# Significance of Polyamines in Mitigating Adverse Effects of Abiotic Stress

**A Hemantaranjan[1]\*, A Nishant Bhanu[2] and Shivani Lalotra[1]**

*[1]Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India*
*[2]Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India*

**\*Corresponding Author**: A Hemantaranjan, Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

## Abstract

Crop yield harmed adversely under abiotic stresses essentially demands its maintenance. Strategies adapted till date draw attention towards accrual of metabolites, which have vital defensive roles. There are certain low-molecular weight compounds like polyamines, like putrescine (Put), spermidine (Spd), and spermine (Spm) are the entrenched examples of metabolites and have enough influence to mitigate adverse effects of abiotic stresses. Polyamines could act as cellular signals in complicated cross talk. This is along with the hormonal pathways that include abscisic acid (ABA) regulation, relevant for responses to abiotic stress. It has been elucidated In the last two decades that NO may act as a link between polyamine-mediated stress responses and other stress mediators, since polyamines induce the production of NO. The γ-aminobutyric (GABA) is another product of Put and Spd catabolism. The improved tolerance is evermore linked with high levels of Put and/or Spd and Spm highlighting potential of polyamines for stress tolerance. The antisense silencing of ethylene biosynthesis genes ACC synthase and ACC oxidase in tobacco favoured the flux of SAM to polyamines, directing to an amplified tolerance to salt, drought and a broad range of existing abiotic stresses. Nevertheless, up-regulation of PA-biosynthetic genes and accumulation of Put are largely ABA-dependent responses that under water stress. Altogether, the mitigating effects of PAs on the oxidative stress of stressed plants both by transforming ROS production and redox status and by regulation of antioxidant systems has been also known due to a direct link between improved PA levels and antioxidant enzyme activity influencing PAs to function as signaling molecules in making active many of the antioxidant enzymes. In addition, spermidine pretreatment could also alleviate the negative impacts of high temperature stress on rice seed grain filling and improve the rice seed quality caused by up-regulating endogenous polyamines and starch metabolism. PAs might regulate the mycorrhizal formation, since AM locates in cortex cells of roots. Put and Spm in trifoliate orange seedlings markedly increased leaf P and K contents and root P, Mg, Fe and Zn contents and so a possible concentration of exogenous PAs will need be conducted in citrus trees for enhanced effects on mycorrhizal development of citrus over soil drenching. Obviously, several perspectives emerge out of the significant roles of polyamines that explicate enough potential in mitigating adverse effects under abiotic stress. Consequently, this issue of exogenous economic dose of different polyamines widely needs devoted research in achieving improved economic crop yield relentlessly under conditions of drought, salinity, heat and other abiotic stresses. Unfortunately, such stresses have been noticed to be extensively expanding with every increasing year.

*Keywords: Polyamines; Abiotic Stress; Putrescine (Put); Spermidine (Spd); Spermine (Spm); Abscisic Acid (ABA); γ-aminobutyric (GABA)*

## Introduction

In order to survive adverse environmental conditions, plants have evolved various adaptive strategies, among which is the accumulation of metabolites that play protective roles. A well-established example of the metabolites that are involved in stress responses, or stress

tolerance, is the low-molecular-weight aliphatic polyamines, including putrescine, spermidine and spermine. Significance of naturally occurring intracellular polyamines (PAs) in relation to the mechanism and adaptation to combat abiotic stress has been well established in plants because of their polycationic nature at physiological pH. Since polyamines are protonated at normal cellular pH, their biological function was initially associated with the capability of binding different anionic macromolecules, viz., DNA, RNA, chromatin and proteins, thus confining them as substances with a structural role, which was later confirmed that polyamines act as regulatory molecules in many fundamental cellular processes [26].

Exposure of plants to the stressful conditions can lead to a substantial difference in potential and actual crop yields, the size of which largely depends on the severity and duration of the environmental stresses in question. Abiotic stresses, such as drought, flooding, extreme temperatures, high salinity, chemical toxicity, nutrient deficiency and others, are regarded as the predominant causes of crop loss and may account for more than 50% reduction of the yield of the major annual and perennial crops worldwide [55]. Maintaining crop yield under adverse environmental conditions is probably the major challenge faced by modern agriculture, where PAs can play important role. Over the last two decades, genetic, transcriptomic, proteomic, metabolomic and phenomic approaches have unravelled a lot of significant functions of different PAs in the regulation of plant abiotic stress tolerance. Much attention has also been devoted in recent years to the involvement of PAs in mitigating different environmental stresses such as osmotic stress, drought, heat, chilling, high light intensity, heavy metals, mineral nutrient deficiency, pH variation and UV irradiation. Further, a progress in disentanglement the molecular functions of polyamines has also facilitated the generation of Arabidopsis transgenic plants resistant to various stresses. Breeding stress-resistant varieties by making use of naturally occurring compounds is a basic prerequisite of sustainable agriculture.

The understanding of how plants adapt to and survive, the abiotic stresses are important for the efficient exploitation of genetic resources with desirable stress tolerance and for developing new approaches to enhance stress tolerance. The present review tries to gather the information on work done so far on effects of different polyamines on abiotic stresses by elucidating first the regulatory functions of polyamine molecules, followed by polyamines and ABA in drought, salt and cold stresses focusing towards polyamines function in stress tolerance besides revealing vital roles of spermidine in enhancing heat tolerance and enumerating citrus mycorrhizal responses to polyamines.

### Regulatory functions of polyamines molecules

The physiological significance of increased polyamine levels in abiotic stress responses is indistinct as yet [18,26]. Complete sequencing of the Arabidopsis genome has facilitated the use of global 'omic' approaches in the identification of target genes in polyamine biosynthesis and signalling pathways. Studies indicate that polyamines may act as cellular signals in intricate cross talk with hormonal pathways, including abscisic acid (ABA) regulation of abiotic stress responses. Molecular functions of polyamines has also facilitated the generation of Arabidopsis transgenic plants resistant to various stresses. Consequently, the use of Arabidopsis has opened new perspectives in functional dissection of the polyamine metabolic pathway and its role in the control of abiotic stress responses [18,44]. Further exploitation of natural variability can open new alternatives for both fundamental and applied plant polyamine research. Certain intricacies have been discussed under the following heads:

### Polyamine conjugation

Two novel acyltransferase genes regulating the accumulation of disinapoyl-Spd and sinapoyl-(glucose)-Spd have been functionally characterized in Arabidopsis seeds [28]. However, genes encoding *N*-hydroxycinnamoyl transferases, which acylate other polyamines, remain to be identified in Arabidopsis. Polyamines are catabolized through the activity of one or more diamine oxidases (DAO, EC 1.4.3.6) and polyamine oxidases (PAO; EC 1.5.3.3). The DAOs occur at high levels in dicots, but genes encoding these enzymes have been identified so far only in few species [12]. The second group of plant PAOs resemble the mammalian Spm oxidase (SMO, EC 1.5.3.3) that catalyses the back-conversion of Spm to Spd with concomitant production of 3-aminopropanal and $H_2O_2$ [32]. The Arabidopsis genome contains five genes encoding putative PAOs [2]. PAO1 and PAO4 catalyse the same reaction as SMO [23,48]. The third class of plant PAO-domain proteins LSD1 acts as a histone demethylase, representing an important regulator of chromatin structure and gene expression [20]. Arabidopsis has four *LSD1*-related genes [22,24].

### Interactions of polyamines with other metabolic routes

Polyamine and ethylene biosynthesis are connected through SAM that acts as a common precursor. Antagonistic effects between these compounds occur during leaf and flower senescence and fruit ripening [57]. Further, polyamines induce the production of NO that may act as a link between polyamine-mediated stress responses and other stress mediators [50]. $H_2O_2$ generated by the action of DAOs and/or PAOs is involved in both biotic and abiotic stress signalling, as well as in ABA-induced stomatal closure [6]. The γ-aminobutyric (GABA) is another product of Put and Spd catabolism [12].

Under dehydration, the levels of GABA, agmatine (a precursor of Put) and some components of the TCA cycle increase [51] along with an increase in Put content [1] that obviously suggests a metabolic connection between these routes in response to stress. Moreover, with increased proline (Pro) levels in response to various abiotic stresses [51], polyamine catabolism is remarkably related to Pro accumulation in response to salt stress [8]. Overall, the polyamine metabolism is connected to several important hormonal and metabolic pathways involved in development, stress responses, nitrogen assimilation and respiratory metabolism.

### Polyamine effects on abiotic stress

Researches in different plant species has shown that polyamine accumulation occurs in response to several adverse environmental conditions, including salinity, drought, chilling, heat, hypoxia, ozone, UV-B and UV-C, heavy metal toxicity, mechanical wounding and herbicide treatment [19]. Though this need to be evaluated whether elevated polyamine levels were a result of stress-induced injury or a protective response to abiotic stress. Enhanced tolerance always correlated with elevated levels of Put and/or Spd and Spm. Antisense silencing of ethylene biosynthesis genes ACC synthase and ACC oxidase in tobacco favour the flux of SAM to polyamines [57], which leads to an increased tolerance to salt, drought and a broad spectrum of other abiotic stresses [56]. Comparable results were obtained by homologous overexpression of polyamine biosynthetic genes in Arabidopsis tending overexpression of *SAMDC1* in Arabidopsis leads to elevated Spm levels and enhanced tolerance to various abiotic stress conditions. Interestingly, high Put levels induced by homologous overexpression of *ADC1* enhance freezing tolerance in Arabidopsis [4], similar to elevated levels of Put by overexpressing *ADC2* produces drought tolerance in Arabidopsis related to reduction of water loss by the induction of stomata closure [3].

### Polyamines and ABA in relation to drought, salt and cold stress

The expression of certain genes is also induced by ABA treatment [52]. The expression of *ADC2*, *SPDS1* and *SPMS* was analysed in the ABA-deficient (*aba2-3*) and ABA-insensitive (*abi1-1*) mutants subjected to water stress, which display reduced transcriptional induction in the stressed *aba2-3* and *abi1-1* mutants compared to the wild type, indicating that ABA modulates polyamine metabolism at the transcription level by up-regulating the expression of *ADC2*, *SPDS1* and *SPMS* genes under water stress conditions [1]. In addition, Put accumulation in response to drought is also impaired in the *aba2-3* and *abi1-1* mutants compared to wild-type plants. All these observations sustain the conclusion that up-regulation of PA-biosynthetic genes and accumulation of Put under water stress are mainly ABA-dependent responses.

It is expected that polyamine responses to salt stress are also ABA-dependent, since both *ADC2* and *SPMS* are induced by ABA. In fact, stress-responsive, drought-responsive (DRE), low temperature-responsive (LTR) and ABA-responsive elements (ABRE and/or ABRE-related motifs) are present in the promoters of the polyamine biosynthetic genes [2]. This strengthens the view that in response to drought and salt treatments, the expression of some of the genes involved in polyamine biosynthesis are regulated by ABA. Free Put levels are increased on cold treatment and this correlates with the induction of *ADC* genes. Reduced expression of *NCED3* and several ABA-regulated genes was detected in the *adc1* mutants at low temperature. Complementation analyses of *adc1* mutants with ABA and mutual complementation of *aba2-3* mutant with Put supported the conclusion that diamine controls the levels of ABA in response to cold by modulating ABA biosynthesis at the transcriptional level [15,14]. Certainly, Put and ABA are integrated in a positive feedback loop, in which ABA and Put equally promote each other's biosynthesis in response to abiotic stress that highlights a novel mode of action of polyamines as regulators of ABA biosynthesis.
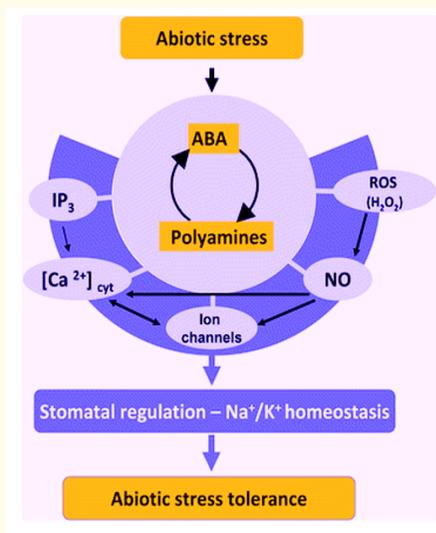
***Figure 1:*** *Basic and simplified model for the addition of polyamines with abscisic acid (ABA), reactive oxygen species (ROS) ($H_2O_2$), nitric oxide (NO), $Ca^{2+}$ homeostasis and ion channel signaling in the abiotic stress response.*

### Polyamines function in stress tolerance

Elevation of endogenous PA levels is one of the metabolic hallmarks of plants exposed to abiotic stresses [26], implying that they are important for protecting plants against unfavourable environmental conditions. On the other hand, under stresses, the precise physiological and molecular mechanisms by which they confer protection remain indefinable [30]. The biological function of the polycationic PAs were initially associated with their capacity to bind anionic macromolecules, such as nucleic acids and proteins, a characteristic that allows PAs to play a role in the regulation of transcription and translation and to function in maintaining membrane stability under adverse conditions [49]. Other than these mechanisms, there is increasing evidence that their role in stress tolerance is associated with modulating antioxidant systems.

Under abiotic stresses, ROS production is prominent, causing excessive ROS build up and oxidative stress, which is toxic to living cells due to lipid peroxidation and membrane damage and can finally result in cell death [9]. PAs play a role in modulating ROS homeostasis in two ways initially by inhibiting the auto-oxidation of metals, which in turn impairs the supply of electrons for the generation of ROS [40]. Next to this step, PAs may affect antioxidant systems, as studies established that priming of plants with polyamines led to increases in endogenous PA contents and concomitant improved tolerance to abiotic stresses, such as drought, heat and cold.

The rise of stress tolerance is simultaneous with the activation of antioxidant enzymes, as for example, exogenous application of Spm to *P. trifoliata* led to an elevation of POD, SOD and CAT activities, accompanied by a significant decrease in ROS levels under dehydration [40]. Exogenous deliver of Spd to rice seedlings mitigated heat-induced damages and increased activities of antioxidant enzymes and levels of antioxidant, accompanied by reduced accumulation of $H_2O_2$ [33]. Conversely, genetic manipulation of PA biosynthetic genes has been demonstrated to promote stress tolerance via modulation of antioxidant machineries. Ectopic expression of *PtADC* in tobacco and tomato also granted improved dehydration and drought tolerance, instantaneous with an extensive repression of ROS generation in the transgenic plants [53]. One more line of evidence supporting the role of PAs in modulating ROS homeostasis is the use of inhibitors of PA biosynthetic enzymes, e.g., the use of D-arginine resulted in a decline in endogenous PA levels and a consequential increase in ROS accumulation [54,59]. This indicates that PAs may assuage the oxidative stress of the stressed plants by regulation of antioxidant systems, together with changes in the ROS production and redox status [41,46].

In fact, there is a direct link between increased PA levels and antioxidant enzyme activity that the PAs may function as signaling molecules in activating the antioxidant enzymes and indeed Spm has been suggested to act as a signaling molecule [31]. A boost in the endogenous PA levels to a positive threshold may help their degradation, generating $H_2O_2$. It is known that $H_2O_2$ plays dual roles in plant responses to abiotic stresses, one being to act as a regulator of signaling cascades at a low cytosolic concentration, which may contribute to the induction of antioxidant enzymes [32,59]. In contrast, the PAs may influence various antioxidant enzymes through regulation of their expression. High transcript levels of antioxidant enzyme-encoding genes detected in tissues treated with exogenous PAs or in the transgenic plants have been overexpressing PA biosynthetic genes [46,60].

## Spermidine and heat tolerance

If high temperatures occur during the seed development, especially at the grain-filling stage, the amylose content of rice seeds is reduced, changing the fine amylopectin structure and producing more chalky grains [7,21]. This also reduces the rice yield and seed quality [25,36]. The occurrence of chalky grains of rice is typically caused by the unusual expressions of genes encoding starch synthase enzymes [34,45].

Most abiotic stressors, including heat, cold, drought and salinity, have easily led to crop yield and quality decline. Since polyamines have been known to be involved in grain filling and they might contribute to build up heat resistance of some cereals, the hybrid rice 'YLY 689' was used to explore the possible effects of exogenous spermidine (Spd) on seed quality under high temperature during the grain filling stage [17]. The content of Spd and Spm in superior grains of rice was significantly higher than that in inferior grains [10]. In this investigation, just after pollination, spikes were treated with Spd or cyclohexylamine (CHA). Interestingly, CHA is its synthesis inhibitor. Very recently, Fu., *et al*. [17]. revealed that when the rice plants were transferred to 40°C for 5-day heat treatment that resulted into significant improvement in the germination percentage, germination index, vigour index, seedling shoot height and dry weight of seeds in case of Spd pretreatment, harvested at 35 days after pollination. In contrast, the CHA significantly decreased the seed germination and seedling growth.

In addition, Spd significantly increased the peroxidase (POD) activity and decreased the malondialdehyde (MDA) content in seeds. Nevertheless, after spraying with Spd, the endogenous content of spermidine and spermine and the expression of their synthetic genes, *spermidine synthase* (*SPDSYN)* and *spermine synthase* (*SPMS1* and *SPMS2*), significantly increased, whereas the accumulation of amylose and total starch and the expression of their related synthase genes, *soluble starch synthase II-3* (*SS II-3*) and *granules bound starch synthase I* (*GBSSI*), also increased to some extent suggesting that exogenous Spd pretreatment could alleviate the negative impacts of high temperature stress on rice seed grain filling and improve the rice seed quality to some extent, which might be partly caused by up-regulating endogenous polyamines and starch metabolism [17]. Recent report also suggests that Spd is involved in heat tolerance in higher plants, which might act by protecting membrane stability and enhancing the reactive oxygen species (ROS) scavenging system. Cao., *et al*. [10] found that the content of Spd and Spm in superior grains of rice was significantly higher than that in inferior grains [10]. The content of Spd and Spm in grains was positively correlated with grain plumpness in the grain filling stage.

## Spermidine synthase (SPDS)

It is recently known that spermidine synthase (SPDS) uses PUT as the substrate for spermidine (SPD) biosynthesis. Spermine and thermospermine are produced from SPD by spermine synthase (SPMS) and thermospermine synthase (TSPS), respectively. Although both aminopropyltransferases (APTs) use the same substrate, they present significant differences that predestine them to produce distinct products [38]. Spermidine synthases (SPDSs) catalyze the production of the linear triamine, spermidine, from putrescine. They utilize decarboxylated S-adenosylmethionine (dc-SAM), a universal cofactor of aminopropyltransferases, as a donor of the aminopropyl moiety. In addition, *At*SPDS1 and *At*SPDS2 are dimeric enzymes that share the fold of the polyamine biosynthesis proteins. Subunits of both isoforms present the characteristic two-domain structure. Smaller, N-terminal domain is built of the two β-sheets, while the C-terminal domain has a Rossmann fold-like topology. The catalytic cleft composed of two main compartments, the dc-SAM binding site and the polyamine groove, is created independently in each *At*SPDS subunits at the domain interface. This is to mention further that the CHA occupies the polyamine binding site of *At*SPDS where it is bound at the bottom of the active site with the amine group placed analogously to the

substrate. The concerted movements are observed by crystallographic snapshots in two compartments of the catalytic cleft, where three major parts significantly change their conformation. These are (i) the neighborhood of the glycine-rich region where aminopropyl moiety of dc-SAM is bound, (ii) the very flexible gate region with helix η6, which interacts with both, the adenine moiety of dc-SAM and the bound polyamine or inhibitor and (iii) the N-terminal β-hairpin, that limits the putrescine binding grove at the bottom of the catalytic site [39].

Hence, overall the endogenous content of spermidine and spermine and the expression of their synthetic genes, *spermidine synthase* (*SPDSYN)* and *spermine synthase* (*SPMS1* and *SPMS2*), significantly increased, whereas the accumulation of amylose and total starch and the expression of their related synthase genes, *soluble starch synthase II-3* (*SS II-3*) and *granules bound starch synthase I* (*GBSSI*), also increased to some extent [17].

### Responses of citrus mycorrhiza to polyamines

Citrus plants are persistently challenged temperature stress (heat, cold), dehydration (drought), high salinity, nutrient stress (Fe deficiency stress) and elevated $CO_2$ [43] in the field. These adverse effects due to abiotic stresses seriously obstruct citrus cultivation. Plant root symbioses with fungi occur in several different forms. Out of many, arbuscular mycorrhiza (AM) is the mutualistic symbiosis between soil-borne fungi (arbuscular mycorrhizal fungi) with the roots of higher plants [37]. AM fungi provide hosts with essential nutrients, such as phosphate, when they have low mobility in the soil solution [35]. In order to elevate mycorrhizal colonization of citrus in field, exogenous polyamines (PAs) have been considered as a practicable measure, because PAs have been linked in plants to various physiological processes, including senescence, biological and physical stress avoidance, cell division, embryogenesis, flower initiation and root development [13]. PAs accumulation is related to adventitious root formation [5]. PAs might regulate the mycorrhizal formation, since AM locates in cortex cells of roots.

The major PAs in higher plants are spermidine (Spd) and spermine (Spm) and their precursor putrescine (Put). In plants, the first step in PA biosynthesis is decarboxylation of ornithine or arginine, catalyzed by ornithine or arginine decarboxylases (ODC, ADC), to yield the Put [1-3]. The total PA concentration and the ratio between individual PAs vary markedly in reliance of plant species and developmental stage [29]. In general, citrus leaves exhibit 26~61 nmol/g Put and 14~349 nmol/g Spm [27], citrus fruits (only *C. reticulate* cv. Ponkan) present 207~280 nmol/g Put, 223~290 nmol/g Spm and 280~320 nmol/g Spd, but Orange fruits (only *C. sinensis* Osbeck, cultivar Brasiliano N.L. 92, a navel orange) contain high levels of Put [47]. In anther of *C. clementina*, cv Nules, Spd is the most abundant among the free PAs detected [11]. Only Spd concentration amongst PAs has direct relationship with citrus cold hardiness [27]. Further, the globular embryos contained more polyamines than embryos at other stages. The transcriptional levels of five key genes involved in PA biosynthesis were increased with maturation of the embryos. Free PAs, Spd and Spm are involved in glycerol-mediated promotion of somatic embryogenesis.

In the three PAs, the stimulated effect was highest in Put-applied trifoliate orange seedlings. There are two explanations; one explanation is that Put is the precursor of Spd and Spm biosynthesis [13]. The other explains is that Put is the most abundant PA in un-germinated spores of *G. mosseae* [16]. Since exogenous PAs can stimulate mycorrhizal infection of citrus, the stimulation would help hosts to uptake mineral from soils. Compared to the sole AMF inoculation, additional Put and Spm in trifoliate orange seedlings markedly increased leaf P and K contents and root P, Mg, Fe and Zn contents [58]. These increases were more significantly in the mycorrhizal seedlings supplied with Put than in the mycorrhizal seedlings supplied with Spm. Positively, the pathway of altered root morphogenesis and increased endogenous IAA level cannot also be excluded [42].

Exogenous PAs have confirmed the enhanced effects on mycorrhizal development of citrus only applied as soil drenching. Consequently, a possible concentration of exogenous PAs will need be conducted in citrus trees.

### Concluding Remarks

Maintaining crop yield under adverse environmental conditions is probably the major challenge faced by modern agriculture, where PAs can play important role. Over the last two decades, genetic, transcriptomic, proteomic, metabolomic and phenomic ap-

proaches have unravelled a lot of significant functions of different PAs in the regulation of plant abiotic stress tolerance. A progress in disentanglement the molecular functions of polyamines has also facilitated the generation of Arabidopsis transgenic plants resistant to various stresses. Polyamines may act as cellular signals in intricate cross talk with hormonal pathways, including abscisic acid (ABA) regulation of abiotic stress responses. Molecular functions of polyamines has also facilitated the generation of Arabidopsis transgenic plants resistant to various stresses, as Arabidopsis has four *LSD1*-related genes. In addition, the polyamine metabolism is connected to several important hormonal and metabolic pathways involved in development, stress responses, nitrogen assimilation and respiratory metabolism. Polyamine accumulation occurs in response to several adverse environmental conditions, including salinity, drought, chilling, heat, hypoxia, ozone, UV-B and UV-C, heavy metal toxicity, mechanical wounding and herbicide treatment. In fact, the up-regulation of PA-biosynthetic genes and accumulation of Put under water stress are mainly ABA-dependent responses and interestingly, ABA and Put equally promote each other's biosynthesis in response to abiotic stress that highlights a novel mode of action of polyamines as regulators of ABA biosynthesis. Furthermore, there is increasing evidence that PAs role in stress tolerance is associated with modulating antioxidant systems. Ectopic expression of *PtADC* in tobacco and tomato also granted improved dehydration and drought tolerance, instantaneous with an extensive repression of ROS generation in the transgenic plants. Increased PA levels may function as signaling molecules in activating the antioxidant enzymes and indeed Spm has been suggested to act as a signaling molecule.

Polyamines have been known to be involved in grain filling and they might contribute to build up heat resistance of some cereals. Exogenous Spd pretreatment could alleviate the negative impacts of high temperature stress on rice seed grain filling and improve the rice seed quality to some extent partly caused by up-regulating endogenous polyamines and starch metabolism [17]. Spd is involved in heat tolerance in higher plants and acts by protecting membrane stability and enhancing the reactive oxygen species (ROS) scavenging system.

In order to elevate mycorrhizal colonization of citrus in field, exogenous polyamines (PAs) have been considered as a practicable measure, because PAs have been linked in plants to various physiological processes, including senescence, biological and physical stress avoidance, cell division, embryogenesis, flower initiation and root development. Exogenous PAs have confirmed the enhanced effects on mycorrhizal development of citrus only applied as soil drenching.

## Perspective and Future Scope

First of all, the causal relationship between PA accumulation and stress tolerance is yet to be determined, despite numerous observations of changes in PA levels in response to abiotic stresses. Secondly, the cellular compartmentation and transportation of PAs is not well understood. In addition, the mode of action of PAs in enhancing stress tolerance has not been definitely established as yet, although several possible models have been proposed. The involvement of PAs in the activation of antioxidant enzymes for ROS detoxification is one example. However, the signaling cascades linking stress responses and PA genes are still far from being well defined. So far, transcription factors (TFs) regulating *ADC* genes have been identified, but those that regulate other PA biosynthetic many genes are unknown. All these open up novel research avenues and in keeping with these unanswered questions, there are several promising areas of future study that includes the sites of PA production and actions in plant cells need to be identified with the cellular localization of PAs and determining their transporters. Nevertheless, the physiological and molecular mechanisms concerning the roles of PAs in stress tolerance need to be elucidated as how PAs contribute to the activation of antioxidant enzymes and ROS removal should be clearly deciphered. All such pieces of information will advance our understanding of PA accumulation and gene expression and can be incorporated with physiological, biochemical, molecular and genetic approaches to better understand the complex regulation of PA synthesis under abiotic stresses, as well as the cross talk between different TF-mediated signaling pathways.

One of the vital issues detailed in this review is the adverse effects due to abiotic stresses that seriously obstruct citrus cultivation and so a possible concentration of exogenous PAs will need be conducted in citrus trees. For this, arbuscular mycorrhiza (AM) is the mutualistic symbiosis and could be stimulated by polyamines for essential nutrient acquisitions both micro- and macronutrients not only in citrus but in many other economically viable fruits cultivated on the globe for human health as well as for earning currencies.

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