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Review

Polyamines and their ability to provide environmental stress tolerance to plants

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Abstract The present review highlights some recent advances regarding the function of polyamines in the environmental stress tolerance of plants. When exposed to adverse environmental stresses, such as salt, drought, low temperature, and ozone, the complex dynamic kinetics of polyamine biosynthesis was observed. Polyamines titers altered in different manners dependent upon several factors, such as plant species, tolerance or sensitivity to stress, and duration of stress. The exogenous addition of polyamines to stress-treated cells or tissues could lead to injury alleviation and growth promotion in most cases, although the effects varied between polyamines and among plant species. Key genes responsible for polyamine biosynthesis have been cloned from a variety of plant species, whose expressions following stress have been investigated on a molecular basis. Overexpression of the genes caused the modification of polyamine biosynthesis in the transformants coupled with enhancement of stress tolerance. All of these results seem to indicate that polyamines are an important component in a plant's response to stress and that they play a significant role in counteracting stress.

Key words: Environmental stress, polyamine, genes for polyamine biosynthesis, putrescine, spermidine, spermine, stress tolerance.

Plants are exposed to a variety of adverse conditions, such as cold, drought, acidity, and heavy metal, among others, which affect their distribution, growth, development, and productivity. Since these conditions produce stress and result in extensive losses to many agriculturally important crops, they have been the main subject of intense research. It is well documented that, under harsh conditions, plants develop various strategies to cope with stress and its negative effects. As a result, they are able to sustain their normal function before being attacked or subjected to an otherwise lethal environment. In order to respond to stress, plants adapt in a multitude of physiological and biochemical ways in addition to inducing an array of functional or regulatory genes (Bartels and Sunkar 2005). The accumulation of some functional substances, such as compatible solute and protective proteins, is an important element of the physiological and biochemical response to the stressful conditions.

Polyamines, mainly diamine putrescine (Put), triamine spermidine (Spd), and tetraamine spermine (Spm), are polycationic compounds of low molecular weight that are present in all living organisms. They have been proposed as a new category of plant growth regulators that are purported to be involved in a large spectrum of physiological processes, such as embryogenesis, cell division, morphogenesis, and development (Bais and Ravishankar 2002; Liu et al. 2006a). In addition, they have been shown to be an integral part of plant stress response (Bouchereau et al. 1999; Walters 2003; Alcázar et al. 2006b). Though the physiological significance of polyamines in stress is not thoroughly understood, much progress has been made. The focus in the present paper is on progress concerning the involvement and potential role of polyamines in plant responses to environmental stresses.

Polyamine biosynthesis and catabolism in plants

The biosynthesis of polyamines in plants has been well documented (Figure 1A). Put is produced either directly from ornithine by ornithine decarboxylase (ODC, EC

Abbreviations: ADC, arginine decarboxylase; DAO, diamine oxidase; dcSAM, decarboxylated *S*-adenosylmethionine; DFMA, α-difluoromethylarginine; DFMO, α-difluoromethylornithine; ODC, ornithine decarboxylase; PAO, polyamine oxidase; PEG, polyethylene glycol; Put, putrescine; SAM, *S*-adenosylmethionine; SAMDC, *S*-adenosylmethionine decarboxylase; Spd, spermidine; SPDS, spermidine synthase; Spm, spermine; SPMS, spermine synthase

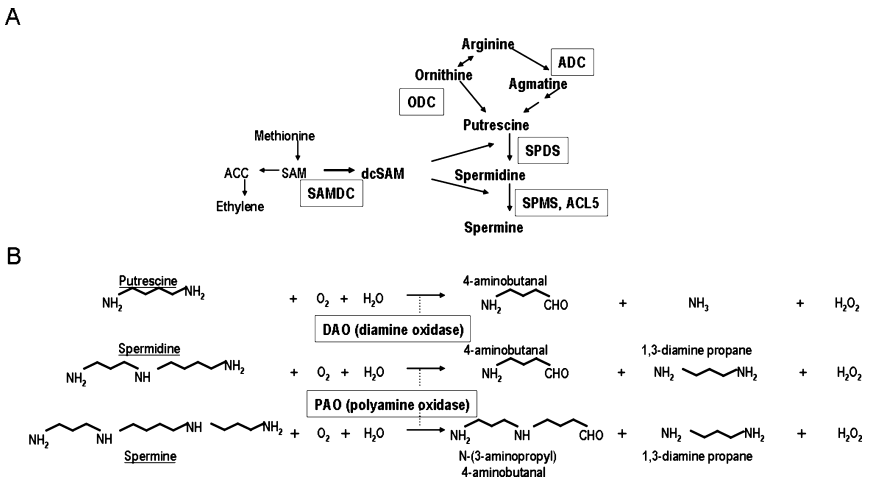

Figure 1. Pathways of polyamine biosynthesis (A) and degradation (B). ADC, arginine decarboxylase; dcSAM, decarboxylated *S*-adenosylmethionine; ODC, ornithine decarboxylase; DAO, diamine oxidase; PAO, polyamine oxidase; SAM, *S*-adenosylmethionine; SAMDC, *S*-adenosylmethionine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase.

4.1.1.17) or indirectly from arginine by arginine decarboxylase (ADC, EC 4.1.1.19) with two intermediates, agmatine and *N*-carbomoylputrescine, and two corresponding biosynthetic enzymes, agmatine iminohydrolase (EC 3.5.3.12) and *N*-carbamoylputrescine amidohydrolase (EC 3.5.1.53) (Malmberg et al. 1998; Martin-Tanguy 2001). Put is converted into Spd via spermidine synthase (SPDS, EC 2.5.1.16) with the addition of an aminopropyl moiety provided by decarboxylated *S*-adenosylmethionine (dcSAM), which is catalyzed by *S*-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50) using *S*-adenosylmethionine (SAM) as the substrate. Similarly, Spm is produced from Spd via spermine synthase (SPMS, EC 2.5.1.22) with the same aminopropyl moiety rendered by dcSAM.

Apart from biosynthesis, polyamine degradation plays an important role in the regulation of cellular polyamine titers, which is primarily ascribed to two amine oxidases, diamine oxidase (DAO, EC 1.4.3.6) and polyamine oxidase (PAO, EC 1.5.3.11, Figure 1B). DAO catalyzes the oxidation of Put to give pyrroline, which is further metabolized to γ-aminobutyric acid (Cona et al. 2006), and PAO catalyzes the conversion of Spd and Spm to pyrroline and 1-(3-aminopropyl)-pyrroline, respectively, along with 1,3-diaminopropane in plants (Martin-Tanguy 2001; Šebela et al. 2001).

Changes in polyamines in response to stress

Since an increase in Put due to potassium deficiency was reported decades ago, changes in polyamines have been extensively investigated when plants are exposed to single or combined stresses (Rowland-Bamford et al. 1989; Tiburcio et al. 1994; Scalet et al. 1995; Nam et al. 1997; Santa-Cruz et al. 1997b; Shen et al. 2000; Mo and Pua 2002; Urano et al. 2003; Kuthanová et al. 2004; Camacho-Cristóbal et al. 2004; Liu et al. 2006b). In many cases, stress led to an accumulation of free or conjugated polyamines, indicating that polyamine biosynthesis might serve as an integral component of plant response to stress (Bouchereau et al. 1999). An increase in polyamine under stress may be caused by *de novo* synthesis or reduced degradation, although the exact mechanism remains a matter of debate. The activation of polyamine biosynthetic enzymes in response to stress might argue in favor of the former notion (Watson and Malmberg 1996; Liu et al. 2006b). Nevertheless, a decrease or negligible alteration of polyamines has also been demonstrated in numerous reports, which shows the complex polyamine biosynthesis under these conditions and that stress affects the polyamine metabolism in different manners. Changes in polyamines under stress could not be predicted and may be affected by several factors, as shown below.

Plant species or cultivars

Under stress, different plant species vary in their response in terms of polyamine fluctuation. Some might accumulate polyamines in response to stress, while others do not or even decrease their endogenous polyamine contents when exposed to harsh environments. When suspensions of tobacco and alfalfa, which differed in thermosensitivity, were exposed to heat, they showed differences in the accumulation of free or conjugated polyamines (Königshofer and Lechner 2002). In addition, cultivars from the same species differing in stress sensitivity might also show a diverse change in the pattern of polyamines under stress. Krishnamurthy and Bhagwat (1989) compared polyamine accumulation of nine rice cultivars with different salt sensitivity, in which they showed that the

salt-tolerant cultivars accumulated high concentrations of Spd and Spm, while the salt-sensitive ones accumulated excessive Put and low levels of Spd and Spm. Liu et al. (2004) reported that, when two wheat cultivars with different degrees of drought tolerance were treated with PEG, a marked increase in free Spd and Spm was observed in the tolerant cultivar, while a significant increase in free Put was detected in the sensitive cultivar. Similar results were also reported in groundnut by Vakharia et al. (2003) and in tomato by Santa-Cruz et al. (1998).

Duration of stress treatment

Several lines of research have demonstrated that changes in polyamines are divergent under stress treatment for different periods. Santa-Cruz et al. (1997b) showed that free polyamine accumulated in *Lycopersicon pennellii* during the first 15 min of salt treatment and decreased thereafter. Similarly, Das et al. (1995), Santa-Cruz et al. (1997a), Tonon et al. (2004), and Legocka and Kluk (2005) reported that a short period of stress increased the polyamine levels, whereas, under longer stress duration, only a small change in the polyamine levels was observed, showing that polyamine accumulation takes place primarily at the onset of stress. In this regard, polyamines were suggested as short-term salt-tolerance traits in tomato (Santa-Cruz et al. 1998) and, thus, can be considered as a biochemical indicator (Sanchez et al. 2005).

Developmental stage of tissues and stress intensity

In addition to the aforementioned factors, the developmental stage of tissue used for stress treatment and stress intensity also influenced the changes in polyamines. Botella et al. (2000) treated tomato plants in various developmental stages with salt and found that an increase in polyamine levels was conspicuous when plants in a later stage were used. In the same research, they also reported that an increase in free polyamines was detected when a higher concentration of salt was applied to tomato plants in the same developmental stage.

Effects of modulating endogenous polyamines on stress response

Changes in cellular polyamines under stress only provide clues on its possible implication in stress response, but they do not provide evidence of its role in counteracting stress. Therefore, in order to understand whether polyamines actually protect cells from stress-derived injuries, an exogenous application of polyamines, which is expected to increase endogenous polyamines, has been attempted before or during stress (Borrel et al. 1996; Velikova et al. 1998, 2000; Navakoudis et al. 2003; Wang et al. 2006). Most of this research demonstrated that exogenous polyamines could, in varying degrees, reverse growth or minimize growth inhibition caused by stress, indicating that polyamines are effective at mitigating stress-derived cell injury. However, the protective effects of individual polyamines are somewhat different. For example, Spm and/or Spd was shown to be effective for reversing the inhibitory effect of acid rain (pH 1.8) on bean (Velikova et al. 1998, 2000), mitigating salt injury on wheat (Iqbal and Ashraf 2005), reducing ozone-derived injury on potato (Ormord and Beckerson 1986), and enhancing copper tolerance in *Nymphoides peltatum* (Wang et al. 2006). In contrast, Tang and Newton (2005) and Ndayiragije and Lutts (2006) reported that Put was more effective for reducing salt-induced oxidative damage in Virginia pine and alleviating NaCl-derived cell damage in rice, respectively. The cause for such a discrepancy may be due to the difference in absorption, transport, and utilization among the plant species.

Except for the identification of the manner in which an increase in exogenous polyamines affects stress response, a decrease of endogenous polyamines was attempted to gain an insight into the roles of polyamines in stress responses. Two methods were used to reduce endogenous polyamines, namely, the use of inhibitors of polyamine biosynthesis, such as α-difluoromethylarginine (DFMA), α-difluoromethylornithine (DFMO), and D-arginine, and the use of a mutant with a defect in polyamine biosynthesis. In general, a reduction of endogenous polyamines led to stress sensitivity and subsequently exacerbated growth impairment. For instance, Rowland-Bamford et al. (1989) reported that DFMA treatment prevented a rise in ADC activity and caused visible injury in barley leaf exposed to ozone relative to the control. An apple callus treated with D-arginine showed more serious growth retardation under salt stress than one without D-arginine treatment, concurrent with a reduction in the Put titer and ADC activity (Liu et al. 2006b). A reduction in endogenous polyamines in the tolerant tobacco plants by a Put inhibitor, 1,4-diamino-butanone, rendered them sensitive to ozone (Navakoudis et al. 2003), which implied that polyamines are important regulators of stress response. However, the application of the inhibitors is limited and met with some limitations due to their stability and specificity. On the other hand, the use of a mutant in terms of polyamine biosynthesis may provide more direct evidence of the role of polyamines in stress tolerance. The *Ds* insertion mutant of the *ADC2* gene (*adc2-1*), one of the two *Arabiodopsis ADC* genes, possessed less Put than the control and was more sensitive to salt stress, which was partially reversed by exogenous Put (Urano et al. 2004). In another report, two non-allelic mutants of *Arabidopsis thaliana*, *spe1-1* (no

information on mutation of *ADC* gene) and *spe2-1* (mutation in *ADC2*), with a defect in Put synthesis due to mutation showed reduced salt tolerance compared with the wild type (Kasinathan and Wingler 2004). Both studies clearly indicated that Put derived from an ADC pathway was important in stress response.

One possible mechanism underlying the positive functions of exogenous polyamines, as reported above, has also been identified; however, nothing definite has been determined. Several lines of evidence have shown that the stimulatory effect of exogenous polyamines may be related to their multi-faceted nature, which includes working as an antioxidant, a free radical scavenger, and a membrane stabilizer (Velikova et al. 2000). First, polyamines act as antioxidants, and they counteract oxidative damage in plants, which, as a consequence, reduce free radicals and alleviate lipid peroxidation (Kramer and Wang 1989; Singh et al. 2002). Verma and Mishra (2005) reported that exogenous Put affected the activities of several antioxidant enzymes, such as superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase, when added to *Brassica juncea* seedlings treated with NaCl, which occurred concomitantly with a reduction of H_2O_2 and lipid peroxidation, implying that the positive effects of exogenous polyamines may be related to its antioxidant properties. In another work by Öztürk and Demir (2003), exogenous polyamines increased the activities of peroxidase and catalase, along with the accruement of proline, an important osmoprotectant. Secondly, due to polycationic nature, polyamines can bind to negatively charged groups in the cell membrane so that phase change following stress treatment may be buffered. In the case of salt stress, a beneficial effect of an exogenous polyamine may also be related to the improvement of the ion balance in salt-treated cells due to its cationic nature, as illustrated by Ndayiragije and Lutts (2006), who showed that exogenous Put at 1 mM clearly decreased both Na^+ and Cl^- accumulation of rice calli exposed to salt. All of these processes could work separately or be incorporated into one strategy to minimize membrane damage and promote cell growth or sustain cell survival in response to stress.

Cloning and expression of polyamine biosynthetic genes under stress

Apart from physiological and biochemical alterations in response to stress, transcriptional change has been an important stress response. It is well documented that stress caused changes in the expression levels of a large spectrum of genes for either functional proteins (membrane proteins and key enzymes for osmolyte biosynthesis) or regulatory proteins (transcription factors and protein kinases). Since polyamine biosynthesis is a complex process involving an array of genes, cloning of the genes and characterization of the expressions during stress treatment can yield an insight into their implication in stress response. Since Bell and Malmberg (1990) first isolated oat *ADC* cDNA, a large number of genes have been cloned from a variety of plant species (Table 1).

The expression of the genes under salt, drought, and chilling has been investigated, and reports available so far indicate the presence of complicated transcriptional profiling. It is noted that the mRNA of some polyamine biosynthetic genes was rapidly induced shortly after stress treatment and underwent either a continuous rise or minor change with a prolonged period of stress; on the other hand, others were only induced when stress was exerted for a certain period. This indicated that the genes were disparately regulated during stress, a phenomenon which could be dependent upon several factors, such as plant species, duration, and intensity of stress and stress sensitivity of the experimental materials (Chattopadhyay et al. 1997; Li and Chen 2000a; Hao et al. 2005a, b; Liu et al. 2006b). In spite of the existence of several polyamine biosynthetic enzymes, the global expressions of a whole set of the genes in response to stress has only been analyzed in a few cases (Urano et al. 2003; Rodríguez-Kessler et al. 2006; Alcázar et al. 2006a; Liu et al. unpublished data). It remains unclear why the genes in the polyamine biosynthetic gene family responded differently in response to stress. One possible reason is the presence of a different response motif in the promoter region, leading to a diverse reaction of the genes under stress, as has been described by Alcázar et al. (2006a), who proposed that the up-regulation of polyamine biosynthetic genes by water stress was an ABA-dependent response. Therefore, the elucidation of responsive motifs therein will shed light on deciphering the underlying mechanism of differential expression profiling.

Improvement of stress tolerance via the transformation of polyamine biosynthetic genes

As described above, the presence of a complex kinetic dynamics of polyamines in response to environmental stresses makes it difficult to draw a general outline concerning the unambiguous role of polyamines in stress tolerance. Recent advances on molecular biology, with emphasis on genetic engineering, make it possible to evaluate directly the involvement of polyamines in stress responses and to provide a powerful strategy to identify the possible role of polyamine in stress responses. There have been several reports on creating transgenic plants harboring polyamine biosynthetic genes in an attempt to enhance stress tolerance. When an oat *ADC* gene was introduced into rice under an ABA-inducible promoter,

Table 1. Tabulation of molecular cloning of key genes involved in polyamine biosynthesis.

Gene	Organism	Accession No.	References	Gene	Organism	Accession No.	References
Arginine dccarboxylase	*Avena saiva*	X565802	Bell and Nelmberg 1990	*S*-Adenosylmethionine decarboxylase	*Brassica juncea*	U80916, X95729	Lec et al. 1997
	Lycopersicon esculentum	L16582	Rastogi et al. 1993			AY536644, AF215665	Hu et al. 2005a
	Pisum sativum	Z37540	Perez-Amador et al. 1995			AY44341	Hu et al. 2005b
	Dianthus caryophyllus	U63832	Chang et al. 1996		*Ipomoea nil*	U64927	Park et al. 1998
	Glycine max	U35367	Nam et al. 1996		*Vicia faba*	AJ250026	Frühling et al. 2000
	Arabidopsis thaliana	U52851	Watson and Malmberg 1996		*Arabidopsis thaliana*	Y07765	Franceschetti et al. 2001
		AF009647	Watson et al. 1997		*Ipomoea batatas*	AF188998	Chang and Chen 2000
	Brassica juncera	AF077547	Mo and Pua 1998		*Triticum aestivum*	AF117660	Li and Chen 2000a
		AF220097, AF220098	Mo and Pua 2002		*Oryza sativa*	AF067194	Li and Chen 2000b
	Vitis vinifera	X96791	Primikirios and Roubelakis-Angelakis 1999			Y07766, AJ251899	Franceschetti et al. 2001
						AB122089	Yamaguchi et al. 2004
	Nicotiana tabacum	AF127540, AF127241	Wang et al. 2000			NM_001069752,	Ohyanagi et al. 2006
		AF321137	Bortolotti et al. 2004			NM_001059748,	
		AB110952	Shimizu et al. 2004			NM_001053939	
	Pringlea antiscorbutica	AY337606, AY337607	Hummel et al. 2004		*Phaseolus lunatus*	AB062360	Arimura et al. 2002
	Malus×domestica	AB181854	Hao et al. 2005a		*Pisum sativum*	U60592	Marco and Carrasco 2002
	Oryza sativa	NM_001063230,	Ohyanagi et al. 2006		*×Citrofortunella mitis*	AF512545	Kang et al. 2003
		NM_001058553			*Glycine max*	AF488307	Tian et al. 2004
		AY604047	Akiyama and Jin 2006		*Malus×domestica*	AB077441, AB077442	Hao et al. 2005b
					Vitis vinifera	AJ567368	Tassoni et al. 2006
Ornithine decarboxylase	*Datura stramonium*	X87847	Michael et al. 1996				
	Nicotiana tabacum	D89984	Imanishi et al. 1998	Spermidine synthase	*Arabidopsis thaliana*	AB006693	Hashimoto et al. 1998
		AB031066	Imanishi et al. 2000			AJ251296, AJ251297	Hanzawa et al. 2002
		AF127242	Wang et al. 2000		*Nicotiana sylvestris*	AB006692	Hashimoto et al. 1998
	Lycopersicon esculentum	AF030292	Alabadi et al. 1998		*Hyoscyamus niger*	AB006690, AB006691	Hashimoto et al. 1998
		AF029349	Kwak and Lee 2001		*Coffea arabica*	AB015599	Hatanaka et al. 1999
	Nicotiana glutinosa	AF323910	Lee and Cho 2001		*Pisum sativum*	AF043108, AF043109	Alabadi and Carbonell 1999 a
	Capsicum annuum	AF480882	Zainal et al. 2002		*Lycopersicon esculentum*	AJ006414	Alabadi and Carbonell 1999 b
		AY078081	Yoo et al. 2004		*Malus×domestica*	AB072915, AB072916,	Zhang et al. 2003
	Glycine max	AJ563382	Delis et al. 2005			AB072917	
	Oryza sativa	NM_001070362,	Ohyanagi et al. 2006		*Oryza sativa*	AB098063	Imai et al. 2004
		NM_001053389			*Cucumis sativus*	AY646352	Wang et al. 2005
S-Adenosylmethionine decarboxylase	*Solanum tubersum*	Z11680	Taylor et al. 1992	Spermine synthase (ACL5)	*Arabidopsis thaliana*	AF184093, AF184094	Hanzawa et al. 2000
		S74514	Mad Arif et al. 1994			AB076743, AB076723	Yoshida et al. 2003
	Spinacia oleracea	X81414	Bolle et al. 1995		*Arabis gemmifera*	AB076744	Yoshida et al. 2005
	Catharanthus roseus	U12573	Schröder and Sohröder1995		*Malus×domestica*	AB204521, AB204522	Kitashiba et al. 2005
	H. chilense×T. turgidum	X83881	Dresselhaus et al. 1996	(SPMS)	*Arabidopsis thaliana*	AY040013	Panicot et al. 2002
	Dianthus caryophyllus	U38526, U38527	Lee et al. 1996		*Malus×domestica*	AB204520	Kitashiba et al. 2005
		U94786	Kim et al.1997				

the resultant transgenic plants showed more up-regulation of ADC activity, polyamine accumulation, and increase in biomass under salt stress than the wild type (Roy and Wu 2001). Similarly, in a recent study, transgenic rice plants expressing the *Datura stramonium adc* gene produced much higher levels of Put under drought stress than the wild type, leading to higher levels of Spd and Spm and improved drought tolerance, in which the transgenic plants exhibited less chlorophyll loss and leaf curling than the wild type (Capell et al. 2004). *SAMDC* genes have been used to produce transgenic plants in terms of increasing stress tolerance. Roy and Wu (2002) reported that the introduction of the Tritordeum *SAMDC* gene into rice resulted in a three- to four-fold increase in Spd and Spm levels in the transformed plants. These transgenic rice plants showed normal growth and development even under NaCl stress, which indicated that the transformants were more stress-tolerant than the wild type. Transgenic tobacco overexpressing a human *SAMDC* gene driven by a constitutive *CaMV35S* promoter showed higher Spd and Put and exhibited tolerance to salt and drought stresses (Waie and Rajam 2003). Interestingly, introduction of a single polyamine biosynthetic gene has been shown to confer tolerance to multiple stresses. For example, Kasukabe et al. (2004) reported that overexpression of *SPDS* from *Curcurbita ficifolia* in *Arabidopsis* enhanced tolerance to chilling, freezing, drought, salinity, osmosis, and paraquat. When the same gene was transformed into sweet potato (*Ipomoea batatas*), the transgenic plants demonstrated more tolerance to salt and drought than the wild type (Kasukabe et al. 2006). More recently, Wi et al. (2006) showed a broad-spectrum tolerance to abiotic stresses in the transgenic tobacco, which showed overexpression of carnation *SAMDC*. These data demonstrated that a transgenic approach involving polyamine biosynthetic genes may be a good strategy to improve crop tolerance against harsh environments so as to meet the requirements of a challenging global environment.

The physiological and/or molecular mechanisms underlying the improved stress tolerance due to gene transformation have been investigated. As illustrated in Figure 2, it seems that the overexpression of polyamine biosynthetic genes altered polyamine biosynthesis, which may play important roles in the acquisition of stress tolerance due to their antioxidant property, cationic nature of polyamines at the physiological pH and /or improvement of ion balance. The production of more polyamines would result in the ability of the polyamines to interact more freely with anionic molecules, such as DNA, RNA, proteins, and membrane lipids (Feuerstein and Marton 1989; Schuber 1989), and prevent the membrane system from denaturing under stress conditions. On the other hand, overexpression of the polyamine biosynthetic genes may influence the transcriptions of an array of stress-related genes in transgenic lines that promote the synthesis of more protective compounds and render stress tolerance. For example, overexpression of *SAMDC* in tobacco has been shown to induce the mRNA levels of several antioxidant enzymes, such as ascorbate peroxidase, superoxide dismutase, and glutathione *S*-transferase in transgenic plants (Wi et al. 2006). Kasukabe et al. (2004), based on

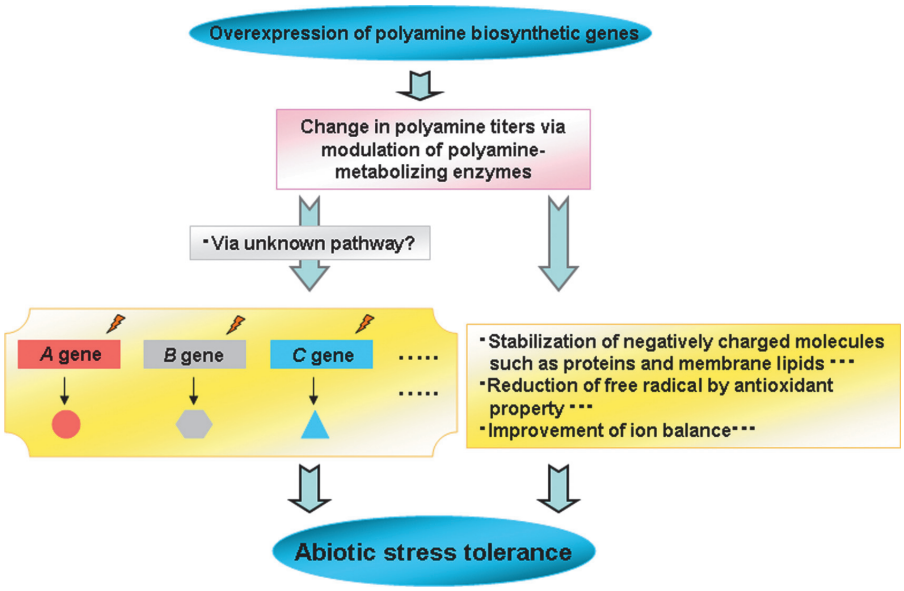

Figure 2. Schematic diagram for a possible regulatory mechanism underlying enhanced stress tolerance via genetic engineering of polyamine biosynthetic genes. Overexpression of polyamine biosynthetic genes leads to changes in polyamine titers (accumulation of individual or total polyamine); on the one hand, through an unidentified pathway, these genes activated or modified the expression of an array of genes that function directly or indirectly in stress tolerance, and, on the other hand, the accumulated polyamines *per se* can act as antioxidants to scavenge excessive free radicals or membrane stabilizers through binding to negatively charged groups, leading to enhanced stress tolerance.

microarray analysis, found that overexpression of the *SPDS* gene in *Arabidopsis* induced the expression of several transcription factors, such as DREB, WRKY, B-box zinc finger proteins, NAM proteins, and MYB, along with stress-regulated genes, such as *low-temperature-induced protein 78* (*LTI78* or *rd29A*). However, it is not clear if these two aspects act in synergy or independently during stress tolerance. Moreover, in any case, it needs to be clarified whether and how crosstalk between changes in polyamine titers and activation of a diversity of genes takes place in order to fend off stress.

Concluding remarks

Polyamines have been regarded as a new type of plant growth regulators and also considered as a secondary messenger. Being an important component of the plant stress response, polyamines are actively researched nowadays. Although the precise role and mode of action of polyamines in plant stress have long been a matter of debate, more and more data have given us evidence that polyamines are important compounds for regulating stress response. Although a final picture for a clear-cut role of polyamine in stress response remains to be established, it is conceivable that, with the cloning of polyamine biosynthetic genes from more plants and the advent of state-of-the-art tools (forward and reverse genetics), the functional significance of polyamine in stress response and defense will be ultimately elucidated. In the long run, they are expected to be used in the same way as farm chemicals to alleviate or mitigate stress-derived injury for crop protection. On the other hand, the polyamine biosynthetic genes will be valuable candidates for genetic manipulation in an effort to create novel germplasms with better stress tolerance to combat adverse environments for the sustainable productivity of agricultural crops.

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