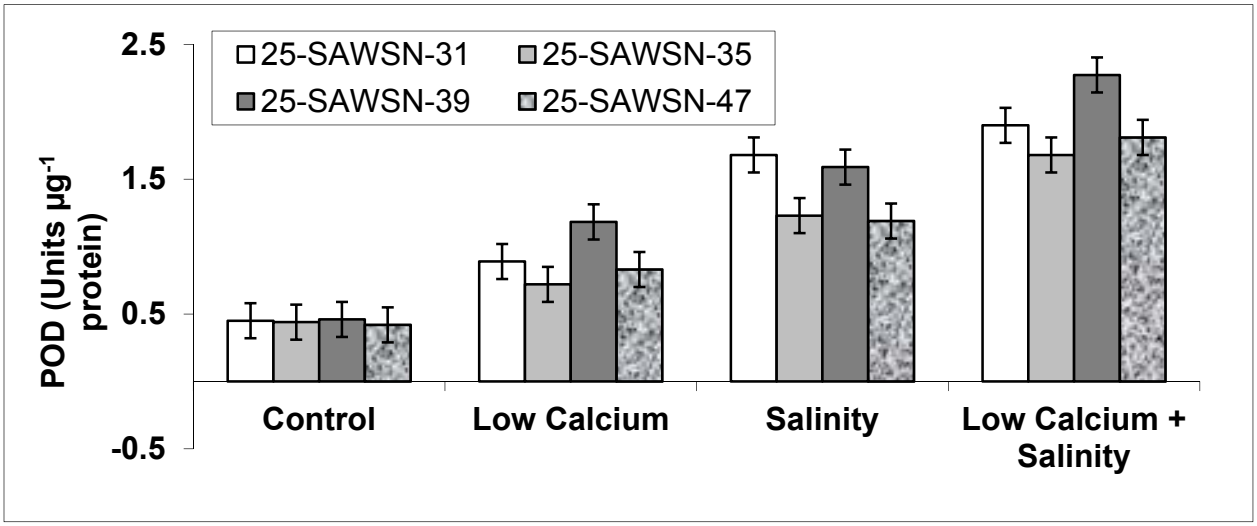


Fig. 4.3.6 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on **POD (Units µg^{-1} protein)** of different wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. The treatment details are same as in Fig. 4.3.1.



4.3.3.7 Catalase activity

Catalase (CAT) activity was increased due to low calcium and salinity and this increase was the maximum under the combined effect of salt stress and low calcium. Significant differences were observed among treatments as well as genotypes. The interaction between genotypes and treatments was also significant. In low calcium treatment CAT activity was significantly higher in 25-SAWSN-39 than all of the other genotypes. In saline treatment, 25-SAWSN-39 showed more CAT activity as compared to 25-SAWSN-47 and 25-SAWSN-35 but it was statistically at par with 25-SAWSN-31. In the saline + low calcium treatment, the maximum CAT activity was found in 25-SAWSN-39 and it was significantly higher than the other genotypes which were statistically at par with one another.

4.3.3.8 Superoxide dismutase

The superoxide dismutase (SOD) activity was increased significantly due to low calcium and salinity and this increase was the maximum under the combined stress of salinity and low calcium. Significant differences were observed among treatments as well as genotypes and there was a significant interaction between genotypes and treatments. In low calcium treatment, SOD activity was the maximum in 25-SAWSN-39 and was significantly higher than the other genotypes. On the other hand it was the minimum in case of 25-SAWSN-47. In saline treatment, 25-SAWSN-39 showed maximum SOD activity and it was statistically at par with 25-SAWSN-31, whereas the minimum SOD activity was found in 25-SAWSN-35 which was statistically similar to 25-SAWSN-47. In the saline + low calcium treatment, the maximum SOD activity was found in 25-SAWSN-39 and it was significantly higher than the other genotypes, whereas it was the minimum in case of 25-SAWSN-47 which was statistically similar to 25-SAWSN-35 and 25-SAWSN-31.

Fig. 4.3.7 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on CAT (Units mg^{-1} protein) of different wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. The treatment details are same as in Fig. 4.3.1.

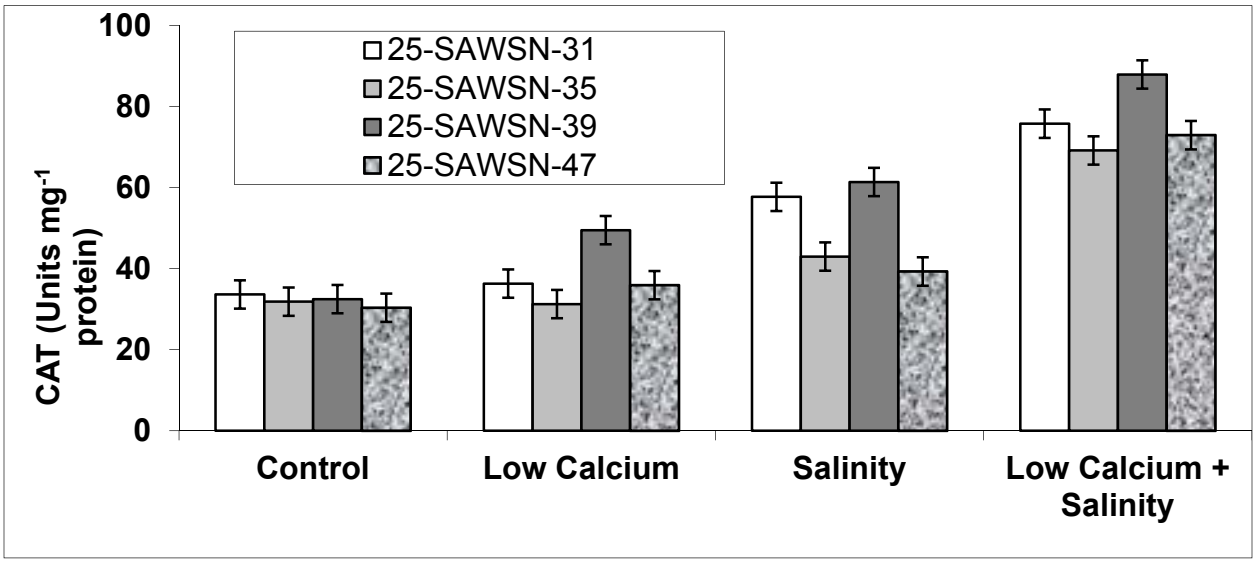
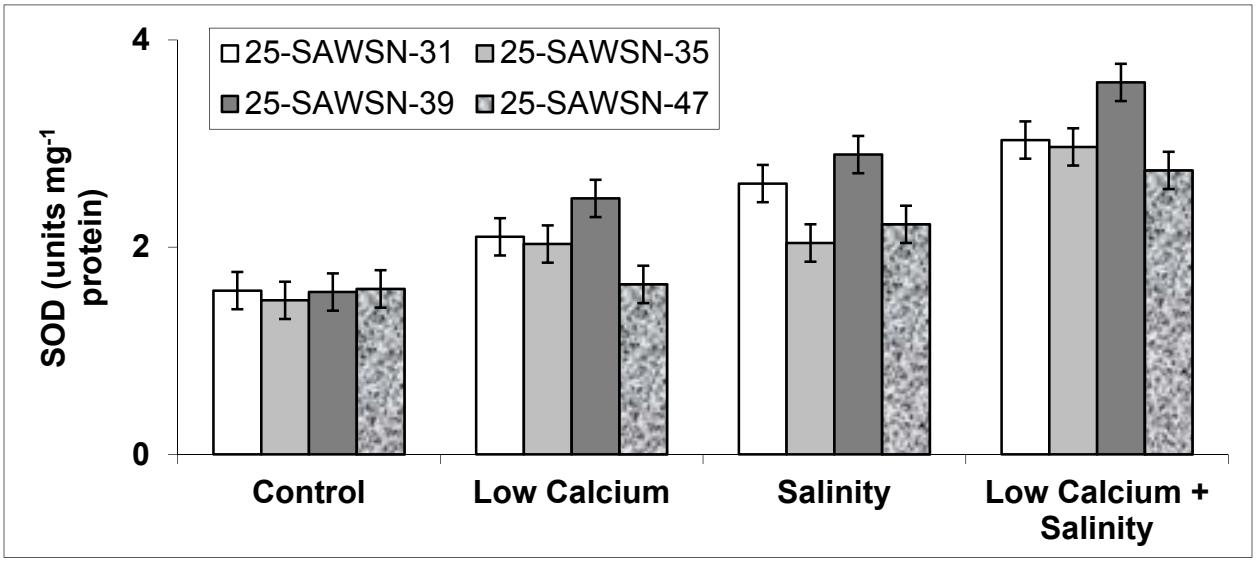


Fig. 4.3.8 Effect of salinity (125 mM NaCl), low calcium (1/4th of control) and their interaction on SOD (units mg^{-1} protein) of different wheat genotypes. Error bars show the values of LSD at $P \leq 0.05$. The treatment details are same as in Fig. 4.3.1.



4.3.4 Discussion

This study investigates the comparative oxidative stress tolerance of the selected wheat genotypes against salinity and low calcium supply. The activities of the antioxidant enzymes namely CAT, POD and SOD were increased under saline conditions alone and in combination with low calcium. Oxygen is necessary for life whereas its reduction leads to the formation of reactive oxygen species (ROS) which disturb plant metabolic processes (Asada, 1999). These ROSs include superoxide, hydroxyl radicals and hydrogen peroxide. The extremely reactive nature of these active oxygen species result in their reaction with important molecules such as DNA, pigments, proteins, lipids, and other essential cellular constituents leading to a series of destructive processes (Mittler, 2002). These reactive oxygen species are produced in low concentrations under normal growth conditions (Polle, 2001) but are over produced under environmental stress conditions (Karpinski *et al*., 2003; Laloi *et al*., 2004). For example, due to osmotic stress stomata are closed leading to reduced supply of CO_2 for photosynthesis and accumulation of superoxide in high concentration in chloroplast which leads to photoinhibition and photooxidation and resultantly cell damage (Ashraf, 2009).

Plants possess specific mechanisms to detoxify these reactive oxygen species. These include the activation of the enzymes of antioxidative pathway such as superoxide dismutase, peroxidase, catalase and those of the ascorbate- glutathione cycle (Noctor and Foyer, 1998; Smirnoff, 2005). The concentration of non-enzymatic antioxidants such as tocopherols, flavones, ascorbic acid and carotenoids is also increased (Johnson *et al*., 2003). Superoxides are dismutated to H_2O_2 and molecular oxygen with the help of superoxide dismutase (Giannopolitis and Ries, 1977). It is an important enzyme for mitigation of oxidative stress in plants. H_2O_2 and some organic hydroperoxides are broken to water and oxygen with the help of catalase enzymes (Ali and Alqurainy, 2006).

Plant species greatly vary with respect to salinity induced increase in the activities of enzymatic and non-enzymatic antioxidants (Zhang and Kirkham, 1995; Nayyar and Gupta, 2006). Similar differences have also been found in some cases between cultivars of a same species (Bartoli *et al*., 1999). The particular role of an antioxidant in detoxification of ROS is affected by the type of species, stress intensity and the plant growth stage at the time of stress.

Salinity stress usually increases the production of antioxidants in plants and the plants with high antioxidant production can better scavenge ROSs and are more salt tolerant (Shalata and Tal, 1998; Garratt *et al*., 2002).

In the present study, SOD activity has been increased under saline environment. There has been found higher increase in the activity of SOD in two salt tolerant lines (Kharchia 65, KRL 19) than in two salt sensitive lines (HD2009, HD2687) of wheat (Sairam *et al*., 2005). Our findings that tolerant genotype 25-SAWSN-39 showed significantly higher activities of SOD as compared sensitive genotypes (25-SAWSN-35, 25-SAWSN-47) agree with the previous results.

The activities of catalase, peroxidase were increased under stress conditions. These results match the findings of Perveen *et al*. (2011) for wheat, Khan and Panda (2008) for rice, Ashraf and Ali (2008) for canola, Davenport *et al*. (2003) for sunflower. Salinity has been found to cause more lipid peroxidation and hence damage to cellular membranes of a salt sensitive wheat line WH-542 than a salt tolerant line KRL-19 (Perveen *et al*., 2011). However, Perveen *et al*. (2011) did not find any genotypic differences with respect to increase in the activities of catalase, peroxidase and ascorbate. In this study we found significantly more activities of catalase, peroxidase under stress as compared to control, and tolerant genotypes 25-SAWSN-39 and 25-SAWSN-31 showed higher levels of these antioxidants as compared to sensitive genotypes 25-SAWSN-35 and 25-SAWSN-47.

4.4 Experiment-IV: Comparative performance of selected wheat genotypes under normal and saline sodic field conditions

4.4.1 INTRODUCTION

This experiment has been carried out to study the performance of the selected wheat genotypes (25-SAWSN-31, 25-SAWSN-35, 25-SAWSN-39 and 25-SAWSN-47) under normal and saline sodic field conditions as the ultimate goal of selection of a genotype is to grow it successfully on a salt-affected field. The genotypes were grown on non-saline as well as on a salt-affected field. The genotypes were compared separately at both the fields and the yield in salt-affected field was compared to the non-saline field as a percent of the later field.

4.4.2 METHODOLOGY

In this study, four wheat (*Triticum aestivum* L.) genotypes, two sensitive and two resistant were used. This study was carried out simultaneously at two sites, one having non-saline and non-sodic soil and the other having a saline and sodic soil (EC_e level of about 15 dS m^{-1} and SAR level of about 35-40 (mmol $L)^{1/2}$). Plants were grown in the field up to maturity using recommended doses of fertilizers and following the recommended agronomic practices. The experiment was continued until maturity. Different parameters like EC, SAR and pH were determined from soil samples taken from fields. Water quality characteristics of irrigation water were measured. At the start of the booting stage, the leaf second to flag leaf was detached and used for ionic analysis (Na^+, K^+, Cl^- and Ca^{+2}) following the methods detailed in Experiment No. 2. The crop was harvested at maturity and data regarding grain and straw yields and different yield components were recorded.

4.4.3 RESULTS

4.4.3.1 Grain and straw yields

The data regarding grain yield under non-saline and saline soil conditions are shown in Fig. 4.4.1. The wheat genotypes did not differ significantly from one another for grain yield production under non-saline conditions. However, the grain yield of different genotypes was reduced to a different extent under saline soil conditions. The grain yield production by different genotypes under saline conditions as a percent of non-saline conditions was 49, 46, 79 and 41 in the case of 25-SAWSN-31, 25-SAWSN-35, 25-SAWSN-39 and 25-SAWSN-47, respectively. The genotypes differed significantly under saline conditions with a significantly higher grain yield production by 25-SAWSN-39 (3557 kg ha^{-1}). The other three genotypes did not differ significantly from one another for grain yield production under saline conditions.

The straw yield of different wheat genotypes was statistically similar under non-saline conditions (Fig. 4.4.2). However, the straw yield of different wheat genotypes differed significantly under saline soil conditions with a significantly higher straw yield production by 25-SAWSN-39 (4510 kg ha^{-1}). The other genotypes were statistically similar to one another under saline conditions. The percent reduction in straw yield was the lowest in the case of 25-SAWSN-39 (15%) followed by 25-SAWSN-31 (41%), 25-SAWSN-35 (46%) and 25-SAWSN-47 (47%) in a descending order.

4.4.3.2 Yield components

As for the grain and straw yield production, the genotypes did not differ significantly for different yield components under non-saline conditions although the yield components of all of the genotypes were reduced under saline conditions compared to the non-saline conditions. This section therefore presents the results regarding the yield components of different genotypes under saline conditions only. The data regarding the number of tillers per meter strip indicated significant differences among the genotypes (Fig.4.4.3). The comparison of genotypes on mean

Fig. 4.4.1 Grain yield (kg ha^{-1}) of different wheat genotypes under normal (EC: 2.0-2.9 dS m^{-1}, SAR: 6.7-85(mmol $L^{-1})^{1/2}$) and saline sodic (EC: 13.7 - 17.3dS m^{-1}, SAR: 28. 7- 32.5 (mmol $L^{-1})^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.

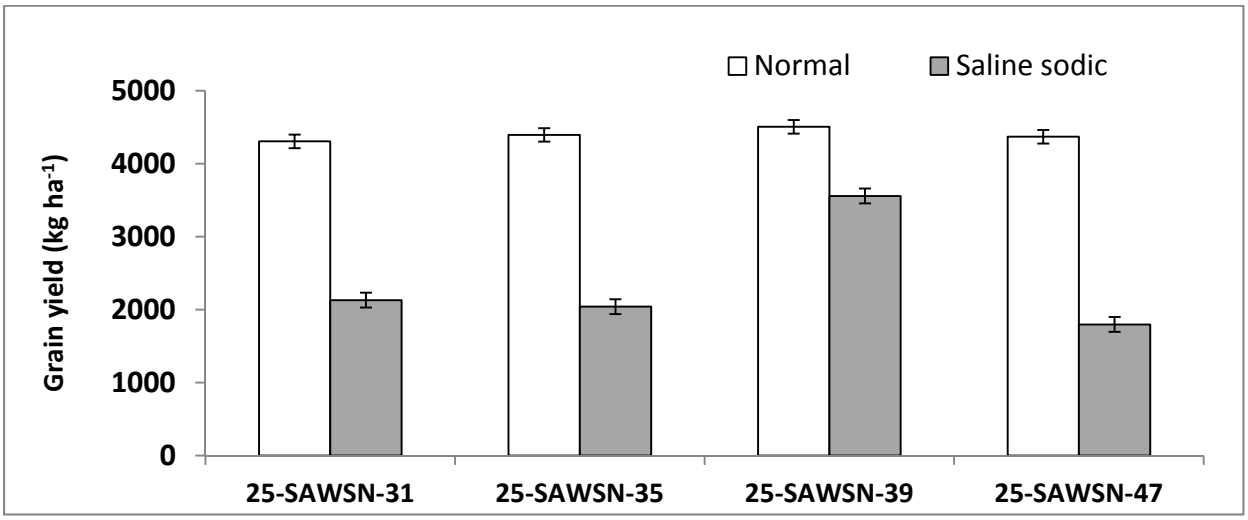


Fig. 4.4.2 Straw yield (kg ha^{-1}) of different wheat genotypes under normal (EC: 2.0-2.9 dS m^{-1}, SAR: 6.7-85(mmol $L^{-1})^{1/2}$) and saline sodic (EC: 13.7 - 17.3dS m^{-1}, SAR: 28. 7- 32.5 (mmol $L^{-1})^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.

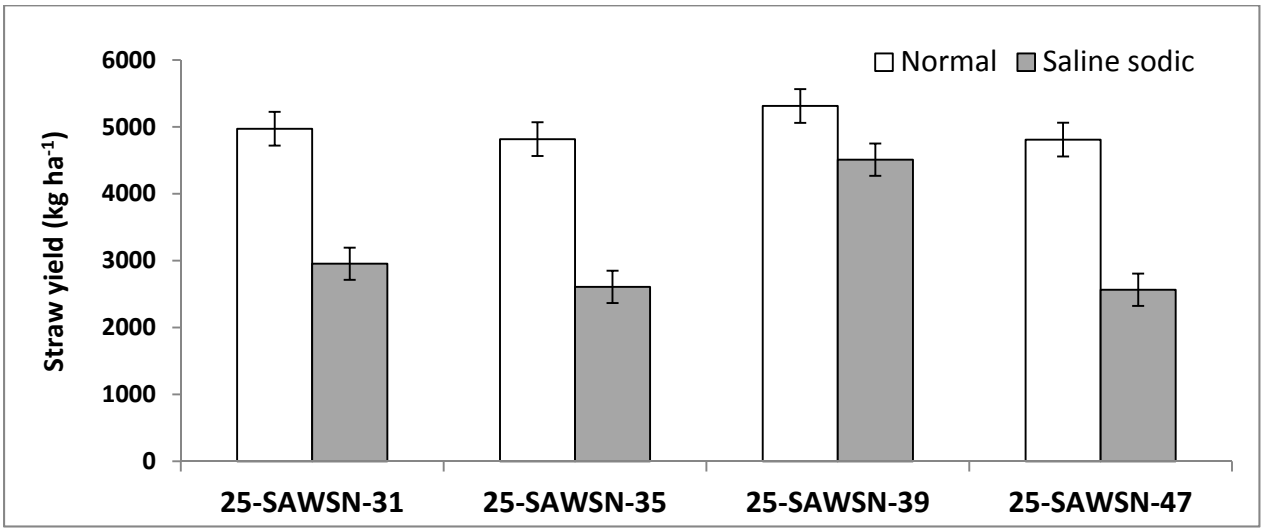


Fig. 4.4.3 Number of tillers m^{-1} strip of wheat genotypes under saline sodic (EC: 13.7 - 17.3dS m^{-1}, SAR: 28. 7- 32.5 $(mmol\ L^{-1})^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.

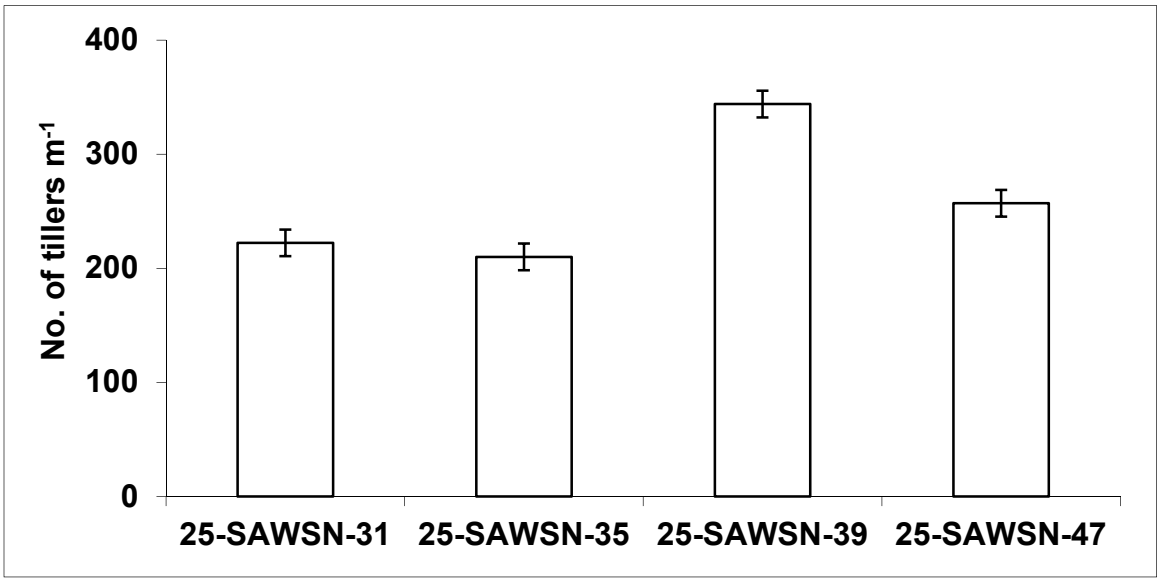
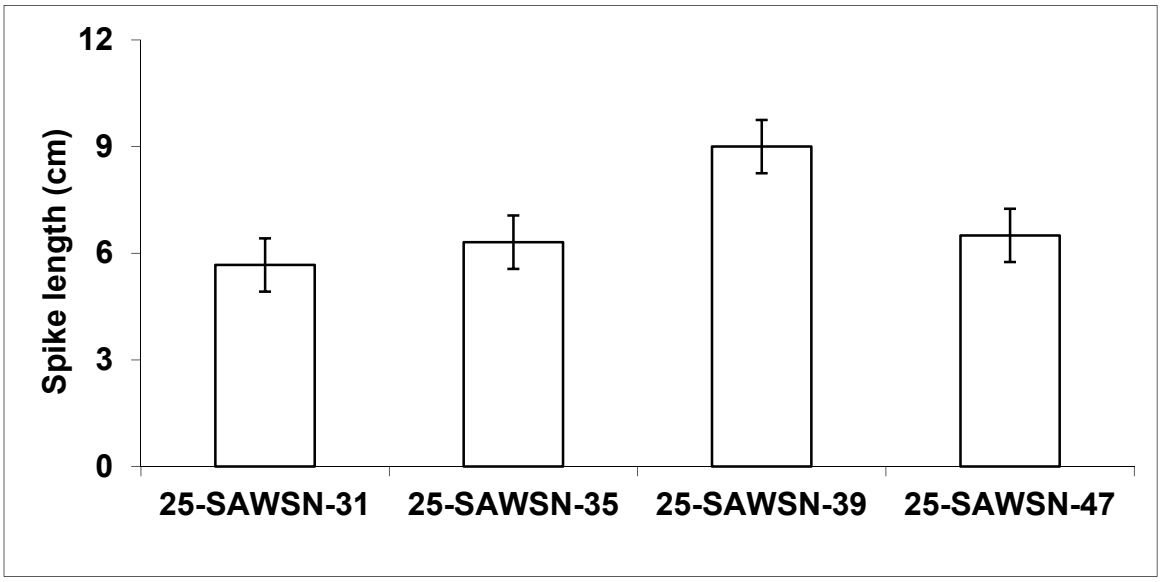


Fig. 4.4.4 Spike length (cm) of wheat genotypes under saline sodic (EC: 13.7 - 17.3dS m^{-1}, SAR: 28. 7- 32.5 $(mmol\ L^{-1})^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.



basis showed that the maximum number of tillers were produced by 25-SAWSN-39 (344) and it was statistically different from the other genotypes whereas the minimum number of tiller per meter strip were produced by 25-SAWSN-35 (210) and it was statistically at par with 25-SAWSN-31. Data regarding spike length showed that genotype 25-SAWSN-39 produced the maximum spike length (9 cm) and it was statistically different from all of the other genotypes. All of the other genotypes were statistically at par with one another. The genotypes did not differ for 100 grain yield (Fig. 4.4.5) and plant height (Fig. 4.4.6).

4.4.3.3 Leaf ionic composition

The data regarding leaf Na^+ concentration showed significant differences among the genotypes (Fig. 4.4.7). The comparison of genotypes showed that leaf Na^+ concentration was the minimum in case of genotype 25-SAWSN-39 and it was statistically different from the other genotypes. All of the other genotypes were statistically at par with one another for leaf Na^+ accumulation. The leaf K^+ concentration also showed significant differences among the genotypes (Fig. 4.4.8). The comparison of genotypes showed that leaf K^+ concentration was the maximum in genotype 25-SAWSN-39 and it was statistically different from all of the other genotypes which were statistically at par with one another. Similar genotypic trend was observed in case of leaf Ca^{2+} concentration (Fig. 4.4.9).

The leaf Cl^- concentration showed significant differences among the genotypes (Fig. 4.4.10). The leaf Cl^- concentration was the maximum in genotype 25-SAWSN-31 and it was statistically different from other genotypes. On the other hand leaf Cl^- concentration was the minimum in case of genotype 25-SAWSN-39 and it also differed significantly from other genotypes. The Cl^- concentration of 25-SAWSN-35 and 25-SAWSN-47 was statistically similar to each other but lower than 25-SAWSN-31 and higher than 25-SAWSN-39.

The leaf K^+: Na^+ ratio and Ca^{2+}: Na^+ differed significantly among the genotypes (Fig. 4.4.11 and Fig. 4.4.12). In both the cases genotype 25-SAWSN-39 showed highest ratio and it differed significantly from the other genotypes. The other three genotypes did not differ significantly from one another.

Fig. 4.4.5 100 grain wt. (g) of wheat genotypes under saline sodic (EC: 13.7 - 17.3dS m^{-1}, SAR: 28. 7- 32.5 $(mmol\ L^{-1})^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.

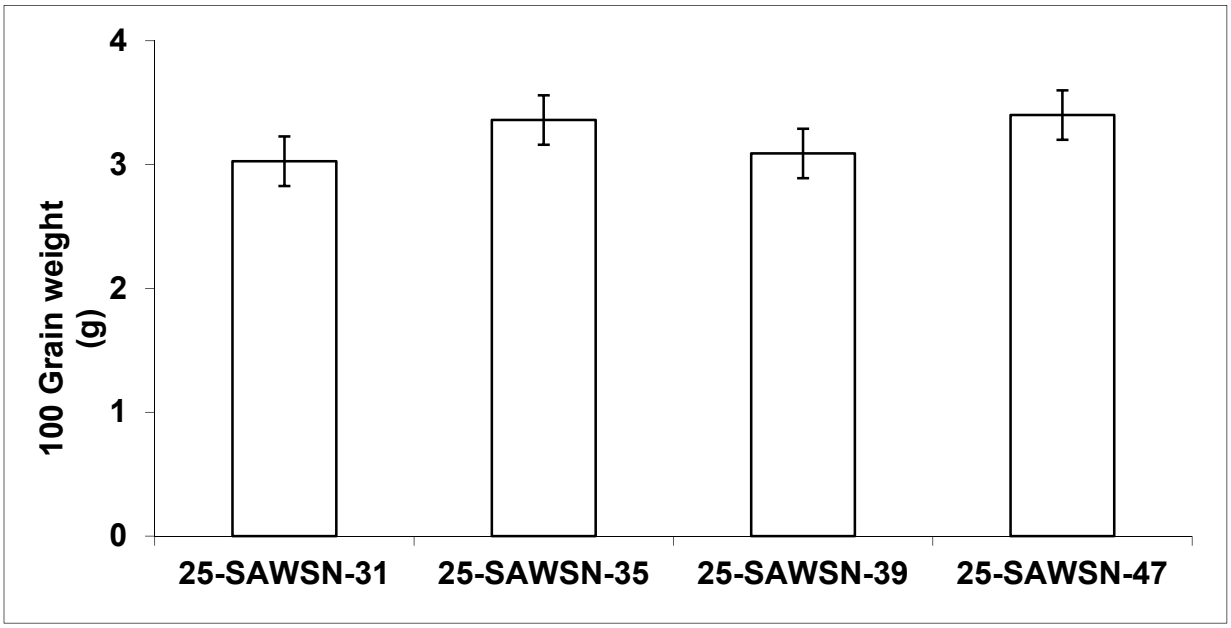


Fig. 4.4.6 Plant height (cm) of wheat genotypes under saline sodic (EC: 13.7 - 17.3dS m^{-1}, SAR: 28. 7- 32.5 $(mmol\ L^{-1})^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.

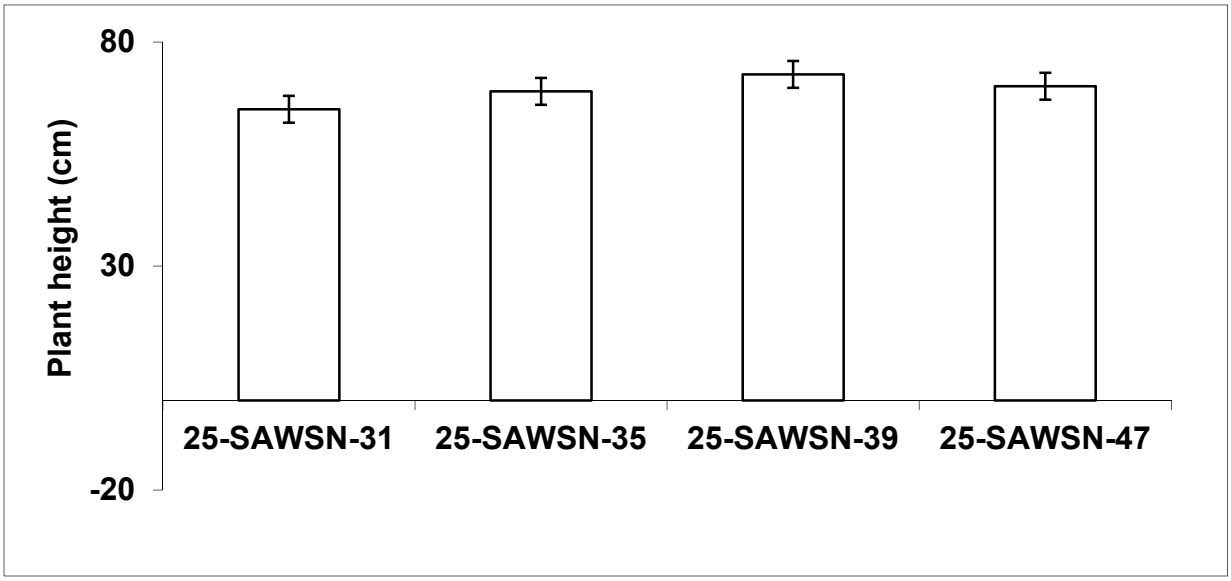


Fig. 4.4.7 Leaf Na^+ concentration (mmol g^{-1} dry wt.) of wheat genotypes under saline sodic (EC: 13.7 - 17.3dS m^{-1}, SAR: 28. 7- 32.5 $(mmol\ L^{-1})^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.

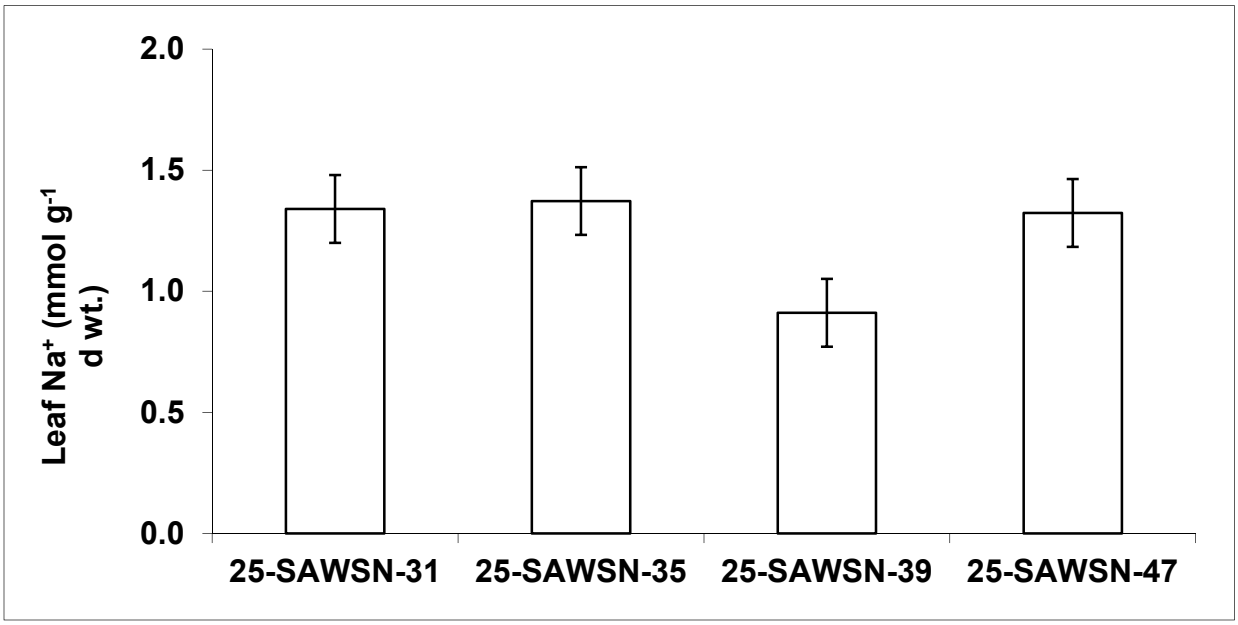


Fig. 4.4.8 Leaf K^+ concentration (mmol g^{-1} dry wt.) of wheat genotypes under saline sodic (EC: 13.7 - 17.3dS m^{-1}, SAR: 28. 7- 32.5 $(mmol\ L^{-1})^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.

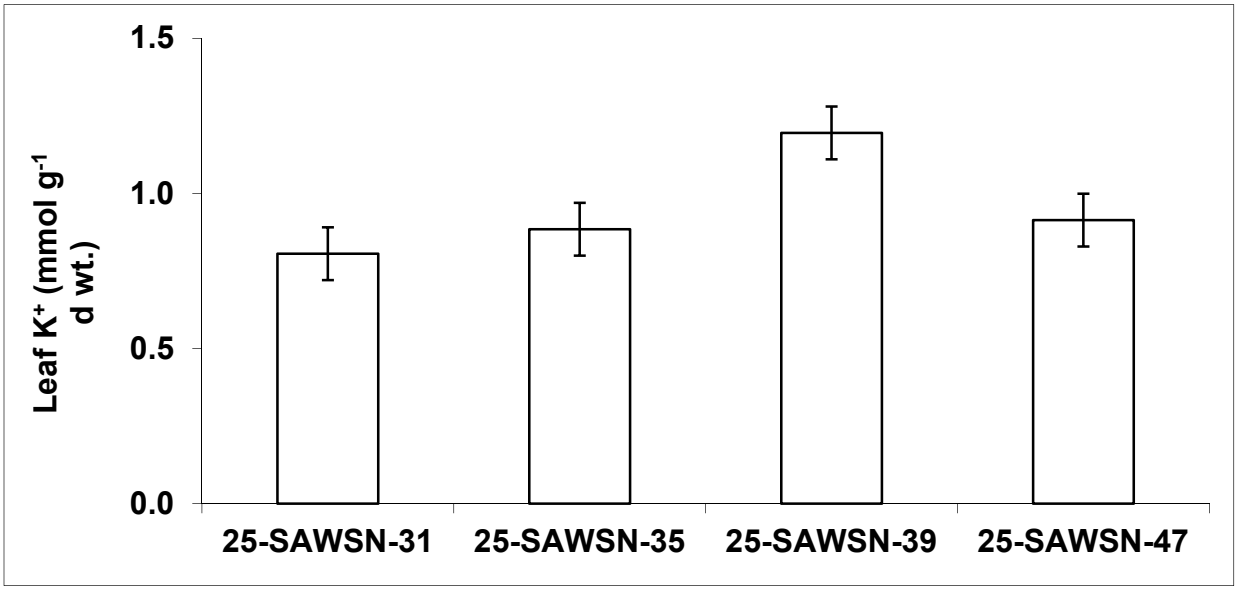


Fig. 4.4.9 Leaf Ca^{2+} concentration (mmol g^{-1} dry wt.) of wheat genotypes under saline sodic (EC: 13.7 - 17.3dS m^{-1}, SAR: 28. 7- 32.5 $(mmol\ L^{-1})^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.

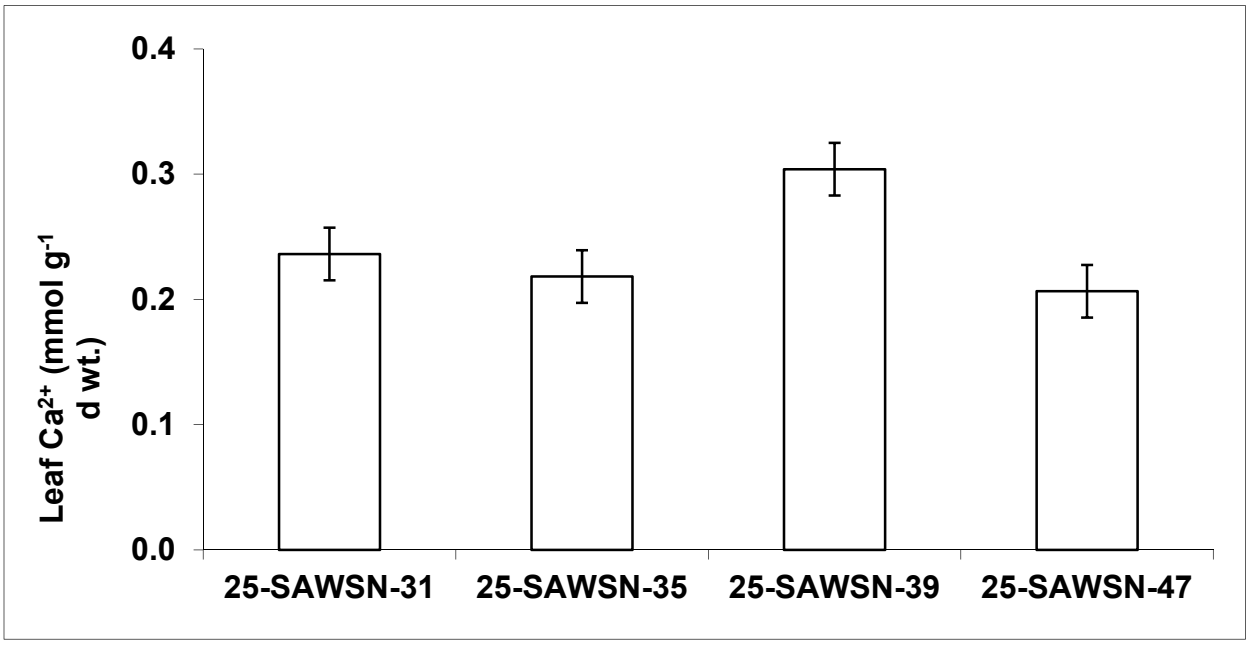


Fig. 4.4.10 Leaf Cl^- concentration (mmol g^{-1} dry wt.) of wheat genotypes under saline sodic (EC: 13.7 - 17.3dS m^{-1}, SAR: 28. 7- 32.5 $(mmol\ L^{-1})^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.

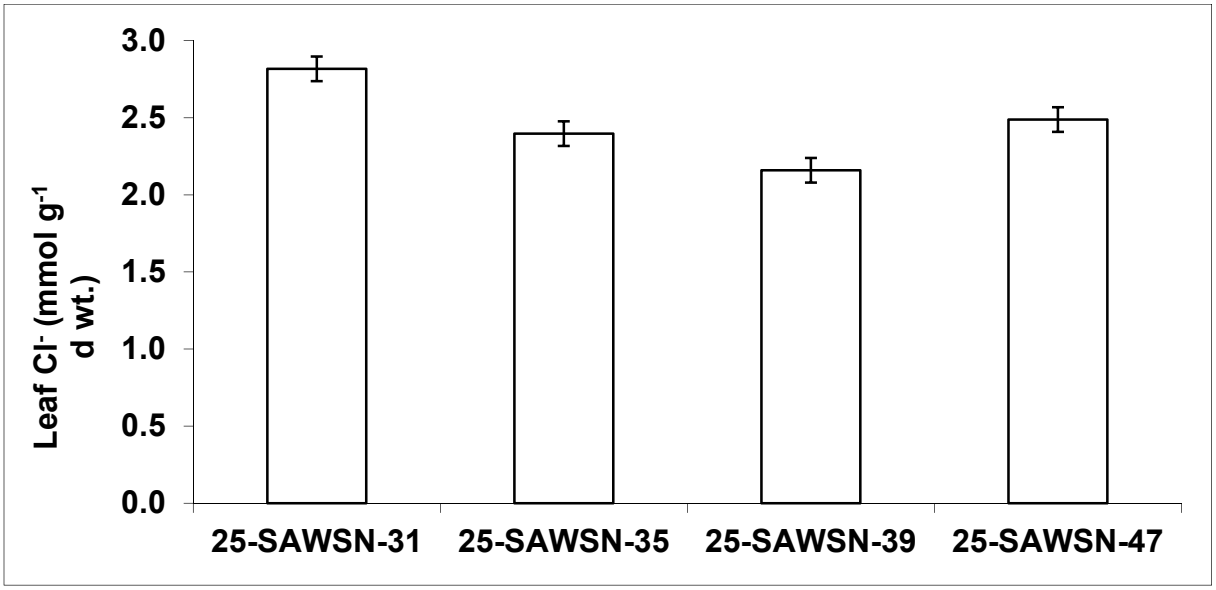


Fig. 4.4.11 Leaf Na^+ concentration (mmol g^{-1} dry wt.) of wheat genotypes under saline sodic (EC: 2.0-2.9 dS m^{-1}, SAR: 6.7-85(mmol L^{-1})$^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.

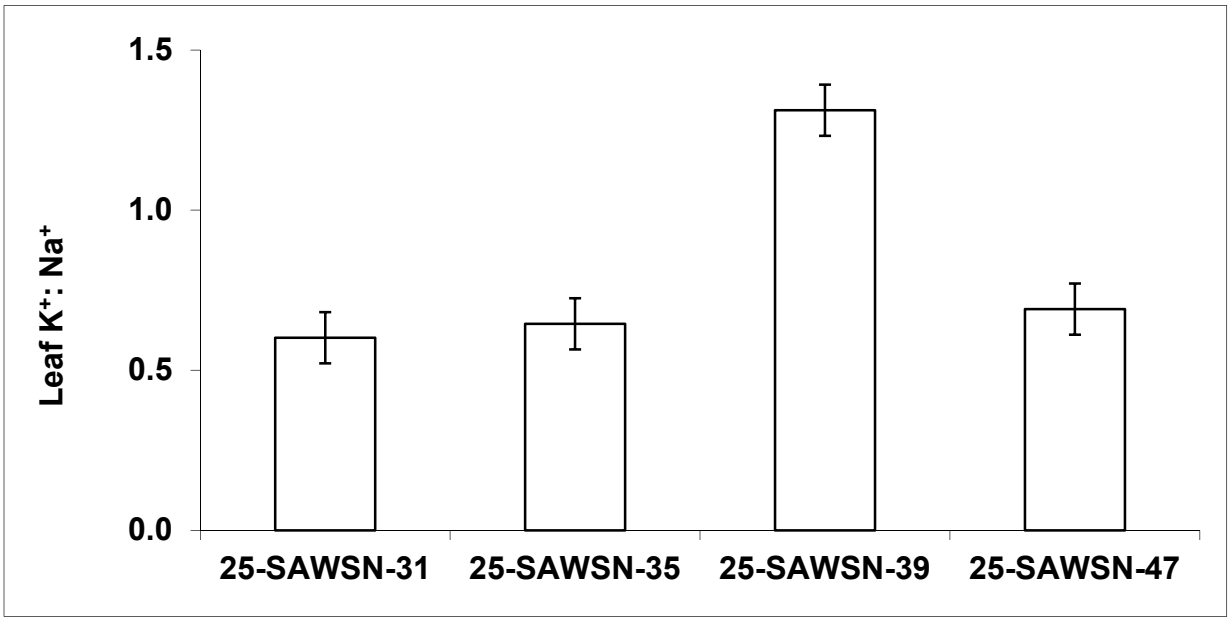
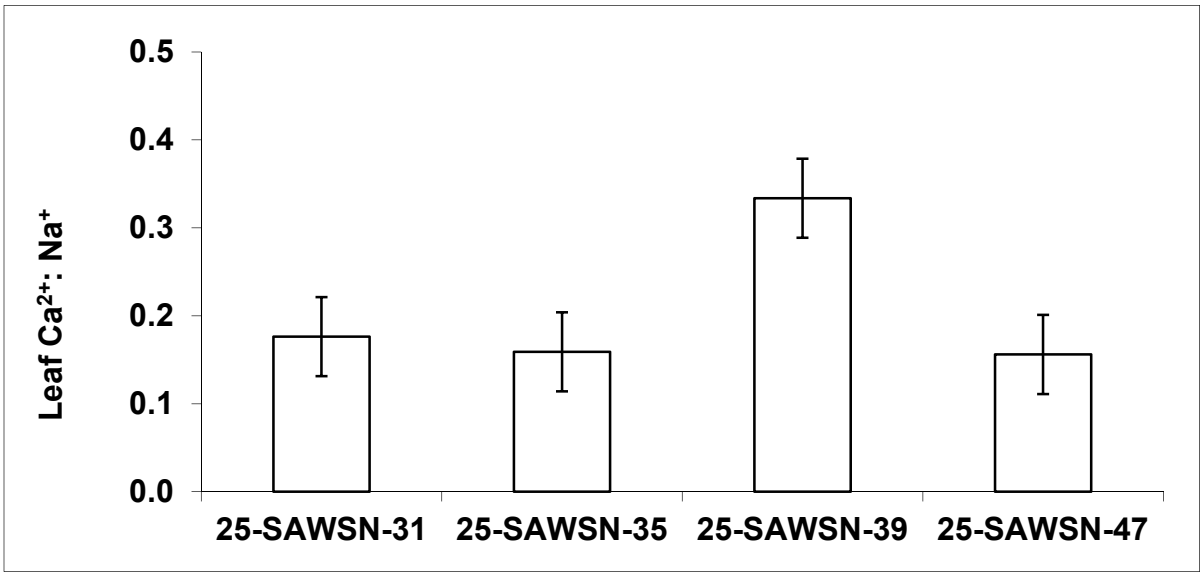


Fig. 4.4.12 Leaf Na^+ concentration (mmol g^{-1} dry wt.) of wheat genotypes under saline sodic (EC: 2.0-2.9 dS m^{-1}, SAR: 6.7-85(mmol L^{-1})$^{1/2}$) field conditions. Error bars show the values of LSD at $P \leq 0.05$.



4.4.4 Discussion

This experiment was conducted to evaluate the performance of selected wheat genotypes in the actual salt affected field conditions. In salt affected soil both salinity and low calcium co-exist. In our solution culture studies the genotypes 25-SAWSN-39 and 25-SAWSN-12 performed better as compared to other genotypes. However in case of salinity and low calcium interaction, 25-SAWSN-39 performed better. Here in the field study again, the performance of the same genotype was better as compared to all other genotypes. So our field experiment confirmed our rest of the experiments.

Plant height, straw yield, grain yield and yield components were reduced with salinity in all of the genotypes. All of these parameters were found maximum in 25-SAWSN-39. The obtained results are in accordance with the findings of Mass *et al*. (1993), Shafi *et al*. (2010) and Ghogdi *et al*. (2012). Due to accumulation of excessive salts, the cells are shrunk. Development of tissues and differentiation of tissues into new cells becomes limited which lead towards suppression of plant growth (Kent and Lauchli, 1985). The reduction in number of tillers may be due to absorption of excessive salts by plants, which ultimately affected the plant growth indirectly by decreasing the amount of photosynthates, water or other growth factors (Munns, 1985; Khathar and Kuhad, 1999). It seems that salt stress might have caused flower sterility, and reduced the transport of assimilates to growing parts. As a result, other yield components like the grain weight were reduced due to salinity. It is reported that reduction of yield under salt stress was used as an indicator of tolerance of the plants to salt stress (Ochiai and Matoh, 2001). Due to higher concentrations of Na^+ and Cl^- in leaves different metabolic processes like photosynthesis and protein synthesis are negatively affected and result in reduced grain weight (Ibrahim, 2003).

High salt concentration disturbs several physiological processes of plants, which results in the reduction of plant growth and development (Taffouo *et al*., 2004). Calcium is required for both cell wall and membrane integrity. Decreased Ca^{2+} availability in salt affected field further reduced plant growth. It might be due to removal of Ca ions from the cell plasma lemma and internal pool (Cramer *et al*., 1985; Lauchli, 1990) and water deficit due to NaCl. The better growth performance of 25-SAWSN-39 might be due to its better adaptation to both salinity and low calcium. The concentrations of Na^+ and Cl^- were lowest in 25-SAWSN-39, while the concentrations of Ca^{2+} and K^+ were the highest.

At higher concentrations, sodium has the ability to displace membrane-associated calcium (Cramer et al. 1985) with the ability to cause Ca deficiency. This effect may be exacerbated when the calcium concentration in the solution is already low. Sodium-induced calcium deficiency has been reported in a number of plant species, including cereals (Ehret et al. 1990; Cramer, 2002). Under field conditions, calcium can be very low in sodic and saline soils, and there is a considerable range in the Na^+: Ca^{2+} ratio in soil solutions (Naidu et al. 1995). But the exact ratio of Na^+: Ca^{2+} affecting the plant growth is not known.

K: Na ratio decreased significantly due to more uptake of sodium as compared to potassium which confirms the findings of Qureshi *et al*. (1991). A positive correlation exists between Na^+ exclusion and salt tolerance of many crops including wheat (Shafi et al., 2010; Ghogdi et al., 2012). When the amount of Na^+ ion in rooting medium is increased, it increases sodium content in leaves with increasing salinity level. This results in passive Na^+ diffusion through damaged membranes and decreased efficiency of exclusion mechanism (Leidi and Saiz, 1997). Potassium influx transporters mediated sodium influx into root cells (Rabhi et al., 2007). High external Na^+ concentration interferes with K^+ absoroption resulting in low root K^+ due to Na^+ antagonistic effect. The preferred uptake of K^+ is an important physiological mechanism of salinity tolerance in many crop plants. The higher K^+: Na^+ and Ca^{2+}: Na^+: ratio was an indicator of better tolerance and more yield attributes of 25-SAWSN-39.

This field study confirms the results of screening study as 25-SAWSN-39 which was tolerant to salinity and calcium stress in solution culture performed better under salt affected field conditions too. Therefore screening of wheat genotypes for salinity should be done with low calcium concentration to better simulate salt affected field condition.

CHAPTER-V

SUMMARY

Salt affected soils are those soils which have higher concentration of soluble salts or exchangeable sodium affecting normal growth of most of the crops. Salt-affected soils include saline, sodic and saline sodic soils. These types of soils are predominantly present in arid and semi arid regions of the globe. In these areas annual rainfall is less than evapotranspirational losses of water. Plants face different types of problems due to the presence of salts. Calcium (Ca^{2+}) is a vital nutrient for all plants. Its capacity to form linkages between molecules gives it a key role in keeping the membranes and cell walls in proper structure and working. Calcium also plays the role of secondary messenger in the cell. The excessive uptake of salts may result in reduced concentration of essential nutrients and their deficiencies. Imbalance of the ions in the cell occurs due to the competition of Na^+ with K^+ and Ca^{2+}, and of chloride and sulphate with nitrate and phosphate. Under salinity and particularly when associated with sodicity, the availability and uptake of Ca^{2+} is reduced that results in the loss of membrane integrity and other disorders associated with Ca^{2+} deficiency in plants.

A wheat genotype efficient in uptake and utilization of calcium under saline conditions may be better able to withstand saline and sodic conditions in the field. Very little information is available on wheat response to salinity and low Ca^{2+} as screening of wheat genotypes has usually been done against salinity alone. The studies reported in this thesis have been planned with the following objectives:

1. To evaluate the performance of different wheat genotypes against salinity and low calcium.
2. To study the physiological and biochemical characteristics of tolerant and sensitive wheat genotypes in response to salinity and low calcium.
3. To study the growth and yield performance of the selected wheat genotypes under salt affected field conditions.

Ten wheat genotypes were screened against salinity (125 mM NaCl) and low calcium in nutrient solution. All of the physical growth parameters including shoot length, root length, shoot and root fresh and dry weights decreased significantly under salinity and low calcium alone as well as under their combined presence. Reduction was more pronounced under the combined stress of salinity and low calcium and different genotypes differed significantly in different treatments. In the saline treatment, the genotypes 25-SAWSN-39 and 25-SAWSN-31 produced more shoot fresh and dry weights, showed less accumulation of Na^+ and Cl^- and higher K^+ and Ca^{2+} whereas the genotypes 25-SAWSN-35 and 25-SAWSN-47 produced less shoot fresh and dry weights, had less accumulation of K^+ and Ca^{2+} and high accumulation of Na^+ and Cl^-. In salinity +low calcium treatment the genotype 25-SAWSN-39 behaved as a tolerant genotype where as 25-SAWSN-31 behaved similar to the sensitive genotype and these differences were due to high accumulation of Ca^{2+} in the 25-SAWSN-39 and vice versa.

On the basis of this evaluation, tolerant and sensitive genotypes were used for further studies. Selected genotypes from the first experiment were grown in nutrient solution in a second experiment under same treatments. Shoot fresh and dry weights of all of the genotypes was decreased with the salinity and low Ca^{2+} supply. However, this reduction was more pronounced in the sensitive genotypes (25-SAWSN-35 and 25-SAWSN-47) than in the salt tolerant genotypes (25-SAWSN-39). In shoot, the concentrations of calcium and potassium decreased, while the concentration of Na^+ and Cl^- increased under saline conditions alone and in combination with low calcium. Salt tolerant genotypes accumulated less Cl^- and Na^+, while more K^+ in the shoot than salt sensitive genotypes. Our results regarding relative water content and membrane stability index showed a reduction pattern under saline and salinity + low calcium conditions. Salt tolerant genotypes showed more relative water contents and membrane stability index than salt sensitive genotypes. Chlorophyll content, photosynthetic rate, stomatal conductance and transpiration rate were also decreased with salinity and low calcium supply, although salt tolerant genotypes showed less reduction compared to sensitive genotypes. Water potential and osmotic potential become more negative under stress conditions. These values were more negative for salt sensitive genotypes than salt tolerant genotypes. Salt tolerant genotypes showed more turgor potential than salt sensitive genotypes. Results of our second study showed that under saline conditions alone and in combination with low calcium, the activities of antioxidant enzymes (CAT, POD and SOD) increased. Tolerant genotypes 25-SAWSN-39

showed higher levels of these antioxidants as compared sensitive genotypes (25-SAWSN-35, 25-SAWSN-47). The behavior of wheat genotype 25-SAWSN-31 was very interesting it could tolerate salinity in the presence of good calcium concentration but could not tolerate salinity and behaved like a salt sensitive genotype under salinity + low calcium treatment. Different physiological and biochemical parameters of the genotype 25-SAWSN-31 supported its growth under saline and saline + low calcium treatments.

The behaviour of the selected genotypes was further tested in the actual salt affected field conditions. In salt affected soil both salinity and less availability of calcium co-exist. In our solution culture studies the genotypes 25-SAWSN-39 and 25-SAWSN-31 performed better as compared to other genotypes under saline conditions. However in the case of salinity and low calcium interaction, 25-SAWSN-39 performed better than all of the other genotypes. Here in the field study again, the performance of the genotype 25-SAWSN-39 was better as compared to all of the other genotypes where as the genotypes 25-SAWSN-31 (which was tolerant to salinity under good calcium concentration) could not perform better under salt-affected field conditions. So the field experiment confirmed the hypothesis that a Ca^{2+} efficient genotype could better tolerate salt-affected field conditions where salinity co-exist with sodicity and low calcium.

On the basis of results of the presented studies, it can be concluded that salinity stress caused a significant reduction in growth of wheat genotypes which was further accentuated by low calcium. Genotypes 25-SAWSN-39 and 25-SAWSN-31 maintained higher K^+: Na^+ ratio and accumulated more of Ca^{2+}, and were ranked as tolerant under salinity treatment whereas 25-SAWSN-35 and 25-SAWSN-47 showed less K^+: Na^+ ratio and accumulated less Ca^{2+} and were considered sensitive genotypes. Tolerant and sensitive genotypes showed different responses to the interactive effect of salinity and low calcium. This interactive effect of salinity and low calcium decreased the growth more than salinity alone. In the combined treatment of salinity + low calcium the genotype 25-SAWSN-39 could maintain its tolerance where as the genotype 25-SAWSN-31 behaved like a sensitive genotype as it could not uptake and utilize calcium efficiently. The genotype 25-SAWSN-39 was also promising under salt-affected field conditions and can be recommended to the farmers and may also be used for the development of more salinity tolerant wheat genotypes by the breeders.

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