Abiotic stresses severely affect the growth, development and ultimately yield of the plant, which results in heavy economic losses and food crisis. Oxidative stress, which is associated with almost all the abiotic stresses is due to over production of toxic reactive oxygen species (ROS) including superoxide ion, hydrogen peroxide and hydroxyl radicals. Salt stress is recognized to bring the structure of mechanical oxygen type and of their scavenger, nonenzymatic or enzymes low molecular group antioxidants, in plant cell antioxidant enzymes have been noticed as the defensive team, whose merged intention is to defend cells from oxidative hurt, important antioxidant enzymes in the metabolism of ROS (reactive oxygen species) produced under salt stress. The function of antioxidant enzymes as the elements of the major tolerance mechanisms expanded in reaction to salt stress. Salinity stress response is multigenic, as a number of processes involved in the tolerance mechanism are affected, such as various compatible solutes/osmolytes, polyamines, reactive oxygen species and antioxidant defence mechanism, ion transport and compartmentalization of injurious ions. The role of genes/cDNAs encoding anti oxidant enzymes involved in regulating other genes/proteins, signal transduction process in defending plant from oxidative stress and strategies to improve salinity stress tolerance have also been discussed.

**Key words :** Stress tolerance, antioxidants, genetic variations, enzymatic and non-enzymatic, ROS scavenging.

**Introduction**

Salinity is one of the environmental stresses, which are expanding throughout the world. The deleterious effects caused by salinity are damage to lipids, proteins and nucleic acids and variations in mechanism of photosynthesis and respiration due to which growth and development of plant is affected. Salt stress leads to severe inhibition of plant growth and development, membrane damages, ion imbalances due to Na⁺ and Cl⁻ accumulation, enhanced lipid peroxidation and increased production of reactive oxygen species like superoxide radicals, hydrogen peroxide and hydroxyl radicals.

**Genetic diversity for salt tolerance in plants**

Most crops are salt sensitive or hypersensitive plants (glycophytes) in contrast to halophytes, which are native flora of saline environments. Some halophytes have the capacity to accommodate extreme salinity because of very special anatomical and morphological adaptations or avoidance mechanisms (Flowers *et al*., 1986). In response to salt tolerance genetic variations exist in plants species and among varieties of a particular species. Among major crops, barley (*Hordeum vulgare*) shows a greater degree of salt tolerance than rice (*Oryza sativa*) and wheat (*Triticum aestivum*). The degree of variation is even more pronounced in the case of dicotyledons ranging from *Arabidopsis thaliana*, which is very sensitive towards salinity, to halophytes such as *Mesembryanthemum crystallinum*, *Atriplex* sp., *Thellungiella salugiinea* (previously known as *T. halophila*) (Pang *et al*., 2010; Munns and Tester, 2008; Abraham, 2011). The variation in salinity tolerance in dicotyledonous species is even greater than in monocotyledonous species. In the last two decades, sumptuous amount of research has been done in order to understand the mechanism of salt tolerance in model plant *Arabidopsis* (Zhang and Shi, 2013).

**Aim of this review**

The main aim of this review is understand the role of oxidative enzymes involved in salinity tolerance in rice. With understanding their role various research perspectives can be solved in future. And it will also be helpful in clumping together the various biotechnological techniques to improve the salt tolerance in rice.
Salinity stress in rice

Rice (*Oryza sativa* L.) is the principal staple food as over 50% of the world’s population depends on it for about 80% of their food requirements (FAO, 2008). Particularly for rice (*Oryza sativa* L.), a species native to swamps and freshwater marshes, secondary salinization is becoming an increasingly serious production constraint (Akbar and Ponnampuruma, 1980). About 120,000 varieties are grown across the world in an extensive range of climatic soil and water condition. Rice is a salt sensitive monocot. In addition to its food values and economic importance rice, with its relatively small genome size together with its complete genome sequence (Sasaki *et al*., 2005) is considered as a model monocot system for various biotechnological, metabolic, genetic engineering and functional genomics development studies worldwide (Bajaj and Mohanty, 2005). However, the yield of rice, especially Asian rice (sativa) is highly susceptible to salinity (Munns and Tester, 2008). In India and especially in coastal rice fields of Maharashtra State, soil salinity is a major stress that reduces the rice productivity to a great extent (Kumar *et al*., 2008).

Mechanism of salinity tolerance in plants

Tolerance to abiotic stresses is connected to modifications of physiological and morphological characteristics these include:

a) modification in plant structural design
b) difference in leaf cuticle width, germination
c) stomatal parameter
d) protein and membrane stability
f) Osmoregulation (glycine betaine and proline)
g) antioxidants (enzymatic and nonenzymatic agents)
h) ion homeostasis and hormonal systems (Cha-um & Kirdmanee, 2010; Hasegawa *et al*., 2000; Singh *et al*., 2008; Vaidynathan *et al*., 2003).

But this review is summarized on regulation of antioxidants under salinity stress conditions.

Generation of ROS under salt stress

ROS are regularly generated in biological pathways, but when ROS are produced in excess then the condition is called oxidative stress. Plants are severely affected by oxidative stress, if ROS are accumulated to large extent. The major damage to some cellular components is caused by ROS are lipids, proteins, carbohydrates and nucleic acids.

Types of ROS

Plants under various abiotic or biotic stress conditions may lead to the overproduction of reactive oxygen species (ROS) such as singlet oxygen (‘O₂), superoxide radical (O₂⁻) hydrogen peroxide (H₂O₂) and hydroxyl radical (OH). But out of these three main type of ROS are superoxide radical, hydrogen peroxide and hydroxyl radical. Atomic oxygen has two unpaired electrons in separate orbits in its outer electron shell. This electron structure makes oxygen susceptible to radical formation. These cytotoxic ROS are incessantly produced throughout usual metabolic procedures in the cytoplasm, peroxisomes and mitochondria and they can devastate usual metabolism during oxidative hurt of nucleic acids, proteins and lipids when they are created in overload (McCord, 2000).

a) Super Oxide : Superoxide (O₂⁻) is the primary ROS, which is formed when the molecular oxygen undergoes one electron reduction. In this reaction, NADPH supplies the electron and NADPH oxidase acts as the reaction catalyst.

b) Hydrogen peroxide : On further reduction of molecular oxygen hydrogen peroxide (H₂O₂) is formed.

The one electron reduction of superoxide first forms peroxide (O₂⁻), which is neutralised by two protons to form hydrogen peroxide. The spontaneous dismutation unlikely occurs at physiological pH when superoxide is in anionic form (O₂⁻) because there is repulsion of superoxides due to negative charge. On the other hand, when the pH is acidic, the proportion of neutral form (HO₂⁻) rises and then the spontaneous dismutation starts to largely participate in hydrogen peroxide formation (Koji *et al*., 2009).

Hydroxyl radical : Hydroxyl radicals are most toxic and formed on further reduction. It is an extremely potent oxidant and reacts with organic molecules at nearly diffusion rates. Hydroxyl radicals are formed by two ways.

1. Haber–Weiss reaction : Under normal conditions, this reaction proceeds at a very slower rate and resulting in the low production of OH⁻ ions.

   \[ H_2O_2 + O_2^- \rightarrow OH^- + OH + O_2 \]

2. Fenton reaction : It is common in biological systems. It occurs in the presence of transition metals like Fe^{2+}, Cu^{2+} etc.

   \[ H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH \]

The antioxidant systems can be divided into three groups:
1) Antioxidant enzymes

2) Lipid soluble, membrane associated antioxidants, e.g. α-tocopherol, β-carotene

3) Water soluble antioxidants (e.g., glutathione and ascorbate)

1) Antioxidant enzymes

An antioxidant is any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substance. Antioxidant enzymes are the most active and efficient protective mechanism. The enzymatic mechanisms are designated to minimize the concentration of O$_2^-$ and H$_2$O$_2$. The enzymes overproduced so far include catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR).

Catalase

Catalase (CAT, EC 1.11.1.6) exists mainly in the peroxisomes and as stress the number of these organelles increase. CAT can have an important role in H$_2$O$_2$ detoxification that can diffuse into the peroxisome from other cell locations, where it is produced (Mittler, 2002). Catalase is one of the most active catalysts produced by nature. It decomposes H$_2$O$_2$ at an extremely rapid rate, corresponding to a catalytic centre activity of about 10$^7$ min$^{-1}$. Depending on the concentration of H$_2$O$_2$, it exerts a dual function (Deisseroth and Dounce, 1970). At low concentrations (<10$^{-6}$ M) of H$_2$O$_2$, it acts “peroxidatically,” where a variety of hydrogen donors (e.g., ethanol, ascorbic acid) can be oxidised. CAT catalyses the hydrogen peroxide breakdown to water:

$$\text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$$

Catalase is unique among H$_2$O$_2$-degrading enzymes in that it can degrade H$_2$O$_2$ without consuming cellular reducing equivalents. Hence, catalase provides the cell with a very energy-efficient mechanism to remove H$_2$O$_2$. Therefore, when cells are stressed for energy and are rapidly generating H$_2$O$_2$ through “emergency” catabolic processes, H$_2$O$_2$ is degraded by catalase in an energy efficient manner.

Superoxide Dismutase

Superoxide dismutases (SOD, EC 1.15.1.1 [18]) are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide.

$$\text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$$

Several common forms of SOD exist: they are proteins cofactored with copper and zinc, or manganese, iron, or nickel. Thus, there are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese) and the Ni type, which binds nickel. In higher plants, superoxide dismutase enzymes (SODs) act as antioxidants and protect cellular components from being oxidized by reactive oxygen species (ROS) (Alscher et al., 2002).

Ascorbate peroxidase

Ascorbate peroxidase (APX) (EC 1.11.1.11) belongs to the class I heme-peroxidases that is found in higher plants, chlorophytes (Takeda et al., 2000). APX detoxify peroxides such as hydrogen peroxide using ascorbate as a substrate. The reaction they catalyse is the transfer of electrons from ascorbate to peroxide, producing dehydroascorbate and water as products.

$$\text{C}_6\text{H}_8\text{O}_6 + \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_6\text{O}_6 + 2\text{H}_2\text{O}$$

APX has a much higher affinity to H$_2$O$_2$ than CAT suggesting that they have different roles in the scavenging of this ROS, with APX being responsible for maintaining the low levels of hydrogen peroxide while CAT is responsible for the removal of its excess (Mittler, 2002). The different isoforms are classified according to their subcellular localization. Soluble isoforms are found in cytosol (cAPX), mitochondria (mitAPX) and chloroplast stroma (sAPX), while membrane-bound isoforms are found in microbody (including peroxisome and glyoxisome) (mAPX) and chloroplast thylakoids (tAPX).

Glutathione reductase

Glutathione reductase (GR) (E.C. 1.6.4.2) is a flavoprotein oxidoreductase which catalyses the reduction of glutathione disulphide (GSSG) to the sulphhydryl form GSH. GR is predominantly found in chloroplast. However, a small amount of the enzyme isoforms is also found in mitochondria, cytosol, and peroxisomes (Edwards et al., 1990 and Jimenez et al., 1997). It acts as a scavenger for hydroxyl radicals, singlet oxygen and various electrophiles. Reduced glutathione participates in glutathione ascorbate cycle in which reduced glutathione reduces dehydroascorbate, a reactive byproduct of the reduction of hydrogen peroxide (Gill et al., 2013).

2) Lipid soluble, membrane associated antioxidants

α Tocopherol

α Tocopherol is the major vitamin E compound found in chloroplasts of leaves and thylakoid membranes. The levels of tocopherol is continuously changing in response to stress and the amount depends upon the type and frequency of stress. A tocopherol is common in leaves while γ tocopherol is more common in roots. They have a role in ROS protection as they can quench singlet oxygen.
(Gill and Tuteja, 2010) and can act as an antioxidant and terminate chain reactions occurring during lipid peroxidation (Garg and Manchanda, 2009).

**Carotenoids**

When plants are under oxidative stress carotenoids are the pigments, which protect the plants. They are efficient antioxidants for scavenging singlet oxygen and peroxyl radicals. Carotenoids have an important protective role during photosynthesis as these molecules can quench the excited states of chlorophyll in order to avoid the production of singlet oxygen. As a consequence, the carotenoid molecules become themselves excited, but this is not a big problem as they don’t have enough energy to form this ROS species (Taiz and Zeiger, 2002).

3) **Water soluble anti-oxidants**

**Glutathione and ascorbate**

Glutathione is abundant in most of the crops found on earth. It is synthesised in both chloroplast and cytosol and occurs at a prominent level in both the compartments. In association with ascorbic acid, it functions as an antioxidant for scavenging free radicals formed during various abiotic stresses. Glutathione is a tripeptide (containing glutamate, cysteine and glycine) that can exist in two predominant forms: the reduced form (usually represented by GSH) and the oxidized form (usually represented by GSSG). It is involved in the sulphur metabolism and in defence reactions against oxidative stress (Potters et al., 2002). Superoxide radical is regularly synthesized in the chloroplast (Elstner et al., 1991) though some quantity is also reported to be produced in microbodies (Lindquist et al., 1991). Scavenging of \((O_2^-)\) by SOD results in the production of \(H_2O_2\), which is removed by APX (Asada, 1992) or catalase (Scanadialas, 1990). However, both \(O_2^-\) and \(H_2O_2\) are not as toxic as the \((OH)\), which is formed by the combination of \(O_2^-\) and \(H_2O_2\) in the presence of trace amounts of \(Fe^{2+}\) and \(Fe^{3+}\) by the Haber–Weiss reaction (Fenton et al., 1889; Haber and Weiss, 1934). Hydroxyl radical can damage chlorophyll, protein, DNA, lipids and other important macromolecules (Frankel, 1985 and Imlay et al., 1986). Thus, fatally affecting plant metabolism and ultimately growth and yield. A schematic presentation of production and scavenging of \(O_2^-\), \(H_2O_2\), and \(OH\) and \(OH\) mediated lipid peroxidation and glutathione peroxidise mediated stabilization of lipids are presented in fig. 1.

**Current status of effect of salinity on antioxidant enzyme activity in rice**

The above explained antioxidant enzymes helps the plants to cope the conditions under various oxidative stress conditions. The activity of various antioxidant enzymes are affected during salinity stress conditions which are as follows.

As in the leaves of the rice plant, salt stress preferentially enhanced the content of \(H_2O_2\) as well as the activities of the superoxide dismutase (SOD), Ascorbate peroxidase (APX) where as it induced in the decrease in catalase (CAT) activity. On the other hand, salt stress has little effect on the activity of glutathione reductase (Lee et al., 2001).

In another experiment plants were subjected to salinity stress and under three different salt concentrations. The sensitive varieties exhibited a decrease in GR activity while the tolerant variety showed increase in GR activity. CAT and APX activities increased with increasing salt concentration. CAT and APX activities were higher in tolerant varieties than in susceptible one (Yasar et al., 2008).

Yaghushi (2014) conducted their experiment on improved cultivar Ghaem and traditional cultivar Sanjego at different salinity levels and found that the activity of antioxidant enzymes CAT, APX, SOD, POD increased in Sanjego will increasing salt concentrations.

Safeena and Bhandara (2009) conducted their experiment to find tolerant and sensitive variety and found that Pokkali high salt tolerant variety showed increase in Superoxide dismutase (SOD) activity and Catalase (CAT) activity while decrease in Peroxidase (POD) content. Moderately At 353 had a slight increase in SOD and CAT but high content of POD. And increase in EC IR 28 resulted in decrease in SOD and CAT activities and increase in content of POD.

Sese et al. (1998) analysed the effect of different salt concentrations on four rice varieties Pokkali, Hitomebore, IR 28 and Bankat. The NaCl concentrations were provided as 0, 6 and 12 dS m\(^{-1}\) for one week and observed their antioxidant response and found that high salt sensitive varieties exhibited a decrease in superoxide dismutase activity and an increase in peroxidase activity under high saliniztion. These varieties also exhibited increase in lipid peroxidation and electrolyte leakage as well as higher Na\(^+\) accumulation in the leaves under salt stress. The salt-tolerant variety Pokkali however, showed only slight increase and decrease in superoxide dismutase and peroxidase activity, respectively, and virtually unchanged lipid peroxidation, electrolyte leakage and Na\(^+\) accumulation upon salinization. On the other hand, the putative salt-tolerant Bankat variety, which showed a slight stimulation in growth rate similar to Pokkali at moderate salinity level, exhibited Na\(^+\) accumulation and symptoms
of oxidative damage during salt stress similar to the salt-sensitive varieties rather than the salt-tolerant one.

Lee et al. (2013) observed that salt stress reversed ROS scavenging response in roots and leaves of two rice cultivars a salt tolerant variety Pokkali and a salt sensitive variety IR 29. Pokkali is known to have higher hydrogen peroxide ($H_2O_2$) scavenging enzyme activities in non-treated seedlings, including ascorbate peroxidase, catalase and peroxidase activities. However, these enzymatic activities were induced to a greater extent in IR-29 by the salt stress. While the level of endogenous $H_2O_2$ was lower in Pokkali than in IR-29, it was reversed upon the salt treatment. Nevertheless, the decreased amount of $H_2O_2$ in IR-29 upon the salt stress didn’t result in a high scavenging activity of total cell extracts for $H_2O_2$, as well as $O_2^-$ and $OH^-$ species.

**Conclusion**

Salinity is one of the major constrain that affect the crop productivity world-wide. Salinity like other abiotic stress leads to the production of ROS in plants, which leads to oxidative stress as these are highly toxic and reactive. ROS are generated in various cellular components during stress conditions and impose their toxic effects on cellular parts and cause oxidative damage. Oxidative damage may have their impact on various cellular parts like lipids, nucleic acids and proteins. Permeability property of plasma membrane is altered to some extent. Cells during abiotic stress conditions have their own defence mechanism to alter the effect of stress. The effect may be altered by the use of enzymatic antioxidants (SOD, CAT, APX, GR etc) or non enzymatic antioxidants (tocopherol, carotenoids, glutathione and ascorbate). The over expression of antioxidant enzymes in plants help to increase tolerance power against salinity stress, it may cause cell death in some plant species. Activity of most of the antioxidant enzymes is increased during stress conditions. Various varieties of rice like CSR 30, CSR 10 and CSR 3 etc. have been developed which are tolerant to salinity stress. In order to make more tolerant varieties gene pyramiding of antioxidant enzymes can be done in which various genes responsible for higher production of antioxidants are introduced, which helps the plant to tolerate stress conditions.

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