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Physiological role of signal molecules in improving plant tolerance under abiotic stress

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Abstract : A wide range of unfavourable environmental conditions may induce stresses in plants that alter plant growth, development and metabolism, and even may lead to plant death. These stresses include mechanical damage, herbicides, UV radiation, salt, low/high temperature, soil drought, flooding, high speed wind, nutrient loss and anaerobic conditions are very important stress factors limiting crop productivity. An unavoidable consequence of aerobic metabolism is production of reactive oxygen species (ROS). ROS include free radicals. In plants, ROS are always formed by the inevitable leakage of electrons onto O_2 from the electron transport activities of chloroplasts, mitochondria, and plasma membranes or as a byproduct of various metabolic pathways localized in different cellular compartments. All ROS are extremely harmful to organisms at high concentrations. When the level of ROS exceeds the defense mechanisms, a cell is said to be in a state of "oxidative stress." The enhanced production of ROS during environmental stresses can poseathreat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway and ultimately leading to death of the cells. The sensing of biotic or abiotic stress conditions induces signaling cascades that activate production of reactive oxygen species (ROS), calcium (Ca^{2+}),nitric oxide (NO), accumulation of hormones such as abscisic acid, , ethylene, jasmonic acid, and salicylic acid. These signals ultimately induce expression of specific subsets of defense genes that lead to the assembly of the overall defense reaction. Plant responds to stresses as individual cells and synergistically as a whole organism. Stress signal is first perceived by the receptors present on the membrane of the plant cells. Following this the signal information is transduced downstream resulting in the activation of various stress responsive genes.

Key words: Abiotic stress, drought, growth, heat stress, salinity, signal molecules, yield.

Introduction

Feeding the world at a time of climate change is one of the major challenges of our generation. World agriculture is facing a lot of challenges like producing 70% more food for an additional 2.3 billion people by 2050 while at the same time fighting with poverty and hunger, consuming scarce natural resources more efficiently and adapting to climate change[1]. However, the productivity of crops is not increasing in parallel with the food demand. The lower productivity in most of the cases is attributed to various biotic and abiotic stresses. Under unfavourable environmental conditions plant growth and development is severely affected which impede the productivity and thus prevent them from reaching their full genetic potential. Indeed, abiotic stresses are the primary cause leading to worldwide crop loss and reducing the average yield for most crops by more than[2] 50%.

Stress can be understood as a stimulus or influence that is outside the normal range of homeostatic control in a given organism. A wide range of unfavourable environmental conditions may induce stresses in plants that alter plant growth, development and metabolism, and even may lead to plant death. These stresses include mechanical damage, herbicides, UV radiation, salt, low/high temperature, soil drought, flooding, high speed wind, nutrient loss and anaerobic conditions are very important stress factors limiting crop productivity[3]: if a stress tolerance is exceeded, mechanisms are activated at molecular, biochemical, physiological, and morphological levels; once stress is controlled, a new physiological state is established, and homeostasis is reestablished. When the stress is retired, the plant may return to the original state or to a new physiological situation[4,5,6].

In the last years, and because of the great interest for both basic and applied research, there has been an important progress in the understanding of the mechanisms and processes underlying abiotic stress adaptation and defense in different plant species[4]. The sensing of biotic or abiotic stress conditions induces signaling cascades that activate production of reactive oxygen species (ROS), calcium (Ca^{2+}),nitric oxide (NO), accumulation of hormones such as abscisic acid, ethylene, jasmonic acid, and salicylic acid. These signals ultimately induce expression of specific subsets of defense genes that lead to the assembly of the overall defense reaction[7]. Plant responds to stresses as individual cells and synergistically as a whole organism. Stress signal is first perceived by the receptors present on the membrane of the plant cells. Following this the signal information is transduced downstream resulting in the activation of various stress responsive genes.

Reactive oxygen species (ROS) are continuously produced during aerobic metabolism as byproducts of different metabolic pathways which are localized in some cellular compartments. However, under unfavourable environmental conditions production of (ROS) may increase and lead to oxidative stress in many plant species[8]. Reactive oxygen species can also play a role of “oxidation signalling molecules”. Signals of ROS have been shown to induce large transcriptional changes and cellular reprogramming that can either protect the plant cell or induce programmed cell death[9] (Fig 1).

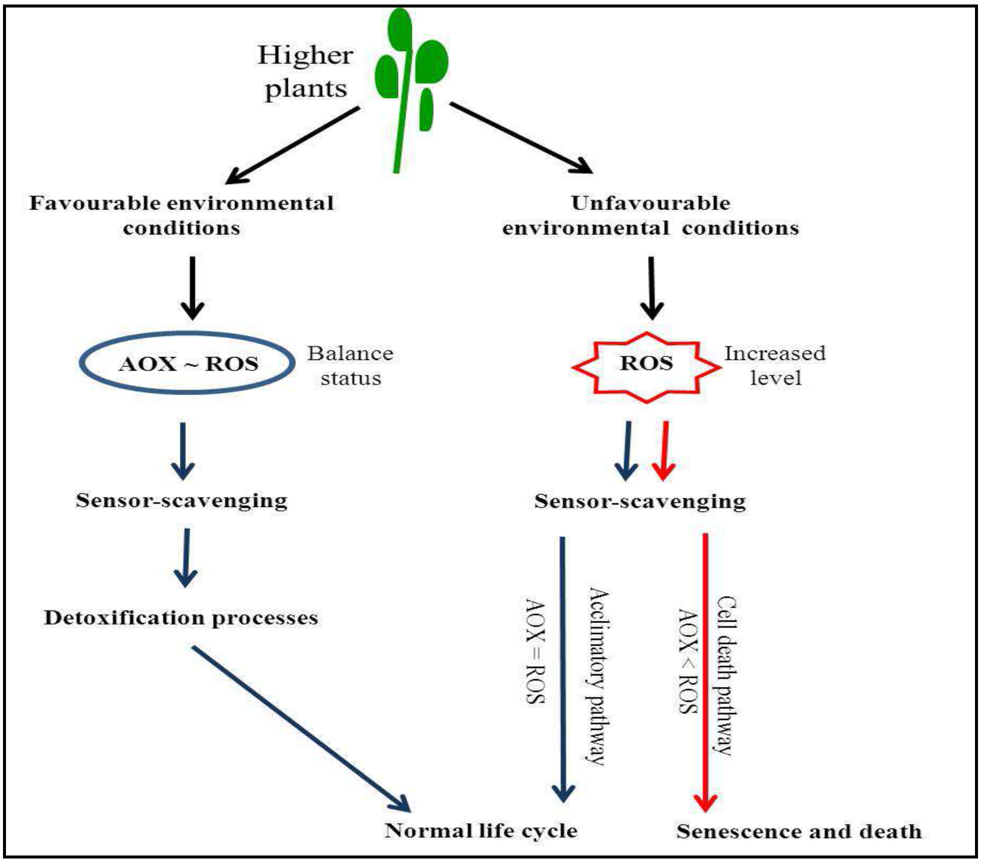

Fig.1. Antioxidants and redox signaling in plants (AOX: antioxidant)

Hydrogen peroxide is a major kind of ROS in plant tissues. It acts as an important signal molecule involved in acclamatory signaling, triggering higher tolerance against various biotic and abiotic stresses at low concentrations, whereas at high concentrations, it is toxic to plant tissues and can trigger programmed cell death[10&11].

Calcium(Ca^{2+}) is a universal signaling molecule[9] and the calcium-sensing (CaS) receptor is of fundamental importance for extracellular calcium signaling and calcium homeostasis[12]. Calcium is an important second messenger in signal transduction pathways[13], mediating various defense responses to the action under environmental stresses[14].

Nitric oxide (NO) is a small, highly diffusible gas and bioactive molecule. Its chemical properties make NO a versatile signal molecule that functions through interactions with cellular targets via either redox or additive chemistry[15].

Plant growth regulators play important roles in the regulation of plant developmental processes and signaling networks as they are involved either directly or indirectly in a wide range of biotic and abiotic stress responses and tolerance in plants[16].

Ethylene gas as plant growth regulators, this hormone has been established to modulate a number of important plant physiological activities, including seed germination, root hair and root nodule formation, and maturation[17] (fruit ripening in particular). On the other hand, although ethylene has also been suggested to be a stress-related hormone responding to a number of biotic and abiotic triggers, little is known about the exact role of stress-related ethylene in plants[18].

ABA is an important phytohormone and plays a critical role in response to various stress signals. Application of ABA to plant mimics the effect of a stress condition. As many abiotic stresses ultimately results in desiccation of the cell and osmotic imbalance, there is an overlap in the expression pattern of stress genes after cold, drought, high salt or ABA application. This suggests that various stress signals and ABA share common elements in the signaling pathway and these common elements cross‑talk with each other, to maintain cellular homeostasis[19].

Jasmonic acid is lipid-derived signaling molecules involved in induced systemic resistance, wounding and stress responses as well as in plant growth and development. A large body of evidence has accumulated showing that jasmonic acid (JA) and its volatile methyl ester methyl jasmonate (MeJA) are signaling molecules in biotic and abiotic stresses[20].

Salicylic acid is (SA) is a phenolic compound involved in the regulation of growth and development of plants, and their responses to biotic and abiotic stress factors[21]. SA mediates the phenylpropanoid pathway. Exogenous application of SA manipulates various physiological, biochemical and molecular processes in plants including antioxidative enzyme activities[22].

1- Abiotic stresses:

Plants have to deal with various and complex types of interactions involving numerous environmental factors. In the course of evolution, they have evolved specific mechanisms allowing them to adapt and survive stressful events. Exposure of plants to abiotic stress induces a disruption in plant metabolism implying physiological costs[23], and thus leading to a reduction in fitness and ultimately in productivity[24]. Abiotic stress is responsible for severe losses in the field. The resulting growth reductions can reach >50% in most plant species[25]. These environmental conditions such as water – logging, drought, high/low temperatures, excessive/extreme soil salinity, inadequate mineral content of soil and phytotoxic compounds (such as ozone, etc.) which cause damage to plant growth and productivity.

Due to sessile organisms, planta are always open to low/high temperature, salinity, drought, flood, oxidative (stress), air pollution/heavy metal poisoning, etc. solely or combinations and their consequences. Plants are unable to dislocate in its own environmental therefore they have to cope with various stresses with internal mechanisms for the tolerance and/or resistance. Since, they have developed various mechanisms to provide resistance or tolerance against them. But this is open that all of the above mentioned stress factors are responsible for shifting physiological and biochemical events from interms of the various anomalous growth regulators, promotors, transcription regulators, Ca^{+2} and Na^{+} ions uptake and balance, etc. in any organism[26&27].

Three main phases may be considered on plant stress events and responses: i) the phase of alarm; ii) the phase of resistance; and iii) the phase of exhaustion[27]. A fourth phase was added, the regeneration phase[28], which occurs only when the stressor is removed before damage being too severe, allowing partial or full regeneration of the physiological functions. The alarm phase starts with the so-called stress reaction, characterized by functional declines due to the stressor factor, offset by restitution counter reactions, in the transition to the phase of resistance. Sensing is the very first event experienced by a plant when one or more environmental factors (biotic or abiotic) depart from their optimum. Stress sensing is a complex issue and there is not a single sensing mechanism common to all stresses. For instance, some stresses directly affect the underground parts of plant bodies (e.g. drought, flooding) whereas other stresses (e.g., photoinbition) affect directly the aboveground structures of plant bodies. It is, thereby, expected that different sensing mechanisms will be involved.

Following sensing, one or more signaling and signaling transduction cascades are activated, preparing restitution counter reactions which will lead to the phase of resistance to stress.

The responses to developmental and environmental cues occur by stimulus-response coupling: the cell perceives a stimulus, a signal is generated and transmitted (signal transduction), and a biochemical change is investigated (the response). The signaling constituents play an important role in the tissue-specific gene expression, metabolism and cell growth and division. Continuous signaling is an integral component in the establishment and maintenance of cellular identity[30].

2- Signal molecules:

Signaling molecules are the molecules that are responsible for transmitting information between cells in plant. The size, shape, and function of different types of signaling molecules can vary greatly. Cell signaling is the release of a signal molecule, a chemical message, from one cell and the detection of this signal by another cell, the target cell. The signal molecule is detected by the target cell through its binding to a specific receptor. Once the receptor has bound the signal molecule, a series of changes is initiated in the target cell that affects many cellular processes.

Plants are sessile and they must be able to sense their natural environment and undergo changes in their physiology and development in response to those environmental cues whether they are adverse or beneficial. Receptor proteins in the plasma membrane sense various environmental stimuli and transduce them to downstream intra- and intercellular signaling networks[31].

Every stressor triggers in the cell a signalling cascade leading to the triggering of specific defence responses. Recognition of the stress stimulus by the cell membrane receptor results in the formation of signalling molecules, which in turn leads to a change in the concentration or modulation of the so-called second messengers and as a consequence—to the triggering of defence response.

Several signaling mechanisms have evolved in plants that involve the use of proteins, calcium ions, hormones, reactive oxygen species and nitric oxide as signaling molecules to cope with abiotic stress. These mechanisms facilitate plants to survive under abiotic stress by activating their defense systems. The pathways by which these stress signals are perceived and responded is an unexplored area of research and there are lots of gaps still to be filled. A good understanding of these signaling pathways can help in raising the plants which can perform better in abiotic stress.

3- Physiological role of signal molecules in plant tolerance under abiotic stress:

3-1 Reactive oxygen species (ROS) as signaling molecule:

Reactive oxygen species are continuously produced during aerobic metabolism as byproducts of different metabolic pathways which are localized in some cellular compartments such as chloroplast, mitochondria and peroxisomes – organelles with a highly oxidizing metabolic activity or with intense rate of electron flow (Fig 2).

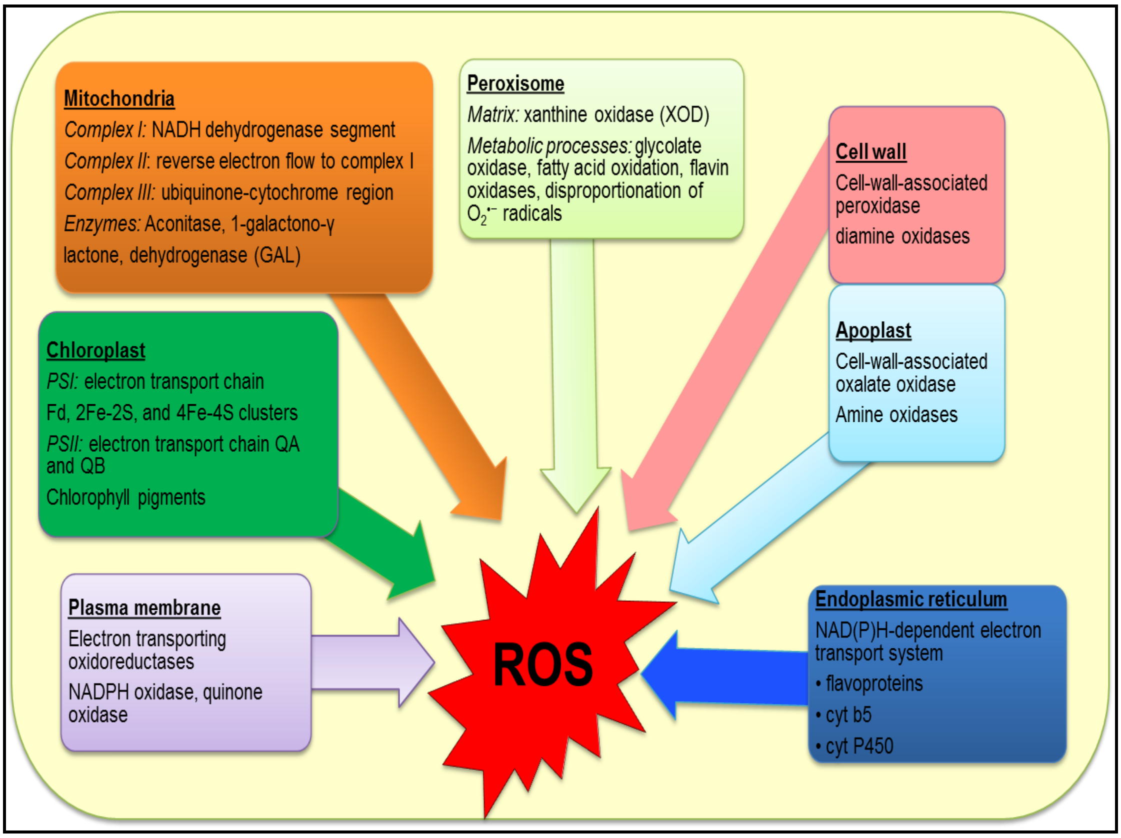

Figure 2: Sites of production of reactive oxygen species (ROS) in plants.

ROS are a group of free radicals, reactive molecules, and ions that are derived from O_2. It has been estimated that about 1% of O_2 consumed by plants is diverted to produce ROS[31]. ROS are well recognized for playing a dual role as both deleterious and beneficial species depending on their concentration in plants. At high concentration ROS cause damage to biomolecules (Fig 3), whereas at low/moderate concentration it acts as second messenger in intracellular signaling cascades that mediate several responses in plant cells.

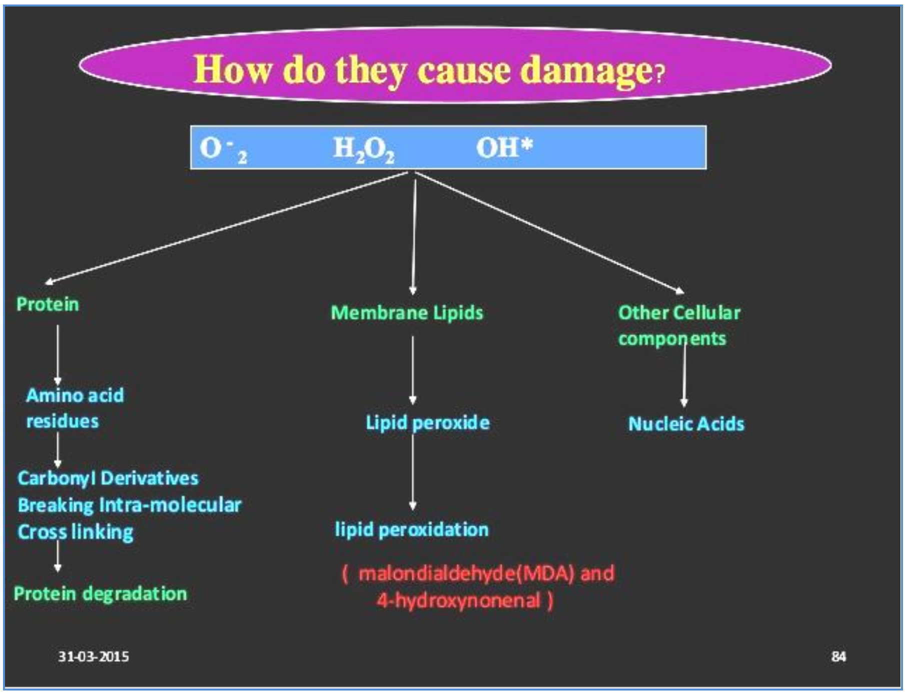

Fig (3): ROS damage

The most common ROS which are produced sequentially in accordance to the degree to which oxygen is reduced:

1. Superoxide ($O_2^{\cdot-}$)is the primary ROS which is formed when:

$O_2 + 1e \rightarrow O_2^{-\cdot}$ (1)

the major site of superoxide radical ($O_2^{\cdot-}$) production is the reaction centers of photosystem I (PSI) and a photosystem II (PSII) in chloroplast thylakoids and in mitochondria.

2. Singlet oxygen ($^1O_2$)is the first excited electronic state of O_2.

$2O_2^{-\cdot} + 2H+ \rightarrow H_2O_2 + O_2$ (2)

It has been proved that singlet oxygen formation during photosynthesis can have damaging effect on PSI and PSII and on whole machinery of photosynthesis.

3. Hydroxyl radicals (OH^-) are the third and highest reactive and most toxic ROS which are formed when the hydrogen peroxide undergoes further reduction. The hydroxyl ion formation occurs in the plant by mainly two ways:

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

$H_2O_2 + O_2^- \rightarrow OH^- + OH + O_2$

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

$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH + {}^{\cdot}OH$

4. Hydrogen peroxide (H_2O_2) is produced by univalent reduction of $O2^{\cdot-}$. H_2O_2 is moderately reactive. It has been proved that excess of hydrogen peroxide leads to oxidative stress. This molecule may also inactivate enzymes by oxidizing their thiol groups. Moreover, H_2O_2 play dual role in plants. At low concentration it can act as a signal molecule involved in acclimatory signaling triggering tolerance to different biotic and abiotic stresses. At high concentration it leads to programmed cell death[31].

The ROS are normally produced in plant cells during the reactions involved in the metabolic processes like photosynthesis, respiration, etc., by different enzymes like NADPH oxidases, cell bound peroxidases, amine oxidase, catalase, and so on, but the antioxidant machinery in the plant cells is strong enough to maintain ROS at a levelwhich does not prove inimical to the plant. However, during almost all the abiotic stresses like drought, salinity stress, pollutants, herbicides, high light stress, metals, heat shock, chilling UV radiations, etc., there is an over production of the ROS[32&33].

ROS-Scavenging Pathways of Plants:

A wide range of unfavorable environmental conditions like mentioned drought, extreme temperatures, salt stress etc. can induce stresses that alter seriously plant metabolism and may increase production of ROS (H_2O_2, $O_2^{\cdot-}$,$_1O_2$, HO·) inducing an oxidative stress in organelles. Plants are unable to escape exposure to these environmental constraints and evolved mechanisms in order to survive. To prevent appearance of these toxic compounds and their consequences plants have a variety of constitutively expressed antioxidant defense mechanisms to scavenge the ROS generated. A lot of researches have been done to emphasize the importance of the cellular antioxidant machinery in protection against various stresses[34]. ROS-scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and associated antioxidant enzymes, glutathione reductase (GR) and antioxidants such as “big three” antioxidants (ascorbic acid, glutathione and the pyridine nucleotides) and many redox-active phenolics, carotenoids and tocopherols are essential for ROS detoxification. The components of cellular “antioxidant machinery” and their role in plant protection against various abiotic stresses have been summarized in Fig.4.

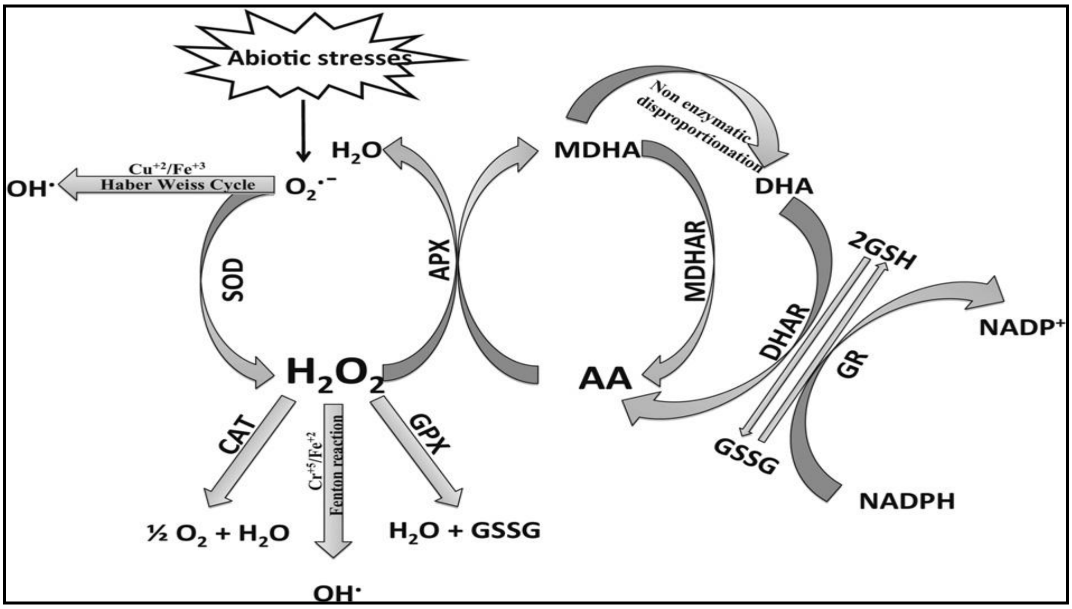

Fig (4): ROS and antioxidant defense mechanism.

Enzymatic antioxidants:

The enzymatic components of the antioxidative defense system comprise of several antioxidant enzymes such as These enzymes operate in different subcellular compartments and respond in concert when cells are exposed to oxidative stress.

Superoxide Dismutase (SOD,1.15.1.1) plays central role in defense against oxidative stressin all aerobic organisms . The enzyme SOD catalyzes the dismutationof $O_2^{\bullet-}$ to O_2 and H_2O_2.

Catalase(CAT EC 1.11.1.6): is a heme-containing enzyme that catalyzes the dismutation of two molecules of hydrogen peroxide to water and oxygen.

Ascorbateperoxidase(APX EC 1.11.1.11): is involved in scavenging of H_2O_2 into water – waterand utilizes ascorbate as an electron donor.

Guaiacol peroxidase (GPOXEC 1.11.1.7).

Glutathione reductase(GR EC 1.6.4.2)

Glutathione peroxidase (GPX EC 1.11.1.9)

Monodehydroascorbatereductase(MDHAR EC 1.6.5.4).

Dehydroascorbatereductase(DHAR EC 1.8.5.1)

In congruent with these findings, it was found that, when canola plants subjected to drought stress the levels of lipid peroxidation (MDA) and H_2O_2 increased and POX, PPO, SOD, CAT and APX enzymes activities increased meanwhile NR activity decreased[35]. Also, on flax plant found that, increasing salinity levels from 2000 to 4000 to 6000 ppm increased lipid oxidation and antioxidant enzymes SOD, CAT, APX of tolerant cultivar (Ariana) of flax plants[36].

Non-enzymatic antioxidants

The non-enzymatic antioxidants refer to the biological activity of numerous vitamins,secondary metabolites and other phytochemicals aimed to protect plants against ROSactivity. Among the most important non-enzymatic antioxidants are ascorbic acid (AA),glutathione (GSH),polyamines, proline, α-tocopherols, carotenoids and flavonoids.

Surprisingly, plants have also evolved a way to exploit lower titer of ROS as signaling component to regulate wide variety of plant processes, including cell elongation, differentiation, morphogenesis and responses to environmental stress[37].

Reactive oxygen species signaling is also highly integrated with hormonal signaling networks processing and transmitting environmental inputs in order to induce plant appropriate responses to environmental constraints[38]. Involvement of hormons such as auxins, cytokinins, ethylene, ABA, jasmonic (JA) and salicylic (SA) acids in signalling together with ROS signaling allow plants to regulate developmental processes and adaptive response to environmental cues.

A protective signaling role of plant hormones may lead to activation of acclimation responses such as stomatal closure, regulation of hydraulic conductivity and developmental processes that affect senescence and abscission[36]. Salicylic acid, similarly as ROS, is involved in both, defence and cell death responses e.g. increased level of ROS can cause SA accumulation which in turn is involved in SA-induced stomatal closure. There is also cross-talk between ROS and ABA. It has been proved that gibberellin (GA) signalling is connected with ROS by stimulating the destruction of DELLA proteins that regulate transcript levels of antioxidant enzymes. The integration of ROS with auxinsignaling networks, caused by recognition of environmental factors as the stress-induced morphogenic response, lead to ROS and auxin metabolism interaction, and in effect tomorphological changes that help to avoid deleterious effect of environmental stresses.

3 – 2 Hydrogen peroxide (H_2O_2) as a Signaling Molecule:

H_2O_2 is a vital cellular component with various functions in the development, metabolism and homeostasis of aerobic organisms. In plants, hydrogen peroxide is one of the major and the most stable ROS and regulates basic processes, such as acclimation, defense and development. Due to its relatively stability and diffusibility through membranes, thus exogenous application of hydrogen peroxide at low concentrations stimulated and enhanced resistance to abiotic stress[39]. H_2O_2 can serve as a second messenger in signal transduction pathways, leading to stress acclimation. Available information suggest that H_2O_2 directly regulates the expression of genes involved in plant defense and the related pathways such as antioxidant enzymes[40]. H_2O_2 is more likely a long distance signaling molecule[41], acting as a translocating second messenger triggering Ca^{2+}fluxes, protein modifications and gene expression[42] (Fig 5). In addition, it was hypothesized that the H_2O_2 produced as a result of the treatment with various signaling molecules could in turn induce the synthesis or activate various transcription factors, which are associated with the induction of antioxidative enzymes[43].

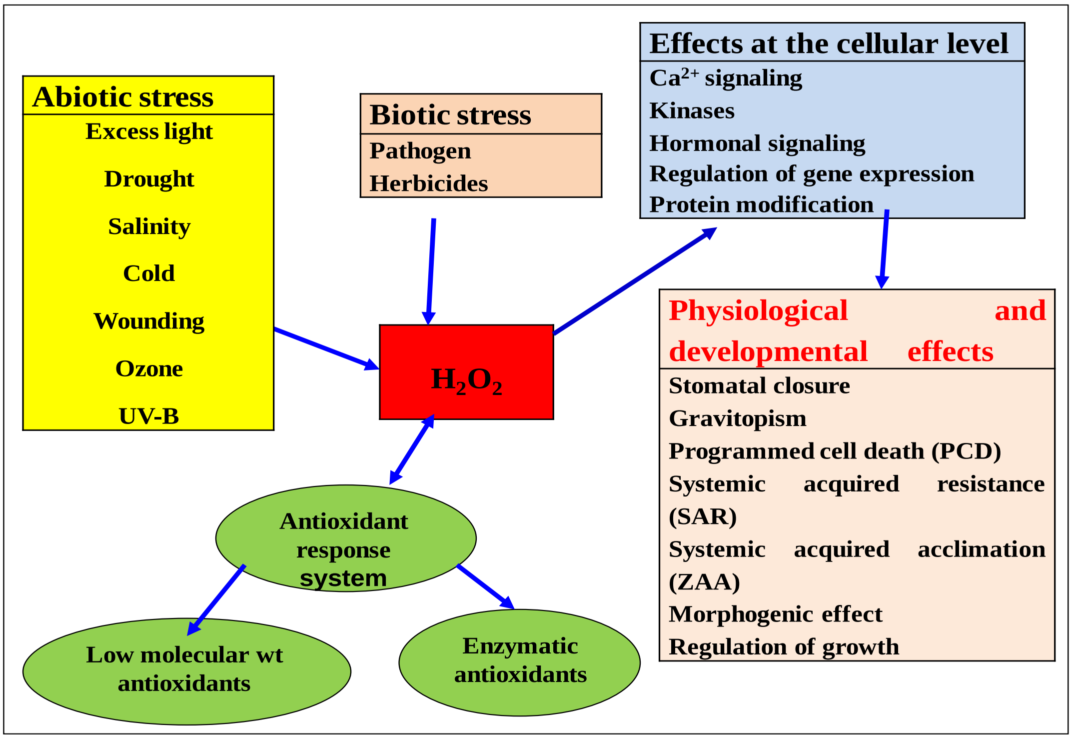

Figure 8. The central role of H_2O_2 in plant responses to various environmental stresses.

Calcium (Ca^{2+}) as a signaling molecule:

Calcium is an essential plant nutrient that plays structural roles in cell wall and membranes (in processes that preserve the structural and functional integrity of plant membranes, stabilize cell wall structures, regulate ion transport and selectivity, and control ion-exchange behaviour as well as cell wall enzyme activities), and regulates plant growth and development[44&45]. Calcium has evolved to be a ubiquitous second messenger in plants that mediates complex responses to developmental and environmental cues. Many external and internal signals can strongly, rapidly and transiently changes cytosolic calcium [Ca^{2+}] through the regulation of diverse calcium transport systems[46]. Regulation of Ca^{2+} level is important for the survival of the cell. The plant cell contains a number of vesicular compartments, which store Ca^{2+} that can be released into the cytoplasm when required. Specific channels/pumps regulate the movement of Ca^{2+} in and out of cells and organelles[47]. Furthermore, calcium-binding proteins (calcium sensors) can provide an additional level of regulation in the calcium signaling. These sensor proteins recognize and decode the information provided in the calcium signatures and relay the information downstream to initiate a phosphorylation cascade, leading to the activation of the signal-induced genes, products of which can directly or indirectly provide the signal response (e.g., stress tolerance). Because calcium appears to be readily displaced from its membrane binding sites by other cations, these functions may become seriously impaired by reduced calcium availability. Increasing the external concentration of calcium largely counteracted this displacement[48]. When plants are challenged with environmental stress, an increase in the concentration of Ca^{2+} often can ameliorate theinhibitory effects on growth. Numerous studiesindicate that a variety of stress conditions, induce cytosolic Ca^{2+} accumulations. Prevailing models for Ca^{2+} function include both membranestabilisation and signaling roles. The role of Ca^{2+} as a second messenger in many biological systems, coupled with these observations, indicates that plants are able to adjust to different environmental stresses by activating a signal transduction system involving Ca^{2+}. However, to avoid toxicity, calcium is maintained at low levels in the cytosol through the activation of calcium pumps and storage in multiple intracellular compartments as well as extracellular spaces. The signal response could also be growth inhibition or cell death, which will depend upon what kinds of genes are upregulated or downregulated[49]. Overall, the calcium-induced response could be a coordinated action of many genes.

Research conducted in tobacco revealed that both oxidative stress caused an increase of intracellular calcium content and induced cell cycle delay[50]. The increased cytosolic free Ca^{2+} by osmotic stress mainly comes from the extracellular and little is from the release of cytoplasmic calcium pool[51]. On the other hand, foliar application of $CaCl_2$ enhanced adaptation of cucumber seedling to low light intensity and suboptimal temperature, as shown by the increase in net photosynthesis Rubisco activity, reduction in stress-induced malondialdehyde content, higher catalase activity and proline content[52].

Nitric Oxide (NO) as a signaling molecule:

Nitric oxide (NO) is a gaseous signalling molecule playing an important roles in physiological processes and response mechanisms to various environmental stresses. NO functions in cellular protection against toxicity of reactive oxygen species (ROS), defense response, and tolerance to abiotic stress. NO also plays important roles in diverse physiological processes in plants, from promotion of seed germination or reduction of seed dormancy[53], regulation of plant maturation and senescence[54], suppression of floral transition[55], and involvement in light-mediated greening to mediation of stomatal movement as an intermediate downstream of ABA signaling, and regulation of multiple plant responses toward a variety of abiotic and biotic stresses, such as drought, salt, heat and disease infection[56]. Both biotic and abiotic stresses alter (promote or suppress) NO production, while externally applied NO donors enhance plant tolerance to specific stresses[57]. The dual function of nitric oxide as a potent oxidant or effective antioxidant mostly depends on its concentration and on the status of the environments. NO clearly purturbs plants normal metabolism when applied at relatively high dose. It has been demonstrated that NO has the capacity to injure membranes, proteins, and nucleic acids in the plant cell[58].

Another important role of NO in abiotic stress responses relies on its properties as a signaling molecule. NO is involved in the signaling pathway downstream of jasmonic acid synthesis and upstream of H_2O_2 synthesis, regulates the expression of some genes involved in abiotic stress tolerance[59] (Fig 6).

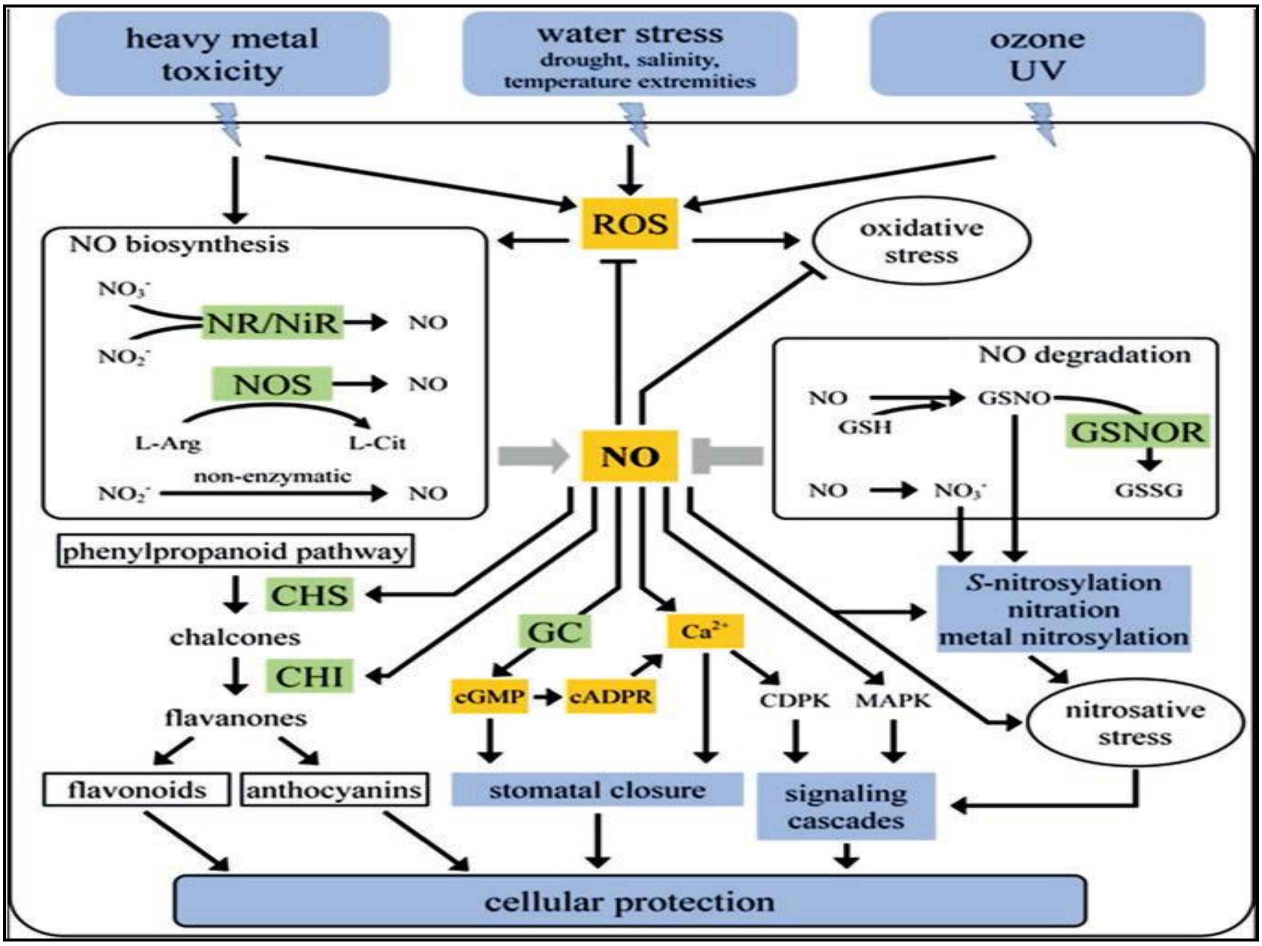

Fig. 6: Schematic representation showing functional role of nitric oxide under abiotic stresses. The signalling molecules include nitric oxide (NO), reactive oxygen species (ROS), calcium ion (Ca^{2+}), cyclic guanosine monophosphate (cGMP), and cyclic adenosine diphosphoribose (cADPR). The enzymes include nitric oxide synthase (NOS), nitrate reductase (NR), nitrite reductase (NiR), *S*-nitrosoglutathione reductase (GSNOR), guanylyl cyclase (GC), chalcone synthase (CHS), and chalcone isomerase (CHI). *Arrows* and *T-bars* indicate activation and inhibition, respectively. [Ultra violet (UV), nitrate (NO3−), nitrite (NO2−), L-arginine (L-Arg), L-citrulline (L-Cit), reduced glutathione (GSH), oxidized glutathione (GSSG), *S*-nitrosoglutathione (GSNO), calcium-dependent protein kinase (CDPK), mitogen-activated protein kinase (MAPK)]

There is a synergistic effect between NO and ROS in ABA biosynthesis. Furthermore, NO influences Ca^{2+} level in response to either salinity or osmotic stress[60]. NO has been emerging to be a key signaling molecule in plant signal transduction pathways and, NO may directly or indirectly interact with other signaling molecules such as H_2O_2, salicylic acid, and cytosolic Ca^{2+}. Furthermore, in plants, NO is also used for other intercellular and intracellular signaling functions such as stomatal closure, germination. And NO was found to increase accumulation of ABA[61].

In recent years, evidences have accumulated showing that exogenous NO can alleviate the harmful effects of environmental stresses in plants such as water stress as it was reported that, SNP sodium nitropurside as NO donor when applied exogenously, eliminated the oxidative stress in *Kosteletzkya virginica* imposed by salt stresses by decreasing MDA contents. Moreover, the activities of CAT and SOD in the presence of SNP undersalt stress were much higher than those under salt stress alone[62].

3-5 Signaling via Phytohormones and Growth Regulators

Phytohormones are critical for plant growth and development and play an important role in integrating various stress signals and controlling downstream stress responses. It is now well established that the stress-induced gene products are also involved in the generation of regulatory molecules like ABA. However, in recent yearsregulation and involvement of other hormones like cytokinins, auxins, ethylene and brassinosteroids have been indicated[63,64,65,66&67]. In this context here we described the role of ABA, SA, JA and ethylene gas, whose production under stress can initiate the second round of signaling.

3-5-1 Abscisic acid (ABA) as signaling molecule:

It is known to be generated as a endogenous messenger during a plant's life cycle to control various physiological processes such as seed dormancy and delays its germination, development of seeds, promotion of stomatal closure, embryo morphogenesis, synthesis of storage proteins and lipids, organ senescence and also defense against pathogens[47]. Various stresses induce ABA synthesis, therefore it is now considered as a plant stress hormone[68]. ABA is generated as a signal during a plant's life cycle to control seed germination and developmental processes. The action of ABA can target specifically guard cells for induction of stomatal closure but may also signal systemically for adjustment towards severe water shortage.

Various transcription factors are known to regulate the ABA‑responsive gene expression[69]. Plants have to adjust ABA levels constantly in response to changing physiological and environmental conditions. The application of ABA to plant mimics the effect of a stress condition. As many abiotic stresses ultimately results in desiccation of the cell and osmotic imbalance, there is an overlap in the expression pattern of stress genes after cold, drought, high salt or ABA application. This suggests that various stress signals and ABA share common elements in the signaling pathway and these common elements cross-talk with each other, to maintain cellular homeostasis[69]. Main function of ABA seems to be the regulation of plant water balance and osmotic stress tolerance.

3-5-2 Jasmonates (JA) as signaling molecule:

Jasmonates are lipid-derived signaling molecules involved in induced systemic resistance, wounding and stress responses as well as in plant growth and development. Many of them alter gene expression positively or negatively in a regulatory networkwith synergistic and antagonistic effects in relation to other plant hormones such as salicylate, auxin, ethylene and abscisic acid[70]. Jasmonate signaling mediate long-distance information transmission. Moreover, the systemic transcriptional response shares extraordinary overlap with local herbivory and wounding responses, indicating that jasmonates may be pivotal to an evolutionarily conserved signaling network that decodes multiple abiotic and biotic stress signals. Recently, it was reported the jasmonic acid mediated adaptation of barley to salinity stress[71]. The JA-pre-treated salt-stressed plants accumulated low levels of Na^+ in the shoot tissuecompared with untreated salt-stressed barley plants after several days of exposure to stress. Also, it was stated that, both jasmonic acid andabscisic acid ameliorate the adverse effects of drought stress on soybean plant by increasing putrescine, sppermidine, spermine and finally total polyamine and increased also endogenous growth regulators as IAA, GA_3, JA, ABA, cytokinins and ethylene contents[72].

3-5-3 Salicylic acid (SA) as signaling molecule:

Salicylic acid (SA) is a phenolic compound involved in the regulation of growth and development of plants, and the irresponses to biotic and abiotic stress factors[21]. Salicylic acid also is counted as an endogenous signal molecule in plant resistance to environmental stresses. Effects of SA on plants are concentration dependent, treatment duration, plant species, age at treatment, and plant organ examined used for pretreatment. SA is involved in the regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, proline (Pro) metabolism, production of glycinebetaine (GB), antioxidant defense system, and plant-water relations under stress conditions and thereby provides protection in plants against abiotic stresses[73]. Apart from its involvement in the induction of defense- related genes and stress resistance in biotic stressed plants[74], SA has been shown to improve plant tolerance to major abiotic stresses such as metal, salinity, osmotic, drought and heat stress[22]. Exogenous application of SA manipulates various physiological, biochemical and molecular processes in plants including antioxidative enzyme activities[75]. Moreover, SA regulates the components of its own signaling pathway besides getting involved in cross-talk with other pathways mediating plant resistance. It has been proposed that SA affects the plant growth under stress through nutrient uptake, water relations, stomatal regulation and photosynthesis[76]. It regulates the activities of various enzymes such as, peroxidase (POD), polyphenol oxidase.The mechanism of action of salicylic acid against stress is relevant to its role in the regulation of antioxidant enzymes and compounds containing active oxygen species in plant. Salicylic acid is readily transmitted from treatment location (leaves and roots) to other locations and subsequently the associated response incidences[77].

The external application of salicylic acid enhanced plant growth and photosynthesis, element content and consequently increased yield components in two cultivars of flax plant under salinity stress conditions[78]. Also, it was stated the beneficial role of salicylic acid in improving drought stress tolerance of flax plant as SA increased yield and yield components of flax plant[79].

Regulatory roles of exogenous salicylic acid (SA) on the antioxidant defense and methylglyoxal (MG) detoxification systems were investigated in rapeseed seedlings[80] it was found that, the ascorbate (AsA) content of the seedlings was decreased significantly with increased salt stress. Salt stress resulted marked raise in the levels of H_2O_2 and lipid peroxidation (MDA). The amount of glutathione (GSH) and glutathione disulfide (GSSG) were increased with an increase in the level of salt stress. However, compared to salt stressed seedlings alone, exogenous SA treatment in combination with salt stress enhanced AsA and GSH contents; GSH/GSSG ratio; and activities of antioxidant enzymes such as monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione *S*-transferase (GST), glutathione peroxidase (GPX) and catalase (CAT).

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