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FREE RADICALS AND THEIR ENZYMATIC SCAVENGER DURING MOISTURE STRESS IN PLANT

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Abstract: Free radicals production is the internal phenomena occur during any kind of stress but the moisture stress is most common problem in plant. Accumulation of excess free radicals imparts adverse effects on metabolic activity and eventually damage of the cell. These are the molecule produced by the acceptance of single electrons by the oxygen in the cell. The produced molecule initiate chain reaction, generate reactive oxygen species, reactive nitrogen species, other free radicals and non radical. The main free radicals *viz.* superoxide (O_2^-**)**, singlet oxygen ($^1O_2^-$), hydroxyl (·OH), alkoxyl (RO•), peroxyl (ROO•), lipid peroxyl (LOO•), etc. which cause damage of biomolecules and leakage in the biomembrane. The molecules which scavenge these free radicals are enzymatic antioxidants viz. Superoxide dismutase (SOD), Catalase (Cat), Guaiacol Peroxidase (GPOX), Ascorbate peroxidase (APX), Glutathione reductase (GR) and Glutathione peroxidase (GPOX) activated for scavenging the excess free radicals and suppression of excess accumulation in the cell.
Keywords: Free radicals, SOD, Cat, GPOX, APX, GR.

Introduction

Water constitutes 80-95% of fresh mass of the plant and provides platform for metabolic activity. Its deficiency adversely affects the growth and yield including damage of chloroplast structure, constrain photosynthesis ultimately reduce biomass formation. Water stress promotes the production of free radicals as well as lipid peroxidation resulting in deterioration of the cell. Free radicals are reactive oxygen species (ROS), reactive nitrogen species (RNS) and other non-radical reactive derivatives. They formed due to presence of unused O_2 and/or homolytic cleavage of chemical bond and initiate chain reaction for further formation of other free radicals. The free radicals viz. O_2^-, $^1O_2^-$, OH^-, and H_2O_2 are most commonly formed in the cell which are beneficial at physiological level but excess become toxic to the cell (Kant, 2020). The O_2^- react with signaling free radical species ($NO^{\cdot}$**)** to produce peroxynitrite ($OONO^-$) an another RNS in the cell. The antioxidant defense systems in plant minimize the damage and save from water stress up to certain extent. The enzymatic defense system includes SOD, Cat, GR, GPOX, GPX as well as APX. These biomolecules produced commonly in the system for curing the plant from regular accumulation of ROS and RNS (Khaleghi et al., 2019).

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Superoxide dismutase (SOD)

It is the Metalloenzyme acts as first line of defense against ROS. It scavenges O_2^- and converts it into H_2O_2 and O_2. Thus, prevent further formation of very dangerous free radicals hydroxyl (·OH) and peroxynitrite ($OONO^-$) in the cell. SOD is of three types: i) Fe-SOD - located in chloroplast ii) Mn-SOD - located in Mitochondria and Cu/Zn SOD -located in chloroplast and cytosol. These are the most effective intracellular enzymatic antioxidants formed where free radicals usually produced due to metabolic reactions (Caverzan et al., 2016).

Catalase

It is a haeme containing enzyme mostly found in peroxisome, mitochondria and cytosol and directly dismutates H_2O_2 into H_2O and O_2. It has three isoforms Cat1, Cat2 and Cat3 they become more active when stress induced ROS especially H_2O_2 accumulate in the cell. Catalase activity in leaves is due to Cat 1 activity which accounts for the 80% of total Cat1 while Cat2 activity accounts for ~20% and it is found in phloem. The Vmax value of catalase is very high and it does not consume reducing power during scavenging of H_2O_2 hence it has lower affinity for H_2O_2 than APX and GPOX (Willekens et al., 1997).

Guaiacol peroxidase (GPOX)

Guaiacol peroxidase (GPOX) is an important enzyme among different peroxidases and scavenges H_2O_2 in cytosol, vacuole, apoplastic region etc. using the reducing substrate guaiacol. Guaiacol is a phenolic compound (o-methoxyphenol) acts as electron donor by oxidizing itself during scavenging of H_2O_2. It can biosynthesize lignin in cell wall as well as decomposes indole-3-acetic acid thus provide defense against the pathogen (Mika and Luthje, 2003).

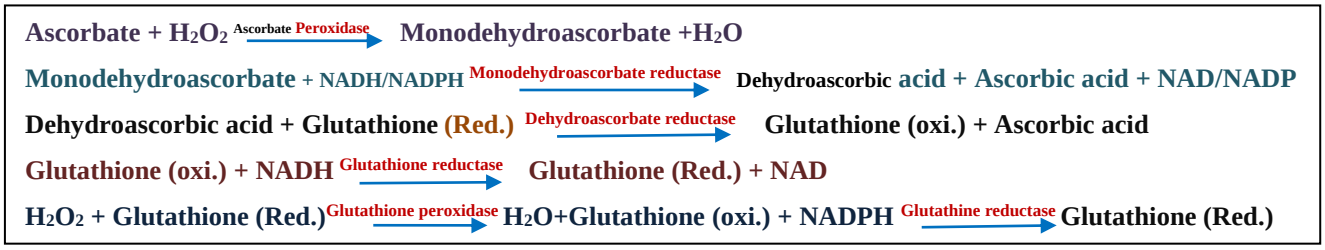
Ascorbate peroxidase (APX)

It is the efficient enzymatic antioxidants which act as main detoxifier of H_2O_2 in plant via ascorbate-glutathione cycle. It is a haeme containing enzyme capable of conversion of H_2O_2 to water by APX using ascorbate (AsA) as electron donor. Secondly, the enzyme is labile in absence of AsA, hence for maintaining the antioxidant system in the cell high level of endogenous AsA is required for activation of APX. It has been reported that below 20 µM concentration of AsA, the enzymatic activity is quickly lost. The enzyme present in different number of isoforms varies its number in different crops. It has eight isoforms reported in rice which are mainly present in cytosol, chloroplast, peroxisome and mitochondria. The Vmax value of APX is very low hence it has very high affinity with H_2O_2 and saturate at micro-

molar range. Increasing its activity during drought and salinity protect the seedling of rice from adverse effects of water stress (Pandey et al., 2017).

Monodehydroascorbate reductase (MDHAR)

It is a flavin adenine dinucleotide and the pyridine nucleotide-binding domain of flavoenzymes. It is generally found in cytosol, chloroplast, peroxisome and mitochondria. It acts together with APX during the dismutation of H_2O_2. It reduces monodehydroascorbate (MDHA) to Dehydroascorbate and Ascorbate by oxidizing NADH/NADPH. It is similar to the iron-sulphur protein reductase of bacteria not ferredoxin-NADP reductase of plant. It is more efficient enzyme in converting monodehydroascorbate to ascorbate as compared to DHAR (Ae Kyung Park et al., 2016).



Dehydroascorbate reductase (DHAR)

It regenerates the ascorbic acid for its recycle in the process of H_2O_2 removal by oxidizing the glutathione. Hence, it maintains cellular ascorbic acid for suppression of H_2O_2. Cysteine residue in the enzyme involve in the regeneration of AsA from reduction of dehydroascorbate and reduces dehydroascorbate to AsA. The oxidized catalytic cysteine residue is reduced again from sulphenic to sulphinic acid using glutaredoxin. Hence, the regeneration of DHAR is essential for continuing the AsA-GSH cycle (Ae Kyung Park et al., 2016).

Glutathione reductase (GR)

It is mainly a chloroplasic enzyme but also present in cytosol and mitochondria. It is an enzyme ubiquitously found in all organisms and plays essential role in ascorbate-glutathione cycle as well as defense against ROS. Glutathione (GSH) is one of the reducing molecule which acts as antioxidants in its reduced state. Glutathione reductase sustains the reduced state of Glutathione and maintains the GSH pool in the cell. Hence, GR and GSH plays crucial role in plant during oxidative stress.

Glutathione peroxidase (GPX)

Glutathione peroxidase is reducing H_2O_2 to H_2O by using reduced glutathione. The oxidized glutathione again reduced by glutathione reductase using reducing molecule NADPH. It generally acts in chloroplast and cytosol (Long He et al., 2017).

Conclusion

The excess production of free radicals under water stress adversely affects the metabolism and ultimately led to dry and decay of the plants. Hence, balance between production and scavenging of free radicals by antioxidants save them from early senescence.

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