5.1 Rationale

Oxygen is essential for survival however, its univalent reduction generates several harmful reactive oxygen species (ROS), inevitable to living cells and highly associated with the wide range of pathogenesis such as diabetes, liver damage, inflammation, aging, neurological disorders and cancer (Halliwell and Gutteridge, 1993; Esterbauer, 1996; Sies, 1997). Oxidative stress, in which large quantities of reactive oxygen species (ROS) are generated, one of the earliest responses to stress. Reactive oxygen species primarily consist of superoxide radical anion (•O₂⁻), hydroxyl radicals (•OH), singlet oxygen (¹O₂) and hydrogen peroxide (H₂O₂). The damaging action of the hydroxyl radical is the strongest among free radicals.

Several environmental conditions like exposure of ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of ROS in the cells. These ROS cause lipid peroxidation, protein peroxidation, DNA damage and cellular degeneration in the cells. Though synthetic antioxidants, BHT, BHA and radioprotector warfarin are being used widely, however, due to their potential health hazards, they are under strict regulation as they are suspected to be carcinogenic (Satio et al. 2003; Rades et al. 2004). Natural antioxidants therefore have gained much importance. Antioxidant principles from natural resources are multifaceted in their multitude/magnitude of activities and provide enormous scope in correcting the imbalance through regular intake of proper diet. Therefore, in the recent years, the interest is centered on antioxidants derived from herbal medicine in view of their medicinal benefits (Umadevi & Ganasoundari, 1995; Winston, 1999; Arora, et al., 2003; Kamat et al., 2004; Kamat, 2007). The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from ageing-related diseases has intrigued scientist for a long
time. Phytoantioxidants, commonly available, less toxic, serving food and medicinal components have been suggested to reduce threat of wide range of ROS (Umadevi & Ganasoundari, 1995; Winston, 1999; Arora, et al., 2003).

It was proved that in dormant seeds, the activity of various enzymes is suppressed. Germinating seeds especially sprouts are known to contain higher concentration of various metabolites of nutritional value and several biochemical constituents & enzymes. (Bewely & Black, 1985).

In view of this and the present understanding about ROS induced multiple diseases, screening of the antioxidant activity of an important medicinal herb N. sativa seeds in different stages of its germination was carried out.

5.2. REVIEW OF LITERATURE

5.2.1 Antioxidant activity of N. sativa

The antioxidant activity of N. sativa seeds and oil was basically dependent on its active constituent thymoquinone and carvacrol (Thippeswamy and Naidu 2005). In vitro studies showed that seed extracts of N. sativa protects erythrocytes against lipid peroxidation, protein degradation, loss of deformability and increased osmotic fragility caused by H$_2$O$_2$. The crude N. sativa oil and its fractions (neutral lipids, glycolipids and phospholipids) showed potent in vitro radical scavenging activity that is correlated well with their total content of polyunsaturated fatty acids, unsaponifiables and phospholipids, as well as the initial peroxide values of crude oil (Suboh et al., 2004).

Thymoquinone, a major component of N. sativa has been shown to exhibit antioxidant property through different mechanisms in several recent reports. For example, it inhibits the production of 5-hydroxyeicosatetraenoic as well as 5-lipoxygenase products (Dakhakhny et al. 2002), both of which are required for the viability of
colon cancer cells. It was shown to work as a scavenger of various ROS including superoxide radical anion and hydroxyl radicals (Mansour et al. 2002; Badary, 2003; Mahgoub et al. 2003). Additionally, it was able to produce significant reductions in hepatic antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase. It has been shown that thymoquinone could inhibit iron-dependent microsomal lipid peroxidation efficiently in rats with doxorubicin-induced hyperlipidemic nephropathy (Badary et al. 2000).

Both *N. sativa* oil and Thymoquinone can partly protect gastric mucosa from acute alcohol-induced mucosal injury which is partly ascribed to their radical scavenging activity. El-Saleh and co-workers have shown that active antioxidant components of black seeds of *N. sativa* plants are capable of rendering protection against the development of methionine induced hyperhomocysteinemia (HHcy) and its associated state of oxidative stress. Under the state of induced HHcy, there were significant increases in the plasma levels of triglycerides, lipid peroxidation and cholesterol as well as in the activities of glutathione peroxidase and SOD although catalase activity was not affected. The total antioxidant status was significantly depressed. All of these effects were almost totally blocked by the prior treatment with thymoquinone (El-Saleh et al 2004).

**5.2.2. Activity of Antioxidant Enzymes during Germination**

Seed germination is a complex process and is characterized by imbibition, after which seeds rapidly increase oxygen uptake and oxidative phosphorylation, processes required to meet the high energy cost of germination (Tommasi et al, 2001). Oxidative phosphorylation and mobilization of food storage generate ROS that can cause structural and functional damage in cells. The enzymes responsible for ROS scavenging are therefore of particular importance for the success of germination. Also it has been shown that seed germination percentage might be related to the
efficiency of free radical scavenging in dry seeds because this scavenging can affect merely seed storage and vigor (Priestley, 1986; Bailly et al., 1998). Some authors have shown that production of ROS during seed germination may be a beneficial biological reaction, one that is linked with germination capacity, seedling development, and protection against parasitic organisms during germination (Schopfer et al., 2001). For these reason there is a growing interest in the functional role of ROS and corresponding scavenging enzymic systems in seed germination (Bailly et al., 2001; Ducic et al., 2003).

Seed germination and post-germination seedling development are well-regulated process in plant physiology involving high metabolic activity and generation of ROS in the cell (Bailly, 2004). ROS affect various aspects of seed physiology, displaying two major functions: as a kind of cytotoxin and as a special role in seed development, dormancy breakage, and in defense against biotic and abiotic stresses (Apel & Hirt, 2004). A series of new roles for ROS has recently been identified: the control and regulation of biological processes, such as cell cycle, programmed cell death and hormone signaling (Gapper & Dolan, 2006). These studies extend our understanding of ROS and suggest a dual role for ROS in plant biology as both toxic byproducts of aerobic metabolism and key regulators of growth, development and defense pathways.

In plant cells, antioxidant enzymes, such as SOD, POD, and CAT, are considered to form a defensive team, whose combined purpose is to protect cells from oxidative damage during growth, development and senescence (Blokhina et al., 2003). Moreover, MDA is considered sensitive marker commonly used for assessing membrane lipid peroxidation (Bailly et al., 1996; Goel & Sheoran, 2003). Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes (Cheeseman et al, 1993). Their destruction can lead to cell death and also to the production of toxic and reactive aldehyde metabolites, known as free radicals. Among these free radicals, malondialdehyde (MDA) is the most important (Paradis & Kollinger 1997). MDA is the main final product of lipid
peroxidation and has been often used for determining oxidation damage (Sevanian & McLeod, 1997) which is indicated by high levels of MDA.

Antioxidative enzymes such as SOD, POD, and CAT are considered to be the main protective enzymes engaged in the removal of free radicals and activated oxygen species (Blokhina et al., 2003; Devi et al., 2005). CAT and SOD are the most efficient antioxidative enzymes (Scandalios, 1993). On the other hand, POD also has a role in very important physiological processes like control of growth by lignification, cross-linking of pectins and structural proteins in the cell wall, and catabolism of auxins (Gaspar et al., 1991). Despite the importance of POD in plant development, their exact relationship to developmental events is often obscured by their extensive polymorphism in a single plant species. It is therefore very important to select POD associated with plant development for purification and further studies (Jackson and Ricardo, 1998).

5.2.2.1. Superoxide Dismutase (SOD) (EC 1.15.1.1)

SOD is the key enzyme in the active oxygen scavenger system and considered to be the first line of defense against ROS (Hamilton & Heckathorn, 2001). This enzyme is present in all aerobic organisms and in all sub-cellular compartments susceptible to oxidative stress (Bowler et al., 1992). In several cases transgenic plants overexpressing SOD showed increased tolerance to oxidative treatments and became more resistant to photoinhibition when exposed to different abiotic stresses (Smirnoff, 1993). Different levels of SOD activity might occur depending on stress intensity, species or genotype, growth conditions, stress period, plant age (Sgherri et al., 2000). Anyhow, a higher degree of protection against oxidative damage should require a fast removal of H$_2$O$_2$ by other scavenging systems, thus minimizing H$_2$O$_2$ toxicity and the formation of the highly toxic hydroxyl radicals (Perl et al., 1993). SOD may catalyze the dismutation of superoxide radical to generate O$_2$ and H$_2$O$_2$, the produced H$_2$O$_2$ removed by POD and CAT (Asada, 1999).
5.2.2.2. Catalase (CAT) (EC 1.11.1.6)

Some workers reported a direct correlation of catalase activity and seedling growth (Nanda, 1950; Chikasne, 1953; Verma and Van Huystee, 1970) while others showed an inverse correlation (Galston, 1951; Harvey, 1974; Prathapasenan et al., 1969). Yokoyama (1956) studied the relation of catalase activity to mitochondrial respiration and pointed out that catalase directly affected the oxidation and reduction of cytochrome-c oxidase system. A rise in enzyme catalase has been reported as germination progresses (Papov, 1965). On the other hand, low moisture content due to water stress reduced the catalase activity in Wheat, Sesamum and Eleusine as the germination advanced (Acharya, 1968).

5.2.2.3. Peroxidase (POD) (EC 1.11.1.7)

Peroxidase catalyses the oxidation of diverse hydrogen donors and peroxidase activity is implicated in many biological events in plants depending on the nature of the donor. This enzyme is known to fulfil many functions in plants. Peroxidase activity is reported to be associated with active differentiation (Harvey 1974; Saxena, 1989). It is well established that high concentrations of auxins promotes the release of ethylene in the regulation of peroxidase activity by growth hormones in many plant species, reported by various authors (Lavee and Galston, 1968; Stuber and Levings, 1969; Bireoka and Galston, 1970; Ritzert and Turin, 1970; Lee, 1972). In cotton plants, auxin—induced evolution of ethylene coincides with the enhancement of peroxidase activity (Sakai and Imaseki, 1971, Fowler and Morgan, 1972).

5.2.2.4. Ascorbate Peroxidase (APX) (EC 1.11.1.11)
Ascorbate is present in chloroplasts, cytosol, vacuole and apoplastic space of leaf cells in high concentrations (Polle et al. 1990; Foyer et al. 1991). It is perhaps the most important antioxidant in plants, with a fundamental role in the removal of hydrogen peroxide (Foyer, 1993). Oxidation of ascorbate occurs in two sequential steps, first producing mono-dehydroascorbate, and if not rapidly re-reduced to ascorbate, the mono-dehydro-ascorbate disproportionates to ascorbate and dehydro-ascorbate. APX activity has mainly been reported from chloroplast and cytosol (Chen and Asada 1989). However some recent studies have also reported its occurrence in mitochondria as well (Gomez et al. 1999).

APX is an antioxidant enzyme that participates in the ascorbate-glutathione cycle and acts in chloroplasts and in the cytosol. It reduces $\text{H}_2\text{O}_2$ to $\text{H}_2\text{O}$ by using ascorbate as reducer agent, protecting thus the plant (Asada, 1992; Meloni et al., 2003). The action of antioxidant systems under drought has been investigated by many authors in several crops, such as spinach (Tanaka et al., 1990), pea (Moran et al., 1994), sorghum and sunflower (Zhang and Kirkham, 1996) and wheat (Sgherri et al., 2000).

5.2.3. ROS generation under stress conditions

Reactive oxygen species are originated under stress conditions. They oxidize photosynthetic pigments, membranes, lipids, proteins, and nucleic acids (Smirnoff, 1993; Alscher et al., 1997). Thus, antioxidants as carotenoids, ascorbate, α-tocopherol, glutathione and flavonoids, as well as antioxidant enzymes such as peroxidases, superoxide dismutase and catalase, can be synthesized in order to protect the plant cells (Tanaka et al., 1990).

Several studies have been carried out to verify the action of some enzymes that indicate different types of environmental stress. Among such enzymes, amylase and ascorbate peroxidase may be included. According to literature, a higher activity of those enzymes has been detected under several stress situations in order to protect the plants against such adversities, favoring thus their survival (Koster, 1991; Hare and Cress, 1997; Nepomuceno, 2001; Taiz and Ziger, 2002).
5.2.4. Heavy metal toxicity in plants and production of ROS

The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms such as (i) uptake and accumulation of metals through binding to extracellular exudates and cell wall constituents; (ii) efflux of heavy metals from cytoplasm to extranuclear compartments including vacuoles; (iii) complexation of heavy metal ions inside the cell by various substances, for example, organic acids, amino acids, phytochelatins, and metallothioneins; (iv) accumulation of osmolytes and osmoprotectants and induction of antioxidative enzymes (v) activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures (Cho et al., 2003).

An overindulgence of both essential and toxic heavy metals has been found to be allied with generation of free radicals. Free radicals or ROS are toxic by-products, generated at low levels in non-stressed plant cells in chloroplasts and mitochondria and also by cytoplasmic, membrane-bound or exocellular enzymes concerned in redox reactions (especially photosynthetic electron transport processes and respiration). An extra amount of ROS occur under stressful conditions and over production of these ROS such as superoxide, H$_2$O$_2$ and OH exhibited that plants exposed to stress conditions including metal stress (Galligo et al., 1999). ROSs is known to spoil cellular membranes by inducing lipid peroxidation interruption of electron transport chain. lipoxygenase, an enzyme that arouse lipid peroxidation, has been reported after cadmium revelation (Smeets et al., 2005). As a consequence, tissues snubbed by oxidative stress generally contain elevated concentration of APX, GPX and CAT and demonstrate an amplified assembly of ethylene (Schutzendübel et al., 2002).

5.2.5. Cadmium Toxicity in Plants
Cadmium is one of the most dangerous heavy metals due to its high mobility and the small concentration at which its effects on plants begin to show (Barcelo and Poschenrieder, 1992). Overnell (1975) reported that cadmium reduced the concentration of ATP and chlorophyll in many species, and decreased oxygen production. Germinating seedlings of mung bean (*Phaseolus vulgaris* L.) treated with different concentrations of cadmium acetate reported to show lowered chlorophyll and heme levels. While the level of lipid peroxides was higher (Somashekaraiah *et al*., 1992).

Cadmium can cause the production of ROS in plants (Foyer, 1997). Evidence was obtained from observation that new isoenzymes of peroxidases were detected in plant tissues exposed to Cd (Van Assche and Clijsters, 1990; Tahlil *et al*., 1999). Other evidence came from the detection of lipid peroxidation and chlorophyll breakdown (Somashekaraiah *et al*., 1992; Gallego *et al*., 1999; Stroinski, 1999). The activities of key enzymes involved in the removal of ROS and hydrogen peroxide, such as catalase, glutathione reductase, and superoxide dismutase, have not been studied in detail. Recently, the effects of Cd on these enzymes in hyper accumulator plants of the genus *Alyssum* (Schickler and Caspi, 1999), sunflower (Gallego *et al*., 1999), and also in radish (Vitoria *et al*., 2001) have been reported, indicating a typical antioxidative defense mechanism.

### 5.2.6. Effect of Salinity on Plants

Soil salinity is a major constraint limiting agricultural productivity on nearly 20% of the cultivated area and half of the irrigated area worldwide (Zhu, 2001). High salinity is one of the most important abiotic stress factors limiting plant growth and productivity of a wide variety of crops (Flowers, 2004; Jaleel *et al*., 2007; Athar *et al*., 2008). The response of plants to excess salinity is complex and involves changes in their morphology, physiology, and metabolism (Parida & Das, 2005). Morphologically the most typical symptom of saline injury to plant is reduction of growth (Azooz *et al*., 2004; Jaleel *et al*., 2008). Of the physiological and metabolic changes possibly occurring as
response to salinity stress are the production of ROS such as $O_2^-$, $O^-\cdot$, $OH^-$ and concomitantly $H_2O_2$ (Misra & Gupta, 2006). ROS have potential to interact with many cellular components, causing significant damage to membrane and other cellular structures, and consequently growth inhibition (Verma & Mishra, 2005; Agarwal & Shaheen, 2007; Gao et al., 2008). Some of the ROS are highly toxic and must be detoxified by cellular responses, if the plant is to survive and grow (Gratão et al., 2005).

Plants under stress display some defense mechanisms to protect themselves from the damaging effect of oxidative stress. Plants with high constitutive and induced antioxidant levels have better resistance to damage ROS (Parida & Das, 2005). The degree of damage by ROS depends on the balance between the product of ROS and its removal by these antioxidant scavenging systems (Demiral & Turkan, 2005; Khan & Panda, 2008). ROS scavenging depends on the detoxification mechanism, which may occur as a result of sequential and simultaneous action of a number of antioxidant enzymes, including CAT, POD, SOD and APX. The activities of these antioxidant enzymes were reported to increase under salinity stress and closely related to salt tolerance of many plants (Azevedo Neto et al., 2006; Koca et al., 2007; Athar et al., 2008).

Present work is the first study to see the level of lipid peroxidation and the expression of the activities of the antioxidant enzymes SOD, CAT, POD, and APX during germination of *N. sativa* seeds. Our aim was to follow the expression of particular parts of antioxidative systems during the different stages of germination of the *N. sativa* in normal and stressed condition.

### 5.3. MATERIALS AND METHODS

#### 5.3.1. Collection of *N. sativa* seeds

Seeds of *N. sativa* were procured in December, 2009 from a local grocery store in Lucknow, India, and surface sterilized with 0.1% sodium hypochlorite solution for 10 min and then rinsed with double distilled water.
After sterilization, the seeds allowed to soak in de-ionized water, 2mM solution of CdCl$_2$ and 100mM solution of NaCl separately.

5.3.2. Germination of *N. sativa* seeds

Seeds of *N. sativa* were grown in glass petri plates having two or three folds of damp blotting paper in distilled water, CdCl$_2$ solution (2mM) and NaCl solution (100mM) at room temperature of about 28°C under control conditions separately. 0.25gm seeds were inoculated in each petri plates under aseptic conditions. The seeds were incubated in dark till sprouting was initiated (3 days) after which the plates were transferred to culture room at a light intensity of 100 µmol m$^{-2}$ s$^{-1}$ and a 14/10 h (day/night) photoperiod till the development of plantlet with two leaves.

5.3.3. Harvest of germinated seeds

The enzymatic activity was analyzed using different germination stages *N. sativa* seeds. Forceps were used to picked out seeds from plates, seeds was put on blotting sheet to absorb extra water. The seeds collected for different experiments were used immediately for preparing enzyme extracts. Three replicates were prepared for each treatment (0.25g per petri dish). All the samples were stored at -80°C until used for further investigation.

5.3.4. Determination of Lipid Peroxidation

The level of lipid peroxidation was determined by measuring the amount of MDA produced by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Fresh sample (0.5 g) was homogenized in 10 mL of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 15000 × $g$ for 10 min. To 2 mL aliquot of the supernatant, 4 mL of 0.5% thiobarbituric acid in 20% TCA were added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath and centrifuged at 10,000 × $g$.
g for 10 min; the absorbance of supernatant was recorded at 532 and 600 nm. After subtracting the non-specific absorbance at 600 nm, the MDA content was calculated using its molar extinction coefficient (155 mM$^{-1}$ cm$^{-1}$) and the results expressed as µmol MDA g$^{-1}$ fresh weight.

5.3.5. Enzyme Extraction

The samples were prepared as described by Mukherjee and Choudhuri (1983) with some modifications. A sample (0.5 g) was finely ground by pestle in a chilled motor; 10 mL of 100 mM phosphate buffer (KH$_2$PO$_4$/K$_2$HPO$_4$) pH 7.0, containing 0.1 mM Na$_2$EDTA and 0.1 g of polyvinylpyrrolidone (PVP) was added to the sample. The homogenate was filtered through cheese cloth, centrifuged at 15000×$g$ for 10 min at 4°C. The supernatant was recentrifuged at 18000×$g$ for 10 min; the supernatant was stored at 4°C for enzyme assay.

5.3.6. Assay of Antioxidant Enzyme Activities

5.3.6.1. Assay of SOD activity

SOD activity was measured according to the method of Gianopolitis and Ries (1977). Three mL of the mixture contained 13 mM methionine, 0.025 mM nitroblue tetrazolium (NBT), 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium bicarbonate and 0.5 mL enzyme extract. The reaction was started by adding 0.002 mM riboflavin and the tubes were shaken and placed under two 15-W fluorescent lamps. Illumination was started to initiate the reaction at 30°C. The reaction was allowed to proceed for 15 min, stopped by switched off the lights and covering the tubes with black cloth. The reaction medium without enzyme developed maximal color, while the nonirradiated reaction mixture served as blanks. Absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT.
5.3.6.2. Assay of CAT activity

CAT activity was assayed by the method of Aebi (1984) in a reaction solution (3 mL) contained 50 mM phosphate buffer (pH 7.0), 30% (w/v) \( \text{H}_2\text{O}_2 \) and 0.5 mL of enzyme extract. The reaction was started by the addition of enzyme extract. The activity of catalase was estimated by the decrease of absorbance at 240 nm for 1 min as a consequence of \( \text{H}_2\text{O}_2 \) consumed. (Havir & McHale, 1987).

5.3.6.3. Assay of POD activity

POD activity was determined according to (Maehly & Chance, 1954) by the oxidation of pyrogallol in the presence of \( \text{H}_2\text{O}_2 \). The reaction solution (3 ml) contained 10 mM \( \text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4 \) pH 7.0, 10 mM \( \text{H}_2\text{O}_2 \), 20 mM pyrogallol and 0.5 mL enzyme extract. The increase in absorbance due to formation of purpurogallin was recorded at 470 nm (Klapheck et al., 1990).

5.3.6.4. Assay of APX activity

The activity of APX was assayed according to (Chen & Asada, 1992). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.5 mM \( \text{H}_2\text{O}_2 \) and 0.1 mL enzyme extract. The reaction was started by the addition of \( \text{H}_2\text{O}_2 \). The activity of enzyme was assayed by measuring the decrease in absorbance at 290 nm for 1 min of ascorbic as ascorbic acid oxidized.

All the enzyme activates were calculated and expressed as unit min\(^{-1}\)g\(^{-1}\) fresh weight.

5.3.7. Statistical Analysis
CHAPTER-5

Every experiment was repeated thrice and all the results were expressed as mean value ±SD for three replications. For each replication plant material was taken by weight from different stages of germination.

5.4. RESULTS AND DISCUSSION

Table 5.1: Activity of different antioxidant enzymes (U/min/g of FW) during various phases of germination of N. sativa in control.

<table>
<thead>
<tr>
<th>DAY</th>
<th>SOD</th>
<th>CAT</th>
<th>POD</th>
<th>APX</th>
<th>LIPID PEROXIDATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>453.0±0.81</td>
<td>3715.10±0.70</td>
<td>16.22±0.33</td>
<td>11.64±0.48</td>
<td>37.99±0.72</td>
</tr>
<tr>
<td>1</td>
<td>446.2±0.48</td>
<td>3302.45±0.49</td>
<td>34.70±0.28</td>
<td>12.56±0.47</td>
<td>36.10±0.55</td>
</tr>
<tr>
<td>2</td>
<td>423.3±0.25</td>
<td>2064.65±0.63</td>
<td>43.20±0.41</td>
<td>25.45±0.35</td>
<td>55.42±0.31</td>
</tr>
<tr>
<td>3</td>
<td>365.5±0.32</td>
<td>1650.80±0.84</td>
<td>46.21±0.40</td>
<td>31.89±0.28</td>
<td>38.34±0.50</td>
</tr>
<tr>
<td>4</td>
<td>351.4±0.59</td>
<td>2890.30±0.42</td>
<td>49.23±0.38</td>
<td>51.58±0.25</td>
<td>29.46±0.48</td>
</tr>
<tr>
<td>5</td>
<td>359.6±0.42</td>
<td>3715.75±0.21</td>
<td>52.24±0.36</td>
<td>64.54±0.34</td>
<td>33.26±0.22</td>
</tr>
<tr>
<td>6</td>
<td>395.7±0.27</td>
<td>4541.55±0.49</td>
<td>61.25±0.35</td>
<td>50.95±0.63</td>
<td>36.41±0.41</td>
</tr>
<tr>
<td>7</td>
<td>407.9±0.65</td>
<td>5779.75±0.35</td>
<td>61.69±0.26</td>
<td>45.29±0.41</td>
<td>36.99±0.29</td>
</tr>
<tr>
<td>8</td>
<td>416.6±0.63</td>
<td>6605.30±0.42</td>
<td>60.21±0.30</td>
<td>45.28±0.39</td>
<td>39.85±0.35</td>
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<tr>
<td>9</td>
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<td>7431.55±0.49</td>
<td>64.76±0.36</td>
<td>31.78±0.45</td>
<td>38.47±0.46</td>
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<tr>
<td>10</td>
<td>497.4±0.70</td>
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<td>38.30±0.42</td>
<td>38.92±0.24</td>
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<tr>
<td>11</td>
<td>474.0±0.28</td>
<td>8256.70±0.14</td>
<td>67.19±0.43</td>
<td>38.80±0.28</td>
<td>40.02±0.50</td>
</tr>
</tbody>
</table>

* The readings were taken in triplicate and values represent Mean ± SD.
* The experiments were repeated thrice
Table 5.2: Activity of different antioxidant enzymes (U/min/g of FW) during various phases of germination of *N. sativa* in CdCl\(_2\) stress.

<table>
<thead>
<tr>
<th>DAY</th>
<th>SOD</th>
<th>CAT</th>
<th>POD</th>
<th>APX</th>
<th>LIPID PEROXIDATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>456.0±0.67</td>
<td>3715.30±0.42</td>
<td>16.29±0.28</td>
<td>12.34±0.44</td>
<td>31.79±0.39</td>
</tr>
<tr>
<td>1</td>
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<td>12.25±0.36</td>
<td>16.86±0.67</td>
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</tr>
<tr>
<td>2</td>
<td>462.3±0.45</td>
<td>1651.60±0.32</td>
<td>10.66±0.23</td>
<td>35.05±0.15</td>
<td>56.90±0.64</td>
</tr>
<tr>
<td>3</td>
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<td>12.24±0.34</td>
<td>42.47±0.47</td>
<td>53.20±0.35</td>
</tr>
<tr>
<td>4</td>
<td>377.4±0.63</td>
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<td>10.19±0.43</td>
<td>67.07±0.55</td>
<td>46.02±0.34</td>
</tr>
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<td>3302.50±0.31</td>
<td>12.19±0.27</td>
<td>83.92±0.23</td>
<td>43.30±0.42</td>
</tr>
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<td>2890.20±0.36</td>
<td>17.88±0.16</td>
<td>66.25±0.02</td>
<td>40.64±0.79</td>
</tr>
<tr>
<td>7</td>
<td>454.9±0.53</td>
<td>3715.70±0.25</td>
<td>16.24±0.36</td>
<td>58.37±0.94</td>
<td>56.61±0.73</td>
</tr>
<tr>
<td>8</td>
<td>423.6±0.52</td>
<td>5367.20±0.41</td>
<td>43.06±0.66</td>
<td>58.84±0.65</td>
<td>61.81±0.61</td>
</tr>
<tr>
<td>9</td>
<td>477.5±0.33</td>
<td>7018.60±0.34</td>
<td>65.68±0.44</td>
<td>41.29±0.49</td>
<td>67.00±0.34</td>
</tr>
<tr>
<td>10</td>
<td>483.4±0.45</td>
<td>7844.20±0.39</td>
<td>75.35±0.50</td>
<td>48.49±0.72</td>
<td>69.93±0.31</td>
</tr>
<tr>
<td>11</td>
<td>483.0±0.58</td>
<td>7431.00±0.28</td>
<td>79.88±0.45</td>
<td>52.44±0.34</td>
<td>71.06±0.62</td>
</tr>
</tbody>
</table>

* The readings were taken in triplicate and values represent Mean ± SD.
* The experiments were repeated thrice
Table 5.3: Activity of different antioxidant enzymes (U/min/g of FW) during various phases of germination of *N. sativa* in NaCl stress.

<table>
<thead>
<tr>
<th>DAY</th>
<th>SOD</th>
<th>CAT</th>
<th>POD</th>
<th>APX</th>
<th>LIPID PEROXIDATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>448.9±0.59</td>
<td>3667.34±0.53</td>
<td>16.53±0.29</td>
<td>12.35±0.22</td>
<td>35.63±0.12</td>
</tr>
<tr>
<td>1</td>
<td>445.3±0.71</td>
<td>3302.81±0.14</td>
<td>30.60±0.63</td>
<td>16.39±0.14</td>
<td>40.71±0.67</td>
</tr>
<tr>
<td>2</td>
<td>443.9±0.67</td>
<td>4541.20±0.26</td>
<td>25.81±0.03</td>
<td>38.18±0.32</td>
<td>61.66±0.09</td>
</tr>
<tr>
<td>3</td>
<td>449.2±0.23</td>
<td>4128.44±0.63</td>
<td>40.53±0.40</td>
<td>57.54±0.53</td>
<td>49.84±0.31</td>
</tr>
<tr>
<td>4</td>
<td>452.0±0.39</td>
<td>5779.81±0.54</td>
<td>57.77±0.01</td>
<td>82.53±0.27</td>
<td>38.31±0.46</td>
</tr>
<tr>
<td>5</td>
<td>473.3±0.39</td>
<td>6064.02±0.31</td>
<td>50.76±0.69</td>
<td>91.71±0.54</td>
<td>40.92±0.61</td>
</tr>
<tr>
<td>6</td>
<td>483.6±0.83</td>
<td>6809.63±0.09</td>
<td>67.63±0.14</td>
<td>76.99±0.34</td>
<td>54.62±0.48</td>
</tr>
<tr>
<td>7</td>
<td>493.1±0.45</td>
<td>6651.47±0.33</td>
<td>68.09±0.28</td>
<td>86.03±0.12</td>
<td>59.18±0.05</td>
</tr>
<tr>
<td>8</td>
<td>484.8±0.21</td>
<td>825.69±0.047</td>
<td>76.03±0.05</td>
<td>89.18±0.66</td>
<td>59.76±0.29</td>
</tr>
<tr>
<td>9</td>
<td>481.0±0.45</td>
<td>8954.21±0.38</td>
<td>91.57±0.49</td>
<td>91.06±0.47</td>
<td>62.27±0.58</td>
</tr>
<tr>
<td>10</td>
<td>485.9±0.36</td>
<td>9082.62±0.28</td>
<td>68.53±0.37</td>
<td>89.69±0.63</td>
<td>68.38±0.11</td>
</tr>
<tr>
<td>11</td>
<td>489.1±0.53</td>
<td>9192.22±0.74</td>
<td>67.87±0.49</td>
<td>91.98±0.69</td>
<td>60.03±0.29</td>
</tr>
</tbody>
</table>

* The readings were taken in triplicate and values represent Mean ± SD.
* The experiments were repeated thrice

**Fig. 5.1:** Level of lipid peroxidation in control, NaCl and Cd-treated *N. sativa* seeds in different phases of germination.

*Integral University, Lucknow.*
5.4.1. Alteration in the Activity of Antioxidant Enzymes during Different Phases of Germination in N. sativa Seed

5.4.1.1. Lipid peroxidation

The percentage inhibition of hydroxyl free radical scavenging activity was seen to be more or less similar during the germination period till the formation of seedling in N. sativa. Maximum inhibition was observed on 2\textsuperscript{nd} day of germination and least activity was seen on 4\textsuperscript{th} day. Inhibition was also seen to increase in the plantlet after complete germination (Table 5.1 & Fig. 5.1). It is well known that ROS induced lipid peroxidation of membranes is a reflection of stress induced damage at the cellular level. The change in MDA
contents, especially in oil rich seeds, is often used as an indicator of oxidative damage (Sung, 1996). In the present study, oxidative damage to tissue lipid was estimated as MDA content. Increased MDA contents in seeds during the early phases of seed germination suggest that lipid peroxidation increases in the starting of germination process. In this study, an opposite trend in the MDA content with the activities of SOD, POD and CAT (Figs. 5.2, 5.3, 5.4) was observed. Elevated MDA contents mediated by free radicals and peroxides are considered to be one of the likely explanations for lipid peroxidation during germination (Schopfer et al., 2001). Our findings indicate that lipid peroxidation occurred during seed germination and early seedlings growth.

5.4.1.2. Superoxide dismutase

The level of antioxidant enzyme SOD was slightly decreased in the samples from first day to fourth day, followed by a continuous increase, reaching maximum at the end of germination process. The change in the activity of SOD was not too significant during germination, so it can be concluded that SOD activity is not correlated with the changes during seed germination. However, its presence in all samples suggests that this enzyme may participate in protection against free superoxide radicals. The control of steady-state ROS levels by SOD is an important protective mechanism against cellular oxidative damage, since O$_2^-$ acts as a precursor of more cytotoxic or highly relative ROS (Mittler et al., 2004). SOD has been established to work in collaboration with POD and CAT which act in tandem to remove O$_2^-$ and H$_2$O$_2$, respectively (Blokhina et al., 2003). Earlier reports showed that increased SOD activities and cellular ROS levels are involved in many life processes of plant including developmental course such as seed germination (Rogozhin et al., 2001; Duèie et al., 2003; Wojtyla et al., 2006). Enhanced SOD activity can be triggered by increased production of ROS or it might be a protective measure adopted by the growing seedlings against oxidative damage. Moreover, the changes of SOD activity in the germinating seeds are
correlated to those of CAT and POD activities (Figs. 5.3 & 5.4). Our findings
are also in agreement with previous reports suggesting the participation of
SOD in the defense mechanism during germination and early seedlings
development (Duèë et al., 2003; Wojtyla et al., 2006). SODs are generally
organized into multi-gene families. Multiple SOD isoenzymes reported for
some plant species are differentially expressed in different organs and at
distinct developmental and physiological conditions (Blokhina et al., 2003;
Mittler et al., 2004). Thus, the changes in SOD isoenzyme patterns reflect a
complex defense against oxidative stress (Blokhina et al., 2003; Mylona et al.,
2007).

5.4.1.3. Catalase

In the activity of CAT enzyme a significant decrease was observed
during the first four days of germination, followed by a sharp increase in later
stages of seed germination. From the obtained results it was seen that the CAT
activity is correlated with the germination. CAT activity was increased in
germinated seeds after 4 days of germination. This observation suggests that
CAT activity in seeds and seedlings may be involved in preservation of
viability during storage and also necessary for seed germination and early
seedling growth. This is in accordance with previous results indicating that
activity of antioxidant enzymes such as catalase is closely related with storage
longevity and germination percentage of bitter gourd seeds (Yeh et al., 2005).
CAT plays an integral role in the removal of ROS produced under various
stress conditions and then for the avoidance of oxidative damage. CAT and
POD, are often considered to keep H$_2$O$_2$ balance in plant tissues (Blokhina et
al., 2003). In oily seeds, CAT is particularly important in the early events of
seedling growth, because it removes H$_2$O$_2$ produced during $\alpha$-oxidation of the
fatty acids (Bailly, 2004). In the present study, increased CAT activity could be
an indication of the cellular evaluated ROS, since the amount of CAT present
in aerobic cells is directly proportional to the oxidative state of the cells (Apel
& Hirt, 2004). The induction of CAT expression has been studied intensively.
during seed germination and post-germination seedling growth in maize and sunflower, and displays a complex regulation mechanism (Bailly et al., 1996; Mylona et al., 2007).

The decrease in the levels of SOD and CAT in early phases of germination could be attributed to the increased utilization of these antioxidants, which were already present in seeds, after which the higher level of these enzymes might be due to the *de novo* synthesis of these enzyme to combat the reactive oxygen species generated excessively during the oxidative stress produced in the process of germination. These results were in compliance with the research of scientists and published data which showed that the level of the antioxidants, SOD and CAT decreased gradually in all the seeds during the first five days of germination. It was found that the activity of SOD and CAT was significantly higher in the seeds treated with 0.05% carbenazim than in the control seeds (p>0.01) (Sangeetha, 2010). Another research which supports the above result is shown by another group of researchers in *P. omorika* seeds. As no changes in enzyme activity were detected in *P. omorika* seeds up to 4th day after the start of imbibition, enhanced activity of SOD and CAT were observed, maximum activity was seen when most of the seeds germinated (Cakmak et al., 1993).

### 5.4.1.4. Peroxidases

Contrary to CAT and SOD activity, POD activity showed significant increase during germination of *N. sativa* seeds. The activity of POD per gm of fresh weight increased continuously from the start of imbibition and was highest in the seedling (on 11th day of germination). In dry seeds there was very less POD activity observe. Specific activity of POD per gram of fresh weight increased continuously till the end of germination and was highest on the 10th and 11th day of germination, when the complete plantlets were developed (Table & graph 5.4). Peroxidase activity showed the most notable changes during germination. Dry seeds exhibited negligible POD activity, but during germination this activity appeared and dramatically increased. From
this fact it could be concluded that POD activity may have a role in the later stages of germination and in seedling development, but not in preservation of dry seeds. Peroxidase activity was not detected even in imbibed seeds before the start of germination in tomato (Morohashi, 2002) and Chenopodium rubrum (Dučić et al. 2003; Mitrović et al., 2005). It is also reported that POD activity is associated with active differentiation during growth and development of seedlings. (Harvey and Oaks, 1974; 1964; Saxena, 1989). In plants, POD is considered to be associated with a number of essential metabolic processes, such as cell elongation, lignification, phenolic oxidation, pathogen defense and defense against stress (Passardi et al., 2005). Moreover, PODs probably play important roles in seed germination, growth, morphogenesis, and even in the final stage of senescence and death (Kawano et al., 2003). Changes in POD activities occur during developmental process in tissue specific manner and differential regulation in response to germination process and plant species has been reported by several researchers (Omidiji et al., 2003; Duèiæ et al., 2003; Wojtyła et al., 2006). Thus, increased POD activity might be involved in the defense system during seed germination and early seedlings development. Plant PODs are generally organized into multi-gene families. Multiple isoenzymes of POD reported for some plants were detected in the same tissue at different developmental stages (Duroux & Welinder, 2003).

5.4.1.5. Ascorbate Peroxidase

APX activity was seen to increase till 5th day of germination after which there was decrease till 9th day of germination. The APX activity was again increased in 10th and 11th day of germination (Table 5.5 & Fig. 5.5). The results are in compliance with the investigation done in wheat seeds, imbibition and germination is associated with enhanced cellular capacity to detoxify H$_2$O$_2$. For this detoxification the operation of ascorbate peroxidase together with the ascorbate-regenerating enzymes appears to be of particular importance (Cakmak et al., 1993).
5.4.2. Changes in Antioxidative Defence System in *N. sativa* Seed grown under CdCl₂ stress during Different Phases of Germination

In plants, Cd toxicity can promote altered metabolism (Bergmann *et al.* 2001), which can include the formation of ROS (Schützendübel *et al.* 2001). The inhibitory effect of Cd on growth of seedlings has been reported for several plant species (Vito´ria *et al.* 2001, Ferreira *et al.* 2002). The results showed that exposure of seeds to Cd-stress reduced the rate of germination and fresh and dry matter production as compared to control. The decrease in hypocotyle growth caused by toxicity of metals was more severe than the decrease in epicotyle growth. (Chapter 2)

5.4.2.1. Lipid peroxidation

In *N. sativa*, the percentage inhibition of hydroxyl free radical was seen to be more or less similar during the germination period till the formation of seedling in the presence of cadmium stress. Maximum inhibition was observed on 2<sup>nd</sup> day of germination and least activity was seen on 4<sup>th</sup> day. Inhibition was also seen to increase in the plantlet after complete germination. Under cadmium stress, percentage inhibition was seen to decrease from 1<sup>st</sup> day to 6<sup>th</sup> day after which it was increased from 7<sup>th</sup> day to 11<sup>th</sup> day till the formation of seedling. The percentage inhibition of hydroxyl free radicals in terms of lipid peroxidation was very high in seeds which germinated under stress of cadmium ions during the whole germination process in each stage. The increased accumulation of lipid peroxides is indicative of enhanced production of toxic oxygen species. The level of MDA (one of the major TBA reactive metabolites) increased in Cd-treated samples. Similar increases of TBARS by Cd exposure have been observed in *Phaseolus vulgaris* (Somashekaraiah *et al.*, 1992; Dalurzo *et al.*1997; and Schickler & Caspi, 1999), *Helianthus annuus* (Brez,1998) and *Pisum sativum* plants (Dalurzo *et al.*1997).
5.4.2.2. Superoxide dismutase

One of the mechanisms involved in preventing the damage caused by ROS requires the synthesis of antioxidant enzymes. In germinating seeds of *N. sativa*, the total SOD activity appeared to be significantly increased. The results showed that the activity of SOD was significantly increased in response to Cd supply. This increase in activity of O$_2^-$ and H$_2$O$_2$-scavanging enzymes would be considered as an evidence for Cd- induced ROS production. The results reported in the literature also indicate distinct responses depending on plant species and time length of Cd treatment. These results are in well agreement with the results presented in the work of Vitoria *et al.* (2001) who found that activity of SOD was increased by Cd in roots and leaves of radish plants. An increased activity of SOD in response to Cd treatment was also reported in pea (Gallego *et al.*, 1999) and *Alyssum* species (Schickler and Caspi, 1999).

5.4.2.3. Catalase

CAT activity in different phases of germination of *N. sativa* seed exhibit considerable decrease by CdCl$_2$ treatments, during the time length of the experiment. However, these changes appeared to be independent of the period of exposure to Cd and were also observed for the control plants. The results observed for CAT could indicate that CAT is not responding to the oxidative stress caused by Cd. However, it could also be argued that Cd at the concentrations and plant species tested is not generating such an oxidative stress. In previous reports, CAT activity from *Agropyron repens* and radish was shown to increase in leaves and roots in response to Cd (Vitoria *et al.* 2001; Brej, 1998). On the other hand, in *Phaseolus aureus* (Shaw, 1995), *Pisum sativum* (Dalurzo *et al.*1997), *Lemna minor* (Mohan & Hosetti, 1997), *Helianthus annuus* (Brej, 1998) and *Amaranthus lividus* (Bhattacharjee, 1998) CAT activity has been shown to decline when exposed to Cd.
5.4.2.4. Peroxidases

There is considerable decline was observed in POD activity in response to Cd toxicity till 8th day after which a significant increase was reported from 9th to 11th day. Peroxidases exist in both the cytosol and the cell wall and decompose H$_2$O$_2$ to H$_2$O and O$_2$; they have been reported to be involved in several physiological and biochemical processes such as cell growth and expansion, differentiation and development, auxin catabolism, lignification, as well as abiotic and biotic stress responses. Decrease in peroxidase activity under Cd stress was observed in our study. Under these conditions, where CAT and peroxidases are diminished, the cell might not be fully competent to remove H$_2$O$_2$ which would accumulate to toxic levels.

5.4.2.5. Ascorbate Peroxidase

A continuous increase in APX activity was also found throughout the germination. It was observed that APX activity was quite low in seeds (12.34 U/min/g of fr. wt.) reaching Maximum on 11th day of germination (52.44 U/min/g of fr.wt.). This activity is approximately 1.5 folds higher, when compared to control. The APX and CAT are the two potent scavengers of H$_2$O$_2$ maintain its level. The induction of APX provides additional defence against metal toxicity and keeps the metabolic activities in germinating seeds functional. Thus, the oxidative damage imposed by cadmium is avoided with an altogether increase in the activity of antioxidant enzymes.

5.4.3. Changes in Antioxidative Defense System in N. sativa Seed grown under NaCl stress during Different Phases of Germination

Resistance to salinity occurs when a plant withstands the imposed stress and this may arise from either tolerance or a mechanism that permits avoidance of the stress. The loss of the ability to scavenge free radicals during
stress is generally attributed to a decrease in the activity of antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) (Pastori and Trippi, 1993; Mittova et al., 2002). Even under optimal conditions many metabolic processes produce ROS. The production of toxic derivatives is increased as a result of all types of abiotic or biotic stresses. Plants possess efficient systems for scavenging active oxygen species, thus protecting them from destructive oxidative reactions (Foyer et al., 1994). As part of this system, antioxidant enzymes are key elements in the defense mechanisms. Many changes have been observed in the activities of antioxidant enzymes in plants grown under salt stress.

5.4.3.1. Lipid peroxidation

The MDA content in the seeds grown under salt stress (about 42.2%) markedly increased in comparing with the control. MDA is a product of peroxidation of unsaturated fatty acids in phospholipids, and the level of lipid peroxidation has been used as an indicator of free radical damage to cell membranes under stress conditions. Therefore, MDA has been widely used as selection to assess salt injury as criterion in various plants (Jain et al., 2001; Katsuhara et al., 2005; Jaleel et al., 2007). Lipid peroxidation can occur in both chloroplasts and mitochondria (Elstner, 1982; Bowler et al., 1992). Our results indicated a significant increase in the level of MDA content in salt treated samples as compared to control. The increase in the MDA content at all the samples may be due to oxidative damage affecting both organelles (chloroplasts & mitochondria). Our results are in compliance with the report of Azevedo Neto et al. (2006), Khan and Panda (2008), stated that it is likely that MDA plays important role in salt tolerance of maize cultivars.

5.4.3.2. Superoxide dismutase

In this study, the activities of antioxidant enzymes SOD showed a progressive increase as the germination proceeds. The results showed that the
activity of antioxidant enzyme SOD in N. sativa seeds in different germinating stages under 100 mM NaCl stress, was associated with the ability to combat with antioxidative stress. The activity of SOD considerably increased from 3\textsuperscript{rd} day to 7\textsuperscript{th} day as compared to control. The maximal activity of SOD in N. sativa came to 493.1 U/min/g of fresh weight in NaCl stress on 7\textsuperscript{th} day of germination which was considerably higher than control. SOD is the key enzyme in the active oxygen scavenger system and considered to be the first line of defense against ROS (Hamilton & Heckathorn, 2001). The significant increase observed in SOD activity in all the salt treated samples suggested that the enzyme may function as a ROS scavenger by converting O$_2^-$ to H$_2$O$_2$ (Alscher et al., 2002; Costa et al., 2005). The increased enzyme activity coincided with enhanced H$_2$O$_2$ in Cassia angustifolia (Agarwal and Pandey, 2004) and Afzal plants (Khosravinejad et al., 2008) in response to increased salt concentration. The activity of antioxidant enzymes has also been reported to increase under saline conditions in the case of salt tolerant cotton (Meloni et al., 2003), shoot cultures of rice (Fadzilla et al., 1997), cucumber (Lechno et al., 1997), wheat shoot (Menogeuzzo and Navari-Izzo, 1999) and pea (Hernandez et al., 1999).

5.4.3.3. Catalase and Peroxidases

CAT and POD activities were also increased markedly in the most of the salt treated samples (Table 5.3). This may be due to the higher production of H$_2$O$_2$ by the enhanced activity of SOD. The CAT and POD destroys the H$_2$O$_2$ produced by scavenging activity of SOD and other reactions (Foyer et al., 1994). POD is thought to be involved in various plant processes, including lignification (Hendriks et al., 1991), oxidation of phenolics (Largrimini, 1991), regulation of cell elongation (Fry, 1986) and detoxification of toxic compounds such as H$_2$O$_2$, which are produced as a result of oxidative stress (Chaparzadeh et al., 2004). Moreover, increasing body of evidence suggests that high salinity levels induce oxidative stress (Savouré et al., 1999). The tolerance of some genotypes to environmental stresses has been associated
with higher activities of antioxidant enzymes. For example, the wild NaCl-tolerant species *Lycopersicon pennellii* had higher activities of SOD, POD and CAT than the cultivated species *L. esculentum* (Shalata & Tal, 1998). Costa *et al.* (2005) suggested that a strong correlation between salt tolerance and POD activity in *Sorghum* genotypes. Agarwal and Shaheen (2007) reported that a higher CAT activity in *Monordica charantia* was associated with tolerance of plant to NaCl.

5.4.3.4. Ascorbate Peroxidase

APX activity increased with increasing days of germination in NaCl rich medium. A higher degree of protection against oxidative damage should require a fast removal of H$_2$O$_2$ by the scavenging systems, thus minimizing H$_2$O$_2$ toxicity and the formation of the highly toxic hydroxyl radicals (Perl *et al.*, 1993). The intercellular level of H$_2$O$_2$ produced under stress conditions is regulated by catalases and peroxidases. Ascorbate peroxidases (APX) can scavenge H$_2$O$_2$ that is inaccessible for catalase because of their high affinity for H$_2$O$_2$ and their presence in different subcellular locations (Noctor *et al.*, 2002). The analysis of the activity of APX in the germinating seeds of *N. sativa* showed a significantly high activity in the samples grown under NaCl stress when compared non-treated control (Fig. 5.5). Regeneration of the oxidized ascorbate is a critical component of the antioxidant scavenging system in plants.

5.5. CONCLUSIONS

In conclusion, the present findings suggest that the changes of MDA content and antioxidant enzymes activity in the germinating seeds of *N. sativa* appear to be more closely related to germination process. Changes in antioxidant enzymes activity might be regulated by different responses of SOD, POD and CAT by changing enzyme activities. From the results obtained
it can be concluded that the expressions of antioxidant enzymes are relatively higher in germinated/sprouted seeds.

Thus, our findings strongly support the hypothesis that POD, SOD and CAT activities are up-regulated as an antioxidant defense system against endogenous oxidant radicals generated during seed germination. From these results it can also be concluded that the application of stress could be well used to promote plants’ innate antioxidant defense potentials.

5.6 REFERENCES


Integral University, Lucknow.


*Integral University, Lucknow.*


and antioxidant mechanism in *Phaseolus vulgaris* after Cd application.


