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Polyamines and Stress Tolerance of Plants

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ABSTRACT

Polyamines are universal organic polycations implicated in a wide array of fundamental processes in plants ranging from triggering the cell cycle, genome expression, signaling, plant growth and development to plant adaptation toward abiotic stresses. Stress-induced accumulation of polyamines often correlates with the improvement of plant tolerance that has been shown by modulation of the polyamine biosynthetic pathway in some transgenic plants. Genes for several key biosynthetic and catabolic enzymes have been cloned from various plant species. Polyamines can modulate functions of RNA, DNA, nucleotide triphosphates, and proteins and protect macromolecules under stress. Polyamines are also the modulators of stress-regulated gene expression and exhibit antioxidant properties. Taken together, these recent findings have promoted intense efforts to characterize in detail the mechanisms of polyamine homeostasis regulation and to elucidate realization of their multifunctional role in plants under environmental stress. However, molecular mechanisms underlying polyamine participation in plant adaptation to stress are not completely understood. Plant adaptation to various abiotic stresses is a complex process involving numerous changes, including the increased expression of many stress-related genes responsible for the accumulation of compatible solutes, expression of antioxidant enzymes, and supression of energy-consuming pathways. Recent reviews did not summarize data concerning the correlation between polyamine functions and other adaptive mechanisms in plants. Therefore in this review, particular emphasis is placed on discussion on protective mechanisms used by polyamines during different stages of the adaptation process.

Keywords: abiotic stress, anabolism and catabolism, cadaverine, physiological role, putrescine, spermidine, spermine
Abbreviations: **ACC**, aminocyclopropan-1-carboxylic acid; **ADC**, arginine decarboxylase; **Cad**, cadaverine; **DAO**, diamine oxidase; **1,3-Dap**, diaminopropane; **Eth**, ethylene; **ODC**, ornitine decarboxylase; **PA**, polyamine; **Put**, putrescine; **LDC**, lysine decarboxylase; **PAO**, polyamine oxydase; **SAM**, *S*-adenosylmethionine; **SAMDC**, SAM decarboxylase; **SAMS**, *S*-adenosylmethionine synthetase; **Spd**, spermidine; **Spm**, spermine; **SPDS**, spermidine syntase; **SPMS**, spermine synthase; **TGase**, transglutaminase

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INTRODUCTION

Polyamines (PAs) putrescine^{+2} (Put), spermidine^{+3} (Spd), spermine^{+4} (Spm), and cadaverine^{+2} (Cad) are low-molecular-weight organic polycations displaying a high biological activity. PAs are present in all compartments of the plant cell, including the nucleus, which indicates their participation in diverse fundamental processes in the cell (Bouche-

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reau *et al.* 1999; Kaur-Sawhney *et al.* 2003). Like hormones, polyamines are involved in the processes of replication, transcription, translation, membrane stabilization, enzyme activity modulation, cell division and elongation, plant growth and development (Galston *et al*. 1997; Walden *et al.* 1997). The concentrations of PAs in the plant (10^{-9}–10^{-5} M) are much higher than those of endogenous phytohormones (10^{-13}–10^{-7} M). The total PA concentration and the ratios between individual PAs vary markedly depending on the plant species, organ, and tissue, and also on the developmental stage. Major changes in PA metabolism occur in response to various abiotic stress conditions (Bouchereau *et al.* 1999). Stress-induced PA accumulation is important in ameliorating plant responses to abiotic stresses. Due to their cationic nature PAs interact with negatively charged macromolecules, such as nucleic acids, phospholipids, and proteins. These ionic interactions, which are reversible, lead to the stabilization of DNA, tRNA, membranes, and some proteins (Bachrach 2005). The pathways of PA biosynthesis in higher plants are well studied; most of the genes for enzymes involved are cloned, and their regulation is studied. Nevertheless, in order to understand properly the role of PAs during plant development under normal and stress conditions, it seems essential to critically analyze and summarize the accumulated experimental data, focusing the compartmentation and enzymology of PA biosynthesis and catabolism, their transmembrane and interorgan transport, and the molecular mechanisms of their protective effects. In this review, we also consider transgenic and mutant plants displaying changed PA metabolism and the usage of its specific inhibitors.

POLYAMINE METABOLISM AND ITS REGULATION

Biosynthesis of polyamines

In plants, Put, Spd, and Spm are most abundant PAs, but the PA metabolic pathways are regulated by a limited number of key enzymes (Tiburcio *et al*. 1997). Put is produced directly from ornithine by ornithine decarboxylase (ODC, EC 4.1.1.17) or indirectly from arginine by arginine decarboxylase (ADC, EC 4.1.1.19) via agmatine (Agm). Conversion of Agm into Put requires two distinct enzymes: Agm iminohydrolase (EC 3.5.3.12) and N-carbamoylputrescine amidohydrolase (EC 3.5.1) (**Fig. 1**).

The formation of Put from arginine with the involvement of ADC is usually associated with the plant responses to stresses, such as drought, salinity, hyperthermia, potassium and sulfur deficits, etc. (Bouchereau *et al*. 1999; Basu *et al*. 2006). The implication of PAs in stress responses has also been revealed by genetic approaches, most of them being used in Arabidopsis. Arabidopsis contains two different genes encoding ADC (*ADC1* and *ADC2*) (Hanzawa *et al*. 2000; Panicot *et al*. 2002). The occurrence of duplicated genes in Arabidopsis seems to be related to differential regulation of gene responsiveness. Thus, Soyka and Heyer (1999) demonstrated the specific involvement of the *ADC2* gene in hyperosmotic stress. Treatment of Arabidopsis leaves with 0.6 M sorbitol induced *ADC2* expression, while *ADC1* transcript levels remained unaltered.

Using molecular biology, it was shown that ADC was localized in essentially all organs of *Nicotiana tabaccum* L. (flowers, stems, seeds, leaves, and roots) (Bortolotti *et al.* 2004; Paschalidis and Roubelakis-Angelakis 2005a, 2005b). Using immunoenzyme approaches, it was demonstrated that the ADC protein was present in two different compartments: chloroplasts in the leaves (photosynthesizing organs) and nuclei in the roots (nonphotosynthesizing organs), which may be related to specific functions of ADC in different cell types (Borrell *et al.* 1995; Bortolotti *et al.* 2004). It was established, for example, that spinach ADC was associated with the LHC of photosystem II (Legoska and Zaichert 1999). PAs synthesized in chloroplasts evidently stabilize photosynthetic complexes of thylakoid membranes under stress conditions.

Like in animals, plant ODC is localized in the nucleus (Hanfrey *et al.* 2001; Kakkar and Sawhney 2002). Physical uncoupling and independent functioning of the two pathways of Put biosynthesis (Bhatnagar *et al.* 2001) may indicate a difference in their functions, whereas chloroplast and mitochondrial membranes can serve as barriers separating ornithine, the product of catabolism, from ornithine used for arginine biosynthesis through ornithine cycle (Bagni and Tassoni 2001).

The ODC pathway functions more actively in plants at early stages of development. In fact, an increased arginase activity catalyzing ornithine formation and tightly associated with chromatin (Bagni and Tassoni 2001) was detected in young plants, whereas in adult plants its activity was suppressed. This means that ODC dominates in PA biosynthesis in the period of active cell division in different organs of *Nicotiana tabacum* L. cv 'Xanthi' (Paschalidis and Roubelakis-Angelakis 2005a) and also in developing tomato fruits (*Licopersicon esculentum* Mill. cv. 'Pearson') due to intense ornithine formation in mitochondria (Galston *et al.* 1997). Moreover, it was established that the ornithine pathway of Put biosynthesis prevailed in roots of *Nicotiana tabacum* L.

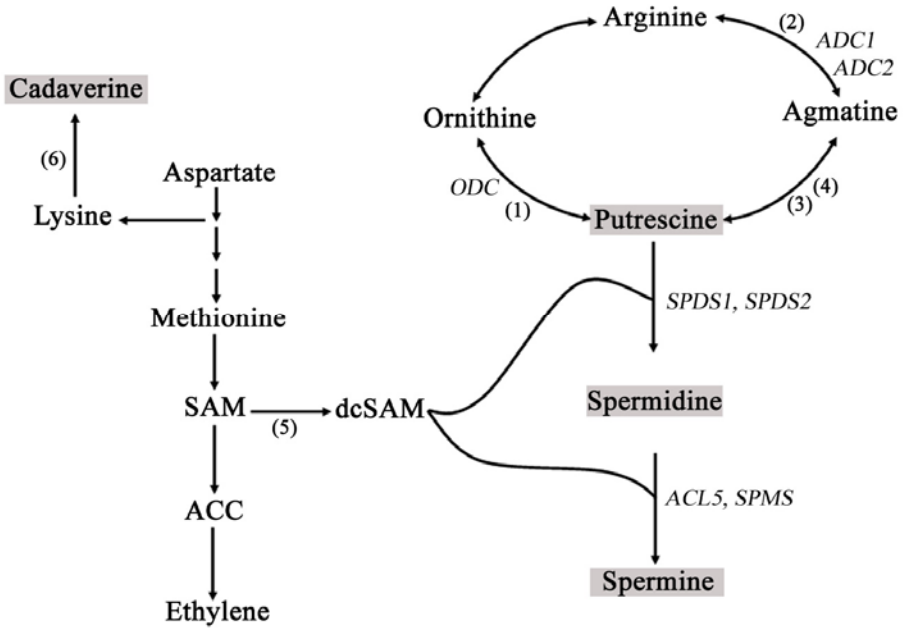

Fig. 1 Pathways of biosynthesis of main plant polyamines (Put, Spd, Spm and Cad). Based on Soyka and Heyer (1999), Hanazawa *et al.* (2002), Panicot *et al.* (2002), Imai *et al*. (2004) and Urano *et al*. (2004). Enzymes involved: (1) ODC, ornitine decarboxylase, (2) ADC, arginine decarboxylase, (3) agmatine iminohydrolase, (4) *N*-carbamoylputrescine amidohydrolase, (5) SAMDC**,** SAM decarboxylase, (6) SPDS, spermidine syntase, (7) SPMS, spermine synthase, (8) LDC, lysine decarboxylase ; ACC, 1-aminocyclopropane-1-carboxylic acid; dcSAM, decarboxylated *S*-adenosylmethionine. The genes involved are *ADC1*, *ADC2*, *ODC*, *SPDS1*, *SPDS2*, *ACL5*, and *SPMS*.

(Paschalidis and Roubelakis-Angelakis 2005a). It is of interest that, unlike other plant species, *Arabidopsis* does not contain *ODC* (Hanfrey *et al.* 2001) and most of the key genes involved in PA biosynthesis are duplicated.

Spd and Spm are synthesized by successive attachment of aminopropyl first to Put and then to Spd (**Fig. 1**). These reactions are catalyzed by aminopropyltransferases, Spd synthase (SPDS, EC 2.5.1.16), and Spm synthase (SPMS, EC 2.5.1.22). Aminopropyl is formed due to decarboxylation of *S*-adenosylmethionine (SAM) by SAM decarboxylase (SAMDC, EC 4.1.1.50), which has a very short half-life (from 5 to 60 min) and is a rate-limiting enzyme of Spd and Spm biosynthesis. SPDS and SAMDC are known to be localized in the cytoplasm (Bouchereau *et al.* 1999; Kaur-Sawhney *et al.* 2003). SAM is produced from methionine and ATP by *S*-adenosylmethionine synthetase (SAMS, EC 2.5.1.6.). It is of importance that SAM is not only the substrate for SAMDC providing aminopropyl for PA synthesis but also a basic donor of methyl groups for numerous reactions of transmethylation and a principal negative regulator of threonine and methionine biosyntheses. *Arabidopsis* contains two different genes encoding SPDS (*SPDS1* and *SPDS2*) and SPMS (*SPMS* and *ACAULIS5* (*ACL5*). It has been demonstrated, that exogenous abscisic acid upregulated SPMS expression (Hanzawa *et al.* 2002; Urano *et al*. 2004) but not *ACL5.* Thus, four enzymes are involved directly or indirectly in the formation of Spd and Spm. Some of these enzymes are encoded by gene families.

In the roots of tomato seedlings, salt stress increased the content of *SAM1* and *SAM2* mRNA, whereas only *SAM1* transcripts accumulated in the leaves (Espartero *et al.* 1994). An increase in the level of *SAM* transcripts in tomato plants was not accompanied by a considerable accumulation of Spd and Spm. This means that mRNA formation was not a rate-limiting step of PA biosynthesis, and post-transcriptional, translational, or posttranslational regulation of gene expression was critical.

For the enzymology of PA synthesis, *Arabidopsis acaulis5* (*acl5*) mutant is of a great interest; the recessive mutation in the *ACL5* gene was manifested in the disturbance of stem elongation. This gene sequencing showed its high homology with sequences encoding the two enzymes of PA biosynthesis, SPDS and SPMS (Hanzawa *et al.* 2000). However, the synthesis of recombinant ACL5 protein in *E. coli* and its immunoenzyme analysis demonstrated that the *ACL5* gene encoded spermine synthase, although it might be that this protein displayed wider substrate specificity, participating in the biosynthesis of other PAs as well (Kaur-Sawhney *et al.* 2003).

A stress-induced activation of the complete pathway of PA biosynthesis with the accumulation of Spd and Spm is observed relatively infrequently (Tiburcio *et al*. 1994). Usually, in sensitive plant species, stress induces the selective accumulation of Put. In contrast, in tolerant plant species, for example in the salt-tolerant rice cultivar (Krishnamurthy and Bhagwat 1989) and salt-tolerant tobacco *NrEs-1* strain, salinity induced a 10- to 15-fold accumulation of Spd as compared to control cells (Shevyakova *et al*. 1984). It is possible that, in sensitive species, stress suppresses the key enzyme of Spd and Spm biosynthesis, SAM decarboxylase (SAMDC). Therefore, the biosynthesis of these two PAs is retarded and their precursor, Put, accumulates actively.

Along with widely occurring PAs, prokaryotes are capable of the synthesis of unusual PAs. Thus, thermophilic and acidophilic bacteria living under extreme conditions contain aminopropyltransferase of wide substrate specificity. This permits them to synthesize PAs with long polymethylene chains and increased the number of NH_2 groups in the molecule (caldopentamine) and also to synthesize some branched PAs (hexamines and heptamines) (Terui *et al.* 2005). Aminopropyl produced at SAM decarboxylation is used for unusual PA synthesis. Plants are also capable of unusual PA synthesis. Unusual PAs were found, for example, in osmotolerant alfalfa plants (Bagga *et al.* 1997).

Cad is a diamine, which is met in plants relatively infrequently; it is derived from lysine, a byproduct of the aspartate pathway of methionine synthesis (Kuznetsov *et al.* 2002) (**Fig. 1**). Its synthesis from lysine is catalyzed by lysine decarboxylase (LDC, EC 4.1.1.18) via pyridoxal phosphate-dependent decarboxylation. Under ornithine deficiency, ODC can use lysine as an alternative substrate for Cad synthesis (Bhatnagar *et al.* 2001).

In some legume species (*Lathyrus sativus*, for example), Cad may be produced by decarboxylation of either lysine or homoarginine via the intermediate production of homoagmatine (Bagni and Tassoni 2001). An enhanced activity of this enzyme is evidently related to the fact that *L. sativus* is a producer of piperidine alkaloids (sparteine, lupinine, anabasine, and others) from Cad. In most plant species with a low Cad concentration in tissues, the activity of LDC is low. Under stress conditions, Cad accumulation in plants evidently compensates a decrease in the content of Put-family PAs (Shevyakova *et al*. 2001; Kuznetsov *et al*. 2002).

As distinct from Put, Spm, and Spm, which are synthesized on thylakoid membranes (Borell *et al*. 1995), Cad is produced in the chloroplast stroma (Herminghaus *et al.* 1991).

Some researchers reported that, in the halophyte *Pulicaria*, Cad was synthesized predominantly in roots (Freadman *et al.* 1989), where, as it is known, plastids are represented by proplastids with the undeveloped membrane system. In another halophyte *Mesembryanthemum crystallinum*, the highest activity of LDC under salinity was also detected in the root system (Kuznetsov *et al*. 2007).

The molecular mechanisms of Cad biosynthesis were studied for tobacco plants transformed with the gene encoding LDC from the enterobacterium *Hafnia alvei*, which is capable of constitutive overproduction of the enzyme. Two different constructs were built for tobacco transformation (Herminghaus *et al*. 1991). In one of them, the gene was under the control of the Tr promoter and did not contain a signal (transport) peptide. The second construct contained the promoter of the Rubisco small subunit gene and a signal sequence providing for the polypeptide transport into chloroplasts, a natural compartment of LDC. The results obtained showed that Cad synthesis in tobacco plants was enhanced only in the second case. When tobacco plants were transformed with the construct containing the gene for LSD from the enterobacterium *H. alvei* under the control of the constitutive 35S CaMV promoter, root culture was obtained with a high LDC activity and an increased level of alkaloids. A direct correlation between the content of Cad and a capacity for alkaloid synthesis was also found for Solanaceae plants (Bagni and Tassoni 2001).

Polyamine catabolism

PA catabolism efficiently regulates the free PA level in the cell; its products can fulfill an important physiological role under both normal and stress conditions (Martin-Tanguy 2001; Paschalidis and Roubelakis-Angelakis 2005b).

Endogenous as well as exogenous polyamine degradation in plants is catalyzed by the two oxidative enzymes: copper-containing diamine oxidase (DAO, EC 1.4.3.6) and flavoprotein-dependent polyamine oxidase (PAO, EC 1.5.3.3). It was believed that the highest DAO activity was characteristic of legumes, and the highest PAO activity, for grasses. Now, this opinion is reassessed because DAO activity was found in grasses as well (Tamai *et al*. 2000). Both enzymes are localized in the cytoplasm and cell walls where they provide hydrogen peroxide required for suberinization and lignification, which confer firmness to the cell walls (Cona *et al*. 2003).

DAO catalyzes oxidation of primary amino groups in many biogenic amines, including mono-, di-, and polyamines with Put and Cad as most preferable substrates. As a result of DAO-catalyzed oxidation of Put, Spd, and Spm, amino aldehydes, hydrogen peroxide, and ammonia are produced. PAO catalyzes oxidation of secondary amino groups

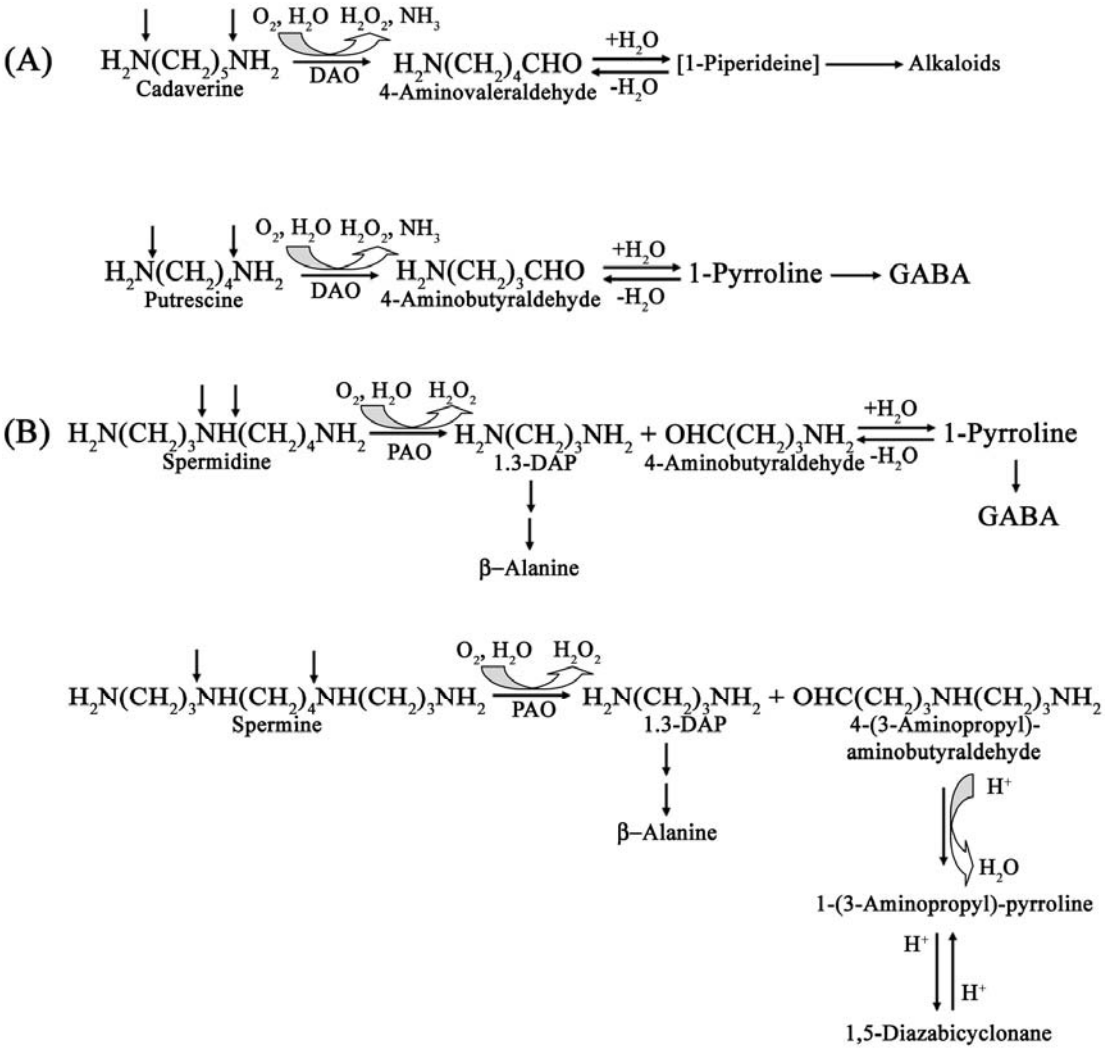

Fig. 2 Pathways of oxidative catabolism of polyamines and their products. (**A**) The oxidation of diamines (cadaverine, putrescine) by diamine oxidase (DAO) leads to the corresponding H_2O_2, NH_3 and aldehyde. Then aldehydes converts to corresponding products. (**B**) The oxidation of polyamines (spermidine, spermine) by flavoprotein-dependent polyamine oxidase (PAO) leads to H_2O_2 and 1.3 Diaminopropane (1.3-Dap). 1,3-Dap converts to corresponding products.

of Spd and Spm (but not other PAs) with the formation of H_2O_2, 1,3-Dap, and amino aldehydes**,** 4-aminobutyraldehyde and 4-(3-aminopropyl)aminobutyraldehyde (**Fig. 2**). The same amino aldehydes are produced as a result of Put and Spd oxidation by DAO. A terminal product of this oxidation, 1,3-Dap, can be a substrate for DAO-catalyzed β-alanine formation.

DAO is a homodimer; its subunits have mol wts of 70-95 kD in pea and 80-95 kD in lentil. Each DAO subunit contains one copper atom and a quinone cofactor. cDNAs of pea DAO were obtained, and, using Southern hybridization, the genes for DAO were identified in many mono- and dicotyledonous plants (Tipping and McPherson 1995). By Northern-blot analysis, it was demonstrated that the level of DAO mRNA increased in darkness, which was correlated with a high enzyme activity. On the other hand, anoxia, low temperature, and other stressors did not affect DAO activity, and the oxygen concentration did not control the transcriptional activity of the DAO-encoding gene (Maccarrone *et al*. 1991).

Recently, some reports appeared demonstrating that N^1-acetylation preceded oxidative PA degradation in plants (Bagni and Tassoni 2001). Acetyl polyamines were identified in sugar beet seedlings, in chloroplasts of Jerusalem artichoke leaves, in maize roots, and in various *A. thaliana* organs (Bagni and Tassoni 2001). This pathway of PA degradation involves various enzymes, including PAO. In plants, both anabolic and catabolic enzymes can play a role in the regulation of PA levels during the cell cycle and cell division/expansion processes (Paschalidis and Roubelakis-Angelakis 2005a, 2005b).

Spontaneous cyclization of amino aldehydes derived from Put results in the formation of 1-pyrroline, which is converted into γ-aminobutyric acid (GABA) by pyrroline dehydrogenase; GABA is a potential modulator of many physiological processes. However, most of GABA accumulated in stressed plants (Turano *et al*. 1993) appears due to gluthamate oxidation. GABA formation in the process of Put oxidative degradation was studied in detached leaves of *Glycine max* (Turano *et al*. 1993). It is well known that, in plants, GABA is metabolized by GABA transaminase and succinyl semialdehyde dehydrogenase into succinate, which enters into the Krebs cycle. By basing on these data, we can suppose that GABA carbon, being involved in the Krebs cycle, enhances the formation of glutamate, which, in its turn, accelerates PA biosynthesis.

Pulse-chase experiments with 1,4-^{14}C-Put introduced into the roots of a halophyte *Limonium tataricum* demonstrated rapid metabolization of this diamine into GABA via the DAO pathway (Duhazé *et al.* 2002). Moreover, the incorporation of ^{14}C into GABA was detected after the introduction of labeled Spd into the roots, which indicated a possibility of Put formation via PAO-catalyzed degradation of Spd and a further conversion of diamine into GABA. As distinct from Put, amino aldehyde, an initial product of oxidation of Cad terminal amino groups, is further converted into 1-piperedine, a precursor of alkaloids.

Investigations of PA catabolism have been focused

mainly on changes in their levels and spectra, leaving the biological significance to be determined. A recent paper of Paschalidis and Roubelakis-Angelakis (2005b) describes the sites and regulation of PA catabolism during cell divi sion/expansion, cell cycle progression, and vascular development in tobacco plants. Gene expression and immunohistochemical analysis revealed that, DAO and PAO in developing tissues precede and overlap with nascent nuclear DNA and also with peroxidases and lignification. The specific activities of the enzymes of PA catabolism increased basipetally in the leaf central and basal, petiolar, and internodal regions throughout development.

The results obtained by Paschalidis and Roubelakis-Angelakis (2005b) permit a supposition that, in stressed plants, developmental changes in PA catabolism are enhanced because DAO and PAO expression and H_2O_2 production occur in the cells destined to undergo lignification (Cona *et al*. 2003).

Polyamine metabolism regulation

Modern knowledge of the mechanisms of the PA synthesis regulation in prokaryotes and eukaryotes are considered in a number of comprehensive reviews (Walden *et al*. 1997; Bouchereau *et al*. 1999; Bagni and Tassoni 2001; Kakkar and Sawhney 2002); therefore, we will not go deeply in these issues.

The principal enzymes of PA biosynthesis are under the complex metabolic and developmental control and affected by stresses; such a control is a necessary condition for the efficient regulation of cell metabolism. Thus, in transgenic rice plants overexpressing ADC, Put enhanced and Spd suppressed the activity of SAMDC, a key rate-limiting enzyme of Spd and Spm biosyntheses (Roy and Wu 2002). In one of rice cultivars, Spd and Spm accumulation was related to the enhanced expression of ADC (Chattopadhyay *et al*. 1997), whereas in salt-tolerant rice and tomato plants, to the enhanced expression of SAMDC (Krishnamurthy and Bagwat 1989). It should be noted that the induction of SAMDC expression resulting in Spd and Spm accumulation is often considered to be more important for plant defense against stress than Put accumulation. SAMDC instability (half-life of 5-60 min) permits a rapid change in the amount of this enzyme in the cell and, consequently, in the PA level in response to new conditions.

The enzymes of PA biosynthesis (ODC, ADC, and SAMDC) are controlled at transcriptional, translational, and posttranslational levels. Recently, Hu *et al*. (2005) showed that a 5′-untranslated leader sequence played a central role in both transcriptional and posttranscriptional control of SAMDC gene expression. This was related to the fact that in plants, as distinct from animals, this gene has no introns in the open reading frame but does have introns in the leader sequence, which are required for the enhanced SAMDC biosynthesis when the level of endogenous Spd is low.

It is believed that all enzymes of PA biosynthesis, including SAMDC, are initially synthesized as inactive precursors (proenzymes), which are subjected to posttranslational processing with the formation of mature enzymes (Xiong *et al*. 1997).

It is of interest that, in detached oat leaves subjected to osmotic stress, processing of ADC inactive precursor was inhibited by Spm treatment (Tiburcio *et al*. 1994; Borrell *et al*. 1995). The authors believe that, when Spm is absent, transcription of ADC gene is enhanced, and inactive ADC precursor is synthesized, which is processed into the active ADC form. ADC catalyzes Put synthesis. In the presence of exogenous Spm, the level of mRNA increased but the number of active enzyme molecules decreased and Put accumulation was blocked.

In some plants, *Arabidopsis* for example, Put can be synthesized only by ADC. The gene encoding ODC and corresponding protein was not found in this plant (Hanfrey *et al.* 2001). However, *Arabidiosis*, like some Brassicaceae plants, contains two genes for ADC (*ADC1* and *ADC2*) (Galloway *et al*. 1998). Using molecular biology, it was shown that mechanical injury to arabidopsis leaves or their treatment with jasmonic acid resulted in the activation of only a single gene for Put synthesis, the *ADC2* gene (Perez-Amador *et al*. 2002). This was accompanied by a transient increase in the content of Put but not Spd or Spm. It is of interest that the sequences of ADC1 and ADC2 proteins were 80% identical and differed only in their terminal fragments. It might be that these proteins differ in their localization, as it was shown for ADC in oat plants (Borrell *et al.* 1995), and fulfill different biological functions (Perez-Amador *et al.* 2002).

Conjugates as a mode of maintaining polyamine homeostasis

Plant physiologists engaged in studing stress pay great attention to PA conjugates with hydroxycinnamic acids (acid-soluble fraction). In this case, PAs produce amide bonds using CoA esters for activation of carboxylic groups with the help of enzymes known as transferases (Martin-Tanguy 1997; Bagni and Tassoni 2001; Martin-Tanguy 2001). One of transferases, putrescine hydroxycinnamyl transferase (EC 2.3.1.138), was isolated from tobacco plants, purified, and characterized (Bagni and Tassoni 2001). This enzyme preferebly utilises 1,3-Dap, Put, Cad, and Spd as substrates, while caffeoyl-, feruloyl-, and cynnamoyl-CoA seem to be the best donors.

PA conjugates with phenolic acids combine the properties of both parent compounds and display a great chemical, metabolic, and functional diversity (Edreva *et al.* 2007). PAs contain highly protonated amino groups, and therefore, at physiological pH values, they are positively charged. In dependence on amino group position in the PA molecule and on the type of substitution, they could confer basic or neutral properties to the conjugates. The two types of these acid-soluble plant conjugates are discerned (Edreva *et al.* 2007): (1) basic, with their character determined by the availability of one protonated free amino group in their molecule. Monosubstituted aliphatic polyamines, such as the feruloylputrescine, are referred to this group and (2) neutral, with no free amino group; disubstituted aliphatic polyamines, such as diferuloylputrescine belong to this group.

Like in PAs, in the conjugates with one protonated amino group, a positive charge could determine their properties as organic cations. In addition, a positive charge provides for electrostatic interactions with negatively charged loci in nucleic acids, proteins, membranes, and cell walls; these interactions are alike to those observed for aliphatic PAs.

Due to electrostatic interactions, such conjugates might modulate the conformational states of macromolecules depending on the charge and also the structure of the cell walls and membranes. In such a way, conjugates could be involved in the control of various biological processes like PAs do (Martin-Tanguy 1997, 2001; Edreva *et al.* 2007). Such interactions are impossible for neutral conjugates. The presence of one free protonated amino group in basic conjugates confers them polarity and water solubility, which can determine their involvement in the maintenance of acid, ionic, and calcium homeostasis. Soluble conjugates are important for the control of the intracellular PA concentrations (Bagni and Pistocchi 1990) and for their interaction with compounds of the cell wall, especially hemicelluloses and lignin (Lam *et al*. 1992). Havelange *et al* (1996) believe that PA conjugates with hydroxycinnamic acids could regulate the intracellular PA pool, serve for PA transport, or even be a substrate for peroxidases.

The formation of PA conjugates with proteins (acid-insoluble conjugates) is based on post-translational covalent binding of PAs primary amine groups to a γ-carboxamide group of protein endo-glutamine residues catalyzed by Ca^{2+}-dependent and Ca^{2+}-independent transglutaminases

(TGase, КФ. 2.3.2.13) (Dondini *et al*. 2003). It is known that PAs can be linked to many molecules by various types of binding. In particular, γ-glutamyl PA derivatives, which bridge two polypeptides, assumes an increasing importance for the post-translational modification of structural, enzyme, or signalling proteins. The binding is highly specific and probably depends on the conformation of substrate molecule, but not on a target sequence of amino acids flanking the glutamine residues. Due to molecular size, putrescine forms the shortest bridge and spermine the longest. In this way, proteins can be assembled to complexes of a higher molecular mass. Some derivatives also confer additional positive charges to the protein: monoderivatives can interact by their free amino group with other molecules by various types of binding (Dondini *et al.* 2003). By isolating chloroplasts from the leaves of *Helianthus tuberosus*, it was confirmed that Rubisco and some antenna proteins of the photosystems are TGase substrates. PAs are linked both to stroma and thylakoid proteins, suggesting that more than one protein with TGase activity could be present in the chloroplast or that a TGase putatively facing the exterior of the thylakoid can also modify stroma proteins (Dondini *et al.* 2003). Such protein modifications with the involvement of PA conjugates could be important for stabilization of molecular complexes of thylakoid membranes in osmotically stressed or senescencent leaves (Legocka and Zaichert 1999). It was reported that TGase activity in the alga *Dunalliella salina* stress condition could be activated under hypersaline stress (Dondini *et al.* 2003).

Since TGases occur not only in chloroplasts, it might be that PA conjugates could fulfill diverse functions under stress conditions.

In various plant species and at various developmental stages, the ratios between free and conjugated PAs could differ sharply under normal growth conditions. According to some researchers (Bagni and Tassoni 2001), free PAs, Put in particular, comprise from 50 to 90% of total PA content in the cell. The smaller PA part is bound with high-molecular-weight molecules (proteins) (Bouchereau *et al.* 1999; Martin-Tanguy 2001). Recently the temporal and spatial distribution of free PAs and their soluble and bound conjugates in tobacco plants was described (Paschalidis and Roubelakis-Angelakis 2005a). According to the studies performed, the total level of conjugated PAs in tobacco stem apex and upper leaves was about 80%; in senescing leaves, when the total level of PAs declined sharply, the proportion of PA conjugates was 50%. The roots displayed similar tendency in the ratio between free and bound PAs. Paschalidis and Roubelakis-Angelakis (2005a) believe that PA synthesis and conjugation are likely an essential part of the homeostatic mechanism that controls the PA levels.

In recent years, conjugated forms of PAs have attracted considerable attention as possible members of the plant defence system against stresses (Matrin-Tanguy 2001; Dondini *et al.* 2003). It was proposed that these conjugates might take part in PA translocation (Havelange *et al.* 1996) and be also important for the regulation of the free PA-titer (Martin-Tanguy 2001). However, published data concerning the content of soluble PA conjugates in stressed plants are rather contradictory (Walters 2000; Sarjala and Taulavuori 2004), which could be determined by species-specificity and the content of phenolic compounds and PAs as substrates for the production of acid-soluble PA conjugates (Martin-Tanguy 2001). In some studies (Biondi *et al.* 2001; Alcázar *et al.* 2006), it was shown that methyl jasmonate and abscisic acid (ABA) stimulated the production of plant secondary metabolites, including hydroxycinnamic acid and PAs. Thus, in barley leaves, the level of free Put, Spd, and Spm and their conjugates increased markedly on the 4th day after treatment with methyl jasmonate, which was accompanied by leaf increased resistance to powdery mildew. It is of interest that, in this study, DAO activation was observed along with the methyl jasmonate-induced increased levels of PAs and their conjugates, which could be a response to the increased level of free Put. On the contrary, in publication of Alcázar *et al*. (2006), transient depletion in the free forms of PAs in *Arabidopsis* occured in parallel to increased insoluble conjugated acid-soluble fraction levels under treatment of ABA. These conjugated form of Spd and Spm increased 8.3- and 16-fold, respectively, in the first hour of treatment. These responses were not observed in non-stressed plants. Thus, authors reasoned that the levels of free Put are subjected to homeostatic control to maintain its level within a non-toxic range.

The multifunctional aspects and different fates of conjugated PAs open a new possibility to establish their role in plant stress response. Conjugation reactions could regulate PA functions, for example, their binding and interaction with nucleic acids. The possibility also exists that PA conjugation to a cinnamic acid could be important in the regulation of the free PA titers and/or in detoxificating phenolic compounds known as growth inhibitors (Martin-Tunguy 2001). It is not excluded that conjugates could represent a storage PA pool used under extreme condions. More data are required to confirm a real role of conjugated PAs in physiological processes during plant adaptation to abiotic stress.

Intracellular and interorgan polyamine transport

In order to understand the physiological role of PAs or any other biologically active compounds, their capacity of interorgan transport should be considered. For tens of years, researchers stated with certainty that the PA polycation nature is incompatible with their long-distance transport. The more so, that, in one of the first studies using ^{14}C-PAs, it was shown that Put and Spd were very poorly transported within the plant (Galston *et al*. 1997). Therefore, it was concluded that PAs could not be growth-regulating compounds and could not exert distant action, as distinct from phytohormones; they were believed to be local modulators of metabolism in the regions of their increased biosynthesis (Galston *et al.* 1997). It was admitted that, in experiments with labeled PAs, their limited spreading along the plant might be an artifact related to the problems of isotope dilution, metabolization, conjugate formation, and possible binding of PA to the cell components, including the components of the transport systems.

However, it was noted later that PAs absorbed by plant tissues could be transported over long distances. The presence of large amounts of PAs in the xylem sap and phloem exudates, which was detected firstly by Friedman *et al.* (1986) and then in our experiments (Shevyakova *et al.* 2001; Kuznetsov *et al.* 2002), is a good argument for interorgan PA transport.

It is of interest that stress factors, such as potassium deficiency, acidic pH, and salinity, enhanced PA interorgan transport (Friedman *et al.* 1986). When *M. crystallinum* organs were subjected to local 2-h heat shock (40°C for roots and 47°C for shoots), we observed Cad and Put translocation in acropetal direction along the xylem and in basipetal direction along the phloem (Shevyakova *et al.* 2001; Kuznetsov *et al.* 2002). Among rapidly transported PAs, Cad played a special role. It accumulated in leaves of the common ice plants in response to salinity (400 mM NaCl) or exogenous ethylene (Eth) teatment (10 μl/l, 4 h). The interrelation between Eth-dependent Cad formation with its subsequent stress-induced transport to roots and the functioning of the system of Eth signal recognition was shown in experiments with the *A. thaliana ein4* mutant insensitive to exogenous Eth. On the basis of experiments performed, a hypothesis was put forward that stress-induced induction and interorgan translocation of Cad was under ethylene control, which formation was characteristic of the plant response to short-term hypothermia or salinity. This means that stress phytohormones, such as Eth and may be ABA, could trigger the interorgan PA translocation in plants.

The mechanisms of transmembrane PA transfer in plants are only poorly studied. First investigations of this

problem were performed with *E. coli* cells. It was established that PA uptake by *E. coli* cells demanded energy, and two transport systems were involved in this process: one system for Put and another, for Spd and Spm (Kashiwagi *et al.* 2000).

E. coli mutants deficient in the PA transport and clones harboring the genes for PA transporters were used for investigation of the molecular properties of the PA transport systems. It was established that, in these mutants, a periplasmic transport system was involved in PA uptake by bacteria; this system was controlled by the two genes (*pPT104* and *pPT79*) for Put and by a single gene (*pPT104*) for Spd. This transport system comprised four protein types (potA, potB, potC, and potD) differing in their localization in the periplasmic space. Expression of all four genes and the synthesis of all four proteins were required for the highest transport activity of this system. The molecular analysis of the *E.coli* system for PA transport permitted a creation of a model of the secondary structure of two transporters and identification the site of Put interaction with a transporter; the mechanisms of regulation of PA synthesis, uptake, and excretion in bacteria were supposed for the first time (Kashiwagi *et al.* 2000).

Recently, several reports appeared simultaneously about identification and the mechanisms of functioning of protein transporters in eukaryotic cells (*Saccharomyces cerevisiae*). The team of Japanese researchers (Uemura *et al.* 2005) established that Gap 1p transporter was localized in the plasma membrane and catalyzed Put and Spm uptake with K_m = 4.6 and V_{max} = 0.59 nmol/(mg protein min^{-1}). The two other proteins, TPO1 and TPO5, catalyzed PA excretion, which was per-formed by TPO1 at acidic pH (5.0) (Uemura *et al*. 2005). TPO1 transport activity increased after its phosphorylation by Ser19 protein kinase C and Thr52 casein kinase. TPO5 transporter encoded by the *YKL174c* gene was resistant to high PA concentrations (120 mM Put and 3 mM Spd). It was more efficient in the transport of Put than Spd. In *S. cerevisiae*, Aouida *et al.* (2005) identified a permease with a high affinity for Put and Spd, which was identical to Agp2p permease catalyzing transport of a set of amino acids. Deletion of the *AGP2* gene reduced sharply the initial rate of Put and Spd uptake and conferred a high resistance toward exogenous PAs to a transporter. *AGP2* is the first gene encoding eukaryotic permease with a high affinity for Spd, which plays a key role in PA uptake by yeast cells.

It was also reported that, in yeast cells, the *YLL028* gene was identified encoding a vacuolar transporter specific for PAs (Tomitori *et al.* 1999). The cells transformed with this gene acquired resistance to PA toxicity, which was suppressed by Bafilomycin A1, the inhibitor of vacuolar H^+-ATPase. In these cells, the vacuolar membrane displayed a highest capacity for PA transport. Some evidence was presented indicating that the membrane protein encoded by the *YLL028* gene was a PA transporter of the tonoplast.

As distinct from prokaryotes and yeast, molecular investigations of plant PA transport systems are early in their development. First studies (Bagni and Pistocchi 1990) were destined to the kinetics of Put transport. Published data are very difficult to interpret because of an extremely high concentration of exogenous diamine (up to 100 mM) and usage of some plant systems (cell suspension, protoplasts, or detached flower petals) that could have transport systems distinct from those in intact plants and tissues. Di Tomaso *et al.* (1992) presented more complete and correct data on the kinetics of Put transport, its subcellular distribution, and excretion, which were obtained on intact maize seedling roots. According to the results of these authors, roots absorbed 0.05 and 1.0 mM Put linearly for 30 to 40 min; the rate of its uptake was 0.35 μmol/g fresh wt. Initially, Put penetrated into the root apoplast, followed by its transport across the plasma membrane. These reports suggest that a portion of the exogenously applied Put is metabolized in maize root cell walls by DAO, but the bulk of the Put is transported across the plasma membrane by a carrier-mediated process, similar to that process for animal systems. It was also shown that Put accumulated in the root-cell vacuoles, which served for this PA storage. From the vacuole, Put could be transported back across the tonoplast and plasma membrane into the apoplast of the cortex and epidermis cells.

A series of studies performed by Italian researchers (Tassoni *et al.* 2002) was destined to general principles of PA specific binding to plasma membrane proteins. This binding can fulfill a dual role: early event of PA signal recognition or binding to a specific transporter. In order to choose between these two possibility, plasma membrane vesicles were isolated from etiolated pumpkin hypocotyls and two Spd-binding proteins (44 and 66 kD) were extracted from them and purified by gel filtration through G-200 Sephadex. No activity of the enzymes of PA biosynthesis (ADC and ODC) was found in vesicles; in contrast, a considerable activity of vanadate-sensitive ATPase, a marker of the plasma membrane, was detected, but this activity was not eluted together with Spd-binding proteins during gel filtration. The authors do not exclude a possibility that such an ATPase activity could correlate with specific Spd binding at the plasma membrane.

Analysis of polyamines in plants

Two main high sensitive methods have been developed for analysis of free PAs in plant extracts based 1) on high performance liquid cromatography (HPLC) of their bensoyl derivatives and 2) on dansylation and thin layer cromatography (TLC) (Flores and Galston (1982).

Put, Cad, Spd, Spm, 1,3-Dap, agmatine and the less uncommon PAs (norspermidine and norspermine) can be completely resolved by reverse phase HPLC. Total PAs are extracted by 5% perchloric acid (PCA) and centrifuged at 10,000 × *g* for 5 min at 20°C. The resuspended pellet and the aliquot of the supernatant were hydrolyzed with 6N HCl for 24 h at 92-94°C in glass soldering ampules in order to release PAs from their conjugated forms (Langebarteis *et al.* 1991). Crude (for free PAs) and hydrolyzed (for PCA-soluble conjugates) supernatant, and hydrolyzed pellet (for PCA-insoluble conjugates). Benzoylation of PAs extracts and analyzis as benzoyl derivatives are performed by reverse phase HPLC as described by Flores and Galston (1982).

Dansylation and TLC analysis described by Flores and Galston (1982) for chromatography of dansylpolyamines on silica gel plates and further for quantitative estimation with spectrophotofluorimeter. Dancyl derivatives of PAs for HPLC have been described by Tassoni *et al*. (2000).

POLYAMINES AND STRESS

Polyamines and plant tolerance

Endogenous content of polyamines in plants and tolerance

Since the time when an active Put accumulation was observed in plants in response to K^+ deficit and salt shock, the investigations of stress-induced changes in PA metabolism and their role in plant responses to abiotic stressors remain to be one of key problems of plant adaptive strategy (Kaur-Sawney *et al.* 2003). Stress-induced accumulation of PAs often correlates with the improvement of plant tolerance (Bouchereau *et al.* 1999). In recent years, the abundance of data concerning stress-dependent PA accumulation appeared. PAs increase survival of various plants under salt stress (Chattopadhayay *et al.* 2002; Roy and Wu 2002; Liu and Moriguchi 2006), chilling stress (Songstad *et al.* 1990; Shen *et al.* 2002), osmotic and acidic stresses (Capell *et al.* 2004), radiation-induced oxidative stress (von Detsch *et al*. 2005), and other stresses.

The application of exogenous PAs (spray of leaves or

root treatment with 1-3 mM) has been useful to identify the association of their stress-accumulation with plant tolerance under abiotic stress. If the synthesis of PA is blocked, cell growth is stopped, and treatment with exogenous PA can restore these processes. Exogenously applied Put (1 mM) could reduce cold-induced (4°C) electrolyte leakage from leaves of wild-type tomato (*Lycopersicon esculentum* Mill) and especially in an ABA-deficient tomato mutant, flacca, to the control level (Kim *et al.* 2002) or could alleviate salt stress (200 mM NaCl) in terms of fresh weight increase of apple *in vitro* callus of *Malus sylvestris* Mill., cv. 'Domestica' (Liu and Moriguchi 2006). All these facts indicate that PAs are tightly involved in stress response and plant tolerance.

Influence of inhibitors on polyamine metabolism and tolerance

Until recently, a principal tool for studing the PA biological role was the use of inhibitors; most of them were initially used in human cancer chemotherapy to suppress PA accumulation in tumors. Most widely used inhibitors of various enzymes of PA biosynthesis or catabolism are difluoromethylornithine (DFMO) for ODC, α-difluormethylarginine (DFMA) for ADC, methylglyoxalbisguanylhydrazine (MGBG) for SAMDC, cyclohexamine (CHA) for SPMS, aminoguanidine (AG) for DAO, and others. These inhibitors are used for switching off separate stages of PA biosynthesis (Walden *et al.* 1997; Bouchereau *et al.* 1999).

The application of DFMA and DFMO helped to establish that ADC and ODC pathways operates in the constitutive Put synthesis under normal conditions, whereas under stress conditions, both ADC and seldom ODC pathways could be activated, resulting in Put accumulation. Plant subjected to different type of stresses show in general a rapid and massive increase of Put levels which can inhibit by application of DFMA (Galston *et al*. 1997). Put reduced cold-induced electrolite leakage from leaves of tomato (*Lycopersicon esculentum* Mill.) but DFMO, a biosynthetic inhibitor of the PA, increased electolite leakage from cold-treated leaves. Furthermore, the DFMO-increased membrane permeability in cold-stressed leaves was completely abolished by the application of Put treatment of leaves (Kim *et al*. 2002). The application of different inhibitors (DFMA, DFMA, MGBG and CHA) in salt-treated (100-300 mM NaCl) leaf discs of *Lycopersicon esculentum* Mill. permitted the appearance of unexpected relations between PA metabolism and osmolyte proline under salt stress (Aziz *et al.* 1998).

The use of inhibitors permitted the elucidation of a compensatory reaction accompanying the switching off some PA biosynthesis, which is of importance for understanding the mechanisms of plant-cell homeostasis, especially under stress conditions (Galston *et al*. 1997). Nevertheless, some limitations of the inhibitory analysis should be mentioned: their possible metabolization in tissues, differences in the rates of their uptake, insufficient specificity determined frequently by differences in the localization of the inhibitor and a target enzyme, injurious effect on membranes, and other drawbacks (Kaur-Sawhney *et al.* 2003).

Tolerance of plant mutants having defects in polyamine metabolism

One of the genetic approaches for the investigation of the mechanisms of PA signal perception and transduction in stressed plants is the biochemical and physiological analysis of mutants displaying different phenotypes. At present, several types of plant mutants with induced changes in PA metabolism were obtained. Among them, there are mutants of tobacco, petunia, tomato, and *Arabidopsis* deficient in PAs and in the genes of their biosynthesis and mutants resistant to PAs and the inhibitors of their biosynthesis. The review of Kakkar and Sawhney (2002) comprises the list of mutants and characteristics of their phenotypic and biochemical defects.

Mutant tobacco lines resistant to MGBG are of interest for establishing the morphogenic role of PA. These mutants display dwarfism and changed morphology of floral organs; they manifest enhanced SAMDC activity and have the expanded PA pool (Fritze *et al.* 1995). In petunia, mutant line with changed flower morphology also showed a high level of endogenous PAs and enhanced ADC activity (Gerats *et al.* 1988). In the leaves of non-flowering tobacco *rmb7* mutant, PA conjugates were not found, which are supposed to be transported to stem apices toward floral buds and induce flowering (Martin-Tanguy 1997, 2001).

Some types of mutants are beneficial for studying the role of PA in stress physiology. Thus, tobacco DFMO-resistant mutant with a high PA concentration was resistant to low pH values inducing an acidic stress in plants (Hiatt and Malmberg 1988). The *flacca*-ABA-deficient tomato mutant is characterized by a high ADC and low ODC activities at late developmental stages, which was accompanied by the reduced total level of PAs. Such a mutant is of importance in the study of interactions between ABA and PAs during adaptation to abiotic factors (Kim *et al.* 2002).

Recently, a group of Japanese researchers described *Arabidopsis* insertion mutants harboring T-DNA for two genes of Spd synthase, *SPDS1*and *SPDS2* (Imai *et al.* 2004). While each mutant allele showed a normal phenotype, *spds1-1 spds2-1* double-mutant seeds were shrunken and had embryos that were arrested morphologically at the heart-torpedo transition stage. This mutation was lethal. These seeds contain a reduced level of Spd and, in contrast, a high level of Put. These data provide the first genetic evidence indicating a critical role of the Spd synthase in plant embryo development. On the basis of these data, we may suppose that a double coding of PA synthesis enzymes in higher plants is essential for plant survival under extreme conditions. At the same time, Imai *et al.* (2004a, 2004b) showed that, as distinct from Spd, Spm was not necessary for *Arabidopsis* normal development. Earlier, it was shown that a disruption of the *ACL5* gene, encoding Spm synthase in *Arabidopsis* and required for stem elongation resulted in a severely dwarfed phenotype (Hanzawa *et al.* 2000). However, exogenous Spm (concentration not specified) could not restore normal stem growth. The authors believe that this is explained by the fact that exogenous Spm did not reach a required intracellular compartment or did not produce a conjugate required for the manifesting of its action.

Tolerance of transgenic plants with a changed polyamine level

At present, other approaches became available for studying the mechanisms of PA biosynthesis as well. One of the promising approaches is the production of transgenic plants harboring the genes encoding enzymes of various pathways for PA biosynthesis. In Kakkar and Sawhney's review (2002), the list of genes controlling PA metabolism in plants, which were characterized and cloned, is presented (**Table 1**). Since the 1990s, studying transgenic plants helped to answer some important questions concerning the control of PA metabolism. Firstly, overexpression or negative regulation of key genes for ODC, ADC, and SAMDC permitted the control of the endogenous Put level. Overexpression of yeast ODC cDNA in tobacco plants or mouse ODC cDNA in tobacco and carrot plants (Bastola and Minocha 1995) increased the level of Put but did not affect the levels of Spd and Spm, as compared to wild-type plants. At the same time, transgenic tobacco leaves expressing human SAMDC cDNA contained much more Spd and Spm and reduced amounts of Put. SAMDC overexpression in transgenic rice plants was accompanied by Spd accumulation and improved salt tolerance as compared to wild-type plants (Roy and Wu 2002). Transgenic rice plants expressing *Datura stramonium ADC* under the control of the monocot *Ubi-1* promotor produced a much higher level of Put under drought stress, only slightly promoting Spd and Spm synthesis and ulti-

Table 1 Genes controlling polyamine metabolic pathways that have been characterized and cloned [Kakkar and Sawhney (2002) with data published within the period of 2002-2006 added].

Gene	Plant name	Reference
ADC, EC 4.1.1.19	Oat	Bell and Malmberg 1990
	Tomato	Rastogi *et al.* 1993
	Pea	Perez-Amador *et al.* 1995
	Arabidopsis	Watson and Malmberg 1996
	Soybean	Nam *et al.* 1997
	Grapevine	Primikirios and Roubelakis-Angelakis 1999
	Carnation	Chang *et al.* 2000
	Populus maximowiczii × *Populus nigra*	Page *et al.* 2005
	Prunus persica	Liu *et al.* 2006
	Vitis vinifera	Moriguchi and Liu 2006
ODC, EC 4.1.1.17	Datura (*Datura stramonium*)	Michael *et al.* 1996
	Tomato	Kwak and Lee 2001
	Glycine max	Delis *et al.* 2005
	Theobroma cacao	Bae and Bailey 2006
SAMDC, EC 4.1.1.50	Potato	Mad Arif *et al.* 1994
	Spinach	Bolle *et al.* 1995
	Periwinkle (*Catharanthus roseus*)	Schroder and Schroder 1995
	Tritordeum (*Hordeum chilense* × *Triticum turgidum*	Dresselhaus *et al.* 1996
	Arabidopsis, rice, maize	Franceschetti *et al.* 2001
	Carnation	Lee *et al.* 1997
	Caenorhabditis elegans	da'Dara and Walter 1998
	Neurospora crassa	Hoyt *et al.* 2000
	Plantago major	Pommerrenig *et al.* 2006
SAM synthase, EC 2.5.1.6	*Arabidopsis*	Peleman *et al.* 1989a, 1989b
	Carnation (*Dianthus caryophyllus*)	Larsen and Woodson 1991
	Parsley (*Petroselinum crispum*)	Kawalleck *et al.* 1992
	Tomato	Espartero *et al.* 1994
	Poplar (*Poplar deltoids* × *P. richocarpa*)	Doorsselaere *et al.* 1993
	Rice	van Breusegem *et al.* 1994
	Petunia	Izhaki *et al.* 1995
	Pea	Gomez-Gomez and Carrasco 1998
	Flax	Lamblin *et al.* 2001
	Plantago major	Pommerrenig *et al.* 2006
Spermidine/spermine synthase, EC 2.5.1.16	*Arabidopsis*	Hashimoto *et al.* 1998; Hanzawa *et al.* 2000
	Plantago major	Pommerrenig *et al.* 2006
Diamine oxidase, EC 1.4.3.6	Lentil (*Lens culinaris medicus*)	Angelini *et al.* 1996
Polyamine oxidase, EC 1.5.3.3	*Medicago sativa*, *Avena sativa*	Koc *et al.* 1995
	Arabidopsis	Totoki *et al.* 2006

mately protecting the plants from drought (Capell *et al.* 2004).

When antisense SAMDC cDNA was inserted into the potato genome, Spd production was sharply reduced, and transgenic tubers displayed a changed phenotype (Kumar and Minocha 1998). In transgenic tobacco plants transformed with the ADC gene from oat under the control of an inducible promoter (Tet-repressor system), increased levels of this gene transcript, ADC activity, and free Put were observed (Masgrau *et al.* 1997). Transgenic plants displayed a changed phenotype: necrotic lesions appeared on their leaves, and growth was retarded, which was induced by a high level of endogenous Put was toxic for plant growth and development. On the other hand, antisense potato transgenes harboring SAMDC cDNA under the control of 35S CaMV promoter displayed an abnormal phenotype (growth retardation, non-flowering plants, leaf chlorosis, etc.) on the background of a decreased SAMDC transcript level, reduced enzyme activity, reduced Put level, but an enhanced Eth evolution. All attempts to obtain transgenic plants with a SAMDC construct in the normal orientation were unsuccessful. This permitted a supposition that constitutive overexpression of this enzyme might be lethal (Kumar and Minocha 1998). At the same time, in order to elucidate specificity in the metabolism and developmental regulation by PAs, it is necessary to change the PA level in various tissues just by expression of sense and antisense constructs under the control of tissue-specific promoters (Martin-Tanguy 2001). In general, the levels of Spd and Spm in cells are least changeable because of the functioning of homeostatic regulation (Kaur-Sauhney *et al.* 2003), which might be related to the supramolecular organization of enzymes involved in their biosyntheses (Blatnagar *et al.* 2002). The transgenic tobacco plants with antisense constructs of cDNAs for senescence-related 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase were more tolerant to abiotic stresses than wild-type plants (Wi and Park 2002). This shows a positive correlation between enhanced PA content and stress tolerance in plants (**Table 2**).

Functioning in plants of two alternative pathways of Put biosynthesis does not exclude a dependence of their regulation on mutual intracellular conversions of their substrates (ornithine and arginine) or their availability. Experiments with the cell line of *Populus nigra* × *maximowiczii* transformed with mouse *ODC* cDNA was destined to elucidate these questions (Bhatnagar *et al.* 2001). In this study, the capacity of plant cells to overexpress a foreign *ODC* gene to maintain a high level of Put by switching on the homeostatic mechanism was demonstrated. This mechanism induced an increased production of ornithine and its precursor glutamate at increased activity of ODC. Earlier, it was shown that transgenic animals, which could not tolerate excessive production of Spd and Spm in their cells, excreted their precursor Put, i.e., PA overproduction induced the cell homeostatic response (Halmekytö *et al.* 1993). In addition, using a transgenic system, it was demonstrated that plant ODC could use as a substrate ornithine synthesized directly from glutamate rather than ornithine produced from arginine in the urea cycle. Thus, the use of transgenic plants helps to decipher compensatory mechanisms in PA metabolism, which could play a great role in the maintenance of PA homeostasis required under stress conditions. The discussed above of ethylene-induced accumulation of Cad in stress-tolerant common ice plants can be interpreted in a similar

Table 2 Transgenic plants containing constructs of PA biosynthetic genes raised through various vectors, and their associated phenotype/biochemical defects [Kakkar and Sawhney (2002) with data published within the period of 2002-2006 added].

Plant	Vector/approach	Phenotype/biochemical alteration	Reference
Tobacco	Overexpression of *S. cerevisiae* ODC using CaMV 35S promoter with doubled enhancer sequence	3-fold increase in ODC activity, increased Put and doubled nicotine levels.	Hamill *et al*. 1990
Tobacco	35S LDC gene construct	Bacterial LDC increased cadaverine and secondary product accumulation. In some transgenic overexpression constructs, the perturbation of the PA pathway was limited by substrate availability, which could be overcome by either exogenous feeding of substrates or targetting to appropriate subcellular compartments.	Herminghaus *et al.* 1991; Fecker *et al.* 1993; Herminghaus *et al.* 1996
Tobacco	Either full length or a truncated mouse ODC cDNA under the control of the 35S promoter	Truncated ODC construct produced higher ODC activities and a 2- to 3-fold increase in Put levels full length ODC produced up to 50% increase in Put, phenotypes similar to MGBG resistant lines such as wrinkled leaves, stunted growth and abnormal flower growth (reduced stamens).	de Scenzo and Minocha 1993
Tobacco	Human SAMDC cDNA using the CaMV 35S promoter	Increased SAMDC activity 9-fold and Spd level 2- to 3-fold; reduced Put level up to 50%, elevated Spm; thick leaves, stems and stunting; most of these abnormalities disappeared after subculture.	Noh and Minocha 1994
Tobacco	Overexpression of oat ADC cDNA using inducible promoter system that could be activated by treating the plants with tetracycline	Primary transformants showed 12- to 20-fold increase in ADC activity after induction; off springs showed 28- to 55-fold increase, phenotype changes were proportional to the Put content; growth inhibition, leaf necrosis, chlorosis, short internodes, thin stems and leaves, and shortened roots; where as no phenotypes were observed if tetracycline-induction occurred after floral growth had begun.	Masgrau *et al.* 1997
Tobacco	Over-expression of the oat ADC cDNA using CaMV 35S promoter with duplicated enhancer sequences and the termination region of CaMV	Transformed plants had 10- to 20-fold increase in ADC activity, but ODC and SAMDC remain unchanged and 20- to 65-fold increase in agmatine levels while Put, Spd and Spm levels were near normal, no phenotypic change observed.	Burtin and Michael 1997
Carrot	Truncated mouse ODC construct	Transgenic cells showed a significant increase in Put level, ODC activity; improve somatic embryogenesis in the auxin-free medium; cells acquired tolerance to DFMA.	Bastola and Minocha 1995
Carrot	Mouse ODC cDNA	Increased Put level via ODC pathway while ADC pathway not significantly affected, high rate of Put catabolism and conversion to Spd and Spm.	Andersen *et al.* 1998
Potato	Sense or antisense SAMDC, using both the 35S constitutive promoter and *Tet*-inducible transcription system	Constitutive over expression constructs failed to survive after the microcallus stage. The 35S antisense construct had 10-28% of the normal SAMDC activity and varying reduction in PA contents. Tet-inducible antisense lines decreased SAMDC activity and PA pools and mRNA levels and increased ethylene (46-fold) evolution after induction. *Tet*-inducible sense lines showed varying increases in SAMDC and PA levels after treatment. Antisense SAMDC plants displayed a range of phenotype after regeneration such ass tunted growth, branched stems, small and chlorotic leaves, small andelongated tubers, poor root growth and no flowering.	Kumar *et al.* 1996
Potato	Transgenic plants containing both sense and antisense SAMDC constructs driven by the tuber specific patatin promoter	Sense transformants expressed higher steady-state levels of the SAMDC-specific transcript andactivity and higher levels of Spd; no overall change in tuber yield. Antisense transformed lines resulted in decrease in SAMDC transcript andactivity and total PA levels, but node decrease in tuber number.	Pedros *et al.* 1999
Arabidopsis	Expression of the ACL5 cDNA under the control of a heat shock gene promoter	Restored the phenotype in a heatshock-dependent manner in *acl5*mutant; inactivation of the gene causes a defect in the elongation of stem internodes.	Hanzawa *et al.* 2000
Pea	With coding sequence for DAO (PSAO-1) in sense and antisense orientation using tissue-specific promoter (pENOD12A)	Transgenic plants showed strong co-suppression of DAO activity in extracts from nodules and epicotyls, however, antisense constructs unaffected, lines showing co-suppression of DAO were less sensitive to the inhibitory effects of exogenous Put.	Wisniewski and Brewin 2000
Poplar (*Populus* nigra × maximowiczii) cells	Mouse ODC cDNA	Transgenic cells showed several-fold higher amounts of Put, a small increase in Spd and a reduction in Spm, whereas exogenously applied ornithine increased Put in both the lines.	Bhatnagar *et al.* 2001
Rice	Oat ADC cDNA in sense orientation under the control of CaMV 35Spromoter	Phenotypically normal transgenic plants showed 2-fold increase in Put level in regenerated plants, but not in seeds.	Capell *et al.* 1998
Rice	Oat ADC cDNA in an antisense orientation for down regulation of ADC and ODC activity	Significant decrease in the levels of Put, Spd but not Spm in the majority of the callus lines. Cell lines having low levels of PAs exhibited normal morphogenic responses. Put level reduced in seeds.	Capell *et al.* 2000
Rice	Oat ADC cDNA constitutively expressed under the control of the maize 1 ubiquitin promoter	No variation in PA levels in vegetative tissue or seeds except in one specific lineage where seeds showed a 10-fold heritable increase in Put accumulation.	Bassie *et al.* 2000
Rice	Oat ADC cDNA under the control of ABA-inducible promoter	Transgenic plants exhibited higher Put (about 200%) and ADC (300-400%) than control plants. Second generation plants showed an increase in biomass under salinity-stressed conditions as compared to non-transformed control plants.	Roy and Wu 2001
Tobacco	Mouse ODC cDNA	Transgenic cells showed several-fold higher amount of Put and the transgenic with their PA metabolism up-graded showed increased tolerance to salt stress. Further, the lines generated had a variable *in vitro* morphogenic potential, which could be correlated to the shifts in their PA metabolism.	Kumria and Rajam 2002

Table 2 (Cont.)

Plant	Vector/approach	Phenotype/biochemical alteration	Reference
Tobacco	Antisense ACC synthase and ACC oxydase cDNA from carnation flowers introduced into tobacco by *Agrobacterium*-mediated transformation	Several transgenic lines showed higher PA contents than wild-type plants. Stress-induced senescence was attenuated in these transgenic plants in terms of total chlorophyll loss and phenotypic changes after oxidative stress with H_2O_2, high salinity, acid stress (pH 3.0), and ABA treatment. Results suggest that these transgenic plants are more tolerant to abiotic stresses than wild-type plants. This showed a positive correlation between PA content and stress tolerance in plants.	Wi and Park 2002
Tobacco	ODC cDNA from *Datura stramonium* plant	Transgenic plant exhibited increases in ODC activity of 25-fold in leaves and 5-fold in flower buds. The increase in Put levels was only1.5-to 2.1-fold in leaves and 1.1- to 1.3-fold in flower buds. No changes to Spd or Spm steady-state levels or to soluble or insoluble hydroxyl cinnamic acid-conjugated PAs were observed. Plant PA homeostatic mechanisms efficiently accommodate increased ODC activity, suggesting that PA biosynthetic control is invested at multiple interdependent steps.	Mayer and Michael 2003
Tice	ADC cDNA from *Daturastramonium* plant	Transgenic plant produce much higher levels of Put under stress, promoting Spd and Spm synthesis on level of steady-state mRNA of genes and ultimately protecting plants from drought.	Capell *et al.* 2004
Arabidopsis thaliana	SPDS cDNA from *Cucurbita ficifolia* plant under the control of the cauliflower mosaicvirus 35S promoter	T2 and T3 transgenic plants exhibited a significant increase in SPDS activity and Spm content in leaves together with enhanced tolerance to various stresses including chilling, freezing, salinity, hyperosmosis, drought, and paraquat toxicity. During exposure to chilling stress (5˚C), the transgenics displayed a remarkable increase in arginine decarboxylase activity and conjugated Spm contents in leaves compared to the wild type. A cDNA microarray analysis revealed that genes for stress-response is transcription factors DREB and stress-protective -proteins (rd29A) were more abundantly transcribed in the transgenic than in the wild type under chilling stress.	Kasukabe *et al.* 2004
Tobacco	PAO cDNA from *Zea mays* and DAO cDNA from *Pisum sativum* under the control of the cauliflower mosaic virus 35S promoter	Both the PAO and the DAO transgenic plants produced high amounts of H_2O_2 only in the presence of exogenously added enzyme substrates. These transgenic plants represent excellent tools to study PA secretion, conjugation and catabolism.	Giuseppina *et al.* 2004
Sweet potato	SPDS c DNA from *Cucurbita ficifolia* under the control of the cauliflower mosaic virus 35S promoter	Transgenic plants showed twice as high Spd content as the wild type counterpart in both leaves and storage roots. Salt and drought stresses suppressed storage root growth, but the transgenic plants were less affected producing significantly larger mass of storage roots and starches than wild plants. SPDS gene is considered useful for gene transfer technology aimlng at improving environmental stress tolerance of sweet potato.	Kasukabe *et al*. 2006

way.

In the opinion of some Arthur Galston coworkers, some discrepancies arising during PA studies with the usage of transgenic plants can depend on various factors: transgene source, effect of its position, recipient plant system, plant material used for transformation, promoter type, and other factors (Kaur-Sauhney *et al*. 2003). A hierarchical PA accumulation in differing transgenic tissues/organs has been studied (Lepri *et al*. 2001). In general, less metabolically active tissues accumulate higher levels of polyamines (Lepri *et al*. 2001). More significant results concerning the control of transgene expression were obtained with inducible or tissue-specific promoters (Mehta *et al*. 2002). Thus, fruit-specific expression of heterologous SAMDC in tomato resulted in ripening-specific accumulation of Spd and Spm which led to an increase in lycopene, prolonged vine life, and enhanced fruit juice quality (Mehta *et al.* 2002). Besides the agronomic interest of this finding, this latter study constitutes one of the most striking evidence regarding the *in vivo* involvement of PAs in a particular developmental process, i.e. fruit ripening.

Polyamines and oxidative stress

Stress-induced oxidative stress is one of the early responses to abiotic factors. Oxidative stress exerts one of the most deleterious effects of environmental stress on plants, which is characterized by the accumulation of harmful reactive oxygen species (ROS) in tissues: $\bullet O_2^-$, H_2O_2, and $OH^\bullet$). These toxic molecules are capable of causing oxidative damage to proteins, DNA, lipids and other molecules (Apel and Hirt 2004). ROS are produced in various cellular components, including chloroplasts, mitochondria, peroxisomes, glyoxysomes, cell wall, plasma membrane, and apoplasts. There are several antioxidant strategies in plants. ROS-scavenging enzymes are superoxide dimutase (SOD), catalase (Cat), glutation reductase (GR), and different peroxidases. Non-enzymatic antioxidants include various plant pigments (carotenoids, tocopherols) and phenolic acids. Numerous reports appeared about stress-induced accumulation of free and conjugated PAs in various plant species. Since the 1990s PA participation in oxyradical detoxification was studied predomonantly in relation to ozone (O_3)-pollution (Bouchereau *et al.* 1999). When exogenous PAs were fed to tomato and tobacco plants, there was a significant suppression of O_3-induced leaf injury (Ormrod and Beckerson 1986). In O_3-treated barley leaves, ADC activity increased before the injury was apparent but when DFMA was applied to the leaves, the rise in ADC activity was prevented and injury caused by exposure to ozone was considerably enhanced (Rowland-Bamford *et al*. 1989). These results suggest that PAs may have a protective role against O_3 damage, but the mechanism involved was not clear. The finding in the work of Drolet *et al.* (1986) showed that PAs may be involved in free radical scavenging. A later study suggested that the protective effect of exogenous free PAs was mediated by their prior conversion to conjugated forms (Langebartels *et al.* 1991).

Free and conjugated polyamines as antioxidants

Antioxidant properties of free PAs were first noted by Drolet *et al* (1986). Then Bors *et al.* (1988) observed that PAs exibited their most significant antioxidant properties when they form conjugates with phenolic acids. Actually, tobacco leaf injury caused by O_3 was weakened by treatment with exogenous free PAs (Put, Spd, or Spm), which caused a 4- to 6-fold increase in soluble conjugated PAs, especially those associated with the cell wall and membrane fractions (Langebartels *et al.* 1991). According to their study, PA

conjugates with coffeic, cinnamic, and ferulic acids displayed a higher constant of binding to ROS than free PAs, which contrasted to early notion about an important role of free PAs as radical scavengers (Drolet *et al.* 1986). It was thus concluded that free PAs could not account for the protection against ozone (O_3) damage as free radical scavengers.

In contrast, reliable proof of free PA functioning as ROS scangers were obtained in experiments performed *in vitro* in a system generating free radicals (Ha *et al.* 1998; Kuznetsov *et al.* 2007). As shown by Kuznetsov *et al.* (2007), when total DNA isolated from leaves of the halophytic plant *M. crystallinum* was incubated in the presence of the $OH^{\bullet}$-generating system, practically no DNA was detected, indicating that the $OH^{\bullet}$ attack led to DNA oxidative degradation. H_2O_2 alone did not attack DNA. The addition of Cad or Spm to the $OH^{\bullet}$-generating system suppressed DNA damage. These PAs inhibited DNA degradation most efficiently at 1-5 mM. The active formation of $OH^{\bullet}$ in plants can occur due to simultaneous accumulation in the cells of H_2O_2 and Fe^{2+} under stress. Recently, it was found that, under salinity conditions, iron accumulated in chloroplasts of leaf parenchymal cells of *M. crystallinum* as ferritin (Paramonova *et al.* 2007).

At the same time, the functional activity of PA conjugates in plant cells is more efficient that those of parent compounds, and often conjugates acquire new properties permitting them to be involved in a wider range of biological processes. Thus, some recent reports contain data about stronger protective antioxidant activity of conjugates than free PAs and phenolic acids (Edreva *et al.* 2007). This means that plants subjected to abiotic stresses not only accumulate free PAs functioning as scavengers of free radicals but also produce their conjugates, which are more efficient antioxidants.

Conjugated polyamines and plant tolerance

Limited information is available concerning the physiological role of PA conjugates in plant responses to various abiotic stresses.

A comparative analysis of the content of free PAs, perchloric (PCA)-soluble and PCA-insoluble conjugated polyamines in mature leaves and roots of the halophytic C_3-CAM species *M. crystallinum* has been carried out with adult plants exposed to salinity (Shevyakova *et al.* 2006a). In adult plants, the process of CAM (Crassulacean Acid Metabolism) induction under salinity is linked with oxidative stress and activation of antioxidant defensive responses (Shevyakova *et al*. 2002; Ślesak *et al*. 2003). It was found that, under normal conditions or salinity (400 mM NaCl), grown leaves contained PCA-insoluble (bound) conjugates of Put, Spd, and especially Spm, which showed a tendency for growth with increased duration of salt treatment (from 1.5 to 48 h).

In roots, all forms of PAs conjugates (PCA-soluble and -insoluble) were detected. In contrast to leaves, in roots the formation of PCA-soluble conjugates of all PAs, except Spm, was decreased under long-term salinity. Under these conditions, the content of bound conjugates of Cad was decreased, but those of Put, Spd, and especially Spm were increased. Thus, under salinity conditions, during functioning of CAM-type of photosynthesis with enhanced ROS formation, *M. crystallinum* could accumulate both free and bound Spm in both leaves and roots, which supports the involvement of this high-molecular polyamine in the development of salt resistance (Shevyakova *et al.* 2006a, 2006b).

A decreased content of conjugated Cad in roots under salinity could be explained by the faster oxidation of free Cad under these conditions. However, the formation of PCA-soluble or PCA-insoluble conjugated Cad was sharply and fast (1.5 h) inhibited by exogenous Cad treatment. This negative effect was removed by exogenous Cad treatment in combination with aminoguanidine (AG), the inhibitor of DAO. Thus, the inhibiting effect of exogenous Cad was induced by the formation of H_2O_2 during oxidative degradation of this diamine by DAO.

Oxidative degradation of polyamines and role of H_2O_2

Only a few publications have focussed on PA oxidative degradation under the effect of abiotic factors, although, as was aforementioned, PAs are one of the sources of hydrogen peroxide, which is one of the most widely spread ROS even under normal conditions. In this sense, the question arises whether H_2O_2 produced in the reactions of PA catabolism contributes much into damaging effects of abiotic stressors on plant cells and whether peroxide can be involved in adaptation processes.

In order to examine the control of the level of endogenous PAs by their oxidative degradation, *Nicotiana tabacum* plants transformed with constructs containing PAO cDNA from *Zea mays* (*MPAO*) and DAO cDNA from *Pisum sativum* (*PcuAO*) were obtained (Rea *et al.* 2004). These studies showed that both types of transgenic plants (MPAO and PcuAO) produced a great amount of H_2O_2 in the presence of exogenous substrates (Spd and Put). In spite of the fact that in tobacco plants both recombinant proteins were actively synthesized and present in the apoplast, like native proteins in wild-type plants, their activities were limited by the low PA content in the intercellular space, which was characteristic of both transgenic and wild-type plants. High activities of DAO and PAO in transgenic plants reduced the level of endogenous free PAs insignificantly. The amount of H_2O_2 produced in the suspension cells from transgenic tobacco leaves after addition of 1 mM Spd into the culture medium was sufficient for triggering the apoptosis program.

These studies with transgenic plants proved experimentally for the first time that modulations in the level of endogenous PAs only slightly depended on their oxidative degradation under normal physiological conditions, which indicates the occurrence of compensatory mechanisms maintaining PA homeostasis in the cells

Since both enzymes of PA oxidative degradation are mainly localized in the apoplast and associated with the cell wall, they are considered H_2O_2-generating systems required for lignification, suberinization, and the formation of cross bridges between the components of the cell wall during plant normal growth and a defensive factor under unfavorable conditions (Cona *et al.* 2003).

Until recently, hydrogen peroxide was often considered only as a toxic metabolite and the cause for programmed cell death (Scandalios 1993). In recent years, our notions about peroxide changed from statement factually claiming its presence in the plant cell to the recognition of its signaling function (Neil *et al.* 2002). Thus, it was established that generation of H_2O_2, a relatively weak oxidizer and a long-living molecule capable of diffusion from the sites of its production to neighboring cells and tissues, could fulfill a signal role in plant adaptation (Neil *et al.* 2002). Plant cells have a rather wide range of peroxide sources: from electron transport chains of chloroplasts and mitochondria to NADPH-oxidase of the plasma membrane; however, these sources differ in their efficiency (Vranova *et al.* 2002). PAs are less studied sources of peroxide. Free and conjugated forms of Spd or Spm are believed to be the most efficient antioxidants, which are considered scavengers of oxyradicals (Drolet *et al.* 1986; Bors *et al.* 1989; Ha *et al*. 1998). The involvement of PAs in oxyradical scavenging is based on the easy oxygen-dependent autooxidation and enzymatic oxidation of amino groups catalyzed by DAO and PAO and also on the PA capability of accumulation under stress conditions. However, a high level of endogenous PAs and plant tolerance to oxidative stress can be based not only on stress-induced but also on the constitutively high PA biosynthesis. In such plants resistant to oxidative stressors, in particular to paraquat (methylviologen), whose breakdown results in

the formation of $\bullet O_2^-$, a high level of constitutive synthesis of both ADC and ODC was found, and the content of PAs was by two to three times higher than in the sensitive cultivar (Ye *et al.* 1997). In this case, as it was shown for a resistant *Conyza bonariensis* biotype, plant pretreatment with paraquat did not induce the accumulation of Put and Spd but activated antioxidant enzymes. Similar pattern was observed for wheat cultivar displaying cross-reactivity to drought and paraquat and for another species of *Coniza (C. canadensis*) resistant to paraquat. Moreover, only in resistant biotypes, the activities of antioxidant enzymes were high. Treatment of such plants with Put improved further resistance to oxidative stress, but this effect was not observed for a sensitive biotype.

The observed involvement of constitutively high activity of enzymes for PA biosynthesis in plant defense against oxidative stress motivates studies of PA metabolism and their role in naturally tolerant ecological groups of plants, such as halophytes, xerophytes, and heavy-metal accumulators.

Polyamines as regulators of antioxidant enzymes

One of the manifestations of the antioxidant effect of PAs is their ability to regulate the expression of genes encoding antioxidant enzymes. The plant defence antioxidant system comprises a wide spectrum of enzymes.

We have discussed above that PAs are the components of the cellular antioxidant systems being ragarded as scavengers of oxygen radicals. However, the mechanisms of their antioxidant action are still poorly understood. It was shown that the involvement of PAs in ROS scavenging is based on their ability to form soluble conjugates with various phenol derivatives. It cannot be also ruled out that functioning of PAs in oxidative stress is mediated by H_2O_2 produced during their oxidative degradation and playing a signal role.

One of the manifestations of the PA antioxidant effect is their ability to regulate the expression of genes encoding antioxidant enzymes. In transgenic plants, Spd-induced oxidative stress was clearly transient with a highest development in 30 min after the addition of exogenous PA; it required novel proteins, putatively with antioxidant activity (Rea *et al.* 2004). Recently, the capacity of PAs to induce expression of antioxidant genes was demonstrated for Spd in the case of peroxidase in tobacco plants (Hiraga *et al*. 2000) and for Cad in the case of SOD in the roots of the halophyte *M. crystallinum* (Aronova *et al.* 2005).

It was shown that high concentrations of exogenous Cad or Spd (5 mM, 2 h) had no direct effect on the activity of SOD isoforms in the leaves of *M. cristallinum*, but exogenouse H_2O_2 could completely inhibit their activities (Aromova *et al.* 2005). However, there were different organ-specific regulatory mechanisms of Cad effects on the cytoplasmic isoform of Cu/Zn-SOD. Cad (1 mM, 24 h) could enhance activity of this isoform in roots, but not in leaves, and, moreover, it could induce the expression of the gene encoding this enzyme. In this study, it was shown that PAs exhibit their antioxidant effects by inducing the expression of the gene encoding SOD, one of the main antioxidant enzymes, at the level of synthesis of its mRNA.

Thus, some data appeared (Hiraga *et al.* 2000; Aronova *et al*. 2005), which make it possible to consider PAs not only as ROS scavengers but also as activators of the expression of genes encoding antioxidant enzymes.

Multifunctional interactions between polyamines of putrescine family and stress hormones

In the first years after PA detection in plant cells and the onset of active investigations concerning their physiological role, a great attention was paid to PA capability of involving in the initiation of cell division and expansion, in plant morphogenesis, flowering, and senescence (Galston *et al.* 1997). Also a great attention was paid to direct and indirect interactions between PAs and growth-inducing phytohormones. Thus, in some plants, auxins, gibberellins, and cytokinins stimulated biosynthesis and increased the content of PAs, whereas exogenous PAs affected the level of endogenous phytohormones. In one of the recent works performed with *Arabidopsis*, it was shown that IAA induced the *ACL5* gene encoding Spm synthase, but ABA or gibberellic acid could not induce this gene (Hanzawa *et al.* 2000). Inactivation of this gene retarded stem elongation and suppressed cell expansion. It is well known that this mechanism can operate during plant adaptation to extreme conditions when the retardation of growth processes is required for plant survival (Hasegawa *et al.* 2000). Regardless of recent reports concerning PA involvement in varions abiotic stresses, little is known about stress signaling pathways regulating PA metabolism.

A typical example of multifunctional interactions in plants operating under stress conditions is those between PAs of Put family (Spd and Spm) and ABA or Eth. Both hormones are considered as stress hormones (Zeevaart and Creelman 1988), although they may fulfill several other functions in the absence of stress.

Interaction between polyamines and abscisic acid

It is well known that ABA concentration increases under water or salt stress as well as under other abiotic stresses (Christmann *et al*. 2005). ABA induces the expression of multiple genes involved in defence against water and salt stresses (Wang *et al.* 2002b; Bartels and Soer 2004). It has also been noted that ABA induces thermotolerance of some plants (Gong *et al*. 1998). Thus, plant adaptation process is, in large part, mediated by ABA.

ABA is synthesized from carotenoid by ABA-synthesizing enzymes induced in root tip cells or parenchyma cells of vascular bundles by drought and salt stresses (Koiwai *et al.* 2004). ABA synthesized in the roots enters the xylem vessels and from here is transported to the leaves (Sauter *et al*. 2002). It is very important for plant stress adaptation because the root systems of any plant are the first barrier immediately counteracting salinity, water deficit, and other environmental stresses. The roots are less capable of basic biosyntheses as compared to leaves, but they could synthesize ABA, PAs (Shevyakova *et al.* 2006b), and various other secondary metabolites, such as phenolic acids, alkaloids, and others.

It was recently established that, in *A. thaliana*, ABA can induce PAs biosyntheses especially under water and salt stresses (Kasinathan and Wingler 2004; Urano *et al*. 2004; Alcázar *et al.* 2006). The application of exogenous ABA was useful to identify stress signaling pathways regulating PA metabolism. Unlike other plant species, *Arabidopsis* does not contain ODC (Hanfrey *et al*. 2001). It was established that *Arabidopsis* contains doubled genes encoding ADC (*ADC1* and *ADC2*), SPDS (*SPDS1* and *SPDS2*), and SPMS (*SPMS* and *ACL5*) (Hanzawa *et al.* 2000; Panicot *et al.* 2002).

The possible role of exogenous ABA in the modulation of PA metabolism under water stress conditions was studied in the *A. thaliana* plants (Urano *et al*. 2003). The application of exogenous ABA induced expression of the *ADC2* but not *ADC1* gene. It was demonstrated that exogenous ABA upregulated *SPMS* expression (Hanzawa *et al.* 2002; Urano *et al*. 2003), but did not affect *ACL5*. In different plant species, SAMS is encoded by three isogenes (*SAM1*, *SAM2*, and *SAM3*), and they are expressed differently in response to ABA treatment, salt stress, and osmotic stress (Espartero *et al.* 1994).

Alcázar *et al.* (2006) also indicated that ABA modulated PA metabolism at the transcriptional level in response to water deficit by upregulating *ADC2*, *SPDS1*, and *SPMS* expression. As was shown in this work, the highest expression of these genes was induced by water stress, with maximum induction after 8-24 h of treatment. Thus, *ADC2* and *SPDS1* expression increased, respectively, 32- and 25-fold

after 8 h, and *SPMS* was induced 75-fold after 24 h. These expression increases were not observed in non-stressed well-watered plants. In water-stressed plants, the free Put level increased up to 1.8-fold. The levels of free Spd and Spm did not increase above the constitutive level during 24 h of stress treatment. In contrast, a transient depletion in free Spd and Spm levels occured during the first hour of water deficit. However, transient depletions in the free forms occured in parallel to increased insoluble conjugated acid-soluble fraction levels. Thus, these conjugated forms of Spd and Spm increased 8.3- and 16-fold, respectively, in the first hour of treatment. These responses were not observed in non-stressed plants.The involvement of ABA in the transcriptional regulation of PA metabolism under dehydration was confirmed by expression profiling anslyses in ABA-deficient (*aba2-3*) and ABA-insentitive (*abi1-1*) mutants. The authors reasoned that the levels of free Put were subjected to homeostatic control to maintain its level within a non-toxic range.

Interaction between ethylene and polyamines of putrescine family

Eth and PAs are regulators of diverse responses manifested during plant growth and development (Wang *et al*. 2002a). Eth content was drastically increased in plants by drought, salt stress, abnormal temperature, wounding, and UV-B irradiation (Rakitina *et al*. 2004). PAs are universal multifunctional regulators of physiological processes during plant growth and development and are also involved in severe stress responses. However, the functions of PAs and Eth in stressed plants are still obscure (Kuznetsov *et al*. 2007). At the same time, it is known that main natural PAs (Put, Spd, and Spm) interact closely with Eth in the regulatory proceess (Galston *et al*. 1997; Kaur-Sawhney *et al*. 2003).

S-Adenosyl-L-methionine (SAM) takes part in the final biothynthetic stages of these PAs, and it is also an Eth precursor (Adams and Yang 1979). It might be the cause for Eth-induced down-regulation of PA synthesis observed by some researchers (Evans and Malmberg 1989). Along with a competition for a common precursor, other interactions could arise between PAs and Eth under stress conditions; they are manifested in mutual inhibition of their biosyntheses, which became a basis for the competition between major PAs and ethylene (Galston et al. 1997). Such an interaction takes an especial place in the coordination of physiological processes because PAs and ethylene often exert opposite effects, and have thus often been termed antisenescence compounds (Bagni and Tassoni 2006). For example, Spd retards senescence, whereas ethylene accelerates it (Galston *et al.* 1997). It was demonstrated that the facultative halophyte *M. crystallinum* responded to heat shock with a decrease of Put family, especially Spd, contents as well as with a fast and transient increase in ethylene evolution (Kuznetsov *et al.* 2001). In contrast, heat shock treatment of cotton plants resulted in PA accumulation in leaves and a transient acceleration of ethylene evolution (Kuznetsov *et al*. 1991). In experiments involving cut carnation var. 'Reiko' and gerbera var. 'Lisa' flowers, Bagni and Tassoni (2006) showed how senescence could be delayed when 10 mM Spd was added to the watering solution, but none when flowers were sprayed.

To understand SAM functional role as an intermediate in PA and ethylene biosyntheses, we should take into account the following points: (1) SAM is actively used in plant cells as a main donor for transmethylation of proteins, nucleic acids, polysaccharides, and fatty acids, and (2) 5-methylthioadenosine (MTA), a byproduct of SAM degradation during synthesis of Spd, Spm, and ACC, can be recycled by MTA nucleosidase into methionine and further into SAM (Wang *et al*. 2002a), i.e., SAM is positioned of major PAs (Spd and Spm), it should be kept in mind that, in stressed plants, the pool of SAM could increase due to stress-induced accumulation of *S*-adenosylmethionine synthetase (SAMS) transcripts (Espartero *et al*. 1994), i.e., under stress conditions, SAM homeostasis coud be maintained to increase the plant adaptive potential.

Stress ethylene and biosynthesis of cadaverine

Some researchers demonstrated that interaction between PAs and Eth could not be limited only by their antagonism. Thus, pea seedlings responded to Eth treatment by a reduced activity of ADC and increased activity of LDC and increased content of Cad (Apelbaum *et al*. 1985). The stimulatory effect of Eth on Cad biosynthesis did not attract attention for a long time, although processes of their biosyntheses are indirectly interconnected because Cad is formed in the side branch of the aspartate pathway resulting in biosynthesis of methionine and SAM. In its turn, SAM is required for the formation of ACC, a precursor of Eth.

A facultative halophyte *M. crystallinum* turned out to be a very convenient model for investigating the interaction between Cad and Eth under stress conditions. In this plant, aspartate, a distant precursor of lysine, is one of the main metabolites produced from oxalacetic acid during CO_2 assimilation in CAM-photosynthesis. It was demonstrated that, in the common ice plants, stress-induced Cad accumulation coincided with the developmental stage when plants transited from C_3- to CAM-photosynthesis (Shevyakova *et al*. 2001; Kuznetsov *et al*. 2002). In this period, the common ice plants responded to heat shock (HS) by a transient Eth evolution and a subsequent interorgan translocation of Cad. Under NaCl salinity, the level of endogenous Eth in plants increased and Cad accumulated in the leaves. To confirm a possible connection of HS-induced Cad translocation from the leaves to the roots with transient Eth evolution, two lines of *A. thaliana* were used as model plants: wild type (Col-0) and a mutant (*ein4*) displaying disturbed Eth reception. It was established that HS-induced interorgan translocation of Cad, as distinct from Put and Spd, was related to the functioning of the Eth reception system. Eth-dependent Cad formation was proven by this diamine accumulation in detached leaves of the common ice plant exposed to the atmosphere of Eth or incubated in the presence of its precursor, ACC (Kuznetsov *et al*. 2002; Shevyakova *et al*. 2004). The phenomenon of Eth-dependent Cad accumulation permitted a study of putative mechanisms of hormonal signal transduction, which were not examined until recently. It was supposed that protein phosphorylation was involved in Eth signal transduction, like in other signaling pathways. All inhibitors tested abolished a stimulatory effect of Eth on the LDC activity, and this was the first unambiguous proof of the involvement of protein phosphorylation/dephosphorylation in the Eth-induced Cad formation in plants.

It is evident from the results obtained that, along with competitive interrelations between major PAs (Spd and Spm) and Eth, which could be manifested under stress conditions, the interaction between Cad and Eth may be rather evaluated as synergistic (**Fig. 3**). The phenomenon observed permits a fresh insight into the problem of compensatory reactions maintaining PA homeostasis required for plant survival under stress conditions. However, Cad accumulation and an increased level of endogenous Eth in the common ice plants in the period of CAM-photosynthesis functioning did not affect expression of the gene encoding a key enzyme of CAM metabolism, PEPC (Shorina *et al*. 2005). Cad accumulation occurred at the later stages of the common ice plant development and was not associated with CAM induction providing for plant adaptation to water deficit. The mechanism of the common ice plant adaptation to salt stress related to Cad accumulation might be as follows: the high Cad concentrations retard cell wall expansion supplying H_2O_2 for suberinization and lignification and thus reducing cell wall permeability for salts. This conclusion arises from the analysis of phenotypical responses of the common ice plant seedlings to various concentrations of exogenous Cad (Kuznetsov *et al*. 2007), Cad-depended

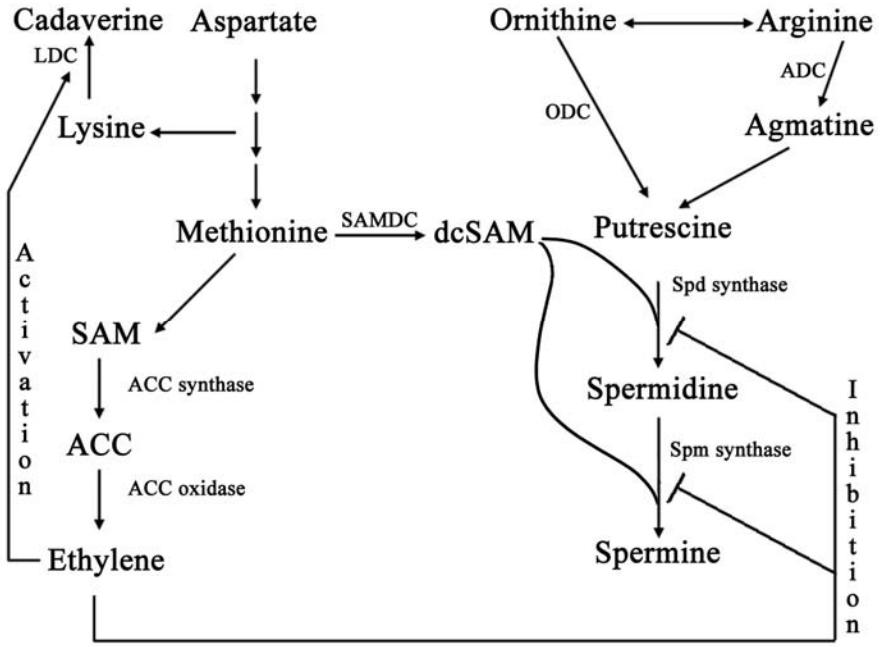

Fig. 3 Polyamine biosynthesis in the common ice plants and its possible regulation by ethylene. Cadaverine synthesis catalyzed by lysine decarboxylase (LDC), which is induced by the ethylene of accumulating in plants under stress. Stress ethylene, on the other hand, can inhibit activity of spermidine syntase and spermine synthase that leads to the reduction of this polyamine content in leaves.

induction of Cu/Zn-SOD gene in roots (Aronova *et al*. 2005; Kuznetsov *et al*. 2007), and exogenous diamine-caused formation of suberine plates in the cell wall (Paramonova *et al*. 2003).

Molecular mechanisms of polyamine protective activity

The abundance of information concerning stress-dependent PA accumulation raises the question as to their possible biological role in the adaptation process.

Most widely accepted and experimentally proved view is that PAs exert their protective action due to their chemical structure, i.e., as polycations. This is largely determined by the shift of their electron density toward nitrogen atoms under physiological pH values; therefore, PAs behave as bases (pK = 9-11). This explains the readiness of PA electrostatic interaction with negatively charged phosphate groups of phospholipids and nucleic acids and with carboxylic groups of proteins and also the PA capability of covalent binding with proteins at the stage of their post-translational modification (Galston *et al*. 1997; Walden *et al*. 1997; Bouchereau *et al*. 1999).

Such defensive properties are ascribed primarily to high-molecular-weight PAs (Spd and Spm) (Kakkar and Sawney 2002) and unusual multipolyamines with longer and often branched molecules, which efficiency is directly related to the increased number of amino groups in their molecules (Bagga *et al*. 1997). PA binding to proteins or nucleic acids not only protects them from degradation but also provides a molecule with the most stable conformation under stress conditions. Thus, Spd and Spm retard cell aging, which is accelerated under stress conditions, due to suppression of the enzymes degrading biopolymers (DNases, RNases, and proteases) and prevent chlorophyll breakdown (Kushad and Dumbroff 1991). Exogenous application of Spd stabilized a native structure of thylakoid proteins D1 and D2, cytochromes, and also a key photosynthetic enzyme Rubisco in oat plants subjected to osmotic stress (Tiburcio *et al*. 1994).

All PAs are capable of binding to A- and B-DNA: in A-DNA, binding occurs mainly to the major groove, whereas in B-DNA, Put and Cad bind to sugar-phosphate backbone and Spd and Spm, which contain more amino groups, bind to both sugar-phosphate backbone and major and minor grooves (Bryson and Greenall 2000). Experiments with B-DNA differing in the guanine to cytosine ratio showed that high-molecular-weight PAs interacted mainly with phosphate groups and did not affect a native secondary structure of DNA, thus providing for normal transcription of stress-induced genes. Such interaction was evidently unspecific and did not almost depend on DNA nucleotide sequence (Deng *et al*. 2000). PAs could inhibit DNA methylation, which permits expression of specific genes responsible for the synthesis of stress proteins (Ruiz-Herrera *et al*. 1995). Spm and to a lesser degree Spd are capable of shifting a dynamic equilibrium between B- and Z-DNA and are involved in DNA spiralization. PA protective role is manifested in their capability of neutralizing the action of ROS dangerous for the cell structures and accumulated under the effect of various abiotic and biotic stresses (Ha *et al*. 1998; Aronova *et al*. 2005; Kuznetsov *et al*. 2007).

Recently, it was found that PAs could substantially affect the conductivity of ionic channels in plants. Thus, Put, Spd, and Spm blocked fast and slow vacuolar channels, including calcium channels, and the effect was proportional to PA charge ($Spm^{+4} > Spd^{+3} > Put^{+2}$) (Dobrovinskaya *et al*. 1999). The capability of biogenic amines to affect stomatal conductivity under stress conditions was also connected with their charges. It was shown that this universal for plants physiological response to stress was based on the PA-induced blockage of potassium channels in the plasma membrane of guard cells, which increased their turgor and, as a consequence, resulted in decreasing the stomatal aperture. In particular, PAs blocked potassium channel in the plasma membrane into the mesophyll cells harboring the *KAT1* gene encoding one of such channels. It is of interest that, in spite of induction of one and the same response by PAs and ABA, the underlying mechanisms are different because ABA inhibits inward potassium channels. PAs also affected stomata closure when penetrated into the cytosol, implying the presence of an intermediate cytoplasmic factor involved in the induction of this response (Alcázar *et al*. 2006). PA control of ionic channels might be adaptive under stress conditions. Thus, potassium channels are efficient regulators of cell stimulation and a major target for extracellular and intracellular factors. Blocking potassium channels with Spd was shown to be a major impulse permitting for adaptation of cell stimulation in response to numerous biological stimuli. It was established, for example, that spinach ADC was associated with LHC of photosystem II (Borrell *et al*. 1995; Legoska and Zaichert 1999). PAs synthesized in chloroplasts evidently stabilize photosynthetic complexes of thylakoid membranes under stress conditions (Borrell *et al*. 1995). The regulatory role of PAs manifesting in the activation of protein and nucleic acid syntheses was demonstrated in both prokaryotes and euka-

ryotes (Bouchereau *et al.* 1999).

Plant cell metabolism is changed to prevent damaging consequences of stressor action (Kuznetsov and Shevyakova 1999). This is attained by realization of two pathways of living organism adaptation to extreme factors operating simultaneously or successively: (1) induction of the synthesis of new macromolecules with new properties, which provide for a normal proceeding of the cell metabolism under stress conditions, and (2) optimization of the intracellular medium for functioning of the enzyme systems due to the accumulation of low-molecular-weight organic compounds with protective and/or osmoregulatory properties. Both pathways of adaptation are directed to the solving the same tasks, namely, organism providing with energy, reductants, precursors of nucleic acids and proteins, and also to the maintenance of cell regulatory system functioning under stress conditions.

In spite of a large progress in the elucidation of mechanisms of PA anabolism and catabolism in the plant cell, a general scheme of the controlling of the PA endogenous level under stress conditions is not yet suggested. Considering the sum of published data, we can state that stressors induce a transient accumulation of free PAs in plants during first minutes and hours of stress; thereafter, days are necessary to maintain PA homeostasis in the cells at the level required for the development of long-term plant adaptation to stress. The time course of changes in PA metabolism in the plant cell can be described as a primary response to rapid disturbances dangerous for plant life: turgor loss and ROS generation. These events activate the signaling cascades inducing a transient Put synthesis in stress-sensitive plant species. In stress-tolerant species, the Spd and Spm levels required for long-term plant adaptation to stress is maintained constitutively by high activities of the genes encoding enzymes of their biosynthesis (Kasukabe *et al*. 2004; Maiale *et al*. 2004). The level of Put decreases because of its consumption as a precursor in these syntheses. In Cad-containing stress-tolerant plant species, increased levels of Spm and Cad are maintained, compensating a reduced level of Put in the cells.

Polyamines and accumulation of compatible stress-induced osmolytes

One of the strategies of plant survival under conditions of abiotic stresses is the accumulation of compatible solutes (proline, citrulline, glycine betaine, mannitol, pinitol, sucrose, and others). Compatible solutes contribute to stress tolerance by acting as osmoregulators, because their high solubility in water acts as a substitute for water molecules under water deficit. High concentrations of compatible solutes can increase the cellular osmotic pressure (Kusnetsov and Shevyakova 1999). Moreover, their hydrophilicity helps maintain the turgor pressure and water content of the cells and protect them against water loss from leaves under drought or salinity. Compatible solutes, because of their extremely hydrophilicity, might also replace water molecules around nucleic acids, proteins, and membranes during water shortage. It is thought that compatible solutes become soluble at high concentations without inhibition of other cellular components. However, their accumulation is tightly controlled because their too high concentrations could be toxic to cells (Yokota *et al*. 2006). Compatible solutes also act as active oxygen scavengers or thermostabilizers (Kaushik and Bhat 2003).

Stress-induced PAs are also often considered as compatible solutes because many their properties (hydrophilicity, protection of macromolecules, active oxygen scavengers, the involvement in the system maintaning the cell pH-stat, and others) are close to those of proline and other osmolytes. However, the stress-induced PA concentrations are by 1-2 orders of magnitude lower than those of proline and other osmolytes, i.e., it is not likely that PAs play a role of osmoregulators. At the same time, some of the products of PA catabolism, β-alanine for example, could be converted into β-alanine betaine. It is required for the osmoregulation in some halophytes, *Limonium tataricum* for example (Duhazè *et al*. 2002).

Many researchers suppose a close correlation between increased levels of cellular proline and PAs. It is found that DFMA (difluoromethylarginine), which blocks Put synthsis from arginine in tomato explants under both control and stress conditions, also inhibits proline accumulation (Bouchereau *et al.* 1999). DFMO (difluoromethylornithine), which blocks Put synthesis from ornithine, does not exert a significant effect on proline content. In accordance with these findings, Put exogenously supplied at low concentrations stimulates the accumulation of proline. PA accumulation in plants subjected to osmotic stress could be required for transduction of the osmotic signal (Erdei *et al*. 1996). PAs were shown to suppress plant responses to osmotic stress (Bouchereau *et al.* 1999). According to our investigations (Radukina *et al*. 2007), one of the reason of inverse correlation between PA and proline accumulation levels could be a deficit of the common precursor in some plant species.

Polyamines as components of stress-signaling systems

PAs are supposed to mediate phytohormone signaling, i.e., fulfill the role of second messengers (Galston *et al.* 1997; Kakkar and Sawhney 2002). Experimental evidence for this PA function was first obtained for animal cells. As early as in the 1983, it was shown for the first time for mouse kidney cortex that a transient PA (Put, Spd, and Spm) accumulation induced by an animal hormone testosterone generated a Ca^{2+} signal via its enhanced exit into the cytoplasm from the reserve membrane pool (Koenig *et al.* 1983). Later, in the work with cultured animal cells, it was shown that, along with the control of the intracellular Ca^{2+} level, PAs were involved in the hormonal signal transduction via their binding to G-proteins, which activated hormone recognition by the receptor (Bueb *et al.* 1992). Spd and Spm could function as blockers of potassium channels in the plasma membrane and ionic channels in the tonoplast (Dobrovinskaya *et al.* 1999).

The role of PAs as second messengers in plants was recently demonstrated in the series of studies by Messiaen and van Cutsem (1999). These authors gave attention to the presence of PAs in the cell walls, where they produced complexes with acidic polysaccharides (pectins); these complexes were considered earlier as one of the factors in the control of pH, thus affecting cell expansion, or in the control of methylesterase activity in the cell walls. It was also known from some studies that pectin fragments (α-1,4-oligogalacturonides), which formation is catalyzed by methylesterases, were capable of modulation of various morphological and physiological processes in the cell walls and at the level of the whole plant, in particular in defensive responses (Bellincampi *et al.* 1995). In the laboratory of Messiaen (Messiaen and van Cutsem 1999), it was demonstrated in cultured carrot cells that a low concentration (10^{-6} M) of a pectin fragment produced a calcium-induced favorable supramolecular conformation, which was recognized by cells as a signal molecule controlling lignification and hydrogen peroxide generation in the cell wall matrix. Messiaen and van Cutsem (1999) supposed that pectin-PA complexes produced in the cell walls helped the recognition of pectins by methylesterases. However, in experiments on PA binding to isolated carrot cell walls and to polygalacturonides, it was found that PAs (Spd^{3+} and Spm^{4+}) with a high affinity for galacturonides and Ca^{2+} blocked the formation of Ca^{2+}-induced supramolecular conformation of pectin fragments, underlying their signaling activity. The results obtained indicate that plant PAs could function as second messengers modulating pectin signal transduction and thus affecting various morphological and physiological processes in the cell walls and protoplasts of plant cells.

However, PA involvement in the maintenance of plant

growth and development and their interaction with phytohormones was not studied properly at the molecular level. Recently, Hanzawa *et al.* (2000) isolated the *ACL5* gene required for internodal elongation of the *Arabidopsis* stem and gibberellin signaling pathway; this gene encodes proteins with PA-synthesizing activity. Thus, in the model system studied, PAs could support phytohormone action as a component of their signaling pathways, and, therefore, they are considered second messengers in accordance with previously made statements (Kakkar and Sawhney 2002).

In recent years, some reports appeared about more complex character of interaction between some phytohormones and PAs (Rakova and Romanov 2005). Thus, it was shown that PAs could block rapid cytokinin-induced effects based on the expression of the genes of cytokinin primary response (Rakova and Romanov 2005). In this work, amaranth seedlings accumulating betacyanine in response to cytokinin treatment and transgenic *Arabidopsis* plants harboring the reporter *GUS* gene under the control of cytokinin-dependent P_{ARRS} promoter were used as model systems. In both systems, all PAs tested (Put, Spd, Spm, and Cad), especially Put and Spm, inhibited the accumulation of amaranthine and activity of the *GUS* gene induced by 5 μM benzyladenine. The PA action manifested at the post-transcriptional level, not affecting the cytokinin-dependent mRNA accumulation. These data showed that PAs did not behave as second messengers of cytokinins in the model system used by the authors, as distinct from earlier suppositions, and did not affect total membrane receptor protein, as was supposed in some works. The physiological role of PA-induced inhibition of cytokinin effects could be in the compensatory regulation of the intracellular cytokinin content when their concentration became excessive.

In contrast to the trustworthy establishment of the PA role in plant defense against various abiotic and biotic stresses, the involvement of PAs in the signal transduction in plants was detected only in some cases (Messiaen and van Catsem (1999), whereas in animals and bacteria, it was reliably shown (Bueb *et al.* 1992).

A great interest was manifested to PAs as modulators of gene expression under oxidative stress. In an exponentially growing culture of *E. coli,* physiological concentrations of Put and Spd significantly increased expression of oxyR and KatG genes responsible for defence against oxidative stress (Tkachenko and Nesterova 2003). It was shown that expression of these genes depended on stimulation PA-induced DNA supercoiling. There are publications, which indicated that PAs interact with DNA phosphate groups, thus protecting the genomic DNA from digestion by DNase and play a crucial role in genomic DNA protection and conformation (D'Agostino *et al.* 2005). PAs are involved in many cellular processes, including chromatin condensation, maintanance of DNA structure, RNA processing, translation, and protein activation (Childs *et al.* 2004). The level of PAs in the cell directly affects the range of genes expressed in response to both growth stimulating and growth inhibiting agents (Lindemose *et al.* 2005). It is demodstrated that PAs affect gene expression at the transcriptional level and this effect is most probably determined by the direct interaction of PAs with DNA and/or transacting protein factors (Wang *et al.* 2002b; Lindemose *et al*. 2005). In plants PAs were found to activate protein phosphorylation and the activities of definite protein kinases (Tassoni *et al.* 1998).

All these properties of PAs can play a crucial role in ameliorating plant responses to abiotic stresses and PA functioning as components of stress-signaling systems.

PA signaling function in plant defense against oxidative stress is the first important process. Despite the continued interest in the role of PAs in plants exposed to biotic stresses, only limited information is currently available.

Recently, it was found that 1 mM Cad added to the nutrient medium for halophytic plant *M. crystallinum* for 2 h induced transcription of the gene for cytoplasmic Cu/Zn SOD form (Aronova *et al.* 2005). The addition of the inhibitor of diamine oxidative degradation, 1 mM AG, along with Cad to nutrient medium did not reduced the level of mRNA, which indicates that non-oxidized diamine affected this gene transcription. Root treatment with 1 mM H_2O_2 increased the level of mRNA as well, but to a lesser degree. This supports a previously suggested hypothesis (Kuznetsov *et al*. 2002) that stress-induced Cad accumulation in the common ice plants and its capability of long-term transport permitted Cad to play a role of a stress signal, which switches on the plant defense mechanism directed, in this case, to the improvement of cell antioxidant activity. In this connection, it should be mentioned that ROS generation by the common ice plant increased sharply during the period of CAM-photosynthesis, which, in its turn, activated antioxidant systems. On the other hand, an enhanced transcription of the genes for antioxidant enzymes did not always correlate with the activity of corresponding enzymes. A possible cause for this discrepancy might be direct effects of peroxide on the activities of peroxide-sensitive enzymes. In the common ice plants, such an enzyme might be Cu/Zn SOD isoform located in the apoplast. We demonstrated that, after the treatment of the common ice plants with low concentrations of Cad and Spm (below 1 mM), PAs behaved as antioxidants, whereas high PA concentrations manifested prooxidant properties due to the active formation of peroxide and increased pH (>7.0) in the apoplast. In this case, PAs facilitated the reverse reaction with the formation of superoxide radical ($\bullet O_2^-$) from H_2O_2. Superoxide radical produced during a burst of PA oxidative degradation could serve a signal for enhanced transcription of the corresponding gene. Switching on such a compensatory mechanism is evidently typical for the functioning of defense systems in stress-resistant plant species.

Recent studies provide evidence that Spm is an endogenous inducer and novel signal transmitter in defense responses against phytopathogens (Takahashi *et al*. 2004). Some authors implicate PAs, emerging intracellular signaling molecules, as potential physiological regulators of the anti-apoptotic action in animal cells (Kutuzov *et al*. 2005).

Investigations of the Takahashi group (Takahashi *et al*. 2004) showed that exogenously appliied Spm specifically activated the expression of five hypersensitive response (HR) marker genes (*HSR203J*, *HMGR*, *HSR201*, *HSR515*, and harpin-induced 1 (*NIN1*)) in tobacco leaves. Induction of these HR marker genes, including *NIN1*, by Spm was suppressed by pretreatment with antioxidants (calcium channel blockers, inhibitor of the mitochondrial permeability transition pore openings (mitochondial dysfunction), and blockers of amine oxidase (AO) or polyamine oxidase (PAO). Free Spm was accumulated in the intercellular spaces during tobacco mosaic virus (TMV)-triggered HR Under these conditions, Spm is catabolized by AO and/or PAO localized in the apoplastic spaces, resulting in the production of H_2O_2 and initiation of Ca^{2+} flux into the cytosol. The downstream reaction of dysfunction of this organelle is the upregulation of HR marker genes by pathways separated into at least two branches, namely, one independent of and the other dependent on the activation of two mitogen-activated protein kinases (MAPKs): salicylic acid-induced (SIPK) and wound-induced (WIPK) protein kinases. Based on the carried out investigations, Takahashi *et al.* (2004) proposed the following model, presented in **Fig. 4**, for Spm signal trunsduction pathways in tobacco in response to pathogen attack, which lead to defence against programmed cell death during HR.

The above described signaling pathways with Spm participating in a plant response to abiotic and biotic stresses are the sole revealed factors, which are required to elucidate the defensive role of Spm.

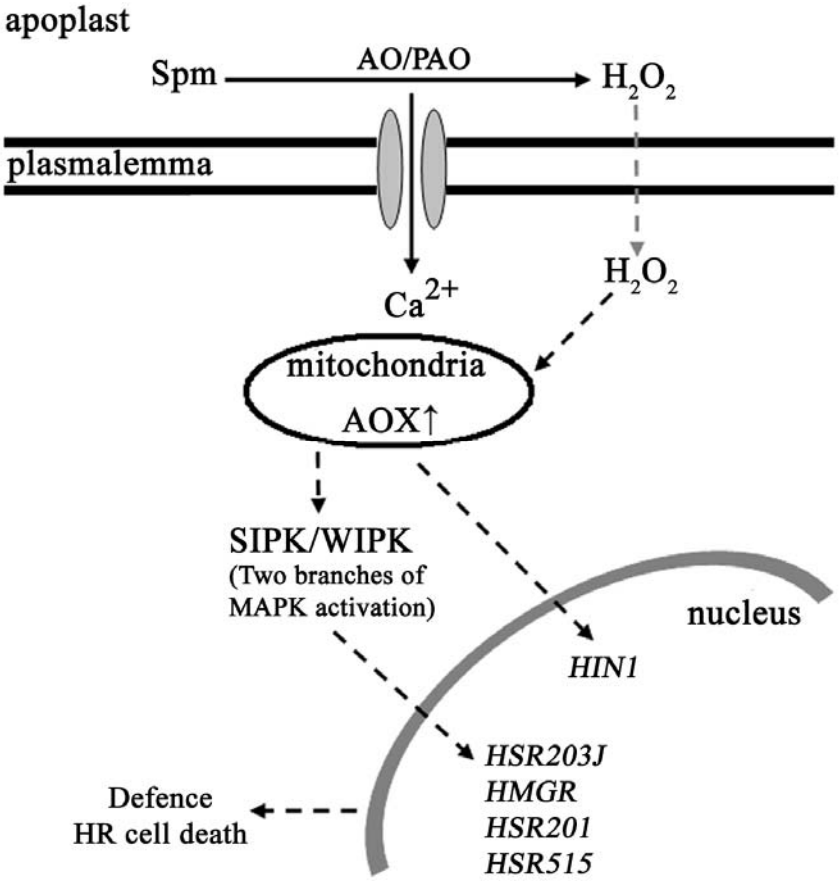

Fig. 4 A model of spermine signal transduction pathways in tobacco during (TMV)-triggered hypersensitive response (HR), proposed by Takahashi *et al*. 2004 (partly modified). Spermine oxidated by amine oxidase(AO) and/or polyamine oxidase (PAO) in the apoplast, resulting in the production of. H_2O_2 and concomitantly initiation of Ca^{2+} flux into the cytosol. The combined events cause mitochondrial dysfunction (malfunction via an unknown mechanism). The downstream reaction of dysfunction of this organelle is the upregulation of a subset of HR marker genes by pathways separated into at least two branches; one independent of and the other dependent on the activation of salicylic acid-induced protein kinase (SIPK/wound-induced protein kinase (WIPK) and the activation of HR marker genes *HSR203J*, *HMGR*, *HSR201*, *HSR515*.

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

Considerable evidence indicates that PAs are involved in a wide range of plant processes, including adaptation to abiotic stresses. However, their precise role in these specific processes remains to be established. The PA biosynthesis pathways are ubiquitous in living organisms but include only a limited number of enzymes. Thus, the PA biosynthesis pathways represent an excellent model to test the hypotheses of PA involving in plant protection against stresses. In recent years, many alternative approaches have been developed to manipulate PA metabolism: specific inhibitors, mutants, and transgenic plants. Taken together, some results concerning the elevated levels of Spd and Spm in stress-tolerant plant species suggest that the levels of these PAs in the cells are under a strict homeostatic regulation due to a supramolecular organization of some of these enzymes. Application of advanced genomic and proteomic approaches will help to elucidate the role of PAs in particular plant processes in stress tolerance (Kaur-Sawney *et al.* 2003).

Much of the recent advances in understanding the role of PAs in plant response to abiotic stress came from studies that employed the stress-tolerant plant genotypes, for example halophytes. It is supposed that halophytes could use specific mechanisms in their responses to stress, which are not used in stress-sensitive plants (glycophytes). Currently, a facultative halophyte *M. crystallinum* (the common ice plant) is widely used as a model plant to search for such mechanisms, in particular those involving PAs. However, a relatively large genome of this plant species limits its genetic analysis, production of mutants and transgenic plants (Cushman and Bonnert 2000).

Recently, several groups of researchers (Gong *et al.* 2005) have reported the usage of salt cress (*Thellungiella halophila*), a close relative of *Arabidopsis* with a genome size approximately twice of that in *Arabidopsis*, as an appropriate halophytic model. In contrast to *Arabidopsis*, *T. halophila* displays extreme tolerance to high salinity, low humidity, and freezing. The analysis of differences in transcript and metabolite profiles supported by the microarray results showed that *Thellungiella* induced genes functioning in protein folding, posttranslational modification, and protein redistribution. This can initiate comparative genomic and proteomic studies to understand the involvement of PAs in plant stress tolerance. Thus, *Thelungiella* can be a valuable model for the study of abiotic stress tolerance as well as an excellent tool for study of molecular mechanisms underlying PA role in stress tolerance.

It is also very important that not much is known about the exact cellular and subcellular localization of PAs and their biosynthetic enzymes in plants, especially under stress conditions, and that remains one of the obstacles in understanding their biological role. There is a gap in information on the translocation of free PAs and their interaction with hormones, on their role in gene expression, and does the bound PAs also play a definite role and other the site of its action in plants experienced stress. The use of molecular approaches, cloning of genes for PA biosynthetic enzymes in particular, production of transgenic plants, isolation and characterization of mutants defective in PA biosynthesis will provide a better understanding of the role of PAs in adaptation of plants to stress conditions. Improvement of crop abiotic stress tolerance by cellular and molecular modifications of PA metabolism is in progress. However, it is reasonable to assume that a thorough comparative study of the expression and function of members of the PAs gene families in extreme halophytes and xerophytes will eventually assist in the breeding of stress-tolerant crop plants.

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