Chapter - SIX

Summary & Conclusions
Growth and development of the plant is influenced by various environmental factors like drought, temperature, salt, and high light. When any of these exceed the optimum tolerance, this results in stress to the plant, which in turn affects its developmental, structural, physiological and biochemical processes (Jaleel et al., 2008a–d; Tuteja et al., 2009). In arid and semi-arid regions, salinity in soil or water is one of the most important abiotic factors that limit plant growth and productivity (Flower, 2004). Sodium chloride is one of the most abundant salts that contribute to soil salinity (Koca et al., 2007). Salinity can affect growth and yield of most crops. High salinity is known to cause both hyper-ionic and hyper-osmotic effects in plants, leading to membrane disorganization, increase in activated oxygen species production and metabolic toxicity (Joseph and Jini, 2011). It invariably leads to oxidative stress in the plant cell due to higher leakage of electrons towards $O_2$ during photosynthetic and respiratory processes leading to enhancement in reactive oxygen species (ROS) generation (Asada, 1999). ROS such as hydrogen peroxide, superoxide ions, singlet oxygen, peroxides etc are toxic molecules for plant metabolism (Apel and Hirt, 2004). All cellular macromolecules including DNA are damaged due to the deleterious effects of ROS (Tuteja et al., 2009). Plant systems are equipped with enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), ascorbic acid (AsA), glutathione etc. They minimize the deleterious effects of ROS. Every compartment of the cell contains one or more antioxidants that act on a particular ROS and detoxifies it (Nobuhiro and Mittler, 2006). Introduction or over-expression of selected genes is the promising way to generate stress tolerant plants (Mathur et al., 2008).

Chickpea is highly sensitive to salinity, like many other leguminous crops (Ashraf and Waheed, 1993). Therefore, identifying sources of tolerance to salinity
will be of great practical importance. Since chickpea is indigenous to arid areas, it may have a degree of adaptation to various environmental stresses. It thus, offers a valuable germplasm for breeding purposes and for the determination of more tolerant cultivars that give minimum depression in yield when grown in saline soils and may be an efficient tool resolving the salinity problem to some extent. On the other hand, DNA markers are being increasingly utilized in cultivar development, quality control of seed production, measurement of genetic diversity for conservation and management, varietal identification and intellectual property production. Among various molecular markers, RAPD are well suited for diversity studies because they are technically simple, relatively inexpensive and requires small quantity of DNA. Biochemical and molecular techniques has the great opportunity to marry together to find out the salt tolerant genotypes in salinity tolerance research. In our study, antioxidant defence system was monitored in eight genotypes of chickpea in response to salt stress. The genetic variability of these genotypes was investigated through isozyme and RAPD profiling. Finally, molecular marker of salt tolerant genotype was developed. The summary of this work is given below-

In chickpea genotypes, biochemical variability was studied in terms of differential induction of antioxidant defense in response to various levels of salt stress. Seeds of chickpea genotypes (SKUA-01, SKUA-02, SKUA-03, SKUA-04, SKUA-05, SKUA-06, SKUA-07 and SKUA-08) procured from Sher-e-Kashmir University of Agricultural Science and Technology, Kashmir, were sown in soilrite. After three days of proper germination, young seedlings of uniform size were transferred to 250 ml plastic containers containing one fourth strength Hoagland's solution (Hoagland and Arnon, 1950). The growth chamber was maintained at a photosynthetic photon flux density of 430 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), 16 h of light, 8 h dark, and a relative humidity of 65%. Fifteen-day-old plants were treated with various level of \( \text{NaCl} \), in the nutrient solution. \( T0=0 \text{mM}, T1=25 \text{mM}, T2=50 \text{mM}, T3=75 \text{mM}, T4=100 \text{mM}, T5=150 \text{mM NaCl} \). Samples were
collected at 1st, 3rd, 5th and 7th days of NaCl treatment for measuring growth performance, activities of enzymatic antioxidant and contents of non-enzymatic antioxidant. Following observations were observed:

Chickpea genotypes showed significant reduction in fresh weights and dry weights in response to NaCl stress, however the maximum reduction was observed in the SKUA-03, SKUA-04 and SKUA-05 while as the minimum reduction was observed in the SKUA-06 and SKUA-07 and were able to tolerate NaCl treatments upto the level of 100 mM. Similar trend was observed in the root, shoot length and leaf area in a dose dependent manner in all the genotypes under NaCl stress. Growth response to salinity is often regarded as a basis of evaluation for tolerance. In our experimental findings, salt tolerant genotypes, possessing inherently the higher growth rate when compared to salt sensitive genotypes.

A decrease in the total chlorophyll content of the chickpea genotypes was observed with the increase in the levels of salt stress. Genotypes SKUA-06 and SKUA-07 showed the minimum total chlorophyll content reduction. There was a decrease in the total chlorophyll content under higher doses of NaCl treatment in SKUA-01, SKUA-02 and SKUA-08 while as genotypes SKUA-03, SKUA-04 and SKUA-05 showed a maximum reduction in total chlorophyll content at all the levels and all days of treatment.

Soluble protein content in SKUA-06 and SKUA-07 increased with the increase in the levels of salt stress while as in SKUA-01, SKUA-02 and SKUA-08 genotypes soluble protein content showed reduction at 75mM, 100mM and 150mM of NaCl stress. However, SKUA-03, SKUA-04 and SKUA-05 showed maximum reduction at all the days of treatment. The decrease in protein content is due to the effects of sodium chloride on protein synthesis (Omar et al., 1993). Furthermore, prolonged stress could affect protein synthesis and provoke its decline (Caplan et al., 1990).
Lipid peroxidation in leaves of all the genotypes was measured as MDA content. The MDA content was maximum in the SKUA-03, SKUA-04 and SKUA-05 and minimum in SKUA-06 and SKUA-07 genotypes at all the levels of salt treatment. While as in SKUA-01, SKUA-02 and SKUA-08 the MDA content increased at 75mM, 100mM and 150mM of NaCl stress. The level of lipid peroxidation, measured as malondialdehyde (MDA) content, has been considered an indicator of salt-induced oxidation in cell membranes and a tool for determining salt tolerance in plants (Hernández and Almansa, 2002). Lipid peroxidation rate was found to increase with increase of salt stress especially in sensitive cultivars (Azevedo Neto et al., 2006; Arora et al., 2008).

Antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) were maximum in SKUA-06 and SKUA-07 genotypes at all the levels of salt treatments. Genotypes SKUA-01, SKUA-02 and SKUA-08 showed maximum antioxidant activity upto 50mM of NaCl treatment there after there was decline. SKUA-03, SKUA-04 and SKUA-05 genotypes showed the minimum antioxidant activities. In the present study, the responses of SOD, CAT, APX, and GR enzyme activities and MDA content suggest that oxidative stress is an important component of salt stress in chickpea genotypes. Often, increase in antioxidant activity is identified as the key in the prevention of salt damage, while sensitive species typically exhibit either no change or a decrease in activity (Hernandez et al., 2000; Shalata et al., 2002).

Non-enzymatic antioxidants like GSH content and total ascorbate content showed large variation under salinity stress when compared to control. The GSH content and total ascorbate content was increased in SKUA-06 and SKUA-07 genotypes by NaCl treatment. While as, genotypes SKUA-01, SKUA-02 and SKUA-08 showed increase upto 50mM of NaCl treatment there after there was decline. In SKUA-03, SKUA-04 and SKUA-05 genotypes the non-enzymatic antioxidants decreased with increase in salt stress. Studies
showed that GSH and total ascorbate content plays a protective role in salinity tolerance by the maintenance of the redox status and is correlated with the stress-protecting mechanism of the plant respectively (Shalata et al., 2001).

– Proline accumulation is one of the most frequently reported modifications induced by salt stress in plants and is often considered to be involved in stress resistance mechanisms. In the present study maximum proline content was observed in SKUA-06, SKUA-07 and minimum in SKUA-03, SKUA-04, and SKUA-05. While as in SKUA-01, SKUA-02 and SKUA-08 genotypes the proline content increased only upto 50mM of NaCl after that there was no significant change when compared to control.

On the basis of growth performances and antioxidant defence system, SKUA-06 and SKUA-07 genotypes of chickpea were identified as salt-tolerant and SKUA-03, SKUA-04, SKUA-05 as salt-sensitive genotypes.

– For isozyme analysis, one salt-tolerant (SKUA-06) and one salt-sensitive genotypes (SKUA-03) were selected. In SKUA-06 all the isoforms of SOD were found in the control but with increase in the level of treatment only MnSOD and Cu/ZnSOD were found. Where as in SKUA-03 only the MnSOD was found with the increase in the level of treatment. A significant decrease in FeSOD after the stress in leaf tissues of chickpea seems to diminish the ability to scavenge oxygen radicals, favoring an accumulation of oxygen radical species, which may be one reason for the increase in lipid peroxidation in leaves. Our results agree with the reports of Eyidogan and Oz (2005) has noted three SOD activity bands (MnSOD, FeSOD and Cu/ZnSOD) in C. arietinum under salt stress. It may be suggested that the synthesis of the new SOD isozyme might contribute to increased SOD activity under salt stress.

– Genetic diversity among the chickpea genotypes was studied by using the RAPD markers and has been correlated with the biochemical observations.
1) The RAPD-based dendrogram of chickpea genotypes displayed the genetic relationships between these accessions.

2) SKUA-06 and SKUA-07 genotypes are salt tolerant showed the similarity co-efficient of 26%.

3) The salt sensitive genotypes SKUA-03 and SKUA-04 with similarity of 16.5%, SKUA-05 showed 14.5% of similarity co-efficient respectively.

On the basis of RAPD data, SCAR (sequence characterized amplified region) marker was developed for the salt-tolerant genotypes of chickpea. Amplification of genomic DNA of all the genotypes of chickpea with SCAR primers produced a DNA fragment of 380 bp in salt-tolerant genotypes only, confirming the specificity of SCAR primer for salt-tolerant cultivar of chickpea.

In conclusion, the present study revealed the variability in salt tolerance behaviour of chickpea genotypes at biochemical and molecular levels. There is clear indication of role of antioxidant defence system in conferring the salt tolerance and sensitiveness in chickpea genotypes. SKUA-03, SKUA-04 and SKUA-05 were identified as the salt-sensitive genotypes, and SKUA-06 and SKUA-07 as salt-tolerant genotypes of chickpea. Isozyme and RAPD profiling also provide sufficient information of variability in the tolerance and sensitive genotypes of chickpea genotypes. RAPD-based sequence characterized amplified region (SCAR) marker was developed for the salt-tolerant genotype (SKUA-06 and SKUA-07) of chickpea. This marker can be helpful in screening salt-tolerant genotypes of chickpea.