2

Signaling Molecules in Plants under Abiotic Stress

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ABSTRACT

Signaling molecules, in recent years, are known to regulate plant metabolism, growth and development, and various responses to environmental stresses. Studies have been directed towards understanding the complex regulatory networks associated with these signaling molecules, and this has been possible through both chemical and biological approaches. The signal pathways either operate independently or may positively or negatively modulate other pathways. Also, there is a crosstalk among various transduction pathways under abiotic stresses in metabolic regulation. Consequence to this, many signals interact in a coordinated manner with each other. Among the numerous molecules in plants that can be grouped as signaling molecules, sugars, nitrate, hydrogen peroxide, abscisic acid, brassinosteroids, jasmonic acid and salicylic acid have been investigated in detail by various workers. Their role, both at physiological and molecular levels, is briefly reviewed in this chapter.

INTRODUCTION

Plants are known to perceive various external environmental factors such as i) environmental factors: temperature, light, wind, chemical molecules, minerals, water status ii) plant microbe interaction, and iii) plant hormones. These stimuli induce a number of reactions in plant cells leading to enhanced or reduced synthesis of enzymes. These external stimuli are converted to physiological responses in plants in two ways: the first, in the nucleus, where some hormones pass through cell membrane and get associated and combined with a receptor, which then moves to nucleus, where it meets a promoter part of a gene, causing synthesis of a key enzyme; the second, in the cytoplasm, in which the stimuli do not pass through plasmalemma instead, interact with a protein receptor present on the surface of plasma membrane. This gives rise to a 'signal molecule' that induces a series of reactions modifying the activity of enzyme. Such post-transductional changes in enzyme are linked to protein kinase action, which causes phosphorylation of protein and amplification of stimuli. This is followed by dephosphorylation and returning to a resting situation for turning off the stimulus. Protein kinase bound to plasma membrane plays an important role in transduction of signals from outside the cell. There are

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However, upon addition of 20 mM sucrose to the culture medium, both the MS and ICL transcripts fell to undetectable levels after the next 48 h (Graham *et al*. 1994). In maize root tips deprived of carbohydrate, the mRNA levels of ribosomal protein genes encoding protein S28 and ubiquitin-fused protein S27a decrease rapidly and become undetectable after 24 h of starvation (Chevalier *et al*. 1996). This effect of carbohydrate starvation on Ubi-S27a and S28 mRNA expression is reversed by supplying the root tips with glucose, sucrose and fructose.

Examples of enzymes and metabolic systems directly or indirectly regulated by sugars

Starch synthase (GBSS)	Potato/detached leaves in dark	S, G, F	Kobman *et al*. 1991
Acid invertase	Sugarcane stem Maize root tips	S, G, F	Kobman *et al*. 1991, Gaylor and Glosziou 1972
Sucrose synthase	Maize (Sm1) roots	S, G, F	Koch *et al*. 1989, Koch 1995
Sucrose phosphate synthase	Transgenic potato tubers	Soluble sugars	Muller-Rober *et al*. 1992
Lipoxygenase (storage protein)	Soybean Vsps	S, G, F	Mason *et al*. 1992
Carbohydrate responsive proteins	Potato tubers/ transgenic tobacco	S	Wenzler *et al*. 1993
Nitrate reductase	*Arabidopsis* leaves light/dark	S, G, F	Vincentz *et al*. 1993
Cytochrome c oxidase	Barley roots	S	Muller-Rober *et al*. 1990
Endoproteases	Maize roots	G	James *et al*. 1993
	Barley roots	S	Dwivedi 2001a

S, sucrose; G, glucose; F, fructose

Thus, studies made so far suggest that sugar affects the expression of genes involved in processes, such as photosynthesis, respiration, lipid metabolism, nitrogen metabolism, sucrose and starch metabolism. We also find sucrose as the most frequently used sugar in studies of plant sugar responses in gene regulation and development. But, in many cases, genes that are regulated by sucrose are often also regulated by glucose and fructose. Sucrose is converted to its hexose components, or more probably a close metabolic derivative before it becomes the signal and controls gene expression (Jang and Sheen, 1997). Hexokinase (HXK) may function in the signal transduction pathway of a sugar-sensing mechanism (Sheen *et al*. 1999). The phosphorylation of hexoses by HXK and the sugar-sensing mechanism has been thoroughly studied in yeast (Rose *et al*. 1991, Sheen 1994). HXK could also be responsible for sensing sugars to repress a number of plant genes (Graham *et al*. 1994, Jang and Sheen 1994). Whereas the sugar-sensing complex in prokaryotes (e.g., *Escherichia coli*) is the phosphotransferase system, the sugar-sensing system in plants comprises a complex formed by HXK and a hexose transporter to serve as the active form of the sugar sensor (Sheen 1994, Sheen *et al*. 1999). Several signaling components like calcium-dependent protein kinase (Ohto and Nakamura 1995), a WD protein (Bhalerao *et al*. 1999) and transcription factors (Rook *et al*. 1998) are proposed to be important in plant sugar signal transduction.

NITRATE AS SIGNALING MOLECULE

Plants, except legumes, utilize nitrate as major source of nitrogen. Therefore, a number of studies have been made to elucidate the role of nitrate in various phases of plant's life starting from germination to yield of different plants/crops. About 2×10^4 megatons of organic nitrogen is produced from nitrate assimilation, which is about 100-fold greater than the rate of annual biological nitrogen fixation. However, a number of studies have shown that nitrogen limits seed yield at maturity. Nitrogen affects the role of the most important substrate inducible rate limiting and the first enzyme of nitrogen metabolism, nitrate reductase. Nitrate worked as a controlling factor in regulation of nitrate reductase activity in corn seedling, and supplying additional nitrate to the wheat crop increased the nitrate level and prolonged NR activity in expanding leaves (Meeker and Hageman 1972). Nitrate also facilitates the rate of uptake of other cations like Ca, Mg and K, which decreased with the supply of ammonium (Cox and Reisenauer 1973).

Nitrate application favors chlorophyll content, leaf number, leaf area, dry matter yield, seed yield, etc. in various crops (Singh *et al*. 1985, Islam *et al*. 1988). Lorenz (1980) while working with over 25 crops including maize, beans, peas, potato, sorghum and certain forage crops, found that nitrate sources of fertilizer resulted in higher accumulation than ammonical sources. Hilhorst (1990) reported that NO_3 acts as dormancy breaking agent during germination via stimulating the pentose phosphate pathway by producing $NADPH_2$, which is an essential criterion for the relief of dormancy in many wild species. Bose and Tandon (1991), Bose and Mishra (1992, 1999) and Pandey and Bose (2006) in their studies reported that nitrate application as seed soaking prior to sowing can improve seed germination, seedling emergence, seedling vigor, nitrogen metabolism in growing crop, vegetative growth and yield too. Bose and Sarma (2000) suggested that nitrate treatments before sowing of maize seed may cause nitrogen economy and also may reduce nitrogen pollution in maize field. Mishra and Srivastava (1983) observed that nitrate is the better inorganic form of nitrogen beside ammonium chloride and ammonium nitrate in improving various growth parameters in maize. Accumulation and assimilation of ammonium require less energy than nitrate, which is a more energy demanding process. Besides this, a number of microorganisms including bacteria, fungi and algae assimilate nitrate.

Therefore, it appears that nitrate either directly improves the regulation in the production of primary metabolites or indirectly regulates some secondary metabolites, which have a controlling effect on plant metabolism. Crawford (1995), in his review, suggested that NO_3^- regulated some plant processes, besides acting as a nitrogen source as nutrition.

Plant is a stagnant organism, therefore, it has developed a number of adaptive mechanisms for protection in adverse ambient conditions, which may impose their effects as biotic and abiotic stress factor. To sustain in such situation, plants show different levels of phenotypic plasticity. Nitrate concentration in soil fluctuates with several biotic and abiotic factors. However, the concentration of nitrate in soil is generally less than plant cell (Novoa and Loomis 1981, King *et al*. 1992). Therefore, the uptake of nitrate by the root is obviously an energy dependent process. A number of transporters of nitrate are present in the plasma membrane, which help in the selective uptake and absorption of nitrate ions by roots. Studies suggest that nitrate has a very elaborative transport system, which involves a high affinity transport system (HATS) and a low affinity transport system (LATS) (Glass and Siddiqui 1995). HATS operates in presence of low concentration (below – 0.5 mM) of nitrate whereas LATS operates at high NO_3 concentration (above – 0.5 mM). An inducible high affinity transport system (iHATS) is also found to operate

at lower concentration of NO_3^- and this system is inducible by NO_3^- ions. The transport system, however, varies with the species (*Sawchez Guerrece et al*. 1998).

A number of studies are made in this direction to know about the inducible NO_3 transport proteins (McClure *et al*. 1987, Ni and Beevers 1990). Two families of genes encoding NO_3 uptake system—the Nrt1 and Nrt2—have been identified in which former is a low affinity and the latter is high affinity transporter (Hirsch and Sussman 1999), whereas CHL1 is a double affinity transporter (Tsay *et al*. 1993). However, the net uptake of nitrate in plants represents the difference between the NO_3 influx and its efflux. Therefore, nitrate uptake is regulated by one of these two, either influx or efflux. In recent years it is evident that the availability of NO_3 to plants can further trigger changes in metabolism and developmental processes (Dehlon *et al*. 1995). This has been found by accessing mutants or transgenic lines, which are defective in NO_3 assimilation and the plants have the capacity to use either ammonium or glutamine.

Nitrate ion has a regulatory role in nitrate assimilatory pathway. It also provides reductants and carbon skeleton for the said pathway by reprogramming the carbon metabolism (Redinbaugh and Campbell 1991, Stitt 1999). Wang *et al*. (2000) identified over 40 NO_3 induced genes in *Arabidopsis* seedlings; many of them encode the enzymes of N and C metabolism. Nitrate can induce root branching, leaf expansion, dry matter production and even yield also. The signaling role of nitrate has generally been discussed so far on the basis of its effectivity towards local change or long range changes in the developmental processes, e.g., when a plant is growing in a patch of nitrate rich soil, the higher lateral root proliferation occurs (Robinson 1994). This gives a positive response to that plant competing with the neighboring plants (Robinson 1994). In order to study the NO_3 signaling, a 'split-root' experiment was done, in which one half of the split root system was deprived of NO_3 and other half was provided with NO_3. The nitrate uptake of +N was upregulated, despite any change in the external NO_3 supply to those roots (Laine *et al*. 1998). This proves that the local presence of nitrate induces nitrate uptake in roots as local signal. Nitrate sensor is thought to be located on external face of plasma membrane (Forde 2002), which is evident from the observation that NIA transcript levels in barley roots are highly responsive to change in external NO_3 supply, whereas tissue NO_3 concentrations change slowly and cytosolic NO_3 concentrations are strongly buffered with the vacuolar NO_3 pool. It may be possible that intracellular NO_3 sensor is sensitive to a little fluctuation in the cytosolic NO_3 concentration (Van der Leij *et al*. 1998). In bacteria, NO_3 signaling is via a His-to-Asp phosphorelay system, and external NO_3 is sensed by means of a pair of transmembrane histidine kinase specified by *Nar Q* and *Nar X* genes (Stewart, 1994). This may operate via ethylene and cytokinin in plants, but no direct evidence is available. It is suggested that NO_3 sensing must be considered as a possible role for one or more of seven NRT2 proteins encoded in *Arabidopsis* genome (Forde 2002).

Plant fertilized with NO_3 may also generate NO via NAD(P)H nitrate reductase as a by-product of nitrogen assimilation. Previously it was supposed that this is restricted to a constitutive NR (Dean and Harper, 1998), but later on it was found in inducible NR, which also gives rise to NO (Tamasaki *et al*. 1999). NO plays a signaling role in plants, but there is no evidence that NO itself is able to induce the expression of NO_3 inducible gene.

NO has a definite role in the control of root growth and senescence (Ribiero *et al*. 1999). NO in animals is synthesized via nitric oxide synthase (NOS) enzyme. The activity of NOS is found in plants. NO production is inhibited by inhibitors of mammalian NOS (Foissner *et al*.

molecules of JA pathway (Staswick *et al*. 2002). JA accumulates in response to plant wounding. ABA along with JA is involved in signal transduction for the wound response in some plants (Peña-Cortes and Willmitzer 1995). Systemin, an 18 amino acid peptide, is shown to move in the phloem and induce JA and JA-responsive genes in the apical part of the plants (Pearce *et al*. 1991).

Examples of JA functions and responsive genes

(-) Seed, pollen germination	?	McConn and Browse 1996
(+) Tendril coiling	?	Weiler *et al*. 1993
(-) Photosynthetic apparatus	(-) rbc L	Reinbothe *et al*. 1993
(+) Vegetative storage protein	(+) *Vsp*	Anderson 1988, Mason and Mullet 1990
(+) Seed development	(+) cruciferin, napin, *Vsps*	Staswick 1989, Wilen *et al*. 1991
(+) Disease resistance	(+) osmotin, thionin	Becker and Apel 1992, Xu *et al*. 1994

SALICYLIC ACID AS SIGNALING MOLECULE

Phenols and phenolic acids are of considerable importance in plant growth regulation and its metabolism (Wain and Talyor 1965). Salicylic acid (SA) is a naturally occurring phenolic (Ibrahim and Shaw 1970), having a benzene ring (aromatic nucleus) bearing carboxylic acid. It is biosynthesized from organic acids. Side chain degradation of trans-cinnamic acid results in the formation of SA in plants (Stumph and Cohn 1981, Ribnicky *et al*. 1998); the former acid is formed from shikimic acid via phenylalanine, which is a precursor for other similar compounds. The accumulation of free SA is also associated with the formation of SA conjugates such as the SA glucoside and GLC ester (Edwards 1994), as well as methyl salicylate (Shulaev *et al*. 1997). Shulaev *et al*. (1997) have shown that methyl salicylate vapour from inoculated tobacco may serve as an air-borne signal that activates resistance in neighboring plant. A survey by Raskin *et al*. (1990) showed that SA is ubiquitously distributed in plants species, but the level of SA differs according to plant species. In the plants like rice, crub grass, green foxtail, barley and soybean, the SA content is about 1μg g^{-1} fresh weight.

SA affects several aspects of growth and development in plants, such as increase in yield and pod number in mungbean (Singh and Kaur 1980), tuber inducing activities in potato (Koda *et al*. 1992), increase in NADH:GOGAT activity, thereby influencing productivity in maize (Singh and Srivastava 1978) and flowering in Lemna (Khurana and Maheswari 1978), *Impatiens* (Nanda *et al*. 1976) and nitrogen content in maize (Asthana and Srivastava 1978). While the concentration of SA is between 1 and 10 mM, it significantly reduces transpiration in leaves of kidney bean (*Phaseolus valgaris*) via regulating the behavior of stomata (Larque 1979). Jain and Srivastava (1981) found that lower concentration of SA significantly increased the *in vivo* activity of nitrate reductase in maize seedlings.

Organogenic tobacco tissue culture supplemented with kinetin and indole acetic acid when applied with SA showed its flowering inducing effects (Lee and Skoog 1965). It is hypothesized that SA induces flowering by acting as a chelating agent (Oota 1975). But, the florigenic activity of benzoic acid (Watanabe *et al*. 1981) and other non-chelating phenolics suggests that there may be other flower inducing mechanisms existing in the plant system.

SA, however, was recognized as an endogenous regulator in plants after the findings that it triggers a dramatic increase in production of metabolic heat and insect attracting chemicals in thermogenic inflorescence of *Arum lilies* and other thermogenic plants (Raskin *et al*. 1987, Raskin *et al*. 1990). Further, endogenous SA is found to play a role in disease resistance. In tobacco, hypersensitive response (HR) to tobacco mosaic virus (TMV) is controlled by a dominant *N* gene in contrast to *n* gene where virus spreads rapidly and systematically. HR leads to systemic acquired resistance (SAR), which is defined as resistance to subsequent pathogen attack. Studies regarding HR and SAR related systemic synthesis of several low molecular weight pathogen related (PR) proteins have been made and it has been found that production of PR proteins in tobacco and other plants can be induced by SA or acetylsalicylic acid (Aspirin) (Yalpani *et al*. 1991). This predicts the signaling activities of SA. Malamy *et al*. (1990) while working with levels of SA in respect to PRI mRNA in TMV inoculated resistant Xanthine (NN) varieties of tobacco found that SA levels increased 50-fold and 10-fold in inoculated in respect to non-inoculated one.

SA was effective in inducing PR proteins in Xanthin (NN) tobacco (Malamy *et al*. 1990). Yalpani *et al*. (1991) reported that high temperature has an effect on loss of hypersensitive responses in inoculated tobacco with TMV. This may be associated with the failure in accumulation of SA. But spraying of SA induced PR-I protein at both 24°C and 32°C. SA is most likely exported from the primary site of infection to the uninfected tissues. It is noted that SA may play a role not only in pathogenesis (Mur *et al*. 1997) but also in UV, ozone, and heat stresses (Dat *et al*. 1998). In these stresses also pathogenesis-related proteins appear, which imply some crosstalk between their signaling pathways (Sharma *et al*. 1996). HS proteins can be induced by UV exposure. UV and heat stress responses were proposed following the characterization of the uvh6 mutant of *Arabidopsis*, which can not grow at elevated temperature (Jenkins *et al*. 1997).

Foyer *et al*. (1997) and Dat *et al*. (1998) noted that O_2 and H_2O_2 levels increased during heat stress in plant tissues. High temperature can alter the integrated systems of enzymatic and non-enzymatic antioxidants involved in detoxification of active oxygen species (AOS) (Paolacci *et al*. 1997). Dat *et al*. (1998) reported that increase in endogenous SA and changes in antioxidants may be involved in heat acclimation in mustard. SA levels were found to increase at 45°C in mustard. This showed that SA accumulation is not inhibited by heat treatment and it represents the thermosensitivity point in N-gene-mediated elicitation of HR, which occurs upstream of SA induction (Mur *et al*. 1997). Heat stress induces O_2^- and its product, H_2O_2, in plant tissues (Doke *et al*. 1994). An increase in level of H_2O_2 has a capacity to stimulate SA accumulation (Chamnongpol *et al*. 1998). Therefore, the heat and SA might have some link, which is operated via H_2O_2 increment (Rao *et al*. 1997). Further the H_2O_2 may be removed by catalase activity or ascorbate peroxidase of the ascorbate glutathione antioxidant cycle (Foyer *et al*. 1997).

Salicylic acid has been evaluated for its valuable effect on cold tolerance in several species including maize, rice and wheat (Janda *et al*. 1999, Tasgin *et al*. 2003), cucumber (Kang and Saltveit 2002), tomato (Ding *et al*. 2002) and banana (Kang *et al*. 2003). It is demonstrated that use of salicylate in *Arabidopsis* plants inhibited its growth (Scott *et al*. 2004). *Arabidopsis* wild type shoots under chilling conditions accumulate salicylate as free and glucosyl SA. *Arabidopsis* plants transformed with the bacterial SA hydroxylase gene *NahG* contained reduced amounts of SA. The *npr1* mutant is a non-expresser of SA-inducible *PR* genes, which displays reduced

de-etiolated dwarf mutants of *Arabidopsis*, as described above, was restored by exogenous supply of brassinolide and not by gibberellin or IAA (Sakurai 1999). Similar observation was made with application of brassinozole (a specific inhibitor of brassinolide biosynthesis) to *Lepidium sativum* plants, which resulted in dwarfism, and exogenous supply of brassinolide restored the dwarfism (Asami *et al*. 2000). The dwarf mutant *lkb* of *Pisum sativum* was BR-deficient and application of brassinolide restored normal growth (Nomura *et al*. 1997).

Brassinosteroids and Crosstalk with other Plant Hormones

Brassinolide is reported to induce expression of several auxin-inducible genes such as the *SAUR*, *GH3*, and IAA gene families in *Arabidopsis* (Goda *et al*. 2002, Mussig *et al*. 2002). *AXR1* is a positive regulator of auxin signaling pathway and is involved in BR-mediated elongation as well as BR-mediated *SAUR-AC1* gene expression in *Arabidopsis*. This demonstrated strong synergistic interaction between auxin and BR and hence for crosstalk between BR and auxin signaling (Nakamura *et al*. 2003). Apart from its relationship with auxin, BR affects expression of genes involved in biosynthesis of other phytohormones. For example, the expression of *OPR3* (encoding12-oxophytodienoate reductase 3), *GA5* (encoding GA 20-oxidase-1) and *VR-ACS7* (encoding ACC synthase), which are involved in the synthesis of JA, GA and ethylene, respectively, are induced by BRs (Yi *et al*. 1999, Mussig *et al*. 2000, Bouquin *et al*. 2001). Investigations in recent years have been focused on BR biosynthesis and signaling both in dicots and monocots. Several genes involved in sterol and BR biosynthesis and BR signal transduction have been characterized by using BR-deficient and BR-insensitive mutants. However, many mechanisms remain unknown. A study of biosynthetic inhibitor of BR will unravel the mode of action of BR. The study of *BRI1*, *DET2*, and *CPD* expression using *in situ* hybridization in different developmental stages will help explore the role of BRs in different physiological functions/processes.

HYDROGEN PEROXIDE AS SIGNALING MOLECULE

Hydrogen peroxide (H_2O_2) is a form of reactive oxygen species (ROS) produced as a result of oxidative stress. It is the most stable one among all the intermediates of the oxygen reduction. The H_2O_2 is directly generated by divalent reduction of O_2 or from superoxide anion during electron transport in photosynthesis and respiration. H_2O_2 is the primary product of many oxidases like xanthine oxidase, uricase and α-hydroxyl acid oxidase. Environmental stress factors like drought and cold lead to electron transport mediated production of H_2O_2 (Bartosz 1997, Dat *et al*. 2000, Hang *et al*. 2004). H_2O_2 functions as signaling molecule mediating responses to various stimuli (Desikan *et al*. 2004). It is best suited as signaling molecule due to its higher stability and longer half-life. Maize seedlings pretreated with H_2O_2 acquire chilling tolerance as compared with control plants (Prasad *et al*. 1994), suggesting that chilling induced ROS acts as a message to cause antioxidant systems in cells.

UV-B induced gene expression takes place through H_2O_2 in the experiment where *Arabidopsis* plants were exposed to UV-B in presence of antioxidants. This led to down-regulation of UV-induced gene *PDF1.2* (A-H-Mackerness *et al*. 1999). This observation and H_2O_2 mediated systemic responses to excess excitation energy stress, suggest that H_2O_2 can also function as a signal during abiotic stresses. H_2O_2 plays an important role in transduction of ABA signals in *Arabidopsis* (Meinhard *et al*. 2002) through regulation of activity of phosphatase: protein

phosphatase 2C (PP2C) enzymes ABI1 and ABI2 act as targets for H_2O_2 modifications of cysteine residues *in vitro*. Both ABI1 and ABI2 are negative regulators of ABA signaling. In presence of H_2O_2 and glutathione both ABI1 and ABI2 are inactivated (Meinhard and Grill 2001, Meinhard *et al*. 2002). These observations suggested that ABI1 and ABI2 are receptors for the H_2O_2 signal in higher plants. Apart from these studies, the H_2O_2 is involved in induction of stomatal closure in response to ABA and elicitors (Allan and Fluhr 1997, Pei *et al*. 2000) as well as that of catalase gene expression in maize cells (Guan *et al*. 2000).

Role of Ca^{2+} in H_2O_2 Signaling Pathway

The study of H_2O_2 homeostasis in *Arabidopsis* indicated a close relationship between intracellular H_2O_2 level and cytosolic Ca^{2+} in response to both biotic and abiotic stresses. Increased level of cytosolic Ca^{2+} favored generation of H_2O_2 (Yang and Poovaiah 2002). The Ca^{2+} influx during H_2O_2 production in oxidative burst activates the plasma membrane-localized NADPH oxidase (Xing *et al*. 1997, Grant *et al*. 2000). Thus, higher cytosolic Ca^{2+} (which has signaling role in stress responses) activates the calcium sensor, calmodulin and passes the signal downstream target 'CAT' (catalase). This finally down-regulated H_2O_2 levels by stimulating the plant catalase activity.

Involvement of Kinases and Phosphatases

H_2O_2 activates a protein phosphorylation cascade, which is a mitogen activated protein kinase (MAPK) cascade, thus providing a linkage between an upstream H_2O_2 signal and downstream gene expression (Desikan *et al*. 1999). Activation of MAPKs by H_2O_2 is an important factor in mediating cellular responses to multiple extracellular signals (stresses) like drought, cold, phytohormones and osmotic stress. This activation of MAPKs leads to activation of signal transduction pathways resulting in nuclear gene expression (Hirt 1997; Fig. 1).

It is shown that H_2O_2 induces activation of a MAPK in *Arabidopsis* suspension cultures (Desikan *et al*. 1999). This MAPK has been identified as AtMPK6. The ANP class of MAPKKKs from *Arabidopsis* is induced by H_2O_2, which in turn activates a specific class of stress-induced MAPKs (Kovtun *et al*. 2000). Transgenic plants overexpressing ANP1 show extra tolerance to heat shock, freezing and salt stress.

H_2O_2 modulates expression of various genes, including those encoding antioxidant enzymes and modulators of H_2O_2 production (Neill *et al*. 2002). Using cDNA microarray technique, a large-scale analysis of *Arabidopsis* transcriptome during oxidative stress was undertaken by (Desikan *et al*. 2001). Out of these transcripts, 113 were induced while 62 repressed by H_2O_2. A comparison of microarray results of oxidative stress with other stresses like UV irradiation and wilting exhibited overlapping expression of some of these genes (Desikan *et al*. 2001). The H_2O_2-induced transcripts encoded proteins having functions such as energy, protein transport, biogenesis and transcription.

ABSCISIC ACID AS A SIGNAL MOLECULE

The level of abscisic acid (ABA) increases many-fold under conditions of stress, thereby leading to altered gene expression as well as altered cell volumes to cause stress-specific physiological responses. ABA is known to regulate gene expression both in a positive and negative manner (Chandler and Robertson 1994), and such regulation of gene by ABA is organ-specific and

(i) Transcriptional events and/or including rRNA
(ii) Posttranscriptional events, such as mRNA stability, translational control, protein activity and protein turnover.

Signal Transduction of ABA: Water Stress and ABA

1. Water stress encompasses both drought and salt stress because plant responses to salt and drought are closely related with overlapping mechanism. In case of drought stress responses, guard cell signaling becomes critical since it is a key denominator within plant water budget (Leung and Giraudat 1998). Role of ABA under water stress has been exemplified by ABA - deficient mutants in *Arabidopsis – abl, aba2* and *aba3*; ABA-deficient mutants for tomato, tobacco and maize have also been produced. Under drought (water deficit), ABA-deficient mutants wilt and die if stress persists.
2. ABA metabolism is governed by osmotic stress at multiple steps. Its biosynthetic genes *2EP, NCED, LOS5/ABA3* and *AAO* are up-regulated by salt and drought stresses.
3. Early responsive genes in salt, drought, cold and ABA regulation have emerged (*CBF*/*DREB* gene family, *RD22BP, AtMy6*). These genes are rapidly induced by either ABA or one or more of stress factors.
4. Availability of ABA-deficient and ABA-insensitive mutants in *Arabidopsis aba1, aba2, abi1* and *abi2* showed that some osmotic stress responsive genes are induced completely independent of ABA, some fully dependent on ABA, and others only partially ABA dependent. For example, osmotic stress induction of *RD29A* gene transcripts accumulation is only partially blocked by *aba1* or *abi1* mutation, suggesting for both ABA-dependent and ABA-independent regulation.

CONCLUSION

There are a number of biomolecules that are involved as signal transduction for external stimuli. There still exists scope for unraveling of signaling mechanism in plants. Such information could be usefully utilized in biotechnological investigations and for generating genotypes that are tolerant or resistant to a particular stress factor. Many of such signal transduction pathways act independently while some have a crosstalk among themselves. Future research needs a focus on defining set of markers for tolerance towards a specific type of stress. This is because there are multiple genes affected by abiotic stress factors, and this suggests that there could not be a single marker for stress tolerance.

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