TOXICITY MEDIATED OXIDATIVE STRESS AND ITS MITIGATION STRATEGIES IN CROP PLANTS

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Received 29 October 2020; accepted 07 January 2021

Highlights

- The paper includes various biotic and abiotic stresses like cold, heavy metals, plant-virus interaction etc. and chemically synthesized stresses like pesticides that occur in plants due to environmental stresses.
- Due to the stresses there is production of ROS and on the basis of the concentration of its release the ROS either helps to combat the stress or elevate it.
- Other than this there are also certain antioxidants released by plants such as AsA, glutathione to escape the stress.
- Plants can also be chemically primed in order to combat various stresses.

Abstract. Oxidative stress occurs in plant due to various environmental stressors like drought, high temperature, pathogen invasion, heavy metals, pesticides etc. when plant faces these conditions, reactive oxygen species (ROS) are produced in the chloroplast, mitochondria, plasma membrane, peroxisomes, ER and cell wall due to the leakage of electrons. Depending upon its concentration the role of ROS is decided if less then it will act as a signaling molecule but if in excess it will damage the cellular machinery of plants as the production of species like free radicals would take place. Though to combat these stress plants have antioxidant defense machinery which include enzymatic and non-enzymatic which lower down the level of ROS. Through genetic engineering more tolerant plants are produced which include modification of key genes like transcription factors. In this review article the molecular physiology of plants is discussed where in the factors contributing to stress including biotic and abiotic factors and various mitigation strategies.

Keywords: ROS, antioxidants, key genes, transcription factors, chemical priming, ETC, free radicals.

Introduction

ROS (Reactive Oxygen species) are produced as a result of stresses in plants which occur through abiotic or biotic factors. Depending upon the release of concentration of ROS the plants get affected. If it is released in intermediate concentration then they act like signaling molecules that help to overcome the stress, but if it is released in high concentration they damage the cellular machinery and metabolism of plants and alter their DNA functioning, lipid peroxidation, oxidation of proteins and nucleic acid by producing free radicals that are highly reactive like singlet oxygen, hydroxyl ion, superoxide ion and mainly $H_2O_2$ production is increased which results in necrosis or apoptosis or interfere with the signaling pathways and disturb biochemical, physiological and cellular structure of plant.

Though to combat ROS production several antioxidants are released like CAT, SOD, GSH, DHAR, GR as well as some phytohormones like JA (jasmonic acid), SA (salicylic acid) and in some conditions plants are also chemically primed for example in heavy metals toxicity in some plants $H_2O_2$ priming is done. Though there are transgenic plants that have shown improvement in the stress with less damage to their metabolic pathways and cellular organization. Some techniques have also been developed by chemical priming in plants to overcome the stress conditions (KrishnaMurthy & Rathinasabapathi, 2013; Sharma et al., 2012; Dumont & Rivoal, 2019). In this review molecular physiology of plants, responses towards...
the stress, various types of genes modification and the techniques to conquer the stresses are elaborated.

1. Molecular physiology of oxidative stress

Reactive oxygen species (ROS) are produced in plants during stress condition but excess release of ROS causes production of free radicals like singlet oxygen, OH- and H$_2$O$_2$ production which causes lipid peroxidation, photo-oxidation and causes damage to proteins and cellular structure of plants as well (Khan et al., 2019). However to combat this stress condition certain antioxidant enzymes like APX, SOD, GPX, GR, carotenoids, phytohormones are also released that lower down the concentration of ROS.

When there is high production of ROS the catalase (CAT) catalyse the dismutation of H$_2$O$_2$ and convert it into H$_2$O and O$_2$ in the cell mainly in peroxisomes and mitochondria and finishes the detoxification started by other enzymes (Kalisz et al., 2019).

ROS produced sometimes results in incompatible responses and then plants exhibit HR responses (Hypersensitive response) and due to this response, it causes programmed cell death within the few hours. It was reported that superoxide anions O$_2^-_2$ were responsible for HR between tobacco and TMV (Hammond-Kosack & Jones, 1996). H$_2$O$_2$ can be produced via some enzymatic sources or photo-oxidation (Caverzan et al., 2016; Krishna Murthy & Rathinasabapathi, 2013). ROS is produced in the chloroplast, mitochondria and plasma membrane due to the leakage of electrons that may be from ETC or any metabolic pathways as well and Oxidative stress causes biotic and abiotic stress in plants causing loss in the yield, growth and development (Sharma et al., 2012).

2. Factors contributing to oxidative stress

Factors affecting the oxidative stress and the behaviors shown by them during stress condition in plants and certain antioxidants that are produced in response to those stresses to combat or lower down the ROS levels are presented in Table 1.

3. Possible effects of various abiotic factors on plants

3.1. Cold

Cold stress decreases the growth and yield of plants, when this stress is induced various symptoms are observed including degradation of various compounds like lipids and chlorophyll and these are degraded because of the H$_2$O$_2$ as it is accumulated in response to the stress and many other symptoms are shown because of the generation of free radicals (Xie et al., 2019).

Table 1. Factors affecting the oxidative stress

| S. No. | Environmental factors | Nature of stresses | Antioxidant enzymes and transcription factors |
|-------|-----------------------|-------------------|---------------------------------------------|
| 1.    | Cold                  | Low temperature affects the seed germination because of the apoptosis or cell tissue damage mainly observed in T. aestivum in the initial level (Hasanuzzaman et al., 2013a). It is one of the abiotic stresses affecting the metabolism of plants including their growth and development (Kalisz et al., 2019). | The gene AtGRXS17 present in transgenic tomato was able to cope up with cold stress and was observed with low ion leakage as compared to its wild type. Antioxidants like APX, GSH. |
| 2.    | Salinity              | It mainly causes osmotic stress because of loss of ions which may result in programmed cell death (Apoptosis) or necrosis. Responses of plants towards stress either activation or SOS (salt overly sensitive) pathway or reactive oxygen species (Bai et al., 2018). | Overexpression of STRK1 in the rice plant when exposed to salinity was observed with increased status of growth and less ion loss. Antioxidant – SOD, APX, DHAR, CAT. |
| 3.    | Drought               | Because of water loss excess ROS is produced and free radicals are generated like singlet oxygen and H$_2$O$_2$ is produced which causes alteration in the physiological, molecular and biochemical pathway and rates of photo-oxidation is increased in plants which ultimately decreases the productivity (Warach et al., 2011). | Overexpression of APX and Cu/ZnSOD in the chloroplast of sweet potato when exposed to drought stress shows early recovery status, less photo-oxidation and less water loss. |
| 4.    | Heavy Metals          | Heavy metal gets accumulated in soil which is taken up by plants and can be due to industrial residues. Metals like Cd, Pb, Fe, Cu etc. effects the metabolic processes of plants which include decrease in net photosynthesis or chlorophyll content and this primarily occurs because toxicity affects the flow of electron carriers from water to PS-2 and it also causes hindrances in formation of plant proteins (De Almeida et al., 2007). | GSH and chemical priming by Mannitol, Sodium Nitroprusside, H$_2$O$_2$. |
| 5.    | High Temperature      | Plant’s receiving 5 degrees more temperature than their optimum temperature can affect their metabolism, cellular structures like plasma membrane, and alteration in the synthesis of proteins usually it gets reduced and translation of HSP(heat shock proteins) gets increased to combat the change the temperature (Bita & Gerats, 2013). | AsA, CAT, SOD, APR (ascorbate peroxidase). |
It was found that when the maize seedlings were exposed to the chilling temperature there were 2 fold increase in the protein carbonyl content which is a sign of oxidative damage. Activity of lipoxygenase and lipid peroxidation were increased in the leaves of maize which shows that peroxidation of membrane lipids mediated by lipoxygenase impart oxidative damage to cold stressed maize leaves.

Change in activity of enzymes of antioxidant defense system is observed in response to the cold oxidative stress (Sharma et al., 2012). In rice plant (Oryza sativa) this stress causes poor germination, seedling injury and especially during early vegetative stages, the coleoptile increases and as the young seedling starts developing leaf rolling, necrosis and chlorosis like damages are observed (Shakiba et al., 2017).

### 3.2. Salinity

Salinity stress like cold stress also causes decrease in the growth and development and this occurs in various ways like either the water uptake capacity of plants will decrease because of the osmotic stress caused by salinity which reduce the soil water potential of plants or there is excess uptake of Na$^+$ or Cl$^-$ ions mainly which creates hindrances in the metabolism of plants and these all ways depend upon various factors pathways like the soil concentration or the tolerance of plants towards salinity (Abogadallah, 2010). Due to some antioxidants that play major role in decreasing the stress for example Glutathione majorly functions in protecting plasma membrane which also helps in decreasing the oxidative stress as well as prevent the formation of free radicals which helps in lipid peroxidation. Glutathione has also been helpful in the excess uptake ions which increases the salt tolerance in plants. This enzyme is mainly found in the tissues of plants (Hasanuzzaman et al., 2017).

When high salinity stress is there because of which there is decrease uptake of phosphorus and potassium and increase in the concentration of Na$^+$ and Cl$^-$ ions which elevate the toxicity in plants resulting in necrosis or Apoptosis and also affects the metabolism and signaling pathways of plants (Khan et al., 2019).

### 3.3. High temperature

Temperature has been affecting plants growth and yield more as there is increase in the global temperature of earth by 0.085 °C (Gray & Brady, 2016).

Al-Khatib and Paulsen (1990) observed that rate of photosynthesis and the activity of thylakoids are largely affected by high temperature in wheat (Triticum aestivum L.). The rise in temperature also causes alteration in the metabolism of plants and germination of seeds, loss of water uptake capability, photosynthesis and in overall it effects plant’s morphology, anatomy, biochemistry and also causes some genetic changes. Responses that occur due to ROS are release of antioxidants and increase in the response of heat shock mechanism.

Increase in the translation of heat shock proteins and excessive release of reactive oxygen species increases the free radicle species in the plant and as well increase in the production of H$_2$O$_2$ which may cause necrosis and at very high temperature can cause programmed cell death within minutes. Alteration in the genes related to osmoprotectants, signaling molecules, regulatory protiens are also observed (Hasanuzzaman et al., 2013b).

### 3.4. Wounds

Stress causes various types of wounds which decrease the productivity of plants like due to increased temperature blistering of stems and leaves, leaf senescence is very common accompanying with spoilage of fruits and roots.

Wound causes many alterations in the cell differentiation, growth and structure of microtubules (Bita & Gerats, 2013).

### 3.5. Drought

Drought is known to be largely affecting crop plants in their productivity as it affects the rate of germination, excessive production ROS which also leads to decrease in the uptake of CO$_2$ as it slows down the photosynthetic carbon fixation. Drought for long duration of time decreases the size of leaf, opening of stomata, it subdue the root growth, suspend the flowering and fruiting drought majorly causes osmotic stress in the plants and at this time phytotomones get accumulated like ABA which is responsible for adaptive responses of plants (Waraih et al., 2011; Lata et al., 2018).

An elevated water stress in the plants will be observed in the different parts of the world as a consequence of climate change. Gray and Brady (2016), Umezawa et al. (2006) suggested that it is very important for plants to tolerate the drought conditions. Currently many theories regarding gene expression, transcriptional regulation and signal transduction have been studied in response to the drought conditions. Although to combat this stress various approaches and technologies have been developed like genetic engineering, breeding and endophytic bacteria they are known to show novel benefits to plants during drought stress except a few. Endophytes live inside the healthy tissue and they enter inside the host similar to like pathogenic microorganism via hydathodes, stomatal opening, wounds etc. and these bacteria live inside the tissue that helps plant in the defense mechanism towards drought by releasing certain phytochemicals and phytohormones like indole-3-acetic acid and also helps in increasing root length and density which eventually increases productivity and growth (Ullah et al., 2019; Ashraf, 2010; Lata et al., 2018).

### 3.6. Heavy metals

There are many metals present in the environment but specifically the metals which are heavier than 5 g cm$^{-3}$ are included in heavy metals which are almost 53 in number.
Among these metals some are toxic like Zn, Co, W, Ni and Cr and some non-toxic like As, Hg, Ag, Sb, Cd, Pb. Excess concentrations of heavy metals result in detrimental effects on plants and often cause oxidative injuries (Schützendübel & Polle, 2002) mycorrhizal fungi and mycorrhiza. Based on their chemical and physical properties three different molecular mechanisms of heavy metal toxicity can be distinguished: (a. Excess concentrations of these metals like Fe causes increase in ROS concentration by production of $O_2^-$ and $HO^-$ radicle species and it is observed in Nicotiana plumbaginifolia plants, Cu ion produce free radicals in chloroplast, intact leaves of Silene cucubalus and Phaseolus vulgaris and many more. Metal toxicity and oxidative injuries to plants are understood for many metals Cu, Fe, Zn but Cadmium (Cd) toxicity is least understood and its excessive concentrations is shown to decrease net photosynthesis and chlorophyll content as well (Gallego et al., 1996).

High Cd concentrations are also observed to cause structural changes and imbalance in redox control in plants because it inactivate the enzyme activity by reacting with SH groups of proteins (El-Kafafi et al., 2011; Canesi et al., 1998). To protect itself from metal toxicity plants release phytochelatins which are cysteine rich oligomers and in stress conditions through active transport mechanism these are transported to tonoplast which are then taken by vacuoles. In some plants increased levels of GSH is also found to prevent plants from Cd stress (Khalidi et al., 2019).

Other than the antioxidants certain chemicals also help the plants to combat the stress conditions like Cu stress can be decreased by coating plants by Sodium Nitroprusside, under Cr stress conditions mannitol is observed to produce certain antioxidants to combat the stress (Xie et al., 2019). Plant under Cd stress can be treated with exogenous GSH under non heat stress conditions which helps in increased levels of GSH in leaves which is obverse to decrease the Cd stress in rice seedlings (Hossain et al., 2015).

4. Chemically synthesized compounds that effects ROS

**Pesticides**

They are the compounds that can be either chemically synthesized in labs or are biological agents used to destroy pest population from crops and is used extensively in agriculture to protect crops from various types of insects, weeds, fungal or bacterial diseases and also helps to prevent or kill the growth of rats, insects etc. in stored food other than the beneficial aspects of these they can also be detrimental for plants, if pesticides are used in excess concentration they produce ROS which interfere in the host metabolism, chemical and physiological pathways due to oxidative stress (Shakir et al., 2018; Lushchak et al., 2018). Production ROS occurs due to the imbalance between pro-oxidants and antioxidants ratio. This imbalance is not only detrimental for plants but also is responsible for many pathophysiological diseases and to plants it may result in necrosis, lipid peroxidation, and modification in DNA structure. To detoxify this several antioxidants are released like GSH, AsA, SOD, CAT etc. (Deyashi & Chakraborty, 2016).

5. Possible effects of biotic factors

5.1. Plant-virus interaction

Viruses are the obligate parasite that is they need host to replicate and perform all its necessary intercellular function so there are various defensive immune responses exhibited by plants to protect themselves from incoming viruses and for viruses to penetrate the host, it have various genes that suppresses the activity of plant like VSRs (viral suppressors of RNA splicing), ETI, R genes (Yang et al., 2018). Plant viruses contain single genome inside its protein coat it may be ssDNA, ssRNA, positive ssRNA (contain various groups of viruses like Bromoviruses, Potexviruses and many more) and negative ssRNA viruses (they include orthotospoviruses) (Garcia-Ruiz, 2018).

Virus infects plants and they develop symptoms towards it and this happens usually when plant defends against the pathogen. There are commonly two ways by which plants respond to the pathogen.

Firstly R genes have to get control over the basal resistance of virus which is detected by plants through PAMP’s by certain recognition receptors called PTI. Now to overcome PTI, pathogen encode proteins and after getting control over plant’s basal resistance, plants produce an increased form of resistance which is often termed as Hypersensitive response (HR) often resulting in necrosis. The HR is produced when R protein activate ETI and then plant identifies these pathogens as Avr (Avirulence factors) and thus HR is the consequence of R proteins mediated resistance (Nakahara & Masuta, 2014), Second is RNA silencing, they efficiently inhibit antiviral proteins. VSRs are present in almost all plant virus interaction as defense mechanism (Burgyán & Havelda, 2011; Burgyán, 2008).

RNA silencing occurs in 3 phases that is Initiation phase, effector phase and Amplification phase. It is involved in various stresses, protection against invading pathogen like viruses, bacteria etc as it can occur at 2 levels TGS (transcriptional gene silencing) and PTGS (post transcriptional gene silencing). At the time of initiation sRNA (small RNAs) are formed as duplexes by dsRNA of length 20 to 30 nucleotide long by RNAaseIII enzyme that are known as Dicers. In plants these DCLs (Dicer like proteins) which are involved in various activities like DNA methylation, RNA degradation, hindrances in translation of proteins occur in various eukaryote groups. In Arabidopsis thaliana DCL 1 and DCL 4 produce sRNAs, microRNAs and small interfering RNAs (21 nucleotides) and DCL 2 and DCL 3 – nucleotide RNAs (22 to 24 nucleotides) (Duan et al., 2012; Csorba et al., 2015).
Chloroplast has also been observed as majorly involved in the plant-virus interaction as CPRG (chloroplast photosynthesis related genes) act as central complex for infection. When virus infects chloroplasts, symptoms are observed which reduce the activity of photosynthesis, so little alteration in the activity of photosynthesis can cause virus infection to proliferate. Symptoms commonly observe like modification in the structure of chloroplast, chlorosis in leaf, changes in the pigmentation. For example very less grana with decrease in the chloroplast size is observed in Nicotiana tabacum when its systemic leaves were infected by CMV on chlorosis (Zhao et al., 2016).

Virus infects the host cell and suppresses RNA slicing thus increases the anti-viral defense mechanism of plant but due to this still host’s gene regulation is observed to altered by micro RNAs and siRNAs thus virus partially still effects the host silencing but in the healthy plant cells if plant could not suppress the silencing the disease will not occur although with coarse of time viruses have evolved many mechanisms that can suppress the action of RNA silencing (Pumplin & Voinnet, 2013).

5.2. Plant-bacterial interaction

Bacteria are present everywhere. The interaction between the plant and the wide arrays of bacterial population is usually defined by the properties of soil like texture, moisture, rhizodepositions and many more. Bacteria’s are present in the plant rhizosphere and is of major importance to plants like it helps in plant growth promotion (Kai et al., 2016). PGPR (Plant Growth Promoting Rhizobacteria) helps in increasing the growth of plant, biofertilization of crops it also protects the plant from pathogens. When the seedlings are treated with PGPR, ISR (Induced Systemic Responses) have been observed (Ryu et al., 2004).

ISR can be defined as the resistance produced by plants or host in response to the incoming pathogen depending upon the defensive hindrances like physical and chemical barriers, activated by biotic and abiotic agents. HR (hypersensitive response) this initiates the cell death in the local tissue of plant where the infection occurred and has specific recognition towards pathogens. ISR is only produced when the infection had been recognized by the host plant and thus it provides an extra defensive layer to the interaction between host and the pathogen (Van Loon, 1997).

SA (Salicylic Acid) also helps in the defense against bacterial infection. SA is required for variety of defenses like production of defensive compounds and PR (pathogenesis-related) gene expression which is related to local systemic acquired resistance (Wildermuth et al., 2001). Many changes are observed in soil microbiome due to environmental stress like drought, high temperature and cold. During drought conditions bacterial concentration is reduced. Bacterial concentration in soil is measured by various methodologies like Microbial DNA quantification or PLFA (Phospho Lipid Fatty Acid). Concentration Gram positive bacteria is measure to be increased especially for Actinomycetes and Firmicutes while gram negative bacteria decreases mainly of phyla – Proteobacteria, Bacteriodetes (Naylor & Coleman-Derr, 2018). Increase in the yield is observed when seedling are coated with PGPR for example – potatoes treated with tubers were seen to be increased by weight thus good yield was produced and overall PGPR increases the production from 10% to 20% (Ryu et al., 2003).

5.3. Plant-fungal interaction

Fungi have many roles in the food web and ecosystems and various interactions are also observed between the plant-fungi like mycorhizzal interaction. It may be beneficial for plants or fungi that may act as parasite and disrupt the host machinery (Zeilinger et al., 2016; Mayer, 1989). Mycorhizae is the symbiotic association of plants in which plant and fungi and both are getting benefit as fungi helps plant in tolerating stress or procurement of the soil resources like nutrients, water and in turn plants deliver carbohydrates to fungi. Over 6000 beneficial fungi are present (Zeilinger et al., 2016).

An experiment performed by (Humphreys et al., 2010) shows that AMF (arbuscular mycorrhizal fungi) when colonized with thylakoid of liverwort increases the growth of plant and helps in asexual reproduction and around 100 to 400 m AMF mycelia also strengthen the plant. Major AM fungal association are from Zygomycetes and of order Glomales but research shows that not all plants are colonized by glomales because they produce vesicles which are of species namely Arum and Paris (Read et al., 2000).

Mycorrhizas can be classified as ectomycorrhizas or endomycorrhizas. For colonization there is Wood-Wide Web which serves as the link between plant roots and soil and the link can be any propagule like hyphae, spores because the fungi is present in the rhizosphere and acting as a symbiont. The colonization steps for the Ectomycorrhiza (ECM) occurs like as attach to the root tip by releasing hyphae mantle and then the epidermal cells they get separated because of the labyrinth like hyphae and which increases the contact surface area of the root cells. While the colonization of endomycorrhiza which is quite simple as they penetrate the epidermal cells and forms coils and show symptomless infection inside the host (Bonfante & Anca, 2009).

In the interaction between plant fungi they secrete various types of molecules or polymers that is either helpful for plants like stress responses, helps in signaling or may act as toxic compounds. Mostly secretions are proteins that are taken into considerations and before the discovery of new proteins other proteins were delivered by certain signals recognized by transit peptides and they were delivered by ER (endoplasmic reticulum) secretory pathway. Now the current discovery of new proteins that does not require recognition of transit peptides for signaling of secreted proteins that are called as LSF (leaderless secretory proteins) these does not require ER pathway to deliver secretory protein because they have small extra-cellular vesicles (EV) to transport the proteins (Vincent
et al., 2020). EV can be any microvessels, exosomes that are covered by the phospholipid bi-layer and they act as messengers or vehicles that transport snRNA, these proteins are usually spherical in shape (Regente et al., 2017; Yañez-Mó et al., 2015). Exosomes as EV are also used in the immune responses and stress tolerant and they also act as defense system before the pathogen attacks (Rutter & Innes, 2017).

When stress conditions occurs in plants then PGPR elicit ISR and was seen in Dianthus caryophyllus with resistance to wilt by species of fusarium and many more (Van Loon, 1997; Peer, 1991).

6. Oxidative stress mitigation strategies in crop plants

6.1. Genetic modification of key genes involved in oxidative stress

Through engineering more tolerant plants are produced by the use of molecular tools for the molecular control mechanisms of abiotic stresses that is related to the stress related genes. These are divides into 3 categories. 1. Genes that are engaged in signaling cascades and in controlling transcription like MAP (mitogen – activated protein) and SOS (son of sevenless) kinases, phospholipases, MyC, and transcriptional factors like HSF (heat shock factor), CBF/DREB (C-repeats binding factor’s / dehydration responsive element binding protein). 2. Genes responsible for the protection of the membranes like chaperons, LEA (late embryogenesis abundant protiens) and free radicle scavengers. 3. Genes specially involved in the uptake of oxidative stress


tolerant and those that are engaged in signaling cascades and in controlling transcription by the use of molecular tools for the molecular control mechanisms of abiotic stresses that is related to the stress related genes. These are divides into 3 categories. 1. Genes that are engaged in signaling cascades and in controlling transcription like MAP (mitogen – activated protein) and SOS (son of sevenless) kinases, phospholipases, MyC, and transcriptional factors like HSF (heat shock factor), CBF/DREB (C-repeats binding factor’s / dehydration responsive element binding protein). 2. Genes responsible for the protection of the membranes like chaperons, LEA (late embryogenesis abundant protiens) and free radicle scavengers. 3. Genes specially involved in the uptake of oxidative stress.

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6.2. Key genes and transcription factors involved in overcoming stress conditions

1. NAC TF’s

The NAC TF family including (NAM, CUC and ATAF) is considered biggest plant specific families. First discovered NAC protein was NAM from petunia and Arabidopsis thaliana Ataf1/2 and Cuc2 proteins (Baillo et al., 2019).

A study was conducted by (Wu et al., 2016) where they observed the overexpression of tobacco lines and NAC 72 mutant as there were high accumulation of \( \text{H}_2\text{O}_2 \) and \( \text{O}_2^- \) before and after the dehydration by histochemical staining with DAB for \( \text{H}_2\text{O}_2 \) and NBT for \( \text{O}_2^- \).

The results were as follows, refer to Table 2.

2. FOXO transcription factor

It is fork head box, have few sub classes for a family of transcriptional proteins that are transcriptionally active and these target genes also encode for antioxidant enzymes that decrease the activity of ROS and that modulate the activation of FOXO proteins and they are of 2 types 1. ROS dependent FOXO modulation and 2. ROS independent FOXO modulation.

In ROS dependent modulation there are various FOXO protein levels a) PTM (post translational modification) b) subcellular localization c) FOXO also interact with co regulators d) gene expression of FOXO and its stability. b) and c) are monitored by post translational modification (PTM). PTM that regulate the antioxidant activity includes mainly 3 process phosphorylation, ubiquitination and acetylatin (Klotz & Steinbrenner, 2017; Wang et al., 2014; Maiese, 2015; Klotz et al., 2015).

ROS generation can also occur through metabolism of Xenobiotics. They are the chemical substance that are foreign to the organism and is not usually naturally produced they may be toxins as well. ROS is responsible for both activation and inactivation of FOXO. FOXO prevent the downstream target genes as they are responsible for programmed cell death, proliferation, inflammation or stress resistance also (Klotz & Steinbrenner, 2017; Wang et al., 2014).

3. SOD (Superoxide Dismutase)

It is the first line of defense against ROS production. Table 3 emphasizes on the different sub classes of SOD and their locations from where they help plant to overcome the stress conditions in plant.

Table 2. Dehydration by histochemical staining was done with DAB (diaminobenzidine) and NBT (nitro blue tetrazolium) it shows blue coloration, it was done to examine the accumulation of 2 ROS species in the overexpression tobacco lines and NAC 72 mutant before and after the staining

| Serial no. | Type of staining | Before | After |
|---|---|---|---|
| 1. | DAB Staining for \( \text{H}_2\text{O}_2 \) | Not much difference is observed in transgenic tobacco lines between wild type and PtnNAC72 (overexpressing lines) before dehydration. | Increase in the levels of \( \text{H}_2\text{O}_2 \) in both but more accumulation is seen in transgenic leaves rather than wild type. |
| 2. | NBT Staining for \( \text{O}_2^- \) | High in both the genotypes. | High in both the genotypes. High accumulation of \( \text{O}_2^- \) in transgenic plants. |

Table 3. Different sub classes of SOD and their locations

| Serial no. | SUB-CLASS | Present in (subcellular locations) |
|---|---|---|
| 1. | Cu/Zn SOD | Chloroplast, cytosol |
| 2. | Mn-SOD | Mitochondria and peroxisomes |
| 3. | Fe-SOD | Chloroplasts |

4. CAT (Catalases)

Catalase involves the removal of \( \text{H}_2\text{O}_2 \) by reducing 2 molecules of \( \text{H}_2\text{O}_2 \) and producing water and oxygen. So
since it helps in the degradation of \( \text{H}_2\text{O}_2 \) it is responsible for ensuring homeostasis intracellularly. This type of antioxidant does not require any substrate to donate electron. They are encoded by specific genes (Hasanuzzaman et al., 2017). This is the most primitive antioxidant that is first discovered. It is the important enzyme for the formation of \( \text{H}_2\text{O}_2 \) (Glorieux & Calderon, 2017). Encoding gene for Catalase is CAT gene.

On the basis of structure and sequence there are 3 subclasses:

a) Monofunctional (includes heme) enzyme – present in almost all aerobes.

b) Bifunctional catalase peroxidase-heme containing and less diversified in environment.

c) Mn containing catalase group – does not contain heme group (Nandi et al., 2019).

6.3. Chemical priming of crop plants

Chemical priming is the use of chemicals like \( \text{H}_2\text{O}_2 \), melatonin, polyamines as stress management for plants to enhance their tolerance against abiotic and biotic stress (Savvides et al., 2016; Nguyen et al., 2018).

The defense mechanisms that are already in plants include the cuticles, cell walls, thorns etc. the one mechanism of defense exhibited by plants to various stress conditions either abiotic or biotic is that they have to go deep inside the plant by penetrating the cell wall after this plant reacts in 2 ways first pre-invasive defense which is usually the closure of stomata and then the accumulation of ROS species occurs in post defensive. Plants recognize these microbes through MAMP’S or PAMP’S (Microbe associated molecular patterns OR Pathogen associated molecular patterns) these pathogens also releases some effectors that mimic the hormones of plant like in infected leaf, the delay in the senescence in observed after the pathogen releases cytokinin and due to this it promoted the growth of pathogen inside the plant. These defensive elicitors are known as PTI (PAMP triggered immunity). PTI represses the plant-hormone (Auxin) that is mediated by micro - RNA. Plants have evolved special R (resistance protiens) that produces hypersensitive responses (HR) this type of triggered response are called Effector triggered Immunity (ETI). ETI is more effective than PTI. Though ETI can be very effective but it is not applicable for many biotrophic pathogens so to make plant more resistant and stronger towards the stresses priming was induced and the treatment is done by natural or chemical compounds (Ahmad et al., 2010; Borges et al., 2014; Jones & Dangl, 2006).

Conclusions and future perspective

ROS plays dual role in plants and are generated by chloroplasts, mitochondria during ETC. If there is excess release of ROS then it will cause lipid peroxidation, damage protiens, create hindrances in the metabolic pathways and signaling in plants and if it is released in moderate concentration then it is beneficial as it helps to conquer the abiotic and biotic stress like cold, drought, salinity, high light etc. during excess release of ROS in response to it there are phytohormones, antioxidants like SOD, CAT etc. which helps is decreasing the stress and now some transgenic plants are also cultivated which are able to conquer a variety of stress and more researches required to know the actual alterations in the growth patterns and change in the productivity so that improved conditions could be monitored.

Acknowledgements

I would like to express my deep gratitude to my mentor Dr. Ramendra Pati Pandey this review article would not have been possible without his guidance and support throughout the work. His willingness to spend time so generously has been highly appreciated and guiding me whenever I needed. Family support is very important for all great milestones to achieve so next I would like to thank my parents for their guidance and support always.

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