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Plant growth regulators in water stress tolerance

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ABSTRACT

The present review provides an insight into the relationship between plant growth regulators and water stress with emphasis on metabolic events that regulate growth regulator balance and physiological responses. Possible mechanisms by which ABA controls stomatal function and growth under stress, and interacts with proteins and important osmo-protectants, have been discussed. ABA involvement in signal transduction and root-shoot communication through its effects on gene and gene products is also included. A brief description of involvement of other growth regulators such as cytokinins, ethylene, polyamines and brasssinosteroids in water stress tolerance is also provided. Salient achievements in exploiting the potential of growth regulators in the resistance to water stress in some horticultural crops are also given. Gaps in existing information on plant growth regulator research in water stress tolerance have been summarized.

Key words: Abscisic acid, brassinosteroids, cytokinins, ethylene, polyamines, water stress

INTRODUCTION

Water deficit stress is a serious and frequently encountered abiotic stress in the terrestrial surface. Its deleterious effects on plant growth and productivity are well documented. Plant responses to water stress are believed to be complex as these operate at various levels of plant organization. Several in-built physiological and biochemical mechanisms provide resistance to plants against stress. An understanding of the processes linked to these mechanisms is vital for optimizing crop growth and productivity under stress.

Plants respond and adapt to water stress by altering cellular metabolism, thus invoking stress tolerance. Alteration in endogenous concentrations of growth regulators along with accumulation of osmolytes, modifications in antioxidant cascade, changes in protein profiles and induction of gene expression in plants under stress are important characteristic metabolic changes that invoke stress tolerance at the cellular level. Alteration in endogenous concentrations of growth regulators under stress helps plants through better turgor maintenance and efficient water usage by influencing stomatal functioning, hydraulic conductivity and morphological adaptation (Fig 1). Progress made in plant adaptation to water stress is an outcome of advances made in analytical techniques on endogenous growth regulator analysis, and, powerful and reliable molecular and genetic techniques.

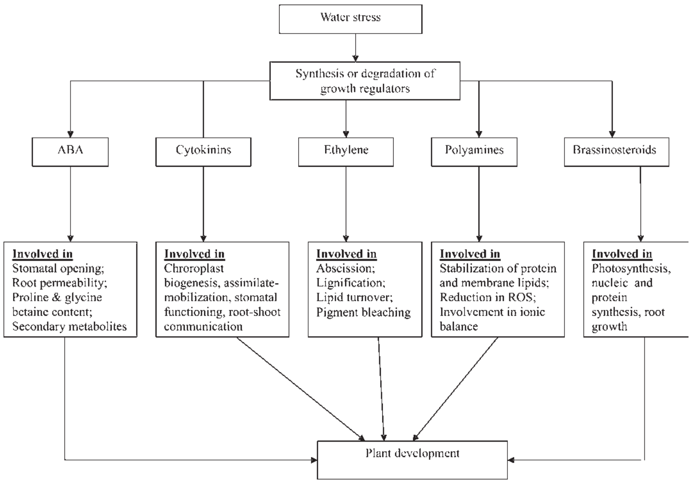

Fig 1. Water stress induced response of growth regulators in plants

The aim of this review is to provide comprehensive information on physiological, biochemical and molecular aspects of growth regulators in stomatal control, signal transduction, induction of proteins and gene expression under water stress. Because of the vast pool of information, emphasis is laid on abscisic acid (ABA). A brief account of other growth regulators such as cytokinins, ethylene, polyamines and brassinosteroids involved in stress tolerance is also provided. Studies on the use of growth regulators for amelioration of water stress in some horticultural crops are also included.

a) Abscisic acid

i) ABA biosynthesis and accumulation

Water stress affects ABA biosynthesis, leading to its accumulation. Evidence for ABA biosynthesis has been obtained by radio label ^{18}O experiments, molecular genetic analysis of auxotrophs and biochemical studies. ABA biosynthesis takes place in the cytosol through the carotenoid biosynthetic pathway (Milborrow, 2001) (Fig 2). Zeaxanthine, produced after cyclization and hydroxylation of *trans*-lycopene via β-carotene, is converted into violaxanthine (Nambara and Marion-Poll, 2005). 9-*Cis*-epoxy carotenoid dioxygenase (NCED) enzyme cleaves violaxanthine to a C_{15} product, *cis*-xanthoxine, and a C_{25} metabolite (Schwartz *et al*, 2003). The ABA is produced from *cis*-xanthoxine via the intermediate abscisic aldehyde through involvement of the enzyme abscisic aldehyde oxidase. During water stress, activities of the enzymes associated with of biosynthesis ABA and relative mRNA are induced in abundance in leaves/roots. Inhibition of catabolism of ABA is also important in stress-induced ABA accumulation. ABA is catabolised in plants into its hydroxylated products, phaseic acid (PA) and dihydrophaseic acid (DPA) (Zhou *et al*, 2004) or converted into the physiologically inactive glucose ester (Boyer and Zeevaart, 1982). Studies have revealed that PA and DPA levels increase in parallel to ABA. However, their levels under stress increase even after the ABA content has reached a plateau. In contrast, upon rehydration of plants, ABA level shows a decrease but PA or DPA levels either increase or remain unaltered. Jia and Zhang (1997) stated that inhibition of ABA catabolism also contributed to ABA accumulation in plants under stress. However, there is no evidence for ABA release from esters under stress.

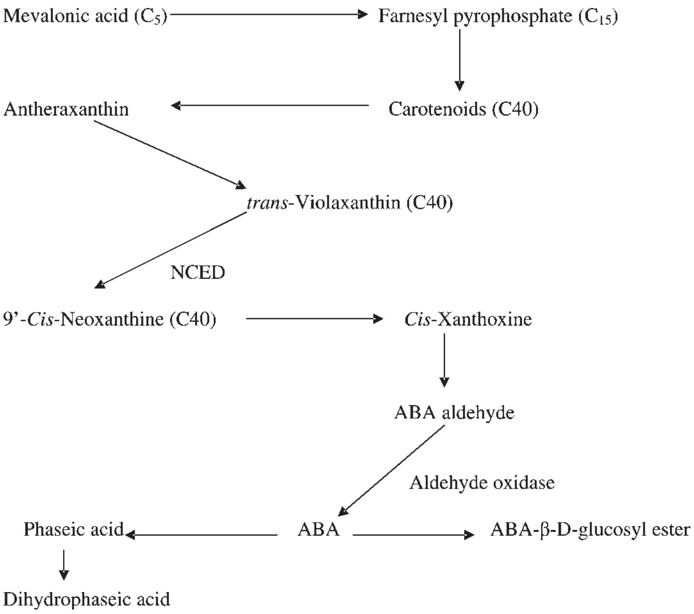

Fig 2. Biosynthetic pathway of ABA (Milborrow, 2001)

Water stress substantially accumulates ABA in a number of plant species including horticultural crops such as tomato, French bean, onion, etc. The enzyme NCED is proposed to be a key enzyme in ABA accumulation (Qin and Zeevaart, 2002). The amount accumulated depends upon factors such as severity of stress, cultivar, species, tissue and the developmental stage. The increased ABA content plays an important role in stress tolerance following its action on stomatal regulation, root-shoot communication, induction in stress proteins and associated genes, osmolyte synthesis, senescence-promotion thereby reducing plant water use, and on maintenance of the antioxidant pool. ABA concentrations are considered a vital tool in selection and breeding of varieties for drought tolerance.

ii) ABA-induced stomatal regulation under water stress

Drought stress induces stomatal closure in the leaves of many plant species. Using this mechanism, plants are able to restrict water loss through transpiration. This response is associated with decline in leaf turgor and/or water potential (Maroco *et al*, 2002). Further, this regulatory mechanism is found to be linked more to the soil moisture content than to leaf water status, thereby suggesting that stomata are responsive to chemical signals produced by dehydrating roots (Davies and Zhang, 1991). Sensitivity of the stomata to ABA varies widely in different species and cultivars, and, is dependent upon leaf-age, temperature, ambient CO_2 concentration, plant nutritional status, ionic status of xylem sap and leaf-water status (Dodd *et al*, 1996). Differences in stomatal response to ABA may be a consequence of differences in the quantity of ABA reaching the active site in the guard cell. Xylem ABA concentration and stomatal conductance showed linear inverse relationship, and the scope of relationship varied diurnally with the most sensitive stomatal closure recurring at lower water potential (Tardieu and Simmoneau, 1998).

The stomatal aperture is regulated by turgor potential of surrounding cells. The guard cell volume is actively responsive to signals produced under stress in order to regulate CO_2 efflux for photosynthesis and transpirational water loss. The ABA increase in guard cells reduces plant water loss through transpiration by promoting stomatal closure (Harris and Outlaw, 1991). The influx or efflux of K^+ , balanced by flux of anions in the guard cell, regulates guard cell volume (Hetherington and Quatrano, 1991). MacRobbie (1991) showed that externally applied ABA evoked efflux of K^+ and anions from the guard cells. Blatt (1990) found very rapid activation of K^+ channel by ABA. Rapidity of this response and lack of modulation by other cytoplasmic factors suggest that ABA is activating this channel directly. Progress is also made in deciphering electrical responses triggered by ABA in the plasmalemma of guard cells (Blatt and Theil, 1993, MacRobbie, 1997, Schoeder, 1992). The cellular electrical changes induced by ABA are an outcome of the depolarization effect which reflects a net influx of cations (Thiel *et al*, 1992). Depolarization is the driving force for K^+ efflux through outward K^+ channel.

Ca^{+2} play an important role in ABA-mediated stomatal closure. Ca^{+2} participate as an intracellular secondary messenger in mediating ABA effects on stomatal aperture and/or plasma membrane channel. ABA is shown to induce an increase in guard cell Ca^{+2} concentrations, which precedes stomatal closure (Irving *et al*, 1992). ABA is also shown to evoke alkalization of the cytoplasm of guard cells (Irving *et al*, 1992), which is necessary in ABA activation of the K^+ channel (Blatt and Armstrong, 1993). The ABA–induced rise in internal Ca^{+2} concentration is contributed by an influx of external Ca^{+2} as well as Ca^{+2} released from intracellular stress (Gilroy *et al*, 1991, McAinsh *et al*, 1991). Inositol 1, 4, 5 – triphosphate is an essential intermediate for triggering cellular Ca^{+2} mobilization.

Protons can directly affect stomatal aperture and/or its sensitivity to ABA. Maintenance of optimum apoplastic pH for stomatal opening is vital for stomatal activity (Wilkinson and Davies, 1997). Feeding artificial sap of pH 7.0 to intact leaves of ABA deficient tomato mutant *Flacca* increased stomatal aperture and transpirational water loss compared to feeding sap buffered to pH 6.0 (Schwartz *et al*, 1994). Other studies also shown revealed that reduced pH sensitizes stomata to ABA (Anderson *et al*, 1994) as guard cells take up ABA more efficiently at more acidic pH and its receptivity to internally located molecular receptors is enhanced.

Patonnier *et al* (1999) gave evidence for involvement of apoplastic sugars in deciding guard cell sensitivity to ABA. There is an increase in the concentration of apoplastic sugars with reduction in soil water potential, concomitant with a decrease in stomatal conductance. Effects of sugars on stomata are specifically on an increase in the anion efflux channel activity of the guard cell. As ABA also induces anion loss and reduces turgor in guard cell, it is imperative that sugars and ABA act synergistically in closure of stomata (Hedrich and Morten, 1993).

Recent studies have depicted H_2O_2 as an important stress signal transduction molecule promotory to stomatal closure (Luan, 2002). Zhang *et al* (2001) showed that ABA increases H_2O_2 production.

iii) Root to shoot communication and involvement of ABA

Several investigators have reported that shoot growth is more inhibited in plants experiencing water stress than is root growth (Munns and Sharp, 1993, Passioura and Gardner, 1990, Sauter *et al*, 2001). Some studies have also found faster root growth in limited soil water environment (Munns and Sharp, 1993). Inhibition of shoot growth and increase in root weight under stress cannot be explained in terms of reduction in photosynthesis, water or nutrient supply.

Investigations have revealed the association of ABA in the process by which root weight increases in response to water stress (Blackman and Davies, 1985, Carmi and Heuer, 1981, Zeevaart *et al*, 1991). Creelman *et al* (1990) and Robertson *et al* (1990) showed that exogenous ABA application caused greater reduction in shoot growth than in root growth. Evidences of sustained increase in root growth have also been found (Biddington and Dearman, 1982; Watts *et al*, 1981). Mutant research also depicted a role for ABA in differential regulation of shoot and root growth. Saab *et al* (1990) reported that ABA-deficient roots grew more slowly at low water potential than the normal ones, while, shoots grew faster. Sharp *et al* (1994) reiterated that exogenous ABA application to ABA deficient plants led to increase in root growth.

The ratio between root and shoot is sensitive to environment and there is coordination among the two via long-distance transport of substrates or through a signal (Munns and Crammer, 1996). Passioura and Stirzaker (1993) opined function of feed-forward signals under adverse soil conditions. In feed-forward controls, plants sense the environment and communicate the status to other plant parts by a signal, and can also provide advance warning of a changing environment. Roots sense soil conditions and send signals to leaves that slow down growth before supply of water/nutrients becomes limiting. The feed-forward signal from roots to the aerial plant parts under water stress is demonstrated to be operating through ABA. Jackson (1993) provided evidence for influence of roots on shoot development via transport of hormones in the xylem.

ABA moves readily in the phloem (Hoad, 1995). It is found in substantial quantities in the phloem exudate, and increases rapidly in plants exposed to soil water deficit (Hoad, 1995). The function of phloem ABA is unclear. Hoad (1975) and Lovey (1984a), employing radio tracer techniques, observed that the ABA synthesized in leaves appeared later in roots and xylem sap. This suggested the possibility of ABA translocation from leaves. Munns and Crammer (1996) suggested that turgor reduction in leaves, under the influence of water stress and thus induced ABA levels, would cause recirculation of ABA in phloem and xylem sap resulting in promotion of early stomatal closure to prevent turgor loss.

Under stress, there is increase in xylem ABA concentration concomitant with reduction in leaf growth (Zhang and Davies, 1990, Hartung *et al*, 1994). Munns (1990) observed a direct relationship between xylem ABA increase and decline in leaf growth. However, Jackson (1993) concluded that ABA in xylem sap was not associated with leaf growth reduction. Munns (1992), using exogenous feeding of ABA to detached shoots at concentrations equivalent or greater to that found in sap of intact plants, observed significant reduction in leaf area at a concentration not found in the intact plant in drying soil. However, this response is species-dependent (Dodd and Davies, 1996, Munns and King 1988, Hartung *et al*, 1994, Bano *et al*, 1993).

ABA action on root are different from that in the shoot. Root expansion is often inhibited by exogenous application of ABA (Barlow and Pilet, 1984, Crammer and Jones, 1996). However, there are contrasting reports of ABA stimulating root growth (Biddington and Dearman, 1982; Watts *et al*, 1981). Saab *et al* (1990) observed that the relationship between ABA and root growth is completely different from that in shoot, in that, higher ABA levels improve rather than reduce root growth at low water potential. Glinka and Reinhold (1971) reported that ABA increased the flow of water by increasing the hydraulic conductivity of roots and enhancing ion uptake, which caused an increase in the water potential gradient between soil and root. External application of ABA increased water-absorbing area of the root which helped the plant to cope with drought conditions. Gaither *et al* (1975) revealed that ABA stimulated growth of excised root tips. Contrasting effects of ABA seen on roots and shoots may be due to differences in receptors, compartmentation control or interaction effects with other molecules. However, this aspect needs further investigation.

ABA involvement in the inhibition of cell expansion in leaves of water stressed plants indicates that ABA is an essential component of long-distance signaling pathway from root to shoot. The pathway may or may not involve interaction with other growth regulators. This suggests the possibility of xylem sap containing compound(s), preferably precursors of ABA or ABA-unrelated compounds, that stimulate the rate of ABA synthesis in growing cells (Dodd and Davies, 1996, Munns, 1992). Other growth regulators, such as cytokinins (Stoll *et al*, 2000) and ethylene (Hussain *et al*, 2000), may act in concert with ABA, either in the xylem sap or in growing cells.

iv) Signal transduction

Signal transduction is molecular description of the regulatory network that relates perception of signal to cellular response (Fig 3.). Interesting researches have been made in signal transduction processes from sensing drought stress signal to the expression of various genes. Regulation of stomata is a well-described response of plants to water stress (Harris and Outlaw, 1991, Kearns and Assmann, 1993). ABA regulates stomatal aperture by promoting stomatal closure or by inhibiting stomatal opening through induction of changes in osmotic potential, mechanical properties of guard cells, or gene expression (Hetherington, 2001). Phosphorylation processes and protein kinases are thought to have an important role in signal transduction cascades in plants (Redhead and Palme, 1996).

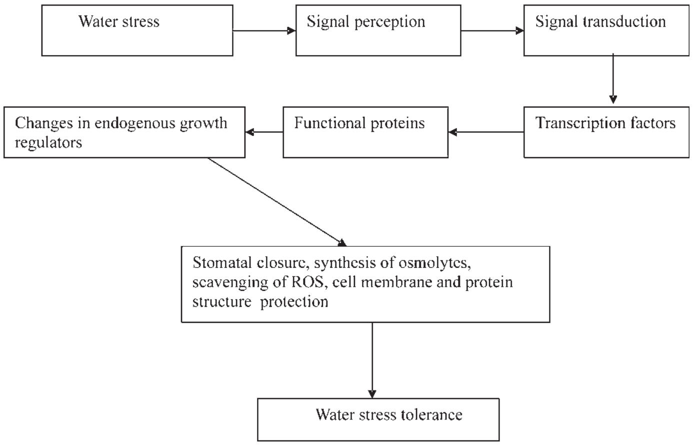

Fig 3. Sequence of events associated with water stress tolerance

ABA-dependent stomatal regulation under stress has also been shown to operate through involvement of cytoskeleton reorganization (Luan, 2002) by virtue of changes in elastic properties of guard cells. Eun and Lee (1997) reported changes in reorganization of the actin structure of guard cell. Guanosine triphosphate protein *AtRac1* was identified in *Arabidopsis* as central component in ABA-mediated disruption of the guard cell actin cytoskeleton (Lemichez *et al* 2001). ABA signal can also be transmitted to the guard cell nucleus to alter the pattern of gene expression, leading to alteration in profiles of protein involved in water transport, ion transport, or carbon metabolism (Dodd *et al*, 1996, Pospisilova and Dodd, 2005). Besides, several genes encoding G-protein, protein kinase and the transcription factor involved in signal transduction pathways are induced by ABA as also by water stress (Palme, 1993).

Mutant research has given important information on ABA involvement in water stress signal transduction pathways (Giraudat *et al*, 1994). *VP1* and *abi1*, *abi2* and *abi3* mutants have been extensively characterized and genes cloned. Among them, the ABI 1 gene product functions in stomatal closure and acts as a negative regulator of ABA–dependent gene expression. The dehydration-inducible ATCDPK1 encoding CDPK, by contrast, functions as a positive regulator. Thus, protein phosphorylation and dephosphorylative processes might be involved in ABA-responsive signaling during stress. In aleurone protoplast, MAPK is induced actively by ABA. A relationship between ABA-induced MAPK activation and ABA-induced gene expression showed involvement of MAPK in signal transduction.

v) Induction of osmo-protectants by abscisic acid

Water stress is found to increase the concentration of compounds (such as proline and glycine betaine in plants) that help in reducing cellular injuries via modifications in the osmo-regulation process. Free proline acts as an important osmo-protectant (Handa *et al*, 1983, Yoshiba *et al*, 1997, Heuer and Nadler, 1998) and as a storage compound for reduced carbon and nitrogen during water stress (Hare *et al*, 1998). Accumulation of proline in the leaves under stress is an important plant adaptation process. Exogenous ABA is shown to up-regulate proline biosynthesis in plants experiencing water stress (Stewart 1980, Ober and Sharp, 1994). Stewart (1980) showed that the metabolic cause of ABA-induced proline is a consequence of stimulated proline biosynthesis from glutamic acid. Pesci (1987) stated that the inhibition of utilization of precursor(s) of proline for protein synthesis does not contribute to proline accumulation by ABA. Verslues and Bray (2005) reported that ABA deficient mutants had less ability to accumulate proline. Applied ABA can also induce proline accumulation in turgid leaves and ABA accumulation precedes that of proline in wilting leaves. Proline is synthesized from glycine via the involvement of an enzyme pyrroline-5-carboxylate synthetase (P5CS) (Yoshiba *et al*, 1997). Savoure *et al* (1997) and Yoshiba *et al* (1997) reported induction of expression of P5CS gene by stress and exogenous ABA both in wild-type and in ABA-deficient (abaI) and ABA insensitive (aba1 and aba2) mutants.

The other osmoprotectant which has gained prominence in ascribing plant tolerance to stress is glycine betaine. ABA is shown to increase its synthesis under water stress conditions (Unayayar *et al*, 2004, Gao *et al*, 2004). Increase in glycine betaine by ABA is found to be the result of induction of betaine aldehyde dehydrogenase enzyme (Gao *et al*, 2004). These observations reveal the enhancement of osmotic protectant pool in stressed plants as an alternate mechanism by which ABA copes with stress responses.

vi) ABA and stimulation of protein expression under water stress

Water stress induces metabolic alteration resulting in synthesis and/or accumulation of a wide range of proteins (Pareek *et al*, 1998, Bray, 1988, 1991, Bartels *et al*, 1996; Cohen and Bray 1990, Piatkowski *et al*, 1990, Plant *et al*, 1991, Yokota *et al*, 2002). An analysis of proteins provides insight into the complexity of stress-response and in stress tolerance mechanism (Ramgopal, 1987, Borkird *et al*, 1991). Studies have shown activation of some proteins by water stress as well as ABA and the information achieved has been useful in describing ABA involvement in cellular signaling processes in plant-stress interactions (Chandler and Robertson, 1994). Water stress alters translatable mRNA and protein species in many plant species. These include a group of small molecular weight proteins such as LEA (Late Embryogenesis Abundant), RAB (Responsive to ABA) and dehydrins (dehydration-induced proteins). Synthesis of ABA is the common dominant factor in induction of all these proteins.

LEA protein accumulates during the development of seed, with a correlative increase in ABA level (Skriver and Mundy, 1990). These proteins are present in the embryo until the seed starts germinating. Bartels *et al* (1996) showed that LEA proteins can be induced in plants by desiccation stress or by treatment with ABA. One of the LEA proteins, α-amylase inhibitor, is induced by drought stress in embryos, concomitant with accumulation of ABA (Nedeva and Nikolova, 1997). Similarly, ABA-induced proteins were seen in aleuronic layers (Hong *et al*, 1992) and leaves and roots (Mundy and Chua, 1988) due to water stress and ABA. Close *et al* (1993) reported that D-11 family of LEA proteins is related to dehydration-tolerance and expression of most of these is found to be regulated by ABA (Hong *et al*, 1992). The ABA-deficient mutant of tomato showed no distinct ABA-responsive proteins when subjected to water stress, compared to the wild type (Bray, 1988). ABA treatment to *flacca* resulted in the synthesis of polypeptides similar to wild type. Studies of Cohen and Bray (1990) employing cDNA probes developed against three of the ABA responsive proteins confirmed the above findings. Singh *et al* (1989) showed that a low water potential environment is required for protein accumulation in response to ABA application. The stress responsive proteins have been thought to function in detoxification of cells during dehydration (Bartels and Sankar, 2005).

vii) ABA - regulated gene expression during water stress

A number of genes that respond to water stress at the transcriptional level have been found to be induced by ABA (Skriver and Mundy, 1990, Delasny *et al*, 1994). It appears that cellular dehydration induced by water stress triggers production of ABA which, in turn, induces expression of various genes. However, not all the genes induced by water stress are responsive to ABA. Thus, there is existence of ABA-dependent and ABA-independent signal transduction cascade between initial signal of stress and expression for specific gene. Genes expressed during stress help in protecting cells from stress injury by producing proteins involved in the signal transduction mechanism (Shinozaki and Yamaguchi-Shinozaki, 1997).

Genes under ABA control have been isolated from different plant species (Skriver and Mundy, 1990). Depending upon the way these have been isolated, the genes have been named either RAB or LEA genes (Galau *et al*, 1986, Mundy and Chua, 1988). These genes have been effectively used as a tool to develop molecular models of ABA action. ABA and water stress regulatory LEA genes have been cloned (Skriver and Mundy, 1990, Ingram and Bartels, 1996). These genes have been found to be transcriptionally regulated (Galau *et al*, 1986). The functions of gene products have been predicted from sequence homology with known proteins and are thought to play a role in protecting cells from water stress. *Cis* and *Trans* factors involved in ABA-induced gene expression have been analyzed extensively (Ingram and Bartels, 1996; Giraudat *et al*, 1994, Chandler and Robertson, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997).

Several genes are induced by water stress in ABA-deficient (aba) and ABA-insensitive (abi) mutants. This revealed that these plants do not require ABA but do respond to it under conditions of stress (Chen and Gusta, 1983, Jayaprakash *et al*, 1998). Analysis of ABA inducible genes revealed that several genes require protein biosynthesis for their induction by ABA, suggesting that two independent pathways exist between ABA production and gene expression during stress. These pathways involve either ABA responsive gene expression or ABA dependent / independent gene expression. Some such genes are *rd29Alilti*, *kin1*, *Cor6.6lkin* and *Cor47lrd17* (Yamaguchi-Shinozaki and Shinozaki, 1993, Izawa *et al*, 1993). The promoter region of the *rd29A* gene was analyzed and a novel *Cis*-acting element responsible for dehydration was identified. A 9-bp conserved sequence, TACGACAT, termed as dehydration responsive element (DRE) is essential for regulation of dehydration-responsive gene expression. The DRE has been demonstrated to function as a *Cis*-acting element involved in induction of *rd29A* expression. DRE-related motifs have been reported in promoter regions of water stress inducible genes such as *Kin 1, Cor 6.6 and rab 17* (Nelson *et al*, 1996; Wang *et al*, 1995). This suggested that DRE related motifs are involved in drought-responsive but ABA-independent gene expression. Two independent families of DREB proteins, DREB1 and DREB2 have been reported to function as trans-acting factors in signal transduction pathways under water stress (Jin *et al*, 1998).

Many changes in mRNA levels observed during stress reflect transcriptional inactivation. Exogenous ABA can also induce these changes. Successes have been made in understanding of transcriptional control mechanisms of ABA and stress induction by identification of *Cis*-acting regulatory sequences and isolating the corresponding nucleotide sequences (Yamaguchi-Shinozaki *et al*, 1989, Iturriaga *et al*, 1996).

ABRE motifs are not involved in ABA regulated stress-inducible genes (Iwasaki *et al*, 1995). The distinct-sequence motif is essential for ABA response. Genes that are induced by ABA and encode other potential transcriptional factors include the box gene, ATHB-07, and several myb homologous genes from *A. thaliana* and *C. plantagineum* (Nelson *et al*, 1996). Comparison of available promoter sequences of ABA and stress-inducible genes revealed that ACGT cores were conserved in many promoter elements of these inducible genes (Shen and Ho, 1996, Iturriaga *et al*, 1996). Existence of ACGT core sequence in the promoter region of these genes suggests that these genes may be mediated by ABA (Izawa *et al*, 1993, Busk *et al*, 1997). A 50-bp ABA responsive element (ABRE) is capable of conferring ABA inducibility. Many ABA-responsive genes contain more than one sequence element with an ACGT core. Involvement of these in ABA or stress-response needs to be investigated. The most efficient, characterized *Cis*-element is the one that contains CACGTC with the G-box ACGT core element (Shen and Ho, 1996). G-box related ABREs have been observed in ABA-responsive genes, though their function needs to be identified.

Several *bZIP* transcription proteins that respond to water stress and ABA treatment have also been identified and these are found to be involved in ABA-dependent pathway (Nakagawa *et al***,** 1996). Em gene is another ABA-responsive gene that has been found to accumulate in response to both ABA and water stress (Morris *et al*, 1990).

b) Cytokinins

Cytokinins are involved in many aspects of plant growth and development such as seed germination, apical dominance, photo-morphogenesis, chloroplast biogenesis, maintenance of assimilate mobilization, translocation and senescence, and in the regulation of stomatal functioning and root to shoot communication under stress. These are synthesized primarily in the roots (Chen *et al*, 1985, Binns, 1994), although some amounts can be synthesized by shoot apex and other plant tissues. Most of the naturally-occurring cytokinins are N^6- substituted adenosine molecules with branched five-carbon side-chain [Zeatin (Z) and isopentenyladenine]. The riboside derivatives and N- and O-linked glycosides of the free bases have also been identified and their biological activity established (Brzobohaty *et al*, 1994, Murti and Upreti, 2000, Binns, 1994). The two pathways for biosynthesis of cytokinins include *de novo* biosynthetic pathway (Chen and Melitz, 1979, Taya *et al*, 1978) and *t*RNA pathway (Skoog and Armstrong, 1970, Hall, 1970). The *de novo* biosynthetic pathway has been found associated for majority of the biologically active cytokinins. The key step in cytokinin biosynthesis is the formation of N^6- (Δ^2 – isopentenyl) adenosine -5'- monophosphate from Δ^2– isopentenyl pyrophosphate and adenosine-5'-monophosphate catalyzed by isopentenyl transferase (IPT) (Renske *et al*, 1992, Mok and Mok, 2001). In the other pathway, *t*RNA is degraded and isomerized to *cis*–zeatin by cis-trans isomerase (Mok and Mok, 2001). Cytokinins are irreversibly degraded by cytokinin oxidases to inactive products that lack the N^6-side chain (Brzobohaty *et al*, 1994, Galuszka *et al*, 2001). Cytokinin levels are also regulated in tissues via their O- and N- glucosylation conjugation reactions (Brzobohaty *et al*, 1994). Conn (1993) reported release of cytokinins from their O-glucosides by the action of specific β-glucosidase present in plants. Cytokinins have also been found to be regulated by other hormones, particularly, auxins (Dunleavy and Ladley, 1995).

Water stress leads to a decline in leaf cytokinin concentration (Naqvi, 1999, Pospisilova *et al*, 2000), although it is difficult to predict the actual changes in any specific cytokinin. Plants under water stress are known to exhibit reduced cytokinin concentration in the xylem sap and this response is usually rapid. Cytokinin activity returns to normal levels upon release of stress. The reduction is presumed to be a consequence of either reduced cytokinin biosynthesis, or enhanced degradation, or both.

Zhu *et al* (2004) reported that changes in the levels of Z and ZR (zeatin riboside) in the xylem sap of apple trunk depended upon drought cycles. During the first cycle of drought and rewatering, levels of Z and ZR in the sap of drought treated-trees decreased significantly, while, in the second, Z continued to decline but ZR did not change significantly. In the third cycle, there was no difference in Z concentration between drought treatments. Masia *et al* (1994) suggested that a decrease in cytokinins transport from root to shoot occurs during the onset of water stress. Pillay and Beyl (1990) reported reduction in cytokinin concentration in a drought-susceptible cultivar of tomato. Upreti *et al* (1998) and Upreti and Murti (2004a) reported

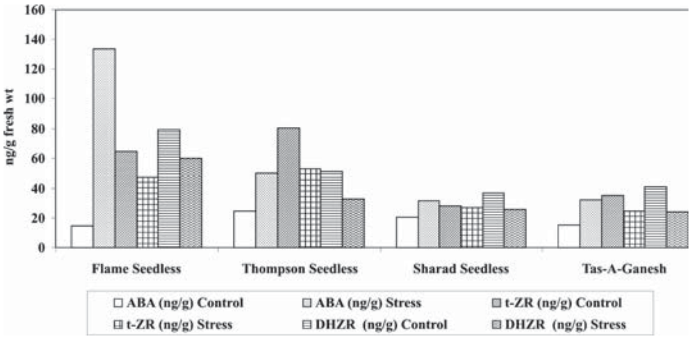

Fig 4. Influence of soil moisture stress on endogenous hormones in grape genotypes

a decline in levels of ZR and DHZR (dihydrozeatin riboside) in stressed leaves of French bean and onion. Satisha *et al* (2005) witnessed a decline in cytokinins in grape genotypes under soil moisture deficit conditions (Fig 4.). Upreti and Murti (2004b) observed that the decline in cytokinin under stress depended upon leaf-age with young leaves showing greater reduction. Water stress led to a decline in root nodulation in bean plants, which is linked to a decline in cytokinins in roots/nodules (Upreti and Murti, 1999a). Stoll *et al* (2000) showed that under partial root-drying there was reduction in cytokinin concentration, concomitant with an increase in the xylem sap pH in grapevine. Goiocchea *et al* (1995) reported a decrease in cytokinins in alfalfa under drought, and this was related with accelerated rate of senescence. In desert-grown almond trees, cytokinins showed peak concentrations in the morning and a rapid decrease in the afternoon; these fluctuations preceded daily variation in stomatal conductance (Fusseder *et al*, 1992).

The precise mechanism and cellular site of cytokinin action are not well understood. Brault and Maldiney (1999) propounded that cytokinins acted at the plasma membrane in association with other signaling molecules. In this context, cytokinins have been shown to antagonize many physiological processes mediated by ABA (Cowan *et al*, 1999). Some important processes induced by ABA and reversed by cytokinins are stomatal closure, leaf senescence and leaf expansion. This antagonism of ABA and cytokinins may be an outcome of metabolic interactions as cytokinins share a common biosynthetic origin with ABA. Cowan and Railton (1987) showed that a range of cytokinins reduced the incorporation of labeled mevalonic acid into ABA. Cytokinins have been shown to exert a response in stomata opposite to that of ABA. This increases stomatal aperture and transpiration in many plant species (Pospisilova *et al*, 2000).

Evidences showing cytokinins overriding the effects of ABA on stomata (Pospisilova *et al*, 2000, Blackman and Davies, 1985) revealed that reduction in cytokinins under stress would amplify shoot response to increasing concentrations of ABA. Davies (1995), thus, conceptualized that cytokinins act as the negative signal in plants undergoing drying. The mechanism of cytokinin action on guard cell involves direct action of membrane hyperpolarization by stimulation of adenylate cyclase activity that leads to increase in intracellular adenosine 3',5'-cyclic monophosphate content, stimulation of guanylate cyclase activity or interaction with a calcium calmodulin system (Pospisilova and Dodd, 2005, Incoll *et al*, 1990 Morsucci *et al*, 1991). The antagonism of cytokinins to ABA-induced stomatal closing may result from interactions in signal transduction pathway of both compounds, perhaps via the involvement of cytosolic calcium (Hare *et al*, 1997).

Stomatal opening is regulated by hydraulic as well as chemical signals, the relative importance of these signals being dependent on the growth-stage and growth-condition (Whitehead, 1998). Both naturally-occurring and synthetic cytokinins increase transpiration rate and increase stomatal aperture (Incoll *et al*, 1990, Incoll and Jewer, 1987). However, stomatal responses to cytokinins are found to be variable. Blackman and Davies (1983) revealed that Z alone did not affect stomatal opening, but partially reversed ABA-induced stomatal closure. In contrast, ZR or kinetin decreased stomatal opening and had no affect on ABA-induced stomatal closure.

Although cytokinin oxidase has been reported long back in the catabolism of cytokinins, little work has been carried out in relation to their involvement in stress tolerance. Manju *et al* (2001) revealed 3-fold increase in the activity of cytokinin oxidase in roots under stress and suggested it to be a regulatory enzyme of cytokinin level in roots of stressed plants.

c) Ethylene

Ethylene is the simplest olefinic gaseous hormone known to regulate a wide range of plant developmental processes. It is biosynthesized by conversion methionine to ethylene via the intermediates S-adenosyl methionine (SAM) and 1-amino cyclopropane-1-carboxylic acid (ACC) and enzymes ACC synthase and ACC oxidase (Fig. 5), (Yang and Hoffmann, 1984). Water stress is found to enhance ethylene level in French bean (Upreti *et al*, 1998, 2000), orange (Ben-Yehoshua and Aloni, 1974), avacado (Adato and Gazit, 1974), *Vicia faba* (El-Beltagy and Hall, 1974) and in many other plant species (Narayana *et al*, 1991, Guin, 1976, Irigoyen *et al*, 1992). The increase in ethylene under stress is of adaptive significance as it helps plants to cope with stress by reducing water-loss through increased senescence of fruits/leaves and reduced growth. The magnitude of ethylene changes under stress depend upon growth stage and stress duration (Upreti *et al*, 2000). The biochemical mechanism that provokes ethylene biosynthesis under stress is still not clearly understood and some reports also show variation in response. Naylor (1972) suggested greater availability of methionine as a result of increased rate of protein breakdown under stress, leading to elevated levels of ethylene. Beltrano *et al* (1997) revealed that increased production of free radicals under water stress facilitated greater conversion of ACC to ethylene. Increase in ethylene level in response to stress is evident primarily by increased synthesis of ACC (Yang and Hoffmann, 1984). Xu and Qi (1993) reported that a slowly developing drought did not promote ethylene or altered ACC levels, while, rapidly developing drought enhanced both ethylene and ACC levels. Narayana *et al* (1991) also reported more ethylene upon rapid loss of water. Upreti *et al* (2000) found increase in ethylene under mild and moderate stress and decline in its concentration under severe stress regimes. Beltrano *et al* (1997) observed slight changes in ethylene in leaves under moderate or severe conditions. Wright (1980) and Hoffmann *et al* (1983) showed that ABA interacted with ethylene metabolism by regulating ACC levels. Also, ABA accumulation in sufficient quantity is found to be inhibitory to ethylene production (Spollen *et al*, 2000).

d) Polyamines

Polyamines are important growth regulatory polycationic molecules known to be involved in a wide range of developmental events including embryogenesis, root development and senescence (Galston and Kaur-Sawhney, 1990; Tiburcio *et al*, 1997) and also in plant responses to stress (Flores 1991; Galston *et al*, 1997; Kumar *et al*, 1997). In plants, polyamines are biosynthesized by decarboxylation of either ornithine or arginine in the reaction catalyzed by enzymes ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) (Fig. 5) (Boucherneau *et al*, 1999). This step leads to formation of putrescine, which in turn, by subsequent addition of aminopropyl moiety, produces spermidine (Spd) and spermine (Spm), respectively, in reactions catalyzed by Spd synthase and Spm synthase. The aminopropyl moiety results from decarboxylation of S-adenosylmethionine (SAM) by the enzyme SAM decarboxylase. The dynamics of polyamines metabolism are complex due to existence of degradation and conjugation pathways and of transport and uptake mechanisms (Martin-Tanguy, 2001, Federico and Angelini, 2001). Besides biophysical effect, through their positive charge at physiological pH, polyamines may be involved in signal transduction pathway, through effects on calcium fluxes (Thomas *et al*, 1993) and interaction with transcriptional factors (Wang *et al*, 1999) and protein kinases (Datta *et al*, 1987). Polyamines also interact with other growth regulators (Altaman, 1989). Polyamines and ethylene synthesis are linked through their common precursor, SAM. Several investigations have revealed that polyamines and ethylene inhibit each other's biosynthesis and action as a result of sharing a common intermediate (Tiburcio *et al*, 1997). Polyamines have also been shown to increase ABA in plants subjected to water stress (Upreti and Murti, 1999b).

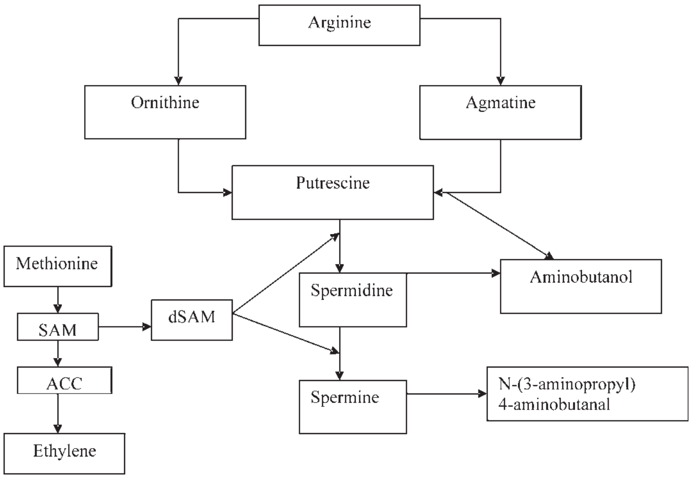

Fig 5. Biosynthesis of ethylene and polyamines (Liu *et al*, 2007)

Water stress leads to accumulation of free or conjugated polyamines in many plant species, indicating that polyamine biosynthesis play an important role in plant response to stress (Boucherneau *et al*, 1999, Liu *et al*, 2007). The increase in polyamines under stress may be a result of their *de novo* synthesis or reduced degradation (Alcazar *et al*, 2006a, Kao, 1997). However, the exact mechanism by which polyamine biosynthesis under stress are altered still remains to be elucidated. There are also some reports of decrease or no alteration in the levels of polyamines, thereby revealing competition in the mechanism of its biosynthesis under stress conditions. Differences in polyamine metabolism under stress depend upon plant species/cultivar, duration of stress, developmental stage, etc. (Liu *et al*, 2007). Upreti and Murti (2005) reported cultivar differences in polyamine changes in French bean under water stress (Fig 6). Moreover, the response of stress on an individual polyamine varied with duration of stress. Putrescine, which increased initially with stress, declined under severe stress regimes. In contrast, spermidine levels consistently declined and spermine levels progressively increased with stress. Spermine level under stress was related to ABA and to stress tolerance of the cultivar. Differential response of water stress to changes in individual polyamines is also shown by Turner and Stewart (1986).

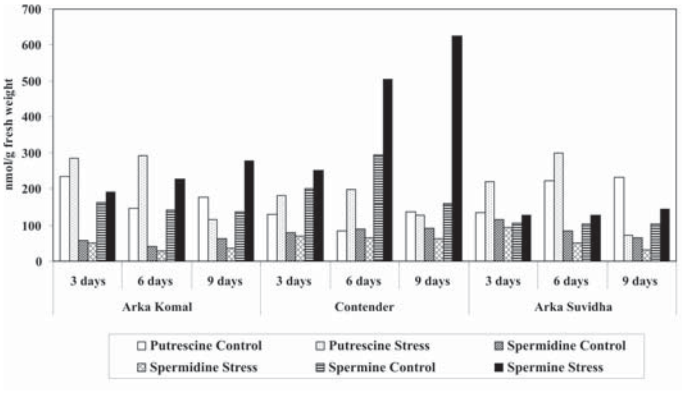

Fig 6. Free polyamines levels in French bean under water stress

Exogenous polyamine applications have been tried for providing evidence of its role in counteracting stress. Polyamine treatment increased endogenous polyamine levels in plants under stress (Tiburcio *et al*, 1997; Bagini and Torrigianni, 1992) and also reversed stress-induced changes in growth and cellular injuries. Stress-mitigating effects of individual polyamines, however, were different, because of differences in their absorption, transport and utilization among various plant species. Several lines of evidences have shown the positive function of polyamines in combating stress as being related to their antioxidative (Ormrod and Beckerson, 1986), free radical scavenging (Schuber, 1989; Malmberg *et al*, 1998), effects on ABA synthesis (Upreti and Murti, 1999b) and membrane stabilizing properties. Evidences provide a role of polyamine in modulation of stomatal aperture, an effect similar to that of ABA, possibly by targeting K^+ Arabidopsis Transporter (KAT1)-like inward K^+ channel in guard cells (Liu *et al*, 2000).

Investigations on gene expression associated with polyamines under drought have been made and reports indicate presence of a complicated transcriptional profiling (Gonzalez de Mejia *et al*, 2003). The mRNA of some polyamine biosynthetic genes is rapidly induced immediately after stress in some species and, in others; it is induced when stress is exerted for a certain period. This indicates that polyamine genes are differentially regulated under stress (Malmberg *et al*, 1998). The possible reason for differential expression of polyamines genes under stress is still unclear. Recent studies of Alcazer *et al* (2006b) depict up-regulation of polyamine biosynthetic genes by water stress as an ABA-dependent response.

e) Brassinosteroids

Brassinosteroids are naturally occurring compounds, well-documented for their role in plant growth and development (Clouse and Sasse, 1998). Their growth-regulatory activity is suggested to be a result of their influence on metabolic processes associated with photosynthesis, and nucleic acid and protein biosynthesis (Sasse, 1997). Brassinosteroids have also been shown to counteract stress effects in plants (Khripach *et al*, 2000). Brassinosteroid biosynthesis is divided into the sterol-specific pathway involving conversion of squaline to compesterol and brassinosteroid specific pathway involving compesterol to brassinosteroid (Agarwal and Gehlot, 2000). In brassinosteroid-specific pathway, compesterol undergoes a series of hydroxylation, reduction, epimerisation and oxidation reactions leading to formation of the oxidised form of brassinolide (Fujijoka and Sakurai, 1997; Choe *et al*, 1997). The last step in brassinosteroid synthesis is C-6 oxidation of castasterone. Brassinosteroids are reported to form 2, 3-glucosyl and acyl-conjugates at 3-position of its moiety (Fig. 7) (Abe *et al*, 1996).

Exogenous application of brassinosteroids is found to stimulate nucleic acid and protein synthesis and activates the ATP driven proton pump. There are also reports that

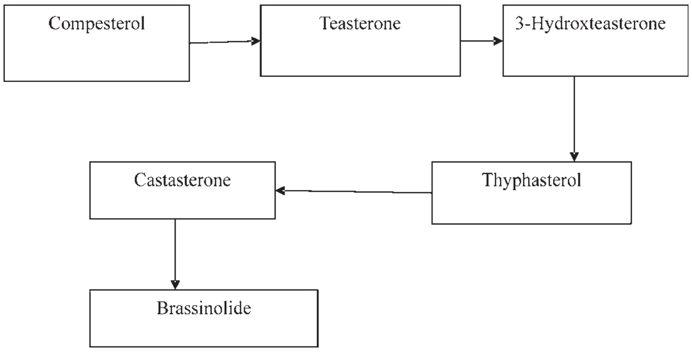

Fig 7. Possible biosynthetic pathway of brassinosteroid (Brosa, 1999)

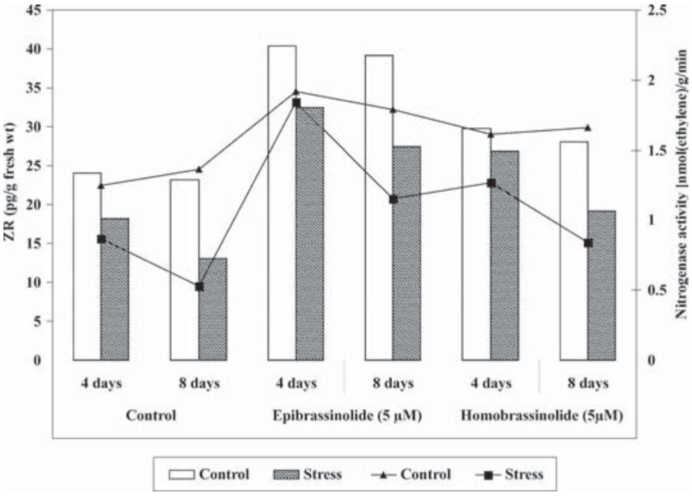

Fig 8. Effect of brassinosteroies on cytocinin-t-ZR content and nitrogenase activity in nodulated roots of French bean under water stress

these interact with other hormones such as auxins, ethylene and cytokinins in evoking physiological responses (Brosa, 1999). Information on changes in brassinosteroid content during water stress is lacking. Foliar application of the epibrassinolide is found to improve plant resistance to water stress by influencing nitrogenase activity and cytokinins levels in the roots (Fig 8.) (Upreti and Murti, 2004c). Xu *et al* (1994) reported a decline in stomatal opening and transpiration rate following brassinolide foliar application, and the treatment enhanced the effect of simultaneously-applied ABA. Rajasekharan and Blake (1999) found delay in stomatal closure induced by water stress following homobrassinolide treatment.

f) Plant growth regulators and stress amelioration in some horticultural crops

Besides conventional breeding and recent transgenic approaches, application of growth regulators in amelioration of water-stress tolerance has been attempted in a wide range of crops. In spite of a good number of studies, commercial success in growth regulator technology in combating stress effects is scant. This is because of the dependence of growth regulator action on factors such as crop species, cultivar, growth stage, stress severity, method of application, sensitivity of tissue, etc.

Potential of ABA as an anti-transpirant compound (to lower plant-water use) has been attempted in bell pepper while transplanting seedlings from greenhouse to the field or for hardening tissue-culture grown plants (Berkowitz and Rabin, 1988). Dipping of roots prior to transplanting exhibited greater survival of seedlings than those dipped in water. This facilitates the nursery industry to minimize maintenance-cost, extended marketing period and reduces the risk of dehydration during storage and transport. This effect of ABA, however, lasts for a short period due to faster breakdown of ABA in plants. To reduce ABA breakdown, Sharma *et al* (2005) employed ABA-analogs in tomato seedlings and found them to be effective in lowering plant water use for a longer period. The effectiveness of ABA-analogs, however, depended upon crop species, as these did not confer any positive effect in marigold (Sharma *et al*, 2005). Moreover, ABA treatment besides lowering transpiration also reduced photosynthesis rate in plants. But, Lovey (1984b) in his studies on grape stated that ABA effects on transpiration were much higher than on photosynthesis. Rajasekharan and Blake (1999) revealed that feeding of ABA through xylem, prior to imposing of water stress in *Pinus bankisana* improved tolerance by manipulating water use efficiency and reducing membrane damage. Pospisilova and Batkova (2004) found ABA treatment to be effective in ameliorating negative effects of water stress in French bean and sugar beet by improving plant water-balance through its effects on stomatal conductance and transpiration rate. Positive effects of ABA were also seen upon rewatering stressed plants.

Water-stress leads to a decline in cytokinin pool in the plants and, hence, the potential of benzyl adenine for mitigating stress response in plants was explored. Rulcova and Pospisilova (2001) witnessed a faster recovery of bean plants from water-stress following application of benzyl adenine. However, effects of the treatment broadly depended upon the concentration of benzyl adenine and were independent of method of application. Pospisilova and Batkova (2004) further observed that the role of benzyl adenine in lowering stress-effects was species-specific. Metwally *et al* (1997) found benzyl adenine and 4-CPPU to be effective in increasing the photochemical activity in stressed and rehydrated beans plants. Upreti and Murti (2000) reported that priming of French bean seeds with benzyl adenine improved seed-germination and seedling-growth under osmotic stress.

Triazole compounds such as cycocel and paclobutrazol have been shown to impart tolerance to water stress in many plant species (Fletcher *et al*, 2000). The precise mechanism by which these impose such effects is not very clear. One possibility is that this occurs through increased production of ABA by inhibiting gibberellin synthesis. When gibberellin synthesis is inhibited, more precursors in the terpenoid pathway accumulate and are diverted to ABA production. Increased ABA helps in plant water-balance, growth reduction and increased antioxidant content/activity (Davis and Curry, 1991). Sankhla *et al* (1989) found soil-drenching treatment with paclobutrazol as important in minimizing water-stress injuries in fruits of ber trees. Still and Pill (2004) found foliar application or seed-priming with paclobutrazol to improve water-stress tolerance in tomato seedlings, by increasing xylem pressure potential and lowering electrolyte-leakage and chlorophyll-loss. Swietlik and Miller (1983) observed an increased plant-water status in apple trees subjected to water stress. Similar effects with paclobutrazol are reported in strawberry (Navarro *et al*, 2007), peach (George and Nissen, 1992) and pea (Wang and Lin, 1992). Paclobutrazol is also found to improve resistance of micropropagated plantlets of chrysanthemum to desiccation (Roberts and Matthews, 1995). Paclobutrazol treatments are also found to induce morphological adaptation to water-stress in landscape plant *Phillyrea angustifolia,* allowing the plants to overcome transplant shock occurring later in transplanting. Paclobutrazol is also stated to improve water stress tolerance in many ornamental perennials and bedding plants (Channey, 2003). Prakash and Ramachandran (2000) reported cycocel as an effective anti-transpirant in brinjal grown under glasshouse conditions. Misra and Pradhan (1972) stated that cycocel and B-9 were effective anti-transpirants for tomato plants grown under water-deficit conditions. Upreti and Murti (2000) found that seed-priming with mepiquate chloride offered good germination in beans under osmotic stress.

Exogenous application of brassinosteroid has gained attention to modulate stress tolerance in the recent past. But there are only few reports that depict their successes in horticultural crops. Upreti and Murti (2004c) reported increase in pod yield in French bean under water stress following epibrassinolide treatment, by checking stress induced decline in root nodulation (Table 1).

SUMMARY

Endogenous growth regulators are vital components of plant growth and development under water stress conditions. Several reports have shown that water stress alters the level of growth regulators, and the resulting balance of growth regulators helps in providing better stress-adaptability to plants through their effect on stomatal functioning, plant water-balance and growth manipulation. There is either increase or decrease in the level of growth regulators in plants under stress. While stressed plants invariably show an increase in ABA and a decrease in cytokinin, the effects of stress on ethylene and polyamines in plants are variable.

Among growth regulators, researches on ABA have received wide attention in view of its involvement in stomatal functioning, osmotic adjustment, root to shoot signaling, gene expression and protein modification. Apoplastic ABA level that regulates stomatal aperture is controlled by synthesis, degradation, delivery and transportation of ABA within the plant. Other factors such as intercellular movement of calcium and potassium, together with pH and sugars, are vital in regulation of ABA-mediated stomatal closure. Water-stress alters protein synthesis and some of these proteins are also sensitive to ABA. The characteristic features of proteins help in establishing ABA-dependent stress perception-response pathway. However, information on ABA-specific proteins associated with stress-responses lacks clarity. Studies on ABA-sensitive and ABA-deficient mutants have indicated a role of ABA in stress adaptation mechanism in plants. At the molecular level, ABA-responsive genes have been identified and their expression has been characterized. Evidences show that some genes are up-regulated while others are down-regulated, resulting in net synthesis of the genomic product offering resistance against stress. However, information regarding ABA interaction with stress-responsive genes and the precise function of ABA-responsive genes still remains unidentified. Documentation of specific genes expression is important in gene-pyramiding associated with water-stress tolerance for developing superior tolerant genotypes. Significant cross-talk and interconnections are involved in stress-signaling. Systematic approaches with genomic analysis will help in resolving the complex network of signaling mechanism and elucidate the stress mechanism.

ABA-dependent signaling is also important in induction of antioxidant-defense response. An interaction between calcium and the reactive oxygen species is

Table 1. Effect of brassinosteroids on nodule number, mass of nodulated roots and pod-yield in French bean

Treatment	Conc. (μM)	Stress (d)	Nodule number		Nodulated root mass (g plant^{-1})		Pod-yield (g plant^{-1})	
			control	stressed	control	stressed	control	stressed
Control		4	39.3	23.0	1.57	1.20	119.7	79.3
		8	46.0	18.0	1.87	0.97	126.5	64.1
Epibrassinolide	1	4	54.0	33.7	2.07	1.60	126.7	95.3
	1	8	58.0	20.3	2.13	1.23	135.3	68.9
	5	4	72.3	50.3	2.90	2.43	157.6	128.7
	5	8	78.3	42.7	3.07	1.50	160.8	89.6
Homobrassinolide	1	4	43.0	29.0	1.90	1.63	126.7	85.4
	1	8	48.7	22.7	2.20	1.13	120.4	67.7
	5	4	64.0	45.3	2.60	2.00	145.8	111.5
	5	8	62.0	35.7	2.70	1.30	148.7	75.6

important in this respect. There are gaps as to how ABA regulates reactive oxygen species or how ABA-induced antioxidant defense is regulated. Answers to these will certainly strengthen knowledge on ABA involvement in stress-tolerance mechanisms in plants. Growth regulators, ABA, cytokinins, polyamines, etc., are synthesized both in leaves and roots of plants and are transported freely in the xylem sap and phloem, and partitioned in different tissues. ABA and cytokinins are interpreted as signals in stress-affected plants. Although there is a great deal of information on regulation of fluxes of these compounds in relation to water-stress, information on links between stress induced changes in soil conditions and generation of the signal is incomplete. There is also insufficient information on long-distance signaling via other chemicals, although some evidences of ABA interacting with ethylene and cytokinin have been provided.

Changes in polyamine content under water-stress are well-understood, but their functional significance in stress responses and defense needs to be elucidated. Brassinosteroids are well known for their functions at various physiological levels but their association in stress-mediation or stress-tolerance has not been fully explored. Exogenous application of growth regulators has been shown to accelerate the rate of plant acclimation to water stress. The effects of treatment, however, have been found to be dependent upon the species, cultivar, growth stage and stress-severity, because of which treatment responses are inconsistent. Systematic efforts are needed to further strengthen the scope of growth regulators in this area of research.

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