Chapter 19
Polyamines: Role in Plants Under Abiotic Stress

Parvaiz Ahmad, Ashwani Kumar, Aditi Gupta, Xiangyang Hu, Khalid ul Rehman Hakeem, Mohamed Mahgoub Azooz, and Satyawati Sharma

Contents

P. Ahmad (✉)
Department of Botany, Amar Singh College, Srinagar 190008, Jammu & Kashmir, India
e-mail: parvaizbot@rediffmail.com; parvaizbot@yahoo.com

A. Kumar • A. Gupta • S. Sharma
Biochemistry Laboratory, CRDT, Indian Institute of Technology, Hauz Khas,
New Delhi 110016, India
e-mail: ashwaniiitd@hotmail.com; 29.aditi@gmail.com; satyawatis@hotmail.com

X. Hu
Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Institute
of Tibet Plateau Research at Kunming, Chinese Academy of Sciences,
Kunming 650204, China
e-mail: Huxiangyang@mail.kib.ac.cn

K. ul Rehman Hakeem
Molecular Ecology Laboratory, Department of Botany, Faculty of Science, Jamia Hamdard,
New Delhi 110062, India
e-mail: Kur.hakeem@gmail.com

M.M. Azooz
Department of Biological Sciences, Faculty of Science, King Faisal University, Saudi Arabia

Department of Botany, Faculty of Science, South Valley University, 83523 Qena, Egypt

M. Ashraf et al. (eds.), *Crop Production for Agricultural Improvement*,
DOI 10.1007/978-94-007-4116-4_19,

Abstract Environmental changes, irrespective of source, cause a variety of stresses in plants. These stresses affect the growth and development and trigger a series of morphological, physiological, biochemical and molecular changes in plants. Abiotic stress is the primary cause of crop loss worldwide. The most challenging job before the plant biologists is the development of stress tolerant plants and maintenance of sufficient yield of crops in this changing environment. Polyamines can be of great use to enhance stress tolerance in such crop plants. Polyamines are small organic polycations present in all organisms and have a leading role in cell cycle, expression of genes, signaling, plant growth and development and tolerance to a variety of abiotic stresses. High accumulation of polyamines (putrescine, spermidine and spermine) in plants during abiotic stress has been well documented and is correlated with increased tolerance to abiotic stress. Genetic engineering of PA biosynthetic genes in crop plants is the way to create tolerance against different stresses. The present review throws light on the role of polyamines in plants.

Keywords Abiotic stress tolerance • Polyamines • ADC • ODC • SAMDC

1 Introduction

Plants are exposed continuously to a variety of adversely changing environmental factors such as heat, cold, light, drought, acidity, alkalinity, oxidative damage and metal damage, which affect plant distribution, growth, development and productivity (Ahmad et al. 2008, 2010a, b). These stressful conditions are associated with the losses in the productivity of many of the agriculturally important crops and therefore, affect the economic returns of the country. Thus, concerted efforts are underway worldwide to understand the mechanism of plant resistance against these stresses. There are several natural ways of self-defense in the plants to cope with these stressful conditions: they can induce several functional or regulatory genes (Bartels and Sunkar 2005) or can undergo different physiological or biochemical changes. The accumulation of some functional substances, such as compatible solutes and protective proteins, is an important element of the physiological and biochemical response of plants to the stressful conditions (Liu et al. 2007; Ahmad and Sharma 2008; Ahmad et al. 2010a, b, 2011). In addition to these responses by the plants, molecules known as ‘polyamines’, have also been known to be an integral part of plant stress response (Bouchereau et al. 1999; Walters 2003a, b; Alcázar et al. 2006b).

Polyamines (putrescine, spermine, spermidine and cadaverine), are the widely distributed of N containing organic molecules, which were discovered more than 100 years ago and hold their significance from the minutest bacteria to multicellular pants, animals and mammals. In addition to their stabilizing effects, which they confer by binding to the intracellular anions (DNA, RNA, chromatin and proteins), they are also known to possess several regulatory functions (Igarasahi and Kashiwagi 2000; Alcázar et al. 2006b, 2010; Kusano et al. 2008). In plants, they have been associated with regulating many physiological processes, such as organogenesis, embryogenesis, floral initiation and development, leaf senescence, fruit development and ripening, and abiotic and biotic plant stress responses (Galston and Kaur-Sawhney 1990; Kumar et al. 1997; Walden et al. 1997; Malmberg et al. 1998; Bouchereau et al. 1999; Bagni and Tassoni 2001; Alcázar et al. 2006b, 2010; Kusano et al. 2008).

Several changes in concentrations of polyamines in plant cells take place while responding to the stressful conditions (Bouchereau et al. 1999; Alcázar et al. 2006b, 2010; Groppa and Benavides 2008). The importance of this process can be exemplified by the fact that the levels of Put may account for 1.2% of the dry matter, representing at least 20% of the nitrogen (Galston 1991) under stressful conditions. Though the exact mechanism of involvement of polyamines during stressful conditions is not fully understood, studies are ongoing to study the molecular mechanisms (Liu et al. 2007; Alcazar et al. 2010). Evaluating the complete genome sequence of Arabidopsis has facilitated the use of global ‘omic’ approaches in the identification of target genes in polyamine biosynthesis and signaling pathways (Alcazar et al. 2010). The advantages of the progress made in these directions have made possible the generation of Arabidopsis transgenic plants, which are resistant to various stresses (Alcazar et al. 2010). Efforts can be made towards the development of such varieties for the agriculturally important crops as well. Such studies add to the economic potential from the agricultural sector touched by the biotechnological advances and hence, further research in these directions is noteworthy.

2 Polyamine Biosynthesis in Different Organisms

The biosynthetic pathways of polyamines have been established for many organisms ranging from bacteria to plants to mammals (Kusano et al. 2007). The synthesis essentially starts from the two amino acid precursor molecules, L-arginine and L-methionine. An overview of the general pathway is given in Fig. 19.1.

In mammals and fungi, putrescine (Put, 1,4-diaminobutane) is produced by a single pathway catalyzed by ornithine decarboxylase (ODC, EC 4.1.1.17) whereas, in plants two alternative pathways operate, namely the ODC-catalyzed reaction, as in mammals and the second is from arginine (Arg), as a result of the action of Arg decarboxylase (ADC, EC 4.1.1.19), via agmatine. A few plant species, including *Arabidopsis thaliana*, lack the ODC pathway (Hanfrey et al. 2001; Kusano et al. 2007). The polyamines in

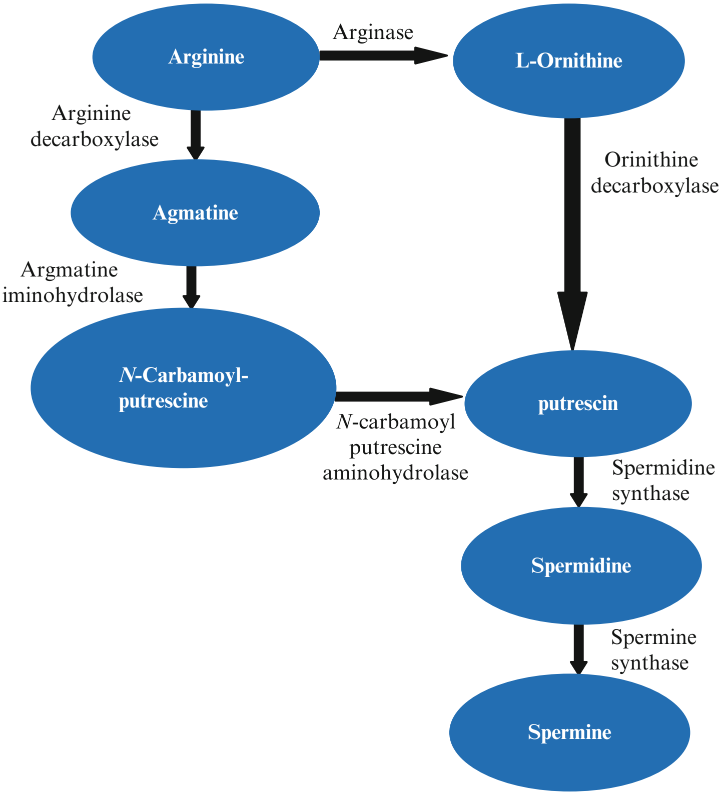

Fig. 19.1 Biosynthetic pathway of polyamines in plants

plants are not only found in the cytoplasm, but also in certain organelles like mitochondria, chloroplasts and vacuoles (Kumar et al. 1997; Kusano et al. 2008). The genes encoding enzymes for the polyamine biosynthesis pathway have been cloned and characterized from various plant species (Bell and Malmberg 1990; Michael et al. 1996; Bagni and Tassoni 2001; Liu et al. 2007; Kusano et al. 2008).

Briefly, starting from arginine, the diamine putrescine is synthesized via ornithine by arginase (EC 3.5.3.1) and ornithine decarboxylase (ODC, EC 4.1.1.17). Putrescine can also be synthesized via agmatine by three sequential reactions catalyzed by arginine decarboxylase (ADC, EC 4.1.1.19), agmatine iminohydrolase (AIH, EC 3.5.3.12), and *N*-carbamoylputrescine amidohydrolase (CPA, EC 3.5.1.53), respectively (Kusano et al. 2008). Putrescine is further transformed to Spd and Spm by successive transfers of aminopropyl groups from decarboxylated *S*-adenosylmethionine (dSAM) catalysed by specific Spd and Spm synthases. The aminopropyl groups are derived from methionine, which is first converted to *S*-adenosylmethionine (SAM) by methionine adenosyltransferase (EC 2.5.1.6), and then decarboxylated in a reaction catalyzed by *S*-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50). The resulting decarboxylated SAM is utilized as an aminopropyl donor (Fig. 19.1).

The use of the polyamine inhibitors have helped in the evaluation of their respective roles. Four commonly used inhibitors of PA synthesis are: (1) difluoromethylornithine (DFMO), an irreversible inhibitor of ODC; (2) difluoromethylarginine (DFMA), an irreversible inhibitor of ADC; (3) methylglyoxyl- bis-guanylhydrazone (MGBG), a competitive inhibitor of *S*-adenosyl-methionine decarboxylase (SAMDC); and (4) cyclohexylamine (CHA), a competitive inhibitor of spermidine synthase. Common oxidases are diamine oxidase and polyamine oxidase (PAO), Each PA has been found to be catabolized by a specific oxidase (Kaur-Sawhney et al. 2003).

3 Polyamine Catabolism

The concentrations of the polyamines in the cells is also maintained by the catabolic pathways (Bagni and Tassoni 2001; Cona et al. 2006). Copper containing diamine oxidases (CuAO, EC 1.4.3.6) and flavine-containing polyamine oxidases (PAO, EC 1.5.3.11) catalyse the oxidative de-amination of PAs. CuAO, which prefers diamine substrates, oxidizes Put and cadaverine (1,5-diaminopentane) with concomitant production of pyrroline, NH_3 and H_2O_2, and the resulting aldehyde is further metabolized to γ-aminobutyric acid via Δ^1-1-pyrroline (Bagni and Tassoni 2001; Kusano et al. 2008). On the other hand, PAO oxidizes Spd and Spm, producing 4-aminobutanal and *N*-(3-aminopropyl)-4-aminobutanal, respectively, in addition to 1,3-diaminopropane and H_2O_2 (Kusano et al. 2007). This means that plant PAOs are involved in the terminal catabolism of polyamines (Kusano et al. 2008). These enzymes are associated with the cell walls of tissues, where lignification, suberization and wall stiffening occur (Slocum 1991). Spermine oxidase (SMO), a FAD-dependent amine oxidase, which directs the back-conversion of spermine to spermidine with concomitant production of 3-aminopropanal and H_2O_2, was initially identified in mammalian cells (Wang et al. 2001; Vujcic et al. 2002; Cervelli et al. 2003; Kusano et al. 2008). Diaminopropane can be converted into β-alanine, whereas pyrroline can be further catabolized to γ-aminobutyric acid (GABA) in a reaction catalysed by pyrroline dehydrogenase (PDH). The γ-aminobutyric acid is subsequently transaminated and oxidised to succinic acid, which is incorporated into the Krebs cycle. Thus, this pathway ensures the recycling of carbon and nitrogen from Put. Far from being only a means of eliminating cellular PAs, the enzymes involved in PA catabolism and the products deriving from their action, have been demonstrated to be involved in important physiological processes (Bouchereau et al. 1999). A simple illustration of the catabolic pathways has been shown in Fig. 19.2.

4 Role of Polyamines in Plants

The first reference of the polyamines in plants can probably be dated back to 1911 when Ciamician and Ravenna demonstrated the presence of putrescine in *Datura stramonium* (Bagni and Tassoni 2001). In plant cells, the diamine putrescine (Put),

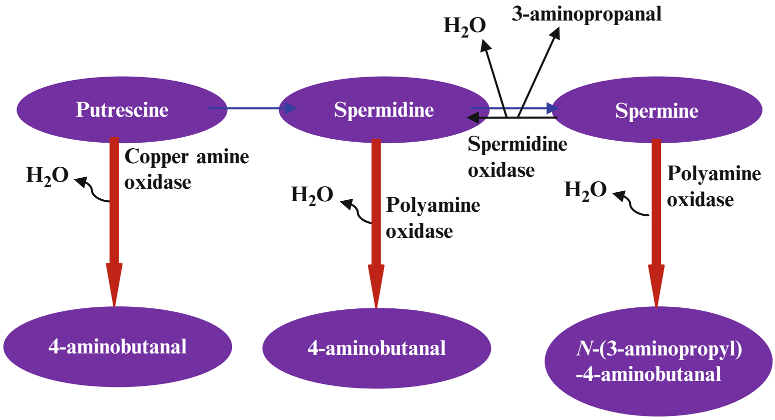

Fig. 19.2 Polyamine degradation in plants

triamine spermidine (Spd) and tetramine spermine (Spm) constitute the major PAs. Cadaverine is also present in legumes. These occur either in the free form or as conjugates bound to phenolic acids and other low molecular weight compounds or to macromolecules such as proteins and nucleic acids owing to their positive charge (Kaur-Sawhney et al. 2003). Besides stimulating DNA replication, transcription and translation, they have contributed to various biological processes in plant morphogenesis, growth, embryogenesis, organ development, leaf senescence, abiotic and biotic stress response and infection by fungi and viruses (Kumar et al. 1997; Walden et al. 1997; Malmberg et al. 1998; Bouchereau et al. 1999; Liu et al. 2000, 2010; Alcázar et al. 2006a, b; Groppa et al. 2008; Kusano et al. 2007, 2008). Their biological activity has been attributed to their cationic nature. Plant polyamines also contribute towards several characteristics of agro-economical importance, such as phytonutrient content, fruiting and fruit quality, vine life, flowering and carnation plants (Kakkar and Rai 1993; Mehta et al. 2002; Piqueras et al. 2002; Matto et al. 2006; Paschalidis and Roubelakis-Angelakis 2005).

Some of the observations suggest that PAs can act by stabilizing membranes, scavenging free radicals, affecting nucleic acids and protein synthesis, RNAse, protease and other enzyme activities, and interacting with hormones, phytochromes, and ethylene biosynthesis (Slocum et al. 1984; Galston and Tiburcio 1991). Because of these numerous biological interactions of PAs in plant systems, it has been difficult to determine their precise role in plant growth and development (Kaur-Sawhney et al. 2003). However, recent use of genomic and proteomic approaches will lead to a better understanding of the functioning in the plants (Kaur-Sawhney et al. 2003; Franceschetti et al. 2004).

The mechanisms involved in the polyamine interactions have been unveiled, at least to some extent with the help of specific polyamine inhibitors, thus explaining their physiological roles in plant growth and development. Clearly, PAs are involved in many plant developmental processes, including cell division, embryogenesis, reproductive organ development, root growth, tuberization, floral initiation and development, fruit development and ripening as well as leaf senescence and abiotic stresses as mentioned above (Evans and Malmberg 1989; Galston et al. 1997; Bais and Ravishankar 2002; Kaur-Sawhney et al. 2003; Cona et al. 2006; Rhee et al. 2007; Groppa et al. 2008; Alcazar et al. 2010). During these developmental processes, changes in the concentrations of the free and conjugated PA's and the enzymes associated with their biosynthesis (ADC, ODC, SAMDC, etc.) take place. Many authors have reported that the increase in the PA levels and the associated biosynthetic enzymes are associated with the rapid cell division in many plant systems e.g., carrot embryogenesis (Montague et al. 1978; Feirer et al. 1984), tomato ovaries (Heimer and Mizrahi 1982; Neily et al. 2011), tobacco ovaries (Slocum and Galston 1985; Franceschetti et al. 2004), and fruit development (Kakkar and Rai 1993; Paschalidis and Roubelakis-Angelakis 2005; Falasca et al. 2010). It has been observed that cells undergoing division (apical shoots, meristems, flowers, etc) contain higher levels of PAs whereas cells undergoing expansion and elongation contain lower levels of PA synthesized via ADC (Kaur-Sawhney et al. 2003). This has been further exemplied as follows: higher levels of endogenous PAs were found in flowers and siliques as compared to their levels in leaves and bolts of certain strains of Arabidopsis; addition of the PA biosynthetic inhibitors, DFMA and CHA to the culture medium, at time of seed germination, inhibited bolting and flower formation and this was partially reversed by addition of exogenous Spd (Applewhite et al. 2000; Kaur-Sawhney et al. 2003). These results clearly show that Spd is involved in flower initiation and development. Similar results have been reported in other plants also (reviewed by Galston et al. 1997; Bais and Ravishankar 2002). The regulation of many important plant hormones such as auxins, gibberellins, ethylene, etc., which play a vital role in plant growth and developmental processes has been correlated with the changes in the PA metabolism. Of the important plant hormones, ethylene is of particular interest as PAs and ethylene are said to play antagonist roles (Kaur-Sawhney et al. 2003). While PAs inhibit senescence of leaves (Kaur-Sawhney et al. 1982), cell cultures of many monocot and dicot species (Muhitch et al. 1983) and fruit ripening (Kakkar and Rai 1993), ethylene promotes all these processes. The most commonly held view is that ethylene is an effective inhibitor of ADC and SAMDC, the key enzymes in PA biosynthetic pathway, and on the other hand, PAs tend to inhibit ethylene synthesis from SAM (Kaur-Sawhney et al. 2003). Plants are exposed to continuous and rapid changing environmental factors (biotic and abiotic) such as light, temperature, water, nutrient availability, and water. These have a major impact on plant growth and productivity and PAs play an important role in these stresses as briefly discussed below.

5 Role of Polyamines in Plant Tolerance to Abiotic Stress

Richards and Coleman (1952) observed the presence of a predominant unknown ninhydrin positive spot that accumulated in barley plants when exposed to potassium starvation. This compound was identified as putrescine. Later on, it was shown that K-deficient shoots fed with L-^{14}C-arginine produced labeled Put in a more rapid way compared to feeding with labeled ornithine (Alcazar et al. 2010). These results suggested that decarboxylation of arginine was the main way of accumulation of Put under K deficiency (Smith and Richards 1962). The relevance of the ADC pathway in plant responses to abiotic stress was later on established by Galston et al. at Yale University (Flores and Galston 1982). It has been observed that polyamines accumulate in plants during various stressful conditions (see Bouchereau et al. 1999; Alcázar et al. 2006b, 2010; Groppa and Benavides 2008). These all reports support the fact that polyamines do play a protective role during the stressful conditions. Several examples have been quoted by Alcazar et al. (2010) in which genetic modification of the genes involved in PA biosynthetic pathway have proven useful in developing plant tolerance against abiotic stresses. The different stress factors have been briefly discussed below.

5.1 Mineral Deficiency

This is one of the most common stress related factors affecting plants almost everywhere. However, studies related to this type of stress are often performed on leaves and/or seedlings, as the external symptoms of deficiency become acute. The accumulation of Put in leaves of K-deficient barley plants was first reported by Richards and Coleman (1952) and subsequent studies by others have established that specific role of Put in maintaining a cation- anion balance in plant tissues. As a result of K starvation, this diamine accumulation (via ADC activation), is widespread among mono- and di-cotyledonous species and may well be a universal response (Bouchereau et al. 1999). The exact reason behind the increase in Put is unclear. The induced high levels of Put might be the cause of the stress injury. Put might also be beneficial for plants. Alternatively, high levels of Put could be one of the many physiological changes induced by mineral nutrient deficiency without any specific significance (Bouchereau et al. 1999). There are several other examples listing the changes in the polyamine content while responding to the mineral deficiencies (Geny et al. 1997). However, the changes differed according to the tissue and the stage of development.

5.2 Cold Stress

The injury due to cold causes alteration in the membrane structure, and the chilling injury involves phase transition in the molecular ordering of membrane lipids (Raison and Lyons 1970). This can cause several deleterious effects like increased membrane permeability and alteration of the activity of membrane proteins. Cold treatment has been reported to increase the levels of Put, and this correlates with the increase in the induction of arginine decarboxylase (ADC) genes (ADC1, ADC2 and SAMDC2) (Urano et al. 2003; Cuevas et al. 2008, 2009). On the other hand, levels of free Spd and Spm remain constant or even decrease in response to cold treatment (Alcazar et al. 2010). The absence of correlation between enhanced *SAMDC2* expression and the decrease Spm levels may be a result of increased Spm catabolism (Cuevas et al. 2008; Alcazar et al. 2010). Boucereau et al. (1999) reported that in the chilling-tolerant-cultivar, chilling induced an increase in free abscisic acid (ABA) levels first, then ADC activity and finally free Put levels. Fluridone, an inhibitor of ABA synthesis, inhibited the increase of free ABA levels, ADC activity and free Put levels in chilled seedlings of a chilling-tolerant cultivar. These effects resulted in a reduced tolerance to chilling and could be reversed by the pre-chilling treatment with ABA. All these results suggest that Put and ABA are integrated in a positive feedback loop, in which ABA and Put reciprocally promote each other's biosynthesis in response to abiotic stress (Fig. 19.1). This highlights a novel mode of action of polyamines as regulators of ABA biosynthesis (Alcazar et al. 2010).

5.3 Thermal Stress

When exposed to heat stress, plants have the ability to synthesize uncommon long chain PAs (caldine, thermine). The levels of free and bound PAs, as well as ADC and polyamine oxidases (PAO) activities, were higher in tolerant than in sensitive cultures of different crop. (Kuehn et al. 1990; Philipps and Kuehn 1991; Roy and Ghosh 1996; Bouchereau et al. 1999). The increased activities of the transglutaminases indicated the high content of the polyamines. This indicates a correlation between heat-stress tolerance, ADC, PAO and transglutaminase activities (Bouchereau et al. 1999).

5.4 Drought Stress

Certain plants during water scarcity tend to accumulate putrescine (Put) which is supported by the fact that transcript profiling under these conditions induces the expression of certain genes involved in the biosynthetic pathway. The expression of some of these genes is also induced by ABA treatment (Perez-Amador et al. 2002; Urano et al. 2003; Alcazar et al. 2010). This throws light upon the fact that up- regulation of PA-biosynthetic genes and accumulation of Put under water stress are mainly ABA-dependent responses (Alcazar et al. 2010).

5.5 Salt Stress

Differences in PA (Put, Spd, Spm) response under salt-stress have been reported among and within species. For example, according to Prakash and Prathapsenan (1988), endogenous levels of PAs (Put, Spd and Spm) decreased in rice seedlings under NaCl stress, whereas Basu et al. (1988) reported that salinity resulted in accumulation of these compounds in the same material (Bouchereau et al. 1999). Santa-Cruz et al. (1997) reported that the (Spd + Spm):Put ratios increased with salinity in the salt-tolerant tomato species (*Lycopersicon pennellii*, Carrel D'Arcy) but not in the salt-sensitive tomato species (*L. esculentum*). In both species, stress treatments decreased the levels of Put and Spd. The Spm levels did not decrease with salinity in *L. pennellii* over the salinization period, whereas they greatly decreased in *L. esculentum*. The effects of different NaCl concentrations on maize embryogenic cells derived from immature embryo cultures of a salt-sensitive inbred line (cv. w64) and a resistant hybrid (cv, Arizona) have also been reported where increased salt concentration remarkably decreased the growth of the calluses and showed a significant increase in the total PA (Put, Spd) content, especially caused by a rise in Put. It has been reported by Bouchereau et al. (1999) that using the inhibitors of Put synthesis, the ADC pathway in tomato plants operates in both stress and control conditions, whereas the ODC pathway is stimulated only under the stress conditions. These findings are further supported by the studies of Urano et al. (2003) who concluded that the expressions of the arginine decarboxylase 2(ADC 2) and spermine synthases (SPMS) during the 24 h stress treatment maintained and hence, increased the levels of Put and Spm. Yamaguchi et al. (2006) also suggested the protective role of Spm when its addition suppressed the salt sensitivity in Spm deficient mutants. Bouchereau et al. (1999) suggested that polyamine responses to salt stress are also ABA-dependent, since both *ADC2* and *SPMS* are induced by ABA. In fact, Alcazar et al. (2006a) reported that 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. This also reinforces 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 (Alcazar et al. 2010). The study of the *Arabidopsis thaliana* flowers by Tassoni et al. (2010) has also supported the hypothesis that polyamine levels (mainly Spm) increase with the increase in the salt concentration and therefore, contribute to plant tolerance during the stressful conditions.

5.6 Osmotic Stress

Osmotic treatments using sorbitol induced high levels of Put and ADC in detached oat leaves (Flores and Galston 1984), whereas, Spd and Spm show a dramatic decrease. Bouchereau et al. (1999) reported that osmotica with widely different assimilation routes, such as sorbitol, mannitol, proline, betaine and sucrose, all induce a rise in Put. These changes are coincident with measurable signs of a stress, such as wilting and protein loss. Tiburcio et al. (1995) reported that when peeled oat leaves are incubated with sorbitol in the dark, they lose chlorophyll and senescence rapidly. Senescence could be delayed by including Spm in the incubation medium.

The senescence-retarding effect of Spm was correlated with increase in the incorporation of labeled precursors into proteins, RNA and DNA. They also concluded that osmotic shock in the dark induces an activation of the pathway catalyzed by ADC. Borrell et al. (1996) have reported the regulation of ADC synthesis by Spm in osmotically-stressed oat leaves using a polyclonal antibody to oat ADC and a cDNA clone encoding oat ADC. Treatment with Spm in combination with osmotic-stress resulted in increased steady-state levels of ADC mRNA, yet the levels of ADC activity decreased. This absence of correlation has been explained by the fact that Spm inhibits processing of the ADC proenzyme, which results in increased levels of this inactive ADC form and a subsequent decrease in the ADC-processed form (Bouchereau et al. 1999). They also showed that in osmotically-stressed oat leaves, degradation of cytochrome thylakoid proteins and the enzyme rubisco can be avoided by addition of Spm to the incubation medium. Thus post-translational regulation of ADC synthesis by Spm may be important in explaining its anti-senescence properties. Interestingly, Masgrau et al. (1997) concluded that the over-expression of oat ADC in tobacco resulted in similar detrimental effects to those observed by ADC activation induced by osmotic-stress in the homologous oat leaf and stem (chlorosis and necrosis). Therefore, optimum levels of polyamines are necessary for the proper growth and development of plants (Bouchereau et al. 1999). Recently, Liu et al. (2010) have investigated the changes in the content and the form of polyamines (PAs) in the leaves of two wheat (*Triticum aestivum* L.) cultivars seedlings, differing in drought tolerance, under the osmotic stress by polyethylene glycol (PEG) treatment. The results suggested that free-Spd, -Spm and PIS-bound Put (perchloric acid insoluble bound putrescine) facilitated the osmotic stress tolerance of wheat seedlings. The important roles of reactive oxygen species in the relationship between ethylene and polyamines (PAs) have also been investigated in the leaves of spring wheat seedlings under root osmotic stress (Li et al. 2010).

5.7 Hypoxia

There has been a lot of work done by Reggiani's group on the role of polyamines under the hypoxic stress conditions. Reggiani et al. (1990) reported that there are many examples available where plant shoots and seedlings of different Gramineae species, when subjected to lack of oxygen, provide evidence of an association between tolerance and the capacity to accumulate Put. Species such as rice and barnyard grass which are adapted to germinate in an oxygen deprived environment, showed a greater capacity of Put accumulation than the anoxia-intolerant species (Reggiani and Bertani 1989). This consideration supports the hypothesis for a role of Put as a protective compound against hypoxia (Reggiani and Bertani 1990; Bouchereau et al. 1999). Reggiani et al. (1989) have reported that Put is required for the anaerobic elongation of rice coleoptiles, but it has no effect on aerobic elongation of rice coleoptiles where auxin is active. This group has also concluded that with a decrease in oxygen concentration, the conjugated Put became predominant in comparison with the free forms (80% at 0.3% oxygen) and there is a negative correlation between Put accumulation (specially under conjugated forms) and shoot elongation (Reggiani and Bertani 1989; Bouchereau et al. 1999). On the other hand, the results of Lee et al. (1996) have indicated that increase in the activities of ADC and ODC, and Put levels are essential for the elongation of *Scirpus* shoots grown under submergence.

5.8 Ozone Stress

Ozone, the protective gas in the upper atmosphere, is known to protect us from the harmful UV rays of the sun. But it is known to have serious effects on the vegetation. Experiments are ongoing throughout the world in this respect. According to Heagle (1989) O_3-stress can lead to a significant decline in net photosynthesis, cause leaf injury and accelerate senescence, even when applied at low levels. Reaction to this stress triggers many biochemical changes in plants such as increase in ABA, peroxidases, phenolic compounds, ethylene and polyamines, which form a part of the plant self-defense mechanism. Rowland-Bamford et al. (1989) observed that the ADC activity in the ozone treated barley leaves increased before the damage became apparent. Many more examples have been quoted by Bouchereau et al. (1999) supporting the protective role of the polyamines during the ozone damage. Though the exact mechanism is not clear, there can be a possibility of PAs being involved in the free radical scavenging (Bors et al. 1989). This is also supported by the fact that the levels of superoxide radical formed enzymatically with xanthine oxidase or chemically from riboflavin or pyrogallol were inhibited *in vitro* by Put, Spd or Spm at 10–50 mM (Drolet et al. 1986). Also, superoxide radical protection was inhibited by PAs when added to microsomal membrane preparations. These findings have been also supported by the fact that PAs tend to inhibit lipid peroxidation (Tang and Newton 2005; Zhao and Yang 2008). These conclusions were, however, disputed by the findings of Langebartels et al. (1991) as mentioned by Bouchereau et al. (1999). Leaf injury, caused by O_3 in the tobacco cultivar Bel W3 could be prevented by feeding Put, Spd or Spm through the root. These exogenous treatments were correlated with a two to three-fold increase in soluble conjugated Put and Spd (monocaffeoyl forms). Conjugated Put and Spd associated with cell wall and membrane fractions were increased four to six-fold. When free PAs were assayed *in vitro* for their radical-scavenging properties, very low rate constants were found. On the other hand, PA conjugates had relatively high rate constants. It was thus concluded that free PAs could not account for the protection against O_3 damage. But assuming their role in the ozone damage, it was suggested that the protective effect of exogenous free PAs was mediated by their prior conversion to conjugated forms. Consistent with this hypothesis, it was found that monocaffeoyl Put, an effective scavenger of oxyradicals, was present in the apoplastic fluid of tobacco leaves exposed to O_3 (Dat et al. 2003). The results of Navakoudis et al. (2003) also support these findings showing that the enhanced atmospheric ozone is the accumulation of polyamines, generally observed as an increase in putrescine level, and in particular its bound form to thylakoid membranes. A study by Schraudner et al. (1990) also discovered a relationship between ethylene emission and PA biosynthesis was found in O_3-treated potato and tobacco plants, the leaves of which show early senescence in response to the pollutant. In the presence of O_3, all compounds of ethylene biosynthetic pathway in tobacco leaves were up-regulated. Put and Spd levels also increased, as did ornithine decarboxylase (ODC) activity (Bouchereau et al. 1999).

6 Polyamine Biosynthetic Genes and Stress Tolerance

The expression of genes responsible for the PA synthesis has benefited the plants to withstand environmental stresses. The over-expression or the down-regulation of the genes for PA metabolism in transgenic plants have been reported by many workers during environmental stress (Kumar et al. 1997; Walden et al. 1997; Malmberg et al. 1998; Capell et al. 1998; Rajam et al. 1998; Roy and Wu 2001; Bhatnagar et al. 2002). The genes which have been reported to be involved in the PA metabolism are *ODC*, *ADC* or *SAMDC*. Bhatnagar et al. (2002) have demonstrated that the cellular levels of Put increases by overexpressing *ODC* or *ADC* cDNA. Panicot et al. (2002a) have also reported that overexpression of *ODC* or *ADC* cDNA increases the Put levels in plants. Cheng et al. (2009) reported that transformation of yeast *SAMDC* in tomato increased Spm and Spd under high temperature stress. Overexpression of *SPDS* in Arabidopsis (Kasukabe et al. 2004); tobacco (Franceschetti et al. 2004) and sweet potato (Kasukabe et al. 2006) plants have conferred tolerance to multiple stresses. Polyamines have been proved to act as antioxidants and protect the plants from oxidative damage and maintain homeostasis in plant cells (Rodrıguez-Kessler et al. 2006). Accumulation of polyamines during environmental stresses in plants has been associated with increase in the levels of antioxidant enzyme activities like SOD, CAT, etc. Increase in MDA content has been observed during temperature stress in tomato, which leads to lipid peroxidation (Cheng et al. 2009). Overexpression of *ySAMDC* in transgenic tomato increases the Spm and Spd levels, which in turn decreases MDA content (Cheng et al. 2009). The overexpression of *SAMDC* gene in transgenic rice and tobacco showed increased levels of PA and confers tolerance to drought and salinity (Roy and Wu 2002; Waie and Rajam 2003). Table 19.1 provides further information about the PA transgenics.

7 Integration of Polyamines with Other Molecules During Stress Conditions

Polyamines affect several physiological processes in plants by activating the biosynthesis of signaling molecules like NO, H_2O_2; they affect ABA synthesis and signaling and are involved in Ca^{2+} homeostasis and ion channel signaling during the abiotic stress conditions. Figure 19.1 summarizes this information.

Table 19.1 Polyamine genes that can be expressed in plants for abiotic stress tolerance

Gene overexpressed	Plant	Response	Reference
ADC	*Oryza sativa*	Salt tolerance	Roy and Wu (2001)
ADC	*Brassica juncea*	Chilling and salt	Mo and Pua (2002)
ADC1, ADC2	*Oryza sativa*	Drought tolerance	Capell et al. (2004)
ADC1, ADC2	*Arabidopsis thaliana*	Freezing	Cuevas et al. (2008)
ADC	*Malus domestica*	Chilling, Salt and Dehydration	Hao et al. (2005)
ADC	*Oryza sativa*	Chilling	Akiyama and Jin (2007)
At ADC2	*Arabidopsis thaliana*	Salt tolerance	Urano et al. (2004)
ADC	*Solanum melongena*	Chilling, Salt and Dehydration	Prabhavathi and Rajam (2007)
ADC	*Zea maize*	Salt	Jimenez-Bremont et al. (2007)
MdADC	*Malus sylvestris* (L.) Mill. var. domestica	Salt tolerance	Liu et al. (2006)
PaADC2	*Pringlea antiscorbutica*	Chilling and salt	Hummel et al. (2004)
Mouse *ODC* cDNA	*Populous nigra* X maximowiczii cells	Stress tolerance	Bhatnagar et al. (2001)
Mouse *ODC* cDNA	tobacco	Salt stress	Kumria and Rajam (2002)
MdSAMDC2	*Malus sylvestris*	Cold and salt	Hao et al. (2005)
MdSAMDC2	*Pyrus communis*	salt	He et al. (2008)
SPDS cDNA from Cucurbita ficifolia	Arabidopsis	Chilling, salinity, drought	Kasukabe et al. (2004)
SPDS cDNA from Cucurbita ficifolia	Sweet potato	Increase in Spd	Kasukabe et al. (2006)
MdSPDS1	*Pyrus communis*	Salt, Heavy metal and osmotic stress	Wen et al. (2008)

Abscisic acid (ABA) is an anti-transpirant that reduces water loss through stomatal pores on the leaf surface in response to water deficit, resulting in the redistribution and accumulation of ABA in guard cells and finally closure of the stomata (Bray 1997). Many authors (Liu et al. 2000; An et al. 2008; Alcazar et al. 2010) have reported that Put, Spd and Spm also regulate stomatal responses by reducing their aperture and inducing closure, and Put modulates ABA biosynthesis in response to abiotic stress. Thus, polyamines are involved in the ABA mediated stress responses which affect the stomatal closure. Polyamines are also linked with reactive oxygen species (ROS) and NO signaling as amino oxidases during the catabolic process generate H_2O_2 which is a ROS (associated with plant defense and abiotic stress) and also there is evidence in which polyamines are reported to enhance the production of NO (Tun et al. 2006). NO is also known to enhance the salt stress tolerance in plants by regulating the content and proportions of the different types of free polyamines. According to Neill et al. (2008), both H_2O_2 and NO are involved in the regulation of stomatal movements in response to ABA, in such a way that NO generation depends on H_2O_2 production. Thus, altogether, the available data indicate that polyamines, ROS (H_2O_2) and NO act synergistically in promoting ABA responses in guard cells (Alcazar et al. 2010).

Polyamines are positively charged compounds, which can interact electrostatically with negatively charged proteins, including ion channels. Indeed, polyamines at their physiological concentration block the fast-activating vacuolar (FV) cation channel in a charge-dependent manner ($Spm^{4+} > Spd^{3+} >> Put^{2+}$), at both whole-cell and single-channel levels, thus indicating a direct blockage of the channel by polyamines (Bruggemann et al. 1998). According to Alacazar et al. (2010), in response to different abiotic stresses, such as potassium deficiency, Put levels are increased drastically (reaching millimolar concentrations), whereas the levels of Spd and Spm are not significantly affected, and this increase of Put may significantly reduce FV channel activity. Bruggemann et al. (1998) have also reported that all PA levels increase in amount, and the enhanced Spm concentration probably blocks FV channel activity under salinity stress. These observations can be explained by the fact that polyamines in plants may thus modulate ion channel activities through direct binding to the channel proteins and/or their associated membrane components (Delavega and Delcour 1995; Johnson 1996; Alcazar et al. 2010). Phosphorylation and dephosphorylation of ion channel proteins are closely related to their activities. Thus, polyamines could also affect protein kinase and/or phosphatase activities to regulate ion channel functions (Bethke and Jones 1997; Michard et al. 2005; Alcazar et al. 2010). However, Zhao et al. (2007) points out that for elucidating the molecular mechanisms underlying polyamine action, identification of ion channel structural elements and/or receptor molecules regulated by polyamines would be of great importance.

Polyamines also tend to maintain Ca^{2+} homeostasis. Several examples have been reported by Alcazar et al. (2010). Yamaguchi et al. (2006, 2007) proposed that the protective role of Spm against high salt and drought stress is a consequence of altered control of Ca^{2+} allocation through regulating Ca^{2+}permeable channels. The increase in cytoplasmic Ca^{2+}results in prevention of Na^+/K^+ entry into the cytoplasm, enhancement of Na^+/K^+ influx to the vacuole or suppression of Na^+/K^+ release from the vacuole, which in turn increases salt tolerance (Yamaguchi et al. 2006; Kusano et al. 2007; Alcazar et al. 2010). Thus, polyamines have a definite role in calcium homeostasis during stress conditions.

8 Conclusions and Future Prospects

Considerable evidence shows that polyamines (PAs) are involved in a myriad of plant processes including DNA regulation, gene transcription, organ development, fruit ripening, leaf senescence and various environmental stresses. The use of the genetic approaches, proteomic approaches and various analytical techniques have made it possible to further understand their mechanisms of action, binding, interaction, transport, signaling, homeostatic control of their metabolic pathways and their defensive role in biotic and abiotic stress conditions, although the exact reasoning is still difficult to interpret. Nevertheless, even this lack of information does not hamper further research into polyamines as they now constitute one of the widely distributed groups of organic molecules in nature with an important contribution towards maintaining plant growth and development, increasing crop production, defensive actions during stress conditions, combating various diseases and more recently acting as biomarkers for cancer detection. Thus, a spectral range of their applications in plants, animals and mammals offer a wide scope into their further research.

Polyamines have now been considered as secondary messengers in addition to being known as vital plant regulators (Liu et al. 2007). Although the exact mechanism of action of polyamines during the stressful conditions is not known, genetic tools have been found useful; traditional quantitative trait loci (QTL) mapping (Alonso-Blanco et al. 2009) and genome-wide association mapping (Nordborg and Weigel 2008) can be used for the identification of the genes underlying the mode of action and regulation of polyamines (Alcazar et al. 2010). Cloning of these genes would be another added advantage as these could be used in the same way as from chemicals to alleviate or mitigate stress derived injury for crop protection. Transfer of such technology to the other crops will help create germplasm which would be better adapted to the harsh stressful conditions and thus contributing to enhanced agricultural productivity.

References

Ahmad P, Sharma S (2008) Salt stress and phyto-biochemical responses of plants. Plant Soil Environ 54:89–99

Ahmad P, Sarwat M, Sharma S (2008) Reactive oxygen species, antioxidants and signaling in plants. J Plant Biol 51:167–173

Ahmad P, Jaleel CA, Salem MA, Nabi GH, Sharma S (2010a) Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress. Crit Rev Biotechnol 30:161–175

Ahmad P, Umar S, Sharma S (2010b) Mechanism of free radical scavenging and role of phytohormones during abiotic stress in plants. In: Ashraf M, Ozturk M, Ahmad MSA (eds) Plant adaptation and phytoremediation. Springer, Dordrecht/Heidelberg/London/New York, pp 99–108

Ahmad P, Ashraf M, Younis M, Hu X, Kumar A, Akram NA, Al-Qurainy F (2011) Role of transgenic plants in agriculture and biopharming. Biotechnol Adv. doi:10.1016/j.biotechadv.2011.09.006

Akiyama T, Jin S (2007) Molecular cloning and characterization of an arginine decarboxylase gene up-regulated by chilling stress in rice seedlings. J Plant Physiol 164:645–654

Alcázar R, Cuevas JC, Patrón M, Altabella T, Tiburcio AF (2006a) Abscisic acid modulates polyamine metabolism under water stress in *Arabidopsis thaliana*. Plant Physiol 128:448–455

Alcázar R, Marco F, Cuevas JC, Patrón M, Ferrando A, Carrasco P, Tiburcio AF, Altabella T (2006b) Involvement of polyamines in plant response to abiotic stress. Biotechnol Lett 28:1867–1876

Alcazar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Carrasco P, Tiburcio AF (2010) Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. Planta 231:1237–1249

Alonso-Blanco C, Aarts MGM, Bentsink L, Keurentjes JJB, Reymond M, Vreugdenhil D, Koornneef M (2009) What has natural variation taught us about plant development, physiology, and adaptation? Plant Cell 21:1877–1896

An ZF, Jing W, Liu YL, Zhang WH (2008) Hydrogen peroxide generated by copper amine oxidase is involved in abscisic acid-induced stomatal closure in *Vicia faba*. J Exp Bot 59:815–825

Applewhite PB, Kaur-Sawhney R, Galston AW (2000) A role for spermidine in the bolting and flowering of *Arabidopsis*. Physiol Plant 108:314–320

Bagni N, Tassoni A (2001) Biosynthesis, oxidation and conjugation of aliphatic polyamines in higher plants. Amino Acids 20:301–317

Bais HP, Ravishankar GA (2002) Role of polyamines in the ontogeny of plants and their biotechnological applications. Plant Cell Tissue Organ Cult 69:1–34

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Crit Rev Plant Sci 24:23–58

Basu R, Maitra N, Ghosh B (1988) Salinity results in polyamine accumulation in early rice (*Oryza sativa* L.) seedlings. Aust J Plant Physiol 15:777–786

Bell E, Malmberg RL (1990) Analysis of a cDNA encoding arginine decarboxylase from oat reveals similarity to the *Escherichia coli* arginine decarboxylase and evidence of protein processing. Mol Gen Genet 224:431–436

Bethke PC, Jones RL (1997) Reversible protein phosphorylation regulates the activity of the slow-vacuolar ion channel. Plant J 11:1227–1235

Bhatnagar P, Glasheen BM, Bains SK, Long SL, Minocha R, Walter C, Minocha SC (2001) Transgenic manipulation of polyamine metabolism in poplar (*Populus nigra* X maximoviczii) cells. Plant Physiol 125:2139–2153

Bhatnagar P, Minocha R, Minocha SC (2002) Genetic manipulation of polyamines in poplar cells. The regulation of putrescine catabolism. Plant Physiol 128:1455–1469

Borrell A, Besford RT, Altabella T, Masgrau C, Tiburcio AF (1996) Regulation of arginine decarboxylase by spermine in osmotically-stressed oat leaves. Plant Physiol 98:105–110

Bors W, Langebartels C, Michel C, Sandermann H Jr (1989) Polyamines as radical scavengers and protectants against Ozone damage. Phytochemistry 28:1589–1595

Bouchereau A, Aziz A, Larher F, Martin-Tanguy J (1999) Polyamines and environmental challenges: recent development. Plant Sci 140:103–125

Bray EA (1997) Plant responses to water deficit. Trends Plant Sci 2:48–54

Bruggemann LI, Pottosin II, Schonknecht G (1998) Cytoplasmic polyamines block the fast-activating vacuolar cation channel. Plant J 16:101–105

Capell T, Escobar C, Liu H, Burtin D, Lepri O, Christou P (1998) Over-expression of oat arginine decarboxylase cDNA in transgenic rice (*Oryza sativa* L.) affects normal development pattern *in vitro* and results in putrescine accumulation in transgenic plants. Theor Appl Genet 97:246–254

Capell T, Bassie L, Christou P (2004) Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. Proc Natl Acad Sci U S A 101:9909–9914

Cervelli M, Polticelli F, Federico R, Mariottini P (2003) Heterologous expression and characterization of mouse spermine oxidase. J Biol Chem 278:5271–5276

Cheng L, Zou Y, Ding S, Zhang J, Yu X, Cao J, Lu G (2009) Polyamine accumulation in transgenic tomato enhances the tolerance to high temperature stress. J Integ Plant Biol 51:489–499

Cona A, Rea G, Angelini R, Federico R, Tavladoraki P (2006) Functions of amine oxidases in plant development and defence. Trends Plant Sci 11:80–88

Cuevas JC, López-Cobollo R, Alcázar R, Zarza X, Koncz C, Altabella T, Salinas J, Tiburcio AT, Ferrando A (2008) Putrescine is involved in Arabidopsis freezing tolerance and cold acclimation by regulating abscisic acid levels in response to low temperature. Plant Physiol 148:1094–1105

Cuevas JC, López-Cobollo R, Alcázar R, Zarza X, Koncz C, Altabella T, Salinas J, Tiburcio AT, Ferrando A (2009) Putrescine as a signal to modulate the indispensable ABA increase under cold stress. Plant Signal Behav 4:219–220

Dat JF, Pellinen R, Van De Cotte B, Langebartels C, Kangasjarvi J, Inze D, Van Breusegem F (2003) Changes in hydrogen peroxide homeostasis trigger an active cell death process in tobacco. Plant J 33:621–632

Delavega AL, Delcour AH (1995) Cadaverine induces closing of *Escherichia coli* porins. EMBO J 14:6058–6065

Drolet G, Dumbroff EB, Legge RL, Thompson JE (1986) Radical scavenging properties of polyamines. Phytochemistry 25:367–371

Evans PT, Malmberg RL (1989) Do polyamines have a role in plant development? Annu Rev Plant Physiol Plant Mol Biol 40:235–269

Falasca G, Franceschetti M, Bagni N, Altamura MM, Biasi R (2010) Polyamine biosynthesis and control of the development of functional pollen in kiwifruit. Plant Physiol Biochem 48:565–573

Feirer RP, Mignon G, Litvay JD (1984) Arginine decarboxylase and polyamines required for embryogenesis in the wild carrot. Science 223L:1433–1435

Flores HE, Galston AW (1982) Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol 69:701–706

Flores HE, Galston AW (1984) Osmotic stress-induced polyamine accumulation in cereal leaves. I. Physiological parameters of the response. Plant Physiol 75:102–109

Franceschetti M, Fornale S, Tassoni A, Zuccherelli K, Mayer MJ, Bagni N (2004) Effects of spermidine synthase over-expression on polyamine biosynthetic pathway in tobacco plants. J Plant Physiol 161:989–1001

Galston AW (1991) On the trail of a new regulatory system in plants. New Biol 3:450–453

Galston AW, Kaur-Sawhney R (1990) Polyamines in plant physiology. Plant Physiol 94:406–410

Galston AW, Tiburcio AF (eds) (1991) Lecture course on polyamines as modulators of plant development 257. Fundacion Jaun, Madrid

Galston AW, Kaur-Sawhney R, Altabella T, Tiburcio AF (1997) Plant polyamines in reproductive activity and response to abiotic stress. Bot Acta 110:197–207

Geny L, Broquedis M, Martin-Tanguy J, Soyer JP, Bouard J (1997) Effects of potassium nutrition on polyamine content of various organs of fruiting cuttings of *Vitis vinifera* L. cv. Cabernet Sauvignon. Am J Enol Vitic 48:85–91

Groppa MD, Benavides MP (2008) Polyamines and abiotic stress: recent advances. Amino Acids 34:35–45

Groppa MD, Rosales EP, Iannone MF, Benavides MP (2008) Nitric oxide, polyamines and Cd-induced phytotoxicity in wheat roots. Phytochemistry 69:2609–2615

Hanfrey C, Sommer S, Mayer MJ, Burtin D, Michael AJ (2001) Arabidopsis polyamine biosynthesis: absence of ornithine decarboxylase and the mechanism of arginine decarboxylase activity. Plant J 27:551–560

Hao YJ, Zhang Z, Kitashiba H, Honda C, Ubi B, Kita M, Moriguchi T (2005) Molecular cloning and functional characterization of two apple S-adenosylmethionine decarboxylase genes and their different expression in fruit development, cell growth and stress responses. Gene 350:41–50

He L, Ban Y, ınoue H, Matsuda N, Liu J, Moriguchi T (2008) Enhancement of spermidine content and antioxidant capacity in transgenic pear shoots overexpressing apple spermidine synthase in response to salinity and hyperosmosis. Phytochemistry 69:2133–2141

Heagle AS (1989) Ozone and crop yield. Ann Rev Phytopathol 27:397–412

Heimer YM, Mizrahi Y (1982) Characterization of ornithine decarboxylase of tobacco cells and tomato ovaries. Biochem J 201:373–376

Hummel I, Gouesbet G, El Amrani A, Aïnouche A, Couée I (2004) Characterization of the two arginine decarboxylase (polyamine biosynthesis) paralogues of the endemic subantarctic cruciferous species Pringlea antiscorbutica and analysis of their differential expression during development and response to environmental stress. Gene 342:199–209

Igarasahi K, Kashiwagi K (2000) Polyamines: mysterious modulators of cellular functions. Biochem Biophys Res Commun 271:559–564

Jiménez-Bremont JF, Ruiz OA, Rodríguez-Kessler M (2007) Modulation of spermidine and spermine levels in maize seedlings subjected to long-term salt stress. Plant Physiol Biochem 45:812–821

Johnson TD (1996) Modulation of channel function by polyamines. Trends Pharmacol Sci 17:22–27

Kakkar RK, Rai VK (1993) Plant polyamines in flowering and fruit ripening. Phytochemistry 33:1281–1288

Kasukabe Y, He L, Nada K, Misawa S, Ihara I, Tachibana S (2004) Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiol 45:712–722

Kasukabe Y, He L, Watakabe Y, Otani M, Shimada T, Tachibana S (2006) Improvement of environmental stress tolerance of sweet potato by introduction of genes for spermidine synthase. Plant Biotechnol 23:75–83

Kaur-Sawhney R, Shih Flores HE, Galston AW (1982) Relation of polyamine synthesis and titer to aging and senescence in oat leaves. Plant Physiol 69:405–410

Kaur-Sawhney R, Tiburcio AF, Altabella T, Galston AW (2003) Polyamines in plants: an overview. J Cell Mol Biol 2:1–12

Kuehn GD, Rodriguez-Garay B, Bagga S, Phillips GC (1990) Novel occurrence of uncommon polyamines in higher plants. Plant Physiol 94:855–857

Kumar A, Altabella T, Taylor M, Tiburcio AF (1997) Recent advances in polyamine research. Trends Plant Sci 2:124–130

Kumria R, Rajam MV (2002) Ornithine decarboxylase transgene in tobacco affects polyamine metabolism, *in vitro* morphogenesis and response to salt stress. J Plant Physiol 159:983–990

Kusano T, Yamaguchi K, Berberich T, Takahashi Y (2007) The polyamine spermine rescues Arabidopsis from salinity and drought stresses. Plant Signal Behav 2:251–252

Kusano T, Berberich T, Tateda C, Takahashi Y (2008) Polyamines: essential factors for growth and survival. Planta 228:367–381

Langebartels C, Kerner KJ, Leonardi S, Schraudner M, Trost M, Heller W, Sanderman H Jr (1991) Biochemical plant response to ozone. I. Differential induction of polyamine and ethylene biosynthesis in tobacco. Plant Physiol 91:882–887

Lee TM, Shieh YJ, Chou CH (1996) Role of putrescine in enhancing shoot elongation in *Scirpus mucronatus* under submergence. Plant Physiol 96:419–424

Li CZ, Jiao J, Wang GX (2010) The important roles of reactive oxygen species in the relationship between ethylene and polyamines in leaves of spring wheat seedlings under root osmotic stress. Plant Sci 166:303–315

Liu K, Fu H, Bei Q, Luan S (2000) Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. Plant Physiol 124:1315–1326

Liu JH, Nada K, Honda C, Kitashiba H, Wen XP, Pang XM, Moriguchi T (2006) Polyamine biosynthesis of apple callus under salt stress: importance of the arginine decarboxylase pathway in stress response. J Exp Bot 57:2589–2599

Liu JH, Kitashiba H, Wang J, Ban Y, Moriguchi T (2007) Polyamines and their ability to provide environmental stress tolerance to plants. Plant Biotechnol 24:117–126

Liu HP, Dong BH, Zhang YY, Liu ZP, Liu YL (2010) Relationship between osmotic stress and the levels of free, conjugated and bound polyamines in leaves of wheat seedlings. Plant Sci 166:1261–1267

Malmberg RL, Watson MB, Galloway GL, Yu W (1998) Molecular genetic analyses of plant polyamines. Crit Rev Plant Sci 17:199–224

Masgrau C, Altabella T, Farras R, Flores P, Thompson AJ, Besford RT, Tiburcio AF (1997) Inducible overexpression of oat arginine decarboxylase in transgenic tobacco plants. Plant J 11:465–473

Matto AK, Sobolev AP, Neelam A, Goyal RK, Handa AK, Segre AL (2006) Nuclear magnetic resonance spectroscopy-based metabolite profiling of transgenic tomato fruit engineered to accumulate spermidine and spermine reveals enhanced anabolic and nitrogen-carbon interactions. Plant Physiol 142:1759–1770

Mehta RA, Cassol T, Li N, Ali N, Handa AK, Matto AK (2002) Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. Nat Biotechnol 20:613–618

Michael AJ, Furze JM, Rhodes MJ, Burtin D (1996) Molecular cloning and functional identification of a plant ornithine decarboxylase cDNA. Biochem J 314:241–248

Michard E, Dreyer I, Lacombe B, Sentenac H, Thibaud JB (2005) Inward rectification of the AKT2 channel abolished by voltage dependent phosphorylation. Plant J 44:783–797

Mo H, Pua EC (2002) Up-regulation of arginine decarboxylase gene expression and accumulation of polyamines in mustard (*Brassica juncea*) in response to stress. Physiol Plant 114:439–449

Montague M, Koppenbrink J, Jaworski E (1978) Polyamine metabolism in embryogenic cells of *Daucuscarota.* I. Changes in intracellular content and rates of synthesis. Plant Physiol 62:430–433

Muhitch MJ, Edwards LA, Fletcher JS (1983) Influence of diamines and polyamines on the senescence of plant suspension cultures. Plant Cell Rep 2:82–84

Navakoudis E, Lutz C, Langebartels C, Lutz-Meindl U, Kotzabasis K (2003) Ozone impact on the photosynthetic apparatus and the protective role of polyamines. Biochim Biophys Acta 1621:160–169

Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro D, Wilson I (2008) Nitric oxide, stomatal closure, and abiotic stress. J Exp Bot 59:165–176

Neily MH, Matsukura C, Maucourt M, Bernillon S, Deborde C, Moing A, Yin YG, Saito T, Mori K, Asamizu E, Rolin D, Moriguchi T, Ezura H (2011) Enhanced polyamine accumulation alters carotenoid metabolism at the transcriptional level in tomato fruit over-expressing spermidine synthase. J Plant Physiol 168:242–252

Nordborg M, Weigel D (2008) Next-generation genetics in plants. Nature 456:720–723

Panicot M, Masgrau C, Borrell A, Corderio A, Tiburcio AF, Altabella T (2002) Effects of putrescine accumulation in tobacco transgenic plants with different expression levels of oat arginine decarboxylase. Physiol Plant 114:281–287

Paschalidis KA, Roubelakis-Angelakis KA (2005) Spatial and temporal distribution of polyamine levels and polyamine anabolism in different organs/tissues of the tobacco plant. correlations with age, cell division/expansion, and differentiation. Plant Physiol 138:142–152

Perez-Amador MA, Leon J, Green PJ, Carbonell J (2002) Induction of the arginine decarboxylase *ADC2* gene provides evidence for the involvement of polyamines in the wound response in Arabidopsis. Plant Physiol 130:1454–1463

Philipps GC, Kuehn GD (1991) Uncommon polyamines in plants and other mechanisms. In: Slocum RD, Flores HE (eds) Biochemistry and physiology of polyamines in plants. CRC Press, Boca Raton, pp 121–133

Piqueras A, Cortina M, Serna MD, Casas JL (2002) Polyamines and hyperhydricity in micropropagated carnation plants. Plant Sci 162:671–678

Prabhavathi VR, Rajam MV (2007) Polyamine accumulation in transgenic eggplant enhances tolerance to multiple abiotic stresses and fungal resistance. Plant Biotechnol 24:273–282

Prakash L, Prathapsenan G (1988) Effect of NaCl salinity and putrescine on shoot growth, tissue ion concentration and yield of rice (*Oryza sativa*). J Agron Crop Sci 160:325–334

Raison JK, Lyons JM (1970) Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. Plant Physiol 45:386–389

Rajam MV, Dagar S, Waie B, Yadav JS, Kumar PA, Shoeb F, Kumria R (1998) Genetic engineering of polyamine and carbohydrate metabolism for osmotic stress tolerance in higher plants. J Biosci 23:473–482

Reggiani R, Bertani A (1989) Effect of decreasing oxygen concentration on polyamine metabolism in rice and wheat shoots. J Plant Physiol 135:375–377

Reggiani R, Bertani A (1990) Polyamines and oxygen availability. In: Flores HE, Arteca RN, Shannon JC (eds) Polyamines and ethylene: biochemistry, physiology and interactions. Rockville, MA: American Society of Plant Physiologists, pp 394–396

Reggiani R, Hochkoeppler A, Bertani A (1989) Polyamines and anaerobic elongation of rice coleoptiles. Plant Cell Physiol 30:893–898

Reggiani R, Giussani P, Bertani A (1990) Relationship between the accumulation of putrescine and the tolerance to oxygen deficit stress in Graminae seedlings. Plant Cell Physiol 31:489–494

Rhee HJ, Kim E-J, Lee JK (2007) Physiological polyamines: simple primordial stress molecules. J Cell Mol Med 11:685–703

Richards FJ, Coleman EG (1952) Occurrence of putrescine in potassium deficient barley. Nature 170:460–461

Rodrıguez-Kessler M, Alpuche-Solís AG, Ruiz OA, Jiménez-Bremont JF (2006) Effect of salt stress on the regulation of maize (*Zea mays* L.) genes involved in polyamine biosynthesis. Plant Growth Regul 48:175–185

Rowland-Bamford AJ, Barland AM, Lea PJ, Mansfield TA (1989) The role of arginine decarboxylase in modulating the sensitivity of barley to ozone. Environ Pollut 61:93–99

Roy M, Ghosh B (1996) Polyamines, both common and uncommon, under heat stress in rice (*Oryza sativa*) callus. Plant Physiol 98:196–200

Roy M, Wu R (2001) Arginine decarboxylase transgene expression and analysis of environmental stress tolerance in transgenic rice. Plant Sci 160:869–875

Roy M, Wu R (2002) Overexpression of S-adenosylmethionine decarboxylase gene in rice increases polyamine level and enhances sodium chloride-stress tolerance. Plant Sci 163: 987–992

Santa-Gruz A, Pérez-Alfocea MA, Bolarin C (1997) Changes in free polyamine levels induced by salt stress in leaves of cultivated and wild tomato species. Plant Physiol 101:341–346

Schraudner M, Trost M, Kerner K, Heller W, Leonardi S, Langebartels C, Sanderman H Jr (1990) Ozone induction and function of polyamines in ozone-tolerant and ozone-sensitive tobacco cultivars. In: Flores HE, Arteca RN, Shannon JC (eds) Polyamines and ethylene: biochemistry, physiology and interactions. Rockville, MA: American Society of Plant Physiologists, pp 394–396

Slocum RD (1991) Tissue and subcellular localisation of polyamines and enzymes of polyamine metabolism. In: Slocum RD, Flores HE (eds) Biochemistry and physiology of polyamines in plants. CRC Press, Boca Raton, pp 22–40

Slocum RD, Galston AW (1985) Changes in polyamine biosynthesis associated with post-fertilization growth and development in tobacco ovary tissues. Plant Physiol 79:336–343

Slocum RD, Kaur-Sawhney R, Galston AW (1984) The physiology and biochemistry of polyamines in plants. Arch Biochem Biophys 325:283–303

Smith TA, Richards FJ (1962) The biosynthesis of putrescine in higher plants and its relation to potassium nutrition. Biochem J 84:292–294

Tang W, Newton RJ (2005) Polyamines reduce salt-induced oxidative damage by increasing the activities of antioxidant enzymes and decreasing lipid peroxidation in Virginia pine. Plant Growth Regul 46:31–43

Tassoni A, Franceschetti M, Bagni N (2010) Polyamines and salt stress response and tolerance in *Arabidopsis thaliana* flowers. Plant Physiol Biochem 46:607–613

Tiburcio AF, Besford RT, Borrell A, Mace M (1995) Metabolism and function of polyamines during osmotically induced senescence in oat leaves and protoplasts. In: Wallsgrove RM (ed) Amino acids and their derivatives in higher plants. Cambridge University Press, Cambridge, UK, pp 205–225

Tun NN, Santa-Catarina C, Begum T, Silveira V, Handro W, Floh EIS, Scherer GFE (2006) Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. Plant Cell Physiol 47:346–354

Urano K, Yoshiba Y, Nanjo T, Igarashi Y, Seki M, Sekiguchi F, Yamaguchi-Shinozaki K, Shinozaki K (2003) Characterization of Arabidopsis genes involved in biosynthesis of polyamines in abiotic stress responses and developmental stages. Plant Cell Environ 26:1917–1926

Urano K, Yoshiba Y, Nanjo T, Ito T, Yamaguchi-Shinozaki K, Shinozaki K (2004) Arabidopsis stress-inducible gene for arginine decarboxylase AtADC2 is required for accumulation of putrescine in salt tolerance. Biochem Biophys Res Commun 313:369–375

Vujcic S, Diegelmann P, Bacchi CJ, Kramer DL, Porter CW (2002) Identification and characterization of a novel flavin-containing spermine oxidase of mammalian cell origin. Biochem J 367:665–675

Waie B, Rajam MV (2003) Effect of increased polyamine biosynthesis on stress response in transgenic tobacco by introduction of human S-adenosylmethionine gene. Plant Sci 164:727–734

Walden R, Cordeiro A, Tiburcio AF (1997) Polyamines: small molecules triggering pathways in growth and development. Plant Physiol 113:1009–1013

Walters DR (2003a) Resistance to plant pathogens: possible roles for free polyamines and polyamine catabolism. New Phytol 159:109–115

Walters DR (2003b) Polyamines and plant disease. Phytochemistry 64:97–107

Wang Y, Devereux W, Woster PM, Stewart TM, Hacker A, Casero RA Jr (2001) Cloning and characterization of a human polyamine oxidase that is inducible by polyamine analogue exposure. Cancer Res 61:5370–5373

Wen XP, Pang XM, Matsuda N, Kita M, Inoue H, Hao YJ et al (2008) Overexpression of the apple spermidine synthase gene in pear confers multiple abiotic stress tolerance by altering polyamine titers. Transgenic Res 17:251–263

Yamaguchi K, Takahashi Y, Berberich T, Imai A, Miyazaki A, Takahashi T, Michael A, Kusano T (2006) The polyamine spermine protects against high salt stress in *Arabidopsis thaliana*. FEBS Lett 580:6783–6788

Yamaguchi K, Takahashi Y, Berberich T, Imai A, Takahashi T, Michael AJ, Kusano T (2007) A protective role for the polyamine spermine against drought stress in Arabidopsis. Biochem Biophys Res Commun 352:486–490

Zhao H, Yang H (2008) Exogenous polyamines alleviate the lipid peroxidation induced by cadmium chloride stress in *Malus hupehensis* Rehd. Sci Hort 116:442–447

Zhao FG, Song CP, He JQ, Zhu H (2007) Polyamines improve K^+/Na^+ homeostasis in barley seedlings by regulating root ion channel activities. Plant Physiol 145:1061–1072