# Chapter 3

# BIOCHEMICAL CHANGES SEEN IN GROUNDNUT DUE TO SALT STRESS

## 3.0 Introduction

Due to increased environmental stress the balance between the production of reactive oxygen species (ROS) and the scavenging activity of antioxidants is upset resulting in oxidative damage. ROS can be important mediators of damage to cell structures, nuclei acid, lipids and proteins. Generation of ROS in plant tissues, such as superoxide anion radicals ($O_2$•-), hydroxyl radicals (•OH), singlet oxygen ($^1O_2$) and hydrogen peroxide ($H_2O_2$), is a common response observed in salt-stressed plants (Vaidyanathan et al., 2003; Azevedo-Neto et al., 2006). So, in addition to its known osmotic and ionic effects, salt stress is also manifested as an oxidative stress (Hernández et al., 1995). These ROS are highly reactive and can alter the normal cellular metabolism through oxidative damage to proteins and nucleic acids, as well as causing peroxidation of membrane lipids (Møller et al., 2007). In order to prevent oxidative damages, plants have evolved a complex antioxidant system, which includes both enzymatic (scavenger enzymes) and non-enzymatic (mainly ascorbate and glutathione) components differentially found in cell compartments (Mittler, 2002). Enzymatic components include superoxide dismutase (SOD), which is a major scavenger of $O_2$•- free radicals, converting them into O and HO. The HO may be scavenged by a variety of peroxidases; including catalase (CAT), ascorbate peroxidase (APX) which uses ascorbate as electron donor in the first step of the ascorbate glutathione cycle, and guaiacol peroxidase (GPX), that decompose $H_2O_2$ by oxidation of co-substrates such as phenolic compounds and/or ascorbate. The capacity to scavenge ROS and to reduce their damaging effects on macromolecules appears to represent an important stress tolerance trait (Amor et al., 2005). A close correlation between the antioxidant capacity and NaCl tolerance has been demonstrated in several crops such as rice (Vaidyanathan et al., 2003), tomato (Mittova et al., 2002) and maize (Azevedo-Neto et al., 2006). In view of the above discussion, we investigated the effects of salt stress on growth and development of groundnut (one of the major sources of income for agriculture in Gujarat) seedlings *in vivo* and *in*

*vitro*, which were associated with changes in antioxidative enzyme system. This knowledge will contribute to improve our understanding on the groundnut's acclimation process to salinity conditions.

### 3.1 Effect of salt stress on biochemical parameter: $H_2O_2$, catalase and paroxidase.

Salt stress induces ionic stress and osmotic stress in plant cells. A direct result of these primary effects is the enhanced accumulation of ROS that are harmful to plant cells at high concentrations.

These are small molecules having unpaired valance shell electrons and are produced continuously as byproduct of various physiological metabolic pathways, such as photosynthesis, photorespiration and $CO_2$ assimilation. They are formed during certain redox reactions and during incomplete reduction of oxygen or oxidation of water by the mitochondrial or chloroplast electron transfer chains.

Apart from salinity, various environmental factors of stresses like air pollution (increased amounts of ozone or sulfur dioxide), oxidant-forming herbicides such as paraquat dichloride (methyl viologen, 1,1 -dimethyl-4,4 -bipyridinium), heavy metals, drought, heat and cold, wounding, UV light, and highly intense light conditions that stimulate photoinhibition also leads to increased ROS production. Due to the unpaired valance shell electrons the ROS are highly reactive and can react readily with neighboring molecules.

ROS were traditionally considered toxic to plants. However, recently it has become apparent that plants actively produce these molecules which may control many different physiological processes such as abiotic and biotic stress response, pathogen defense and systemic signaling.

Under non-stress environmental conditions, ROS can modulate usual events such as DNA synthesis, enzyme activation, selective gene expression and regulation of the cell cycle, hence the indirect control of cellular differentiation, growth, development, and death (Franck et al., 2000).

Thus ROS may act as damaging, protective or signaling factors and the nature of its role depends on the delicate equilibrium between ROS production and scavenging at the proper site and time (Gratão et al., 2005).

## 3.2 Damages caused by ROS

Higher accumulation of ROS in cell leads to damages in the form of change in membrane permeability, increase in rate of lipid peroxidation and thus spreading of ROS effects like increase in rate of mutation, indirect role in chlorosis etc. These changes could culminate in cell death.

In plants, ROS represent the products of successive single-electron reductions of oxygen (Eqn. 1; see Fig. 3.1). Ground state $O_2$ is a triplet state molecule and is relatively unreactive, but it can accept a single electron from a variety of reducing agents, particularly if it gets converted to the singlet state (for instance, by the transfer of energy from photochemically excited chlorophyll), if the reducing agent is photochemically excited, or if the oxygen and reducing agents are in the presence of an appropriate enzyme. The product of this reduction is superoxide, $O_2^-$, which under acidic conditions protonates to the hydroperoxyl radical (Eqn. 2 in Fig. 3.1). Subsequent reductions will form hydrogen peroxide ($H_2O_2$), hydroxyl radical, and water.

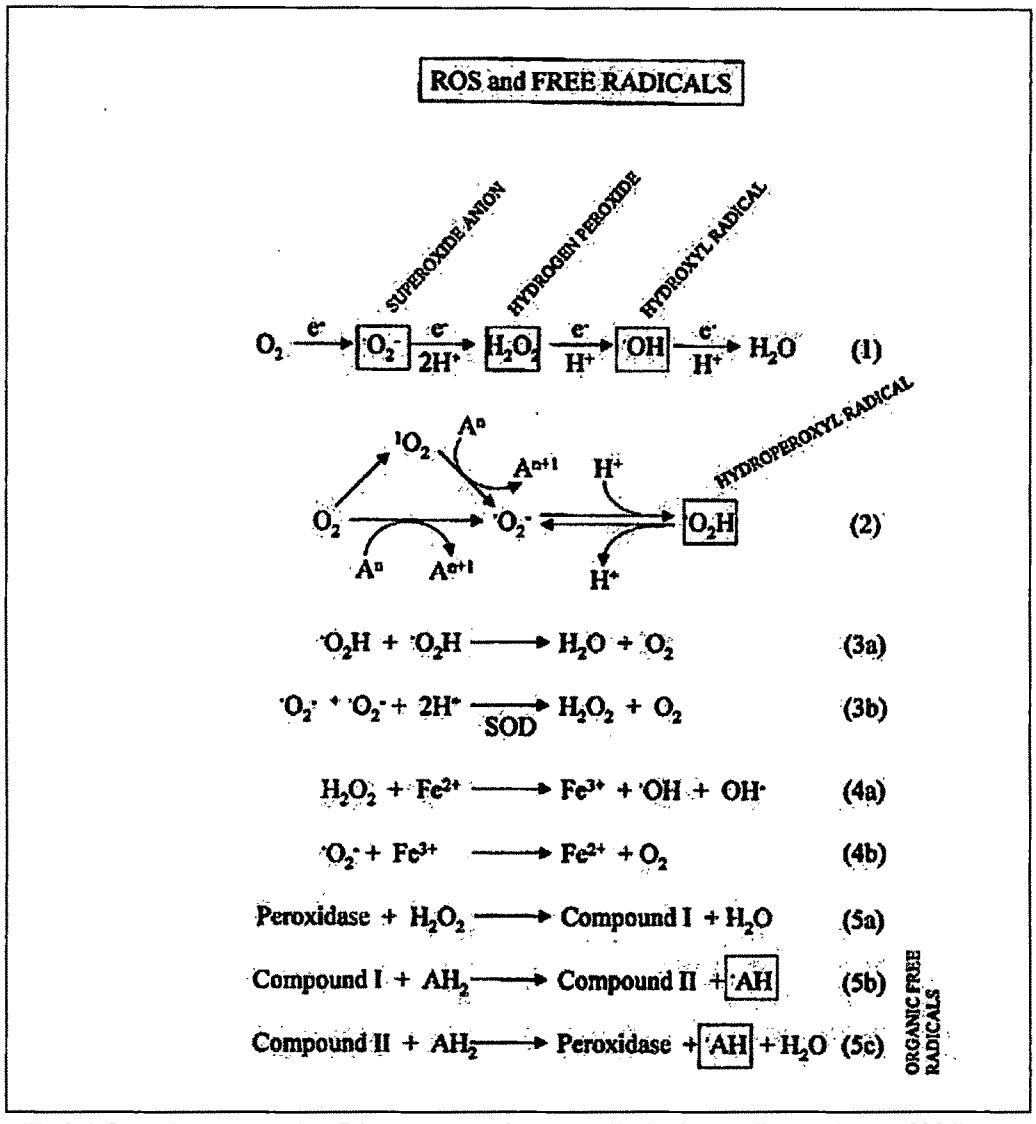


*Fig.3.1.* Reactions producing ROS and organic free radicals. (Cassells and Curry, 2001)

In cells, the appearance of $H_2O_2$ and hydroxyl radical generally does not occur through the sequence of reactions shown in Eqn. 2. Instead, once superoxide has been synthesized, interconversions of the various ROS may account for the appearance of the more reduced forms. For instance, disproportionation of hydroperoxyl radical, or of superoxide in the presence of superoxide dismutase (SOD), forms hydrogen peroxide (Eqn. 3a,b in Fig. 3.1). The combination of hydrogen peroxide and superoxide in the presence of iron or other transition metals forms hydroxyl radical (the Fenton reaction - Eqn. 4a,b in Fig. 3.1). Thus, in the oxidative burst, an enzymatic synthesis of superoxide may lead to the appearance of the other ROS. However, alternative mechanisms for the synthesis of $H_2O_2$ are possible.

Plants scavenge and dispose of the reactive molecules by use of antioxidant defence systems present in several subcellular compartments. There are other ways in which the synthesis of ROS could serve to defend plant cells against pathogens. $O_2^-$ and $H_2O_2$ are required for lignification. When the apoplastic matrix is modified, peroxidase-catalysed reactions that generate free radicals lead to the cross-linking of cell wall proteins, polyphenolics, and cutin, forming walls that better resist penetration by pathogens (Pedreno et al., 1995; Franck et al., 2000). In certain systems, ROS, including $H_2O_2$, superoxide, and possibly hydroxyl radicals may serve as extracellular signals, stimulating defense responses in other parts of the plant.

ROS, especially $HO^-$, are highly destructive to lipids, proteins and nucleic acids. Peroxidation of membrane lipids and other critical cell components can also occur through the production of organic free radicals. Hydroxyl radical is extremely reactive, with a life-time in the cell of nanoseconds. In cells it can oxidise - abstract $H^+$ from almost any available biological molecule, forming an organic radical. Normally, the production of hydroxyl radical is limited by the presence of SOD, which keeps superoxide concentration low. Catalase or peroxidases, which remove $H_2O_2$, also restrict the production of hydroxyl radical. On the other hand, free radicals may be formed also by peroxidases, using $H_2O_2$ as initial oxidant (Eqn. 5a,b,c; Fig. 3.1). The summary of the harmful effects of ROS when the pro- and anti-oxidant balance is perturbed in oxidative stress. (Fig. 3.3).

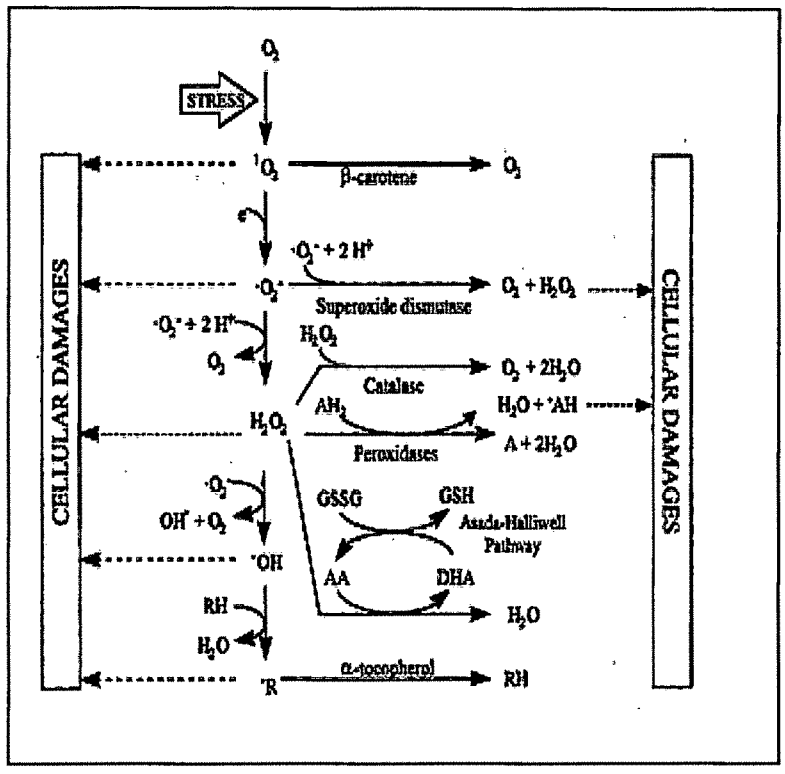


Fig. 3.2. Defense systems (enzymes, antioxidants) against ROS

Source : (Gasper et al., 2002).

Two types of scavenging-pathways / antioxidant defense systems exists in plant cells in protection against oxidative stress and damage caused by ROS 1. Non- enzymatic and 2. Enzymatic (Fig. 3.2 and 3.3). The major antioxidant species in plants are ascorbate (vitamin C), reduced glutathione (GSH), α-tocopherol (vitamin E), and carotenoids; polyamines and flavonoids also may provide some protection from free radical injury (Foyer and Noctor, 2005). Furthermore, the metal chelators, such as metallothionins (MT) and ferritins (FT), due to their metal-binding activity play an important role in metal metabolism and detoxification (Briat et al., 1999).

The ascorbate-glutathione cycle is the major antioxidant pathway in plastids, where ROS are generated during normal biochemical processes that include photosynthetic transfer of electrons. The photosynthetic apparatus receives additional protection from oxidative damage by the exothermic production of the xanthophyll zeaxanthin (Gaspar et al.,2002).

These compounds and enzymes are not distributed uniformly, so defence systems vary among specific subcellular compartments.

ROS are produced in root nodules of nitrogen-fixing plants and are scavenged by enzymatic antioxidants. Regulation of the concentrations of antioxidants and antioxidant enzymes, necessarily in a coordinated manner, constitutes an important mechanism for avoiding oxidative stress.

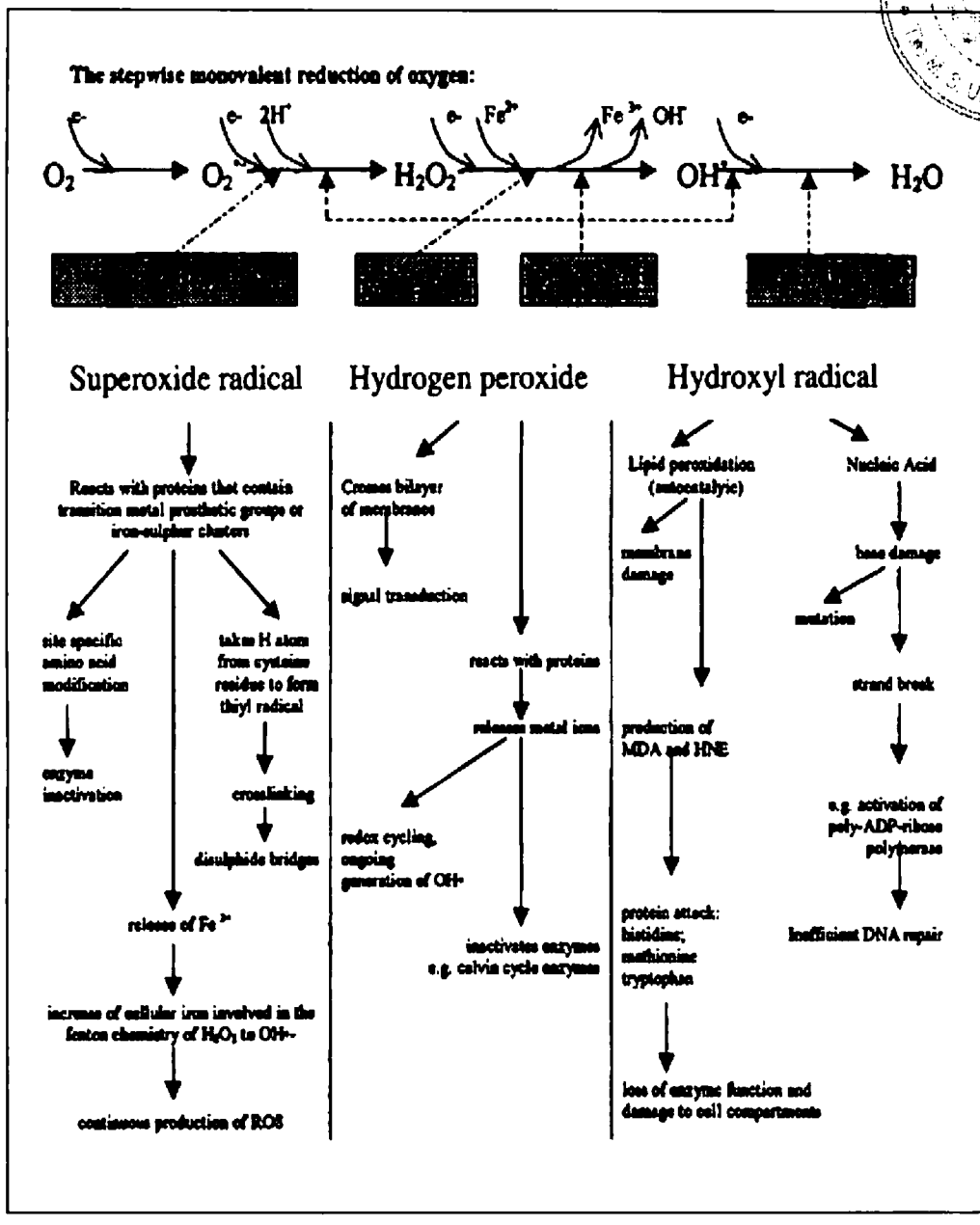


Fig. 3.3. ROS and its harmful effect.

The upper section shows the ROS produced constitutively in the cell, and the natural antioxidants and enzymes used to minimise their toxic effect. The lower section gives selected examples of the harmful effects of ROS when the pro- and anti-oxidant balance is perturbed in oxidative stress. (Gasper et al., 2002)

## 3.3 Materials and Methods.

### 3.3.1 Effect of salt stress on seed germination and growth of groundnut *in vivo.*

The pot experiment was planned and carried out to find out the level of natural salt tolerance and to study the effect of different concentrations of salt stress on groundnut germination.

Salinity is a complex parameter and is contributed by number of salts out of which sodium chloride (NaCl) is dominant salt and is major among all followed by Sodium Sulphate which is of secondary importance and then magnesium and calcium chloride which are of third importance. Cooke et al. (1993) stated that, because of the great solubility of NaCl it is taken down slope, and it characterizes the soil nearest to a playa, while the other salts are deposited to higher altitudes in soil.

Therefore, to provide salt stress, different concentrations of NaCl solution were used in all the experiments. Five different concentrations 10 mM, 25 mM, 50 mM, 100mM and 200 mM were taken along with the control.

Mature dry seeds from the pods of groundnut variety GG-20 were used for the experiments.10 seeds each were taken per treatment. Seeds were washed with 0.1 % bavistin and then immersed separately in flask containing different NaCl concentrations as mentioned above for 4 hours. Along with five doses double distilled water was taken as control without NaCl.

These seeds were planted in separate pots containing mixture of normal soil and Farm yard manure in equal proportion.

The pots were kept in dry open space to facilitate normal germination process. Each pot were irrigated separately with the particular NaCl solution twice a day. Control pot was irrigated with normal double distilled water.

Various parameters like days required for germination, % germination, shoot height, no. of leaves, no of days required for flowering etc. were noted.

### 3.3.2 Effect of salt stress on *in vitro* seed germination and growth of cultures.

The effect of NaCl stress was also tested with different concentration for the germination of seeds and its growth *in vitro.*

Mature dry seeds were washed under running tap water for 25 min. It was then subjected to, treatment with a solution of detergent Extran for 10 min, treatment of antifungal agent Bavistin for 10 min and finally washed thoroughly in sterile double distilled water and then surface-sterilized in 0.1% aqueous mercuric chloride for 10 min, rinsed 3–4 times with sterile double distilled water. The seeds were left soaked 4-6 hours in sterile double distilled water.

Seeds were inoculated in test tubes containing MS medium fortified with B5 vitamins (Gamborg et al., 1968), 100 mg/l myo-inositol, 30 g/l sucrose, BAP 8.88 µM. Based on the results obtained in *in vivo* germination experiment, NaCl 25mM , 50mM, 75mM , 100mM , 125mM , 150mM were taken as treatment along with control. Respective amount of NaCl was separately weighed and added in medium before autoclaving.

The media were solidified with 0.65% agar. The pH was adjusted to 5.8 before autoclaving at 121 C for 20 min. The cultures were incubated at 25 ± 28 °C under 80 mmol photon $m^2$/s light intensity with a photoperiod of 16/ 8 h.

Two seeds were inoculated per test tubes and ten test tubes per treatment. The experiment were performed in triplicate.

### 3.3.3 Role of Hydrogen Peroxide in Plants

Hydrogen Peroxide is one of the major reactive oxygen species (ROS) in plant tissues. It is produced in chloroplast and mitochondria via electron transport chain, where oxygen is reduced to super oxide which is further dismutated into $H_2O_2$ spontaneously or catalyzed by super oxide dismutase (SOD) (Asada, 1999; Moller, 2001). The dismutation of superoxide into hydrogen peroxide and oxygen constitute the first line of cellular defense to

prevent undesirable biological oxidation by oxygen radical generated during cellular metabolism.

$H_2O_2$ production in plant cells is also catalyzed by glycollate oxidase in peroxisomes (Noctor et. al., 2002) membrane bound NADPH oxidase (Jiang and Zhang, 2003) and oxalate oxidase. When plants are subjected to environmental stress, it accumulates and leads to oxidative damage (Asada, 1999).

Accumulating evidences suggests that $H_2O_2$ is a key signalling molecule involved in plant response to biotic and abiotic stresses, such as pathogen attack, extreme temperature, drought, excessive radiation, ozone and wounding.

Therefore it is important to determine $H_2O_2$ concentration in plant tissue.

**3.3.3.1 $H_2O_2$ Estimation:** (Patterson et al., 1984)

**Reagents required:**

5% TCA

Activated charcoal

17M Ammonia solution

colorimetric reagent

10 mg 4- Aminoantipyrin

10 mg phenol

5 mg peroxidase dissolved in 100 ml of glucose solution.

**Method:**

0.5 gm fresh tissue was weighed and rinsed with distilled water. Tissue was then grinded to powder in a mortar with pestle together with 5ml 5% TCA and .15gm activated charcoal and glass powder in dark condition. The mixture was centrifuged at 10,000g for 20 min at $4^0$C. Supernatant is adjusted to pH-8.4 with 17M Ammonia solution and filtered. 1ml of colorimetric reagent was added to 0.5ml sample extract and incubated for 10 min at 30 $^0$C. The absorbance was taken at 505nm by spectrophotometer.

### 3.3.4 Role of catalase in plants

Catalase is one of the most potent catalysts known. The reactions it catalyses are crucial to life. It is an antioxidant enzyme that converts $H_2O_2$ to water in the peroxysomes (Fridovich, 1989; McCord and Fridovich, 1969). In this organelle, $H_2O_2$ is produced from ß-oxidation of fatty acids and photorespiration (Morita et al., 1994). Higher activity of CAT and APX decrease $H_2O_2$ level in cell and increase the stability of membranes and CO2 fixation because several enzymes of the Calvin cycle within chloroplasts are extremely sensitive to $H_2O_2$. A high level of $H_2O_2$ directly inhibits CO2 fixation (Yamazaki et al., 2003).

Thus Catalase protects the cell from the deleterious effects of hydrogen peroxide accumulation. Multiple isoenzymes of CAT have been studied in higher plants, three main CAT isoforms have been characterized (Scandalios, 2005).

Catalase also uses Hydrogen Peroxide to oxidise toxins including Phenols, Formic Acid, Formaldehyde and Alcohols.

The reaction catalyzed is:

$2H_2O_2$ to $2H_2O + O2$,

$H_2O_2$ is a powerful oxidizing agent and is potentially damaging to cells. By preventing excessive $H_2O_2$ build up Catalase allows important cellular processes which produce $H_2O_2$ as a byproduct to take place safely.

### 3.3.4.1 Catalase Estimation: Aebi (1984)

**Reagents required:**

1) Extraction buffer

Na-PO4 buffer 50mM of pH 7.0

DTT 1mM

EDTA 0.1mM , 1% PVP

2) Assay buffer:

Tris HCl buffer 75mM; pH – 8.2

**Method**

0.5 gm of fresh tissue was weighed and rinsed with distilled water and kept it in deep fridge for 30 min. The tissue was grinded to powder with 5 ml of extraction buffer and a pinch of glass powder in chilled condition with precooled mortal and pistel. Centrifuged at 10,000 rpm for 15 min. at 4◦c. The supernatant was removed and used as crude enzyme extract. 100µl of enzyme extract was added with 875µl of assay buffer along with 25µl of $H_2O_2$. The absorbance was taken at 240 nm for I min.

Catalase activity was measured according to Aebi (1984) as described above. Reaction started by adding $H_2O_2$ and decrease in absorbance recorded at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of $H_2O_2$ decomposed.

### 3.3.5 Role of Peroxidases in plants

Peroxidases are haem-containing enzymes that use hydrogen peroxide as the electron acceptor to catalyse a number of oxidative reactions. Most haem peroxidases follow the reaction scheme:

$Fe3+ + H_2O_2 \rightarrow [Fe4+=O]R'$ (Compound I) + $H_2O$

[Fe4+=O]R' + substrate --> [Fe4+=O]R (Compound II) + oxidised substrate

[Fe4+=O]R + substrate --> Fe3+ + $H_2O$ + oxidised substrate

In this mechanism, the enzyme reacts with one equivalent of $H_2O_2$ to give [Fe4+=O]R' (compound I). This is a two-electron oxidation/reduction reaction where $H_2O_2$ is reduced to water and the enzyme is oxidised. One oxidising equivalent resides on iron, giving the oxyferryl intermediate, while in many peroxidases the porphyrin (R) is oxidised to the porphyrin pi-cation radical (R'). Compound I then oxidises an organic substrate to give a substrate radical.

Peroxidases are found in bacteria, fungi, plants and animals and can be viewed as members of a super family consisting of three major classes.

Of these, Class III comprises the secretory plant peroxidases, which have multiple tissue-specific functions e.g., removal of hydrogen peroxide from chloroplasts and cytosol; oxidation of toxic compounds; biosynthesis of the cell wall; defence responses towards wounding; indole-3-acetic acid (IAA) catabolism; ethylene biosynthesis; and so on Hiraga et al., 2001). The wide spectrum of peroxidase activity, coupled with the participation in various physiological processes, is in keeping with its relative lack of specificity for substrates and the occurrence of a variety of isozymes.

Plant peroxidases are monomeric glycoproteins containing 4 conserved disulphide bridges and 2 calcium ions. The 3D structure of groundnut peroxidase has been shown to possess the same helical fold as class I and II peroxidases.

**3.3.5.1 Peroxidase Estimation**

**Reagents required:**

1M NaOH

0.1M Phosphate buffer

5.33% Pyrogallol solution

Sudstrate H2O2 solution

Enzyme solution

**Method**

2.4 ml of 0.1M Phosphate buffer, 0.33 ml of Pyrogallol solution and 0.20 ml of $H_2O_2$ solution was pipetted into a 10 mm cuvette and it was equillibrate in water bath until temperature reaches 20◦c. The enzyme was added at zero time and mixed properly. The total reaction volume was made to 3ml and the recording of increase in absorbance was started immediately at 420 nm.

## 3.4 Result and Discussion

### 3.4.1 Effect of salt stress on seed germination and growth of ground nut *in vivo*

As shown in table 3.1, average 3 days were required for germination of seeds in control as well as low NaCl treatment. While the germination was delayed for the seeds under 100 mM treatment. There was no germination at all for the seeds under 200 mM treatment. All other parameters were more or less similar for initial 3 NaCl treatments i.e. 10, 25 and 50 mM concentrations.

After subjecting plants with different salt concentrations height of plant treated with 25mM were not affected. Whereas in case of plants treated with 50mM, 100mM and 125mM shows significant decrease in plant height with respect to control. It is known that salinity induces a change in the signals of root origin, which changes the hormonal balance of the plant, and this affects root and shoot growth(Munns et al., 1986) . Another reason for that is, at higher salt concentration especially Na+ becomes toxic to plant by inactivating the enzymes like rubisco and disturbs the ion homeostasis that leads to cytotoxic effect in cell In turn leading to death of plant cells and thus decrease in shoot length.

| **Observations** | **Control** | **10 mM** | **25 mM** | **50 mM** | **100 mM** | **200 mM** |
|---|---|---|---|---|---|---|
| Pretreatment | 4 hrs presoaking in 10 ppm GA3 with respective salt concentration | | | | | |
| Sowing date | 28/01/2010 | 28/01/2010 | 28/01/2010 | 28/01/2010 | 28/01/2010 | 28/01/2010 |
| No. of replicates | 10 | 10 | 10 | 10 | 10 | 10 |
| Avg. no. of Days required for germination | 3 | 3 | 4 | 6 | 8 | |
| Avg. 'no. of seeds germinated | 10 | 9 | 9 | 7 | 4 | 0 |
| % seed germination | 100.00 | 90.00 | 90.00 | 70.00 | 40.00 | 0.00 |
| Avg. No. of compound leaves after 10 days | 3-4 | 3-4 | 2-3 | 2 | 2 | 0 |
| Avg. no. of Days required for 1st flowering | 19 | 20 | 21 | 21 | 60 | - |
| Avg. No. of flowers after 3 weeks | 8 | 6 | 4 | 1 | 0 | 0 |
| Ave. No. of compound leaves after 21 days | 9 | 9 | 6 | 5 | 3 | 0 |

Table 3.1: Effect of different level of NaCl stress on seed germination and plant growth & development.

**Fig .3.4: Ground nut plants under different level of NaCl stress Control, 10mM to 200 mM from L to R.**


Fig. 3.5 : Ground nut plants under NaCl stress Control and 10mM on 22nd day.

There was no significant difference in the germination rate of plants treated with 10 mM, 25mM and 50mM of NaCl. However the germination rate was retarded at concentration 100mM.There was no germination at concentration 200 mM as compared to control. Abscisic acid in plant plays major role in germination (Leung et al.,1998). It is known that at higher salt concentration there will be increase in abscisic level that will lead to decrease in activity of α-amylase. Thus inhibiting the germination of seedlings.

A significant decrease of leaf area was observed in plants under high salt stress. Higher concentration of salt leads to accumulation of Na+ in the cells. That leads to toxicity to the cell, in turn reduction in leaf area exposed for photosynthesis. With the increase of NaCl concentration there was reduction in the number of leaves and yellowing of leaves.

These parameters were also more or less same for the treatment up to 50 mM NaCl treatment compared to control however germination, number of leaves, morphological growth and days required to flower were severely affected at the 100 mM treatment. Hardly a flower was observed in any plant that to after 60 days of germination which took 3 times more duration than the normal control plants.

Based on these observations it was concluded that ground nut can naturally withstand salinity up to 50 mM without any aberration.


Fig. 3.6 : Ground nut plants under NaCl stress 25mM and 50mM on 22nd day.


Fig. 3.7: Leaves size of plants under different level of NaCl stress.


Fig. 3.8 - Ground nut plants under NaCL stress 100mM on 22nd day.

### 3.4.2 Effect of salt stress on *in vitro* seed germination and growth of cultures.

The germination of seeds in *in vitro* condition was observed to be severely more affected compared to *in vivo* condition. The germination started on third day of incubation. Within 12 days of incubation seedlings grown to the height of 4 cm with 3 functional compound leaves each in control as well as 25 and 50 mM NaCl treatments. However, for higher dose 75 and 100 mM, the germination was delayed by 4-5 days. Shoot height also was shorter with no germination at all in 125 mM treatment for up to 10 days.

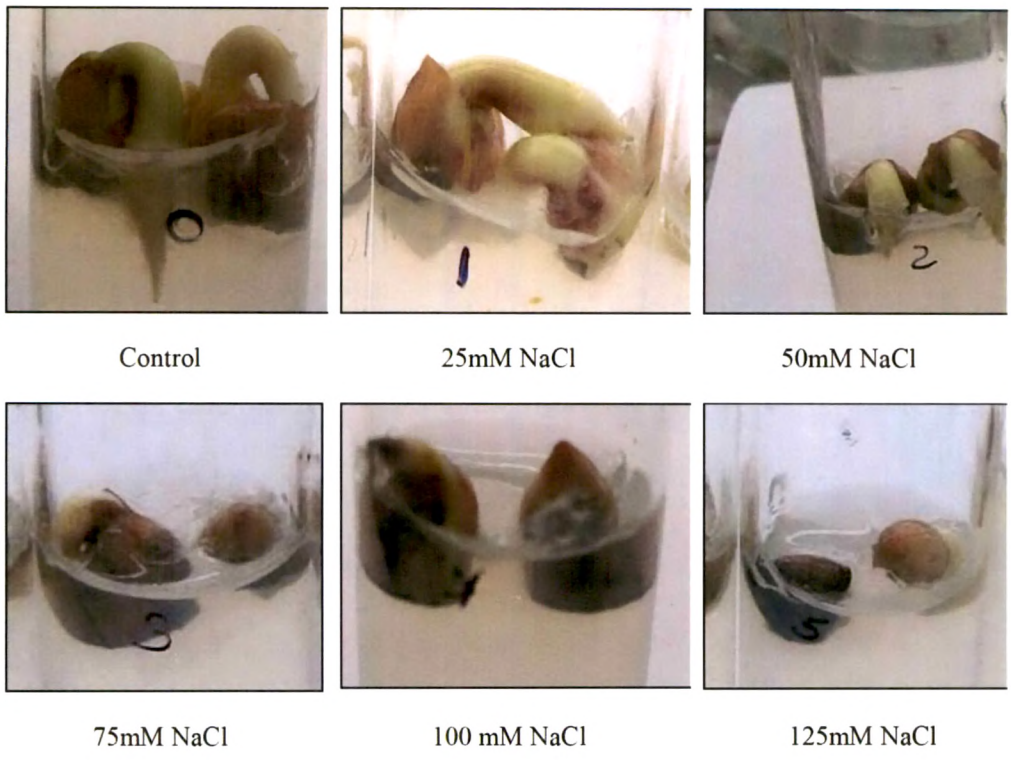


Fig. 3.9: *In vitro* germinating seeds on MS medium on 3rd day of inoculation with different level of NaCl stress.

### 3.4.3 Effect of salt stress on $H_2O_2$ release

$H_2O_2$ level was measured by Zhu et al.2006.The results of hydrogen peroxide estimation showed an increase in $H_2O_2$ level under high salt stress. At the increasing concentrations of salt many reactive oxygen species are produced including hydrogen peroxide as result of free radical chain process in plants.

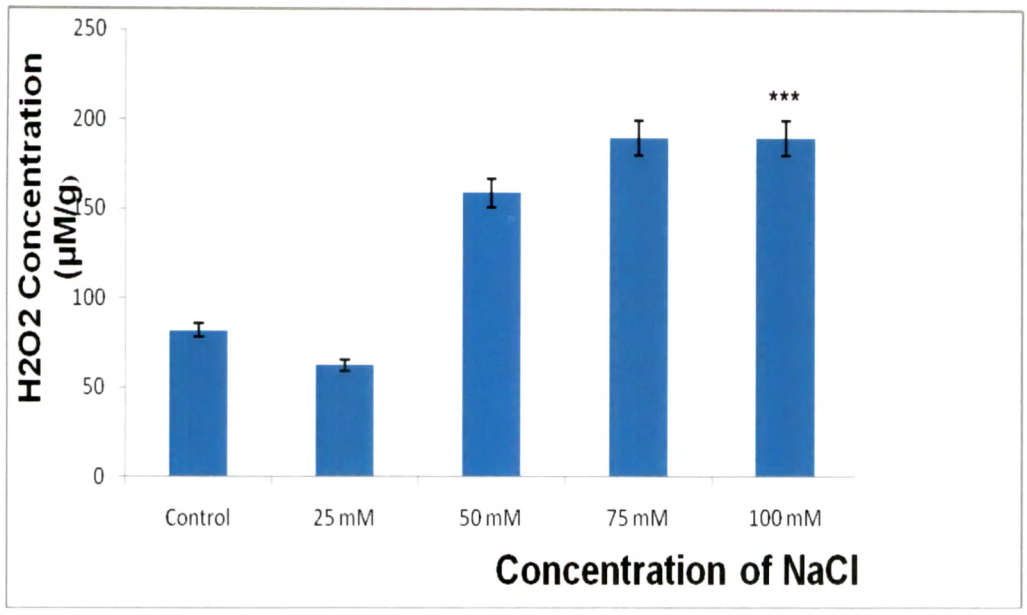


Fig. 3.10: Release of $H_2O_2$ in different concentration of NaCl stress.

Values are reported as Mean + SEM, *** indicates significantly different at $p<0.05$ and $p<0.001$ respectively as compared to the corresponding control. N=5

### 3.4.4 Effect of salt stress on catalase release

The activities of catalase increased with the increase in the concentrations of NaCl. The increase in the activities of POD and CAT with the increase of NaCl concentration has been shown in *S. sieb* by Li (2009).

Catalase is involved in scavenging of reactive oxygen species. Results show that increasing activity with increased concentration of salt. At 75 and 100 mM NaCl the CAT activity increases 2-3 folds compared to that of control. The plant combat salt stress by increasing catalase activity. But at higher concentration of salt as 75mM and 100mM there was no significant change in activity and It seems that, CAT activity was not adequate for the complete scavenging of $H_2O_2$ and thus to combat salt stress. Although its activity increased compared to control.

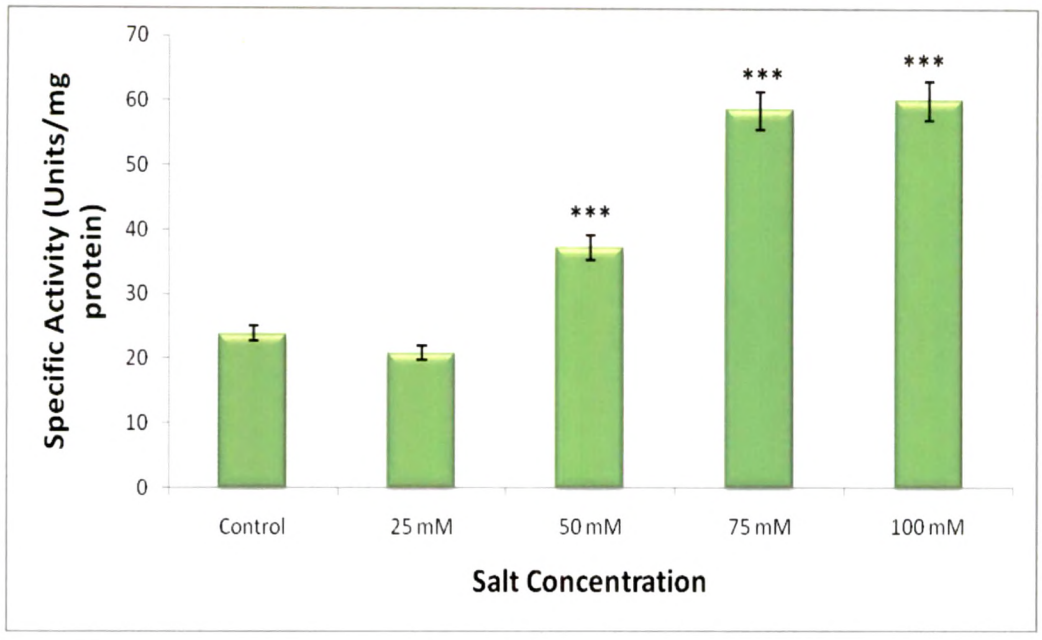


Fig. 3.11: Catalase activity at different concentration of NaCl stress.

Values are reported as Mean ± SEM, *,** and *** indicates significantly different at $p<0.05$ and $p<0.001$ $p<0.001$ respectively as compared to the corresponding control N=5

### 3.4.5 Effect of salt stress on Peroxidase release

Peroxidase activity increased with increased exposure to salt indicating that the hydrogen peroxide produced during salt stress could be effectively removed by it. Peroxidase activity increase with respect to salt concentration. As compared to catalase, there was increase in peroxidase activity at 75mM and 100mM. So it can be concluded that at very high salt concentration peroxidase rather than catalase plays major role in combating salt.

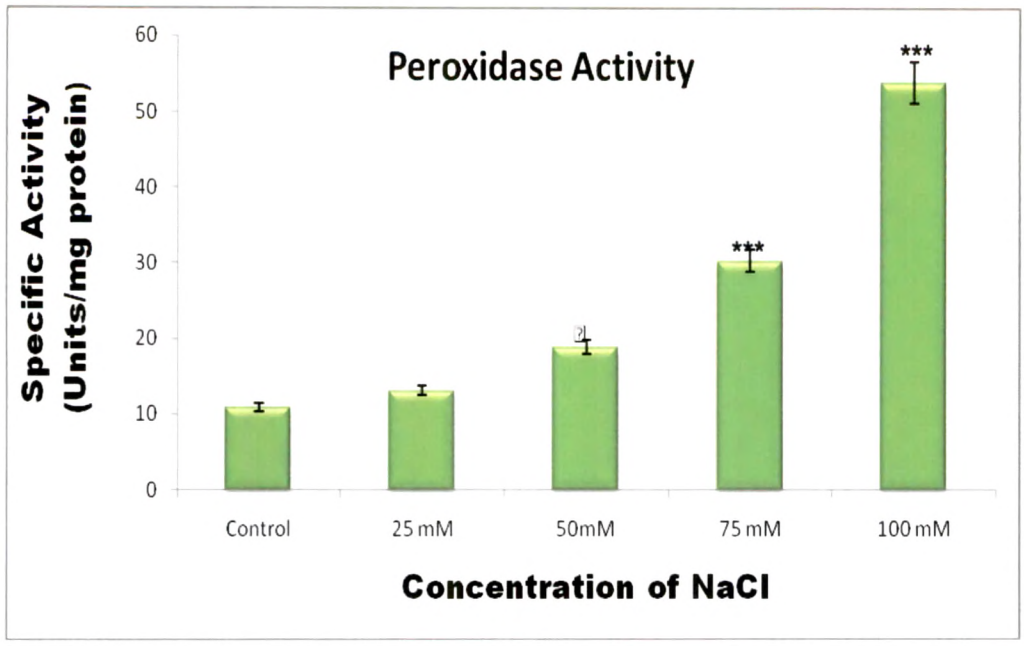


Fig. 3.12: Peroxidase activity at different concentration of NaCl stress.

Values are reported as Mean + SEM, *** indicates significantly different at $p<0.05$ and $p<0.001$ $p<0.001$ respectively as compared to the corrosponding control. N=5

It has been shown, that high salinity leads to programmed cell death (PCD) in higher plants which could be regarded as a salt adaptation mechanism (Huh et al., 2002). The status of mitochondrial permeability transition pore (PTP) and levels of reactive oxygen species (ROS) play key roles in regulating apoptosis in animal cells. Similar role of mitochondria has been observed in plants mediating animal like apoptosis cell death. It is known that at higher salt stress there will be increase in generation of reactive oxygen species that lead to change in mitochondrial transition pore permeability, and in turn release of cytochrome C leads to apoptosis like cell death. It has been shown that at higher salt concentration of salt stress ROS species will damage biomolecules like DNA resulting in oligonucleosomal fragements generation (DNA laddering) (Lin et al., 2006). But when plants are exposed to very high salt concentration that causes severe damage to cells, necrosis will occur, and this gives DNA smear on gel. Using the above mentioned method of DNA isolation, smeared DNA was obtained. Hence, there is a need of improvement of isolation technique in order to be able to study DNA fragmentation.