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Polyamines, All-Purpose Players in Response to Environment Stresses in Plants

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ABSTRACT

The polyamines (PAs), putrescine (Put), spermidine (Spd) and spermine (Spm), are small aliphatic amines that are ubiquitous in all living organisms. PA metabolism in higher plants is involved in both biotic and abiotic stresses including nutrient deficiency, salitniy, hyperosmosis, temperature stress, drought, pH, hypoxia, paraquat, environmental pollutants, and wounding. PA biosynthetic pathway has now been revealed at molecular level in plants and more and more molecular evidences support the roles of PA in plant stress responses, to which transgenic approaches to modulate PA biosynthetic genes have provided further evidence. Transcriptomic and proteomic approaches will help to elucidate the roles of PA in signaling network under environment stresses.

Keywords: abiotic stress, biotic stress, Putrescine, Spermidine, Spermine

Abbreviations: ABA, abscisic acid; **ADC**, arginine decarboxylase; **Cad**, cadaverine; **DAO**, diamine oxidase; **Dap**, diaminopropane; **DFMA**, α-difluoromethylarginine; **DFMO**, α-difluoromethylornithine; **HCAA**, hydroxycinnamic acid amide; **HR**, hypersensitive response; **MGBG**, methylglyoxal bis-(guanylhydrazone); **NO**, nitric oxide; **ODC**, ornithine decarboxylase; **PA**, polyamine; **PAO**, polyamine oxidase; **PEG**, polyethylene glycol; **ROS**, reactive oxygen species; **SAMDC**, *S*-adenosylmethionine decarboxylase; **SPDS**, spermidine synthse; **SPMS**, spermine synthase; **TMV**, tobacco mosaic virus; **UV-B**, ultraviolet-B

CONTENTS

INTRODUCTION

Polyamines (PAs) are low molecular weight polycations found in all living organisms, except two orders of Archaea, Methanobacteriales and Halobacteriales (Hamana and Matsuzaki 1992). In plants, the most common PAs are the diamine putrescine, the triamine spermidine and the tetramine spermine, which have been implicated in a wide range of plant growth and developmental processes including cell division, embryogenesis, morphogenesis, fruit development,

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fruit ripening, leaf senescence and response to environmental stresses (Bouchereau *et al*. 1999; Bagni and Tassoni 2001; Kaur-Sawhney *et al*. 2003; Bagni and Tassoni 2006). During the past few years, a number of genes encoding PA biosynthetic enzymes have been isolated in different plants. Numerous transgenic plants with perturbed PA metabolism have been produced to elucidate their cellular functions. Furthermore, the updated transcriptomic and proteomic approaches have been employed to PA function research; however, the precise roles of PAs remain largely elusive. With the recent reestablishment of the roles of reactive oxygen species (ROS) in the stress response and the relationship between the nitric oxide biosynthesis and PA titers, the roles of PA played in the stress response are attracting more attention than ever, which provides a good chance to make a retrospect to the past studies on the relationships between PAs and stresses, including biotic and abiotic stresses.

PA BIOSYNTHESIS AND CATABOLISM

In plants, the first step of PA biosynthesis is the formation of putrescine (Put), and there are normally two pathways for Put biosynthesis, i.e. ornithine and arginine pathway (**Fig. 1**). In the ornithine pathway, Put is formed directly from the decarboxylation of ornithine, by ornithine decarboxylase (ODC; EC 4.1.1.17). In the arginine pathway, Put is formed indirectly via decarboxylation of arginine by arginine decarboxylase (ADC; EC 4.1.1.19). The resulting intermediate agmatine is subsequently converted to *N*-carbamoylputrescine by agmatine iminohydrolase (also known as agmatine deiminase) and finally converted to Put by *N*-carbamoylputrescine amidohydrolase. Spermidine (Spd) and spermine (Spm) are formed by the successive transfer of an aminopropyl moiety from decarboxylated *S*-adenosylmethionine onto Put and Spd, respectively, which is correspondingly catalysed by the aminopropyltransferase enzymes Spd synthase (SPDS; EC 2.5.1.16) and Spm synthase (SPMS; EC 2.5.1.22). Recently, ACL5 showed thermospermine synthase activity rather than Spm synthase (Knott *et al*. 2007). A decarboxylated *S*-adenosylmethionine is produced from the decarboxylation of *S*-adenosylmethionine (SAM) by SAM decarboxylase (SAMDC; EC 4.1.1.50). A SAM is also the precursor for ethylene biosynthesis, thus PA and ethylene biosynthesis may compete for the utilization of SAM pools in the cell. Some evidence from the opposite accumulating patterns of these two components supported the postulation; however, poor correlations between them were also reported as revealed in some cases, in which treatment with PAs resulted in the stimulation of ethylene (Pennazio and Roggero 1990). Uncommon polyamine cadaverine (Cad, 1,5-diaminopentane) is produced from the decarboxylation of lysine by lysine decarboxylase (EC 4.1.1.18), which is mainly found in the Leguminosae and in the flowers of *Arum lilies* (Smith and Wilshire 1975).

With the last missing link filled, all the genes in the PA biosynthesis pathway of plants have been revealed (Illingworth *et al*. 2003; Janowitz *et al*. 2003). Moreover, several genes encoding PA biosynthetic enzymes have been cloned from different plants as reviewed by Liu *et al*. (2007). Taking *Arabidopsis* for example, there are six enzymes responsible for the PA biosynthesis encoded by 10 genes. *Arabidopsis* has two copies of *ADC* (*ADC1* and *ADC2*), *SPDS* (*SPDS1* and *SPDS2*) and *SAMDC* (*SAMDC1* and *SAMDC2*) (Hashimoto *et al*. 1998; Franceschetti *et al*. 2001; Urano *et al*. 2003). There is one single gene respectively for *Spm synthase*, *thermospermine synthase*, *agmatine iminohydrolase* and *N-carbamoylputrescine amidohydrolase* (Hanzawa *et al*. 2000; Panicot *et al*. 2002b; Janowitz *et al*. 2003; Piotrowski *et al*. 2003; Knott *et al*. 2007).

Several intensive attempts have not found the existence of *ODC* in *Arabidopsis* genome (Hanfrey *et al*. 2001; Allen 2002; Urano *et al*. 2003). As for *Arabidopsis*, the detection of ODC activity showed quite discrepancy: several researchers failed to detect any activity, and even though there was an uncertain result from Watson *et al*. (1998), Tassoni *et al*. (2003) could detected putative ODC activity repeatedly, these authors showing that most of the putative ODC

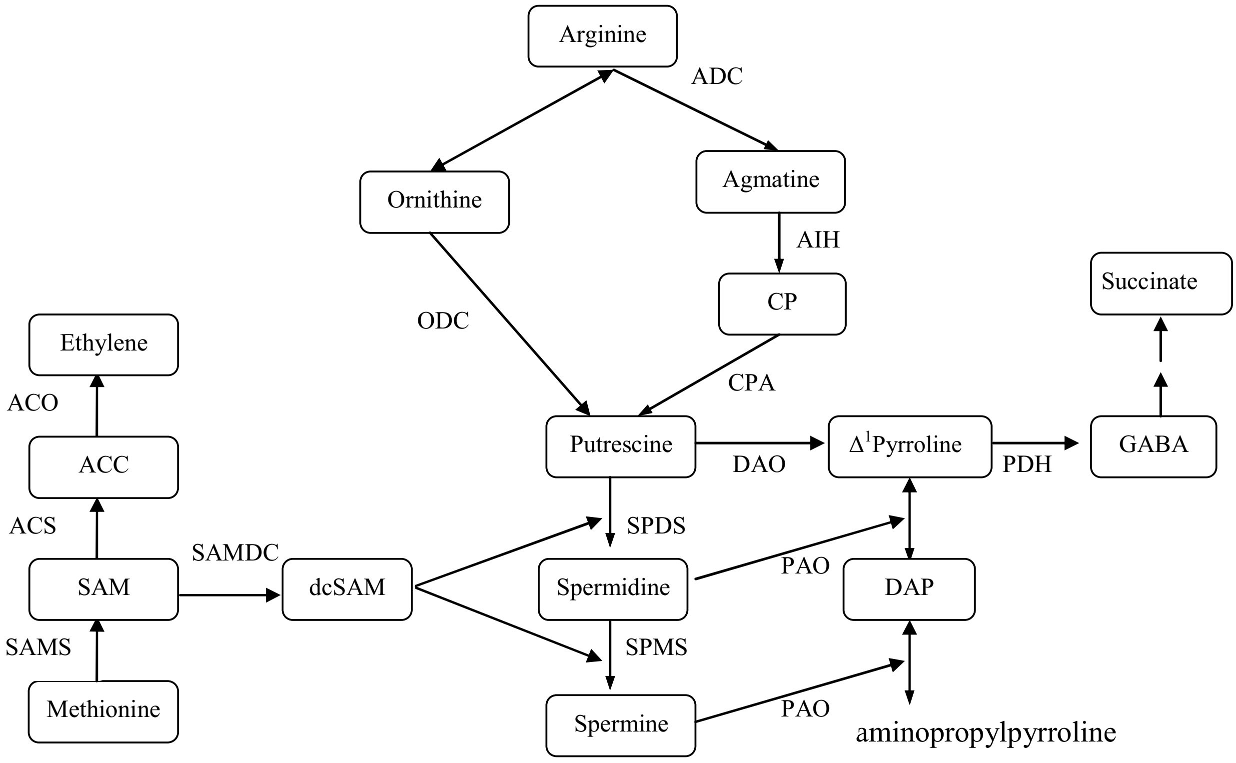

Fig. 1 The biosynthetic pathway for ethylene and major polyamines (putrescine, spermidine and spermine) in plants. ADC, arginine decarboxylase; ODC, ornithine decarboxylase; AIH, agmatine iminohydrolase; CPA, *N* -carbamoylputrescine amidohydrolase; SAM, *S*-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylic acid; SAMDC, *S*-adenosylmethionine decarboxylase; dcSAM, decarboxylated *S*-adenosylmethionine; SPDS, spermidine synthase; SPMS, spermine synthase.

activity was localized in the pelleted fraction, generally consisting of chloroplasts, organellar membranes, nuclei and mitochondria. Based on the putative ODC activities they decteded, Tassoni *et al.* (2003) postulated the presence of an enzyme with low similarity to the known plant *ODC* gene, while Allen (2002) had showed a slim chance for the postulation. As of writing, there are 19 plant species with partial or full sequences of *ODC* in Genebank.

Put is oxidatively deaminated by the action of copper-containing diamine oxidases (DAO; EC 1.4.3.6), while Spd and Spm is oxidized by flavoprotein-containing PA oxidase (PAO, EC 1.5.3.11) (Bagni and Tassoni 2001). DAO preferentially oxidize putrescine and other diamines, and it can also act on spermidine. The function of DAO is to convert Put into pyrroline, ammonia and hydrogen peroxide (H_2O_2). PAO oxidizes Spd to pyrroline, 1,3-diamine propane (Dap) and H_2O_2, and Spm to aminopropylpyrroline, Dap and H_2O_2. Recent experiment data suggested that the production of H_2O_2 in the cell wall is a mediator of several physiological events such as programmed cell death, lignification, wall stiffening and cellular defense (Lamb and Dixon 1997; Pennell and Lamb 1997). The first complete cDNA sequence of *PAO* in monocot was obtained by Tavladoraki *et al.* (1998) from maize. While Kitashiba *et al.* (2006) firstly presented a complete *PAO* cDNA sequence of dicot from apple. DAP can be catabolized to alanine, while pyrroline can be further converted into γ-aminobutyric acid catalyzed by pyrroline dehydrogenase. γ-Aminobutyric acid is transaminated and oxidized to form succinic acid, which can then enter Krebs cycle ensuring the recycling of carbon and nitrogen from Put (Flores and Filner 1985). Thus, PA catabolism is not simply a degradative process, contributing to PA homeostasis, amine oxidases and the products deriving from their action have been showed to be involved in several important biological processes (Martin-Tanguy 2001; Cona *et al.* 2006). For example, recent experiment data suggested that the production of H_2O_2 in the cell wall is a mediator of several physiological events such as programmed cell death, lignification, wall stiffening and cellular defense responses to biotic and abiotic stresses (Pennell and Lamb 1997; Lamb and Dixon 1997; Rea *et al.* 2002; Walters 2003). γ-Aminobutyric acid accumulation has been reported to be a common response to various environmental stresses (Kinnersley and Turano 2000).

CONJUGATION OF PA

In plant cells, PAs often occur as free molecular bases and at the same time they may exist in the conjugated form with phenolic acids and also in the bound form with various macromolecules such as proteins (reviewed in Martin-Tanguy 1997, 2001). PAs are most commonly conjugated to cinnamic acids such as *p*-coumaric, ferulic and caffeic acids, and the resulting conjugates are known as hydroxycinnamic acid amides (HCAAs) (Smith *et al.* 1983; Walters 2003). Post-translational covalent linkage of PAs to proteins is catalyzed by transglutaminases (EC; 2.3.2.13). The different plant species may contain varied proportions of free and conjugated PA (Bagni and Tassoni 2001). In some species such as tobacco and barley, up to 90% of the PA pool is in the conjugated form (Torrigiani *et al.* 1987, Coghlan and Walters 1990). However, less than 10% of PA contents in carrot cell cultures are conjugated (Minocha and Minocha 1995). In plant cells, the "bound" PAs correspond to the acid-insoluble fraction, and the acid-soluble fractions consist of free plus conjugated PAs. The results from Slocum and Galston (1985) and Flores and Filner (1985) suggested that PA conjugation might be a way of regulating the free PA pool in the plant cell. In totacco, the enzyme involved in the conjugation process was characterized whereas the enzyme for the hydrolysis of PA conjugates was not yet identified (Bagni and Tassoni 2001). Besides the importance for the regulation of PA concentration inside the cell, the conjugates may be involved in the regulation of certain growth and developmental events and defense mechanisms against biotic and abiotic stresses (Bors *et al.* 1989; Bagni and Tassoni 2001; reviewed in Martin-Tanguy 2001).

LOCALIZATION AND TRANSPORT OF PA

Since PAs are relatively small, soluble, diffusible molecules at physiological pH, it is difficult to carry out their immobilization in the cell (Galston and Kaur-Sawhney 1990; Kakkar *et al.* 1998). Thus, little information is known about the subcellular localization of PAs in plants. Early reports on the entirely different metabolic fate for the endogenous and exogenous arginine in PA biosynthesis and the different metabolism for endogenous and exogenous PAs indicate that enzymes and substrates in the PA metabolic pathways are functionally compartmented in the cell (Galston and Kaur-Sawhney 1990). ADC and ODC were hypothesized to have different functions by a separate localization within cellular compartments. The use of antibodies produced against oat ADC revealed that oat ADC is localized in chloroplasts (Borrell *et al.* 1995). Recently, it has been demonstrated that in soybean both *ODC* and *ADC* transcripts were found in most plant organs examined. Nevertheless, the localization of *ADC* and *ODC* transcripts in expanding cells of root cap, cortex parenchyma and central cylinder could provide evidence that expression of both genes could be involved in cell expansion (Delis *et al.* 2005).

It was demonstrated that only PAs in the free form are translocated possibly due to that PAs conjugated to cinnamic acids are sequestered in the vacuoles and probably unable to enter the cytosolic fluid of the sieve tubes (Antognoni *et al.* 1998). Considerable amounts of PAs have been detected in the phloem of *Ricinus communis* seedlings, suggesting translocation between different organs (Antognoni *et al.* 1998).

FUNCTION OF PA

PAs are aliphatic polyacations, and Put, Spd and Spm possess two, three and four positive charges, respectively, at physiological pH owing to the protonations of their amine groups as shown in **Fig. 2** (Cohen 1971). PAs can readily bind to the negatively charged phospholipids head groups or other anionic sites on membranes, thus affecting the stability and permeability of such membranes (Marton and Morris 1987), through which the membrane-localized enzymes can be affected (reviewed in Slocum *et al.* 1984). They are also able to bind to cellular polyanions such as DNA, RNA, protein and cell wall components, and thereby affect the synthesis, structure and function of the macromolecular (Tassoni *et al.* 1996; Messiaen *et al.* 1997; Tassoni *et al.* 1998; Hou *et al.* 2001). The reversible protonation of the multiple amino groups of PAs may function to elevate the levels of organic acid or H^+ ions under some acid condition. Thus it was postulated that PA metabolism may facilitate a buffering mechanism to maintain cellular pH and ion homeostasis (Smith 1971; Young and Galston

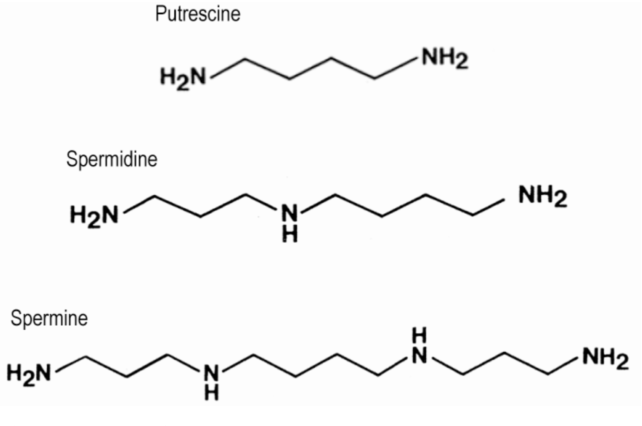

Fig. 2 Formulae of polyamines.

1983). PAs function as free radical scavengers directly or indirectly (Ha *et al.* 1998). PAs have an antioxidative property by quenching the accumulation of $O_2^{\cdot-}$ probably through inhibition of NADPH oxidase (Papadakis and Roubelakis-Angelakis 2005).

INVOLVEMENT OF PA IN ENVIRONMENTAL STRESS OTHER THAN POLLUTANTS

Nutrient deficiency

Since the discovery by Richards and Coleman (1952) on the accumulation of Put in barley leaves under conditions of potassium (K) and magnesium deficiency (Smith 1973), starvation in rice culture (Shih and Kao 1996) and K-deficient oats (Young and Galston 1984) have also been shown to stimulate Put accumulation. Geny *et al.* (1997) reported that PAs (especially conjugated and wall-bound forms) in different organs of grapevine (*Vitis vinifera* L. cv. 'Cabernet Sauvignon') cuttings were strongly influenced by K^+ supply before visual deficiency symptoms appeared. Nevertheless, the changes in PA titers induced by K nutrition differed according to tissue and stage of development. Watson and Malmberg (1996) showed that *A. thaliana* responded to K deficiency by increasing ADC activity to 10-fold more than unstressed plants with a corresponding increase in Put levels of up to 20-fold whereas Spd and Spm levels did not increase proportionately. They further showed the increase in ADC activity induced by K deficiency did not appear to involve changes in mRNA or protein abundance, suggesting the posttranslational regulation mechanism for ADC activation. Enhanced ADC activity and PA titers were observed in phosphate deficient suspension-cultured rice cells. A further experiment indicated that Put accumulation might be one of the causes for inhibition of cell growth under phosphate starvation conditions (Shih and Kao 1996). In contrast, the addition of K^+ leads to a decrease of PA titers, especially for Put levels (Reggiani *et al.* 1993). A number of other ionic treatments caused less changes in PA titers (Young and Galston 1984).

Salinity

Excessive soluble salts (mainly NaCl) in the soils are harmful to most plants. It was reported that about 20% of irrigated land in the world is affected by salinity (Yeo 1999). Understanding the mechanisms of plant salt tolerance will lead to effective means to create salt-tolerant crops through better convention breeding or genetic engineering (Xiong and Zhu 2002).

PA accumulation under stress has been well documented in several plant species. However, their role in stress responses is still elusive and even contradictory (reviewed by Bouchereau *et al.* 1999; Sanchez *et al.* 2005). During salt stress, PA contents have been reported to change dependent on the tissue, species, salt concentration and duration of the experiment (Ashraf and Harris 2004; Kasinathan and Wingler 2004).

Short-term salt treatment, i.e. in a time frame of hours, induces Put accumulation in mung bean (*Vigna radiata* (L.) Wilczek), rice and tomato (Friedman *et al.* 1989; Basu and Ghosh 1991; Santa-Cruz *et al.* 1998). Under long-term salt stress, high titers of Spd and/or Spm, rather than Put, are correlated with the response of a plant to salinity (Krishnamurthy and Bhagwat 1989; Santa-Cruz *et al.* 1997; Sanchez *et al.* 2005; Wen *et al.* 2007).

Krishnamurthy and Bhagwat (1989) reported that salt-tolerant rice cultivars were effective in maintaining high concentrations of Spd and Spm, with a negligible increase of Put content, whereas salt-sensitive ones were characterized by excessive accumulation of Put and low levels of Spd and Spm in the shoot system under saline condition. Thus, it was deemed that saline resistance in rice in these cultivars was due to the great increase in Spd and Spm versus the little increase in Put. A similar situation was reported in barley (*Hordeum vulgare* L.) cultivars ('J4', salt-tolerant; 'KP7', salt-sensitive; Liu *et al.* 2006a), and lettuce (*Lactuca sativa* L.) cultivars (Zapata *et al.* 2003).

Recently, Liu *et al.* (2006b) investigated the involvement of the ADC pathway in the salt stress response using apple (*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf. cv. 'Orin') callus. Salt stress (200 mM NaCl) caused a higher increase of free Put levels compared with the control, with increased conjugated Put limited to the early stage under salt stress. Accumulation of Put was in accordance with the induction of ADC activity and transcript level of *ADC*, whereas ODC activity showed a pattern opposite to that of ADC and no *ODC* transcript level was detected. It was also showed exogenous Put could effectively alleviate salt stress-induced damage. The authors suggested a possibility of different compartmentation for endogenous and exogenous Put to explain why the salt-induced internal Put could not function to alleviate the stress injury.

Urano *et al.* (2004) presented direct evidence that Put biosynthesis under stress conditions (150 mM NaCl) is controlled by the induction of *AtADC2* in *Arabidopsis* using a *Ds* insertion mutant of the *AtADC2* gene (*adc2*-1). In the *adc2-1* mutant, free Put content was reduced to about 25% of that in the control plants, and Spd and Spm showed no difference compared to the control. In control plants, free Put increased twice but not Spd and Spm. However, Put did not increase under salt stress in the *adc2*-1 mutant. The stress-derived damage in the *adc2-1* was alleviated by the addition of exogenous Put (2 mM).

Rodríguez-Kessler *et al.* (2006) investigated the response to salt stress of genes in the PA biosynthesis pathway (*Zmodc*, *Zmadc*, *Zmsamdc*, *Zmspds2A* and *Zmspds2B*) in maize (*Zea mays* L. cv. 'Cafime') leaves. The results showed that only *Zmodc*, *Zmspds2A* and *Zmspds2B* were up-regulated by salt stress; whereas the other two genes were not much affected by salt treatment. Concerning salt-stress, the induced PA response has been generally assumed to rely mainly on *ADC* gene activation (reviewed in Bouchereau *et al.* 1999). This is one of the seldom cases where *ODC* transcript was found to be involved in salt stress.

Dondini *et al.* (2000, 2001) demonstrated the possible role of transglutaminase-induced PA conjugation in the assembly of chloroplast proteins in cells affected by salt stress using a PA-deficient strain (PA^-vs) of the halo-tolerant *Dunaliella salina*, a unicellular green alga. The PA^-vs provides an ideal system for the detection of PA-conjugated proteins since conjugation sites are available for linkage by exogenous radioactively labeled PAs. In the PA^-vs acclimated to high salinity, transglutaminase activity exhibited an increase of two-fold or more. The chloroplast proteins, ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit, and CAB, namely: LHCII, CP 29, CP 24, and CP 26 were identified by incubating chloroplasts with labelled PAs. It was reported that the post-translational modification of purified Rubisco large subunit by PAs favours the polymerisation to the active octameric form and its stabilisation (Margosiak *et al.* 1990). Furthermore, the phosphorylation of the CAB affects its position in the thylakoid membrane, and consequently affects stacking (Allen 1995), while the addition of positive charges by conjugated with polyamine may have an effect opposite that of phosphorylation. Therefore, the transglutaminase induced polyamine conjugation to chloroplast proteins may contribute to the protective mechanism against stress for polyamines.

Legocka and Kluk (2005) presented evidence that NaCl and sorbitol treatment trigger organ-specific changes in PA levels and in the activity of ADC in lupin (*Lupinus luteus* L. var. 'Ventus') seedlings. After exposed to salt stress for 24 h, the free-Put levels decreased in roots and cotyledons by about 48% and 54%, respectively, and increased in hypocotyls and leaves by about 27% and 73%, respectively. The free Spd titer decreased in roots, and elevated in hypocotyls and leaves. At the same time, ADC activity was enhanced by 66% in roots, whereas no difference was observed in leaves compared to the control. The authors concluded that the stresses induce PA biosynthesis in the roots, as well as Put accumulation in the leaves, which possibly indicate translocation of Put from the roots to the shoot. It was also shown that a higher level of PAs bound to microsomal membranes was observed in roots and leaves when subjected to NaCl and sorbitol treatment, suggesting the important role in stress tolerance for the PAs bound to microsomal membranes.

A study in rice suggested the important role for Spm in salt stress tolerance (Maiale *et al.* 2004). *Arabidopsis* double knockout mutant plant (*acl5*/*spms*), incapable of producing Spm, showed higher sensitivity to salt stress than wild-type plants. This phenotype was cured by exogenous Spm but not by Put and Spd, suggesting a strong link between Spm-deficiency and NaCl-hypersensitivity (Yamaguchi *et al.* 2006).

Among different PAs used to counteract salt-induced damage, Put was most effective as observed in pine (Tang and Newton 2005), barley seedling (Zhao and Qin 2004). While Roy *et al.* (2005) clearly demonstrated that the deficit of salt-sensitive rice cultivars, e.g. high accumulation of Na^+, loss of K^+ salinity stress-induced sharp inhibition of plasma membrane-bound H^+-ATPase activity, could be overcome by exogenously supplied Spd (1 mM, 16 h).

One of the ''hallmark ''cellular responses to salt-stress is a massive K^+ efflux, which reduces the intracellular K^+ pool, and significantly impair cell metabolism (Shabala *et al.* 2007). Shabala *et al.* (2007) showed that treatment by 1 mM PAs substantially reduced NaCl-induced K^+ efflux from the pea (*Pisum sativum* L.) leaf mesophyll, most likely by blocking the non-selective cation channels. It was suggested that the observed stress-induced elevation of endogenous PAs titers under saline conditions may represent an important adaptive mechanism, in which the uptake of Na^+ and leakage of K^+ from mesophyll cells are reduced, thus assisting plants in their adaptation to salinity.

Hyperosmosis

Cereal (oat, barley, corn and wheat) leaves subjected to the osmotica show a rapid increase in ADC activity, a massive accumulation of Put, and slow conversion of Put to the higher PAs, Spd, and Spm (Flores and Galston 1984). By contrast, in the plants of dicot genera like *Nicotiana*, *Capsicum*, *Datura*, *Trigonelia*, and *Vigna*, Put titer as well as ADC and ODC activities decline while Spd and Spm titers increase under osmotic stress (Tiburcio *et al.* 1986a, 1986b).

Drought-tolerant wheat cultivar (*Triticum aestivum* L., cv. 'Yumai No. 18') showed marked increases of free Spd and free Spm and bound Put levels in leaves after polyethylene glycol (PEG) 6000 treatment for 7 days, while drought-sensitive cultivar showed a significant increase of Put level. Exogenous application of methylglyoxal bis-(guanylhydrazone) (MGBG) (1 mM) resulted in reduction of free Spd and free Spm levels in leaves and aggravation of PEG-induced injury to drought-tolerant wheat cultivar, while exogenous Spd treatment resulted in an increase of free Spd and free Spm content in leaves and an alleviation of PEG-induced injury to drought-sensitive wheat cultivar (*T. aestivum* L., cv. 'Yumai No. 9'). It was proved that conjugated PAs in leaves has no relationship with the osmotic stress tolerance of wheat seedlings. All these findings collectively suggested that free Spd, free Spm and bound Put play important role in the osmotic stress tolerance of wheat seedlings (Liu *et al.* 2004). It was further elucidated that this tolerance is associated with the activity of H^+-ATPase and the contents of non-covalently conjugated (NCC)-Spd and NCC-Spm together with covalently conjugated (CC)-Put and CC-Spd in the plasma membrane of roots (Liu *et al.* 2005b). By contrast, it was reported that Put, Cad and Spm titers were generally increased in leaves and roots of wheat (*T. aestivum* L. cv. 'Sappo') plants exposed to 0.4 M mannitol for five days, whereas Spd was reduced in first leaves and roots of these plants (Foster and Walters 1991).

In rape (*Brassica napus* L. var. *oleifera* cv. 'Samourai') leaf discs exposed to osmotic stress, Put and Dap (an oxidation product of Spd and/or Spm) increased while Spd titers decreased. These results suggested that detected changes of PA titers responsive to the stress involved not only a rise in Put biosynthesis, but also a stimulation of PA oxidation (Aziz *et al.* 1997). This result concerning Spd change responsive to the mannitol stress seems to be contradicted with a previous report by this research group, where increased Spd titers were detected under similar condition (Aziz and Larher 1995). Larher *et al.* (1998) showed later that exogenous PAs, especially Spm suppress proline accumulation induced by osmotic stress. The effect of PA suppression could be involved in the proline synthesis rather than in proline degradation.

ROS was initially recognized as toxic by-products of aerobic metabolism, removed by means of antioxidants and antioxidative enzymes. However it has recently become established that ROS plays an important signaling role in numerous plants growth and development processes including response to biotic and abiotic environmental stimuli (Bailey-Serres and Mittler 2006). The study by Li *et al.* (2004) suggested that through reducing ROS levels, PAs inhibited ethylene production in the deeply and moderately osmoticly stressed wheat (*T. aestivum* L. cv. 'Ganchun 20') leaves and that through promoting ROS levels, ethylene promoted PA oxidation and hence reduced its content in the deeply osmoticly stressed wheat leaves.

Feirer *et al.* (1998) showed that ADC is the enzyme primarily responsible for biosynthesis of Put in osmotically stressed *A. thaliana*, and it was further demonstrated *AtADC2* is the crucial gene responsible for the induction using an *Arabidopsis* mutant line carrying an insertion of the *En-1* transposable element at the *AtADC2* locus by Soyka and Heyer (1999), which is the first report of a genetically mapped mutation in the PA biosynthetic pathway in plants.

High temperature

Many crops cultivated worldwide are exposed to severe heat stress during their life cycle, resulting in a reduction in yield and quality of fruits (Maestri *et al.* 2002). In order to protect from severe damage and survive the stress, plants adopted a set of responsive mechanism characterized by elevated synthesis of heat shock proteins (HSPs). In rat hepatocarcinoma cells subjected to heat stress, Spd depletion achieved by specific inhibitor α-difluoromethylornithine (DFMO) impairs the DNA binding capacity of selected heat-shock transcription factors, which may be reversed by exogenous Spd. HSPs synthesis in heat-tolerant tobacco cells could be detected up to 46°C, but ceased at 40°C in heat-sensitive alfalfa cells. It was postulated that PAs might directly affect HSPs production at the level of protein synthesis or indirectly by influencing the properties of the cell membranes (Königshofer and Lechner 2002). Under conditions of Spd depletion, the DNA binding capacity of the transcription factors such as activator protein 1 and HSP 70 induced by heat shock were impaired in the rat hepatocarcinoma cells and reversed by exogenous Spd application (Desiderio *et al.* 1999). However, the significance and the specificity of the effects of polyamine depletion are difficult to assess because polyamine influences many biological processes. The precise mode of PAs in the regulation of HSPs synthesis warrants further investigation.

The levels of free and conjugated PAs, as well as ADC and PAO activities, were found higher in tolerant rice (cv. 'N22') callus than that in sensitive rice (cv. 'IR8') callus under non-stressed conditions. Heat-stress resulted in higher levels of free and bound PAs in callus of the heat-tolerant callus than that in the heat-sensitive callus. Furthermore, uncommon polyamines, norspermidine and norspermine, were detected in tolerant cultivar, which increased under stress, while they were not detected in sensitive cultivar under normal or stressed condition. It was concluded that under heat stress, a tolerant rice cultivar had the capacity to maintain or increase its total PA pools and to shift these pools to uncommon PAs while the sensitive cultivar does not have this capacity (Roy and Ghosh 1996). Under heat stress treatment, uncommon PAs were also detected in the heat-tolerant tobacco cells while they were absent in thermo-sensitive alfalfa line. These findings suggested important function of uncommon and conjugated PAs for plants under heat stress.

Exogenous application of PAs (0.1 mM or 1 mM) could enhance the recovery growth of mung bean (*V. radiata* Wilczek cv. 'ML-131') after heat stress (50°C for 2 h) with the effect order as: Put, Spd and Spm (Basra *et al.* 1997). However, Königshofer and Lechner (2002) found that heat-tolerant tobacco lines exhibited moderate ratios of free Put to free Spd while excessive accumulation of free Put characterized the more heat-susceptible alfalfa cell line. The authors hypothesized that excessive accumulation of free-PAs might decrease membrane integrity and influence finally the level of expression of HSP-encoding genes, thereby resulting in less tolerant to heat stress.

Low temperature

Low temperature is one of the most severe environmental stresses, which not only affects the growth and distribution of plants, but also causes serious damage to a number of crops (Heino and Palva 2003). An increase in Put levels after exposure to low temperature has been reported in a number of plant species (Nadeau *et al.* 1987; Sarjala *et al.* 1997; Bouchereau *et al.* 1999; Martínez-Romero *et al.* 2003). In hardened Scots pines, Put concentration showed a positive correlation with frost resistance, whereas Spd and Spm show no correlation. Thus, the increased Put concentration was attributed to an increased state of hardening (Sarjala *et al.* 1997).

It was reported in rice seedlings of a chilling-tolerant cultivar (*O. sativa* L. cv. 'Tainung 67'), levels of Put in both shoots and roots and levels of Spd and Spm in shoots increased after exposure to chilling (Lee *et al.* 1997). In chilling-sensitive rice cultivar (*O. sativa* L. cv. 'Taichung Native 1'), Put levels in shoots increased slightly after exposure to chilling while those of roots decreased drastically. A rise in SAMDC activity and a parallel increase in Put and Spd levels were observed in the leaves and choloroplasts of the chilling-stroken spinach, and it was further demonstrated that the enhanced SAMDC activity with a consequential rise of Spd in chloroplast is crucial for the cold acclimation of photosynthetic apparatus of spinach leaves (He *et al.* 2002).

Wang (1987) showed that cucumber (*Cucumis sativus* L. cv. 'Victory') seedlings induced a substantial increase (2-fold) in the Spd level when exposed to chilling at 5 °C. Consistent with the above findings, Shen *et al.* (2000) found that in chilling-tolerant cucumber cultivar 'Jinchun No. 3', Spd content increased markedly upon chilling and there was little effect on Put and Spm during chilling stress. On the other hand, the chilling-sensitive cultivar 'Suyo' did not show any increase in PA titers upon chilling stress. Though the increase of Spm content was slight, pretreatment of Spm was effective in alleviating chilling injury of the chilling-sensitive cultivar. The authors further demonstrated that Spd may act as a cellular membrane protectant against chill-induced lipid peroxidation through prevention of superoxide-generating NADPH oxidase activation. Expression of *SAM synthetase 2* was found up-regulated during chilling stress by a comparative proteomic analysis (Yan *et al.* 2006), which may contribute to the increase of Spd and Spm titers in some instances.

It was reported that in a chilling-tolerant rice cultivar 'Tainung 67', chilling induced an increase of free abscisic acid (ABA) levels first, then ADC activity and finally free-Put levels. A series of evidences seem to suggest that one of the strategies of rice seedlings to resist chilling-stress is to raise the contents of ABA that consequently enhances the ADC-mediated Put synthesis (Lee *et al.* 1997). On the contrary, Kim *et al.* (2002) showed that both ABA and Put were protective against cold stress and exogenously applied ABA decreased the endogenous level of Put in the tomato leaves. Furthermore, the DMFO-increased electrolyte leakage in cold stressed leaves was completely abolished by the application of ABA. These results suggest that ABA does not exert its role via Put in response to cold stress in tomato leaves. Exogenous Spd (1 mM) treatment did not affect the contents of ABA in chilled leaves of chilling-sensitive cucumber cultivar, and ABA content did not increase in leaves of chilling-tolerant cultivar during chilling, suggesting that ABA does not mediate the Spd effect (Shen 2000). The evidence from rice research did not support this opinion. A root-specific induction of *OsSPDS2* was observed in chilling stressed rice. Moreover, *OsSPDS2* was up-regulated by ABA treatment, suggesting possible involvement of ABA in *OsSPDS2* gene regulation (Imai *et al.* 2004).

Drought

Drought is one of the most severe abiotic stress factors limiting crop productivity worldwide (Sharp *et al.* 2004). PA metabolism has also been reported to play an important role in plant drought tolerance (Nayyar *et al.* 2005; Yang *et al.* 2007; Yamaguchi *et al.* 2007). Spd delayed senescence in drought-stressed jack pine (Rajasekaran and Blake 1999). When chickpea (*Cicer arietinum* L. cv. 'GPF2') and soybean (*Glycine max* (L.) Merrill cv. 'Brag') genotypes were exposed to water stress, they experienced severe stress injury characterized by increased electrolyte leakage and inhibited root and shoot growth rate. Furthermore, Put, Spd, and Spm began to rise at the 2nd day of stress and peaked at the 4th day in soybean and on the 5th day in chickpea. Thereafter, PA levels began to decline significantly, which was accompanied by the accentuated stress injury. Exogenous Put and Spd (0.1 mM) markedly mitigated the stress-induced injuries, suggesting important role of PAs in counteracting drought stress, which was further corroborated by the evidence that the treatment by α-difluoromethylarginine (DFMA), DFMO and cyclohexylamine (an inhibitor for SPDS) increased the stress injury and severely impaired the growth (Nayyar *et al.* 2005).

Drought-resistant rice cultivars had higher SAMDC and SPDS activities and more free Spd and free-Spm titers in the leaves than drought-susceptible ones under water stress, implying that both free-Spd and free-Spm are involved in rice tolerance to drought (Yang *et al.* 2007). Furthermore, the changes in ADC activity and Put levels under drought stress and their relationship with drought resistance of rice cultivars varied greatly with the duration or severity of the stress. The authors further proposed that a high level of free Put at an early stress stage and insoluble-conjugated Put during the whole stress period helps the plant to adapt to stress.

Arabidopsis double mutant plant (*acl5*/*spms*), which cannot produce Spm, is hypersensitive to drought stress. This damage could be recovered by Spm (1 mM) pretreatment but not by Put and Spd (1 mM), suggesting that drought-hypersensitivity exhibited by the mutant is due to Spm deficiency (Yamaguchi *et al.* 2007). It was further showed that absence of Spm causes blockage or slow response of stomatal closure during drought-stress, and that the mutant plant lost more water compared to wild type plants, which may account for its hypersensitivity to drought. Among the three PAs, Spm is the most efficient to contribute to intrinsic gating and rectification of inward rectifier K^+ channels (Oliver *et al.* 2000). Spm was also the most efficient PA to block fast-activating vacuolar channels, which function in release of K^+ and Ca^{2+} from the vacuole to the cytoplasm. Besides these, the mutant plant was impaired in Ca^{2+}-homeostatis. Based on the above collected knowledge, the authors proposed a model describing the role of Spm during drought-stress: Under drought, elevated Spm content may modulate the activities of certain ion channels and raise cytoplasmic Ca^{2+} concentration, which will inactivate the K^+ inward rectifier at the plasma membrane, thereby stimulating stomatal closure (Yamaguchi *et al*. 2007). Anisul Islam *et al*. (2003) also presented evidence to show the important role of Spm to prevent water stress and protect membranes in *Pinus strobus* L. under drought. Exogenous Spm to pines under drought cause a decline in transpiration rates, enhanced photosynthesis and promoted osmotic adjustment, which would help to maintain turgor.

ABA is produced *de novo* under water deficit conditions and plays a major role in response and tolerance to dehydration. In drought-tolerant *Populus popularis*, initial elevation of xylem ABA concentrations reduced PA levels and enhanced ethylene synthesis simultaneously at the onset of water stress or the ABA treatment. The inhibitory effect of ABA on PA synthesis was more pronounced in the drought-sensitive *Populus* × *euramericana* (Chen *et al*. 2002). On the contrary, *AtADC2*, *AtSPDS1* and *AtSPMS* are highly induced by drought stress in *Arabidopsis*, and these responses are mostly impaired in ABA-deficient (*aba2-3*) and ABA-insensitive (*abi1-1*) mutants. These results indicate that ABA modulates PA metabolism at transcriptional and metabolite levels in response to drought stress (Alcázar *et al*. 2006a).

pH stress

The Put content increased upon acid stress in a tobacco cell line that is resistant to DFMO (Hiatt and Malmberg 1988). ADC activity increased in a tissue-dependent manner in response to acid stress in soybean hypocotyls, which was preceded by the increase in its mRNA content (Nam *et al*. 1997). Enhanced Put accumulation under acid stress was also observed in the leaf segments of wheat and oat, which was concomitant with ion efflux, and a decrease in both macromolecular synthesis and the activities of superoxide dismutase and catalase (Shen *et al*. 1994). Xie *et al*. (1999) described a increased Put accumulation in four tree species of *Robinia pseudocacia* Linn., *Liriodendron tulipifera* Linn., *Platanus acerifolia* Willd and *Gardenia jasminoides* Ellis under acid stress, which was prevented by the supplement of D-arginine. Applying exogenous Put to acid medium could mitigate the decrease of catalaze activities induced by acid stress. A treatment of simulated acid rain (pH 1.8) induced lipid peroxidation and increased the level of H_2O_2 in bean leaves, which was prevented by pretreatment with Spd and Spm (1 mM) (Velikova *et al*. 2000). The protective effect of Spm was higher than that of Spd, which is coincided with the survival test of *Escherichia coli* K-12 in extreme acid or base (Yohannes *et al*. 2005). In the survival test, 10 mM Spm increased survival by 2-fold, and Put increased survival by 30% at pH 2; however, *E. coli* survival was decreased 100-fold by 10 mM Spm, Put, Cad, or Spd at pH 9.8. It seems there have no reports on the response of PAs in high pH stress in plants.

Hypoxia

Hopoxia often occurs in cells when respiratory activity exceeds oxygen availability, and in roots and aerial organs as a consequence of poor soil drainage, soil compaction or submergence (Fukao and Bailey-Serres 2004). In plants, oxygen deficit dramatically reduces the cellular ATP production, which brings about deleterious effects such as slow down in ion pump activity, a drop in cytoplasmic pH, accumulation of toxic end products from anaerobic respiration and ROS, etc. (Fukao and Bailey-Serres 2004; Liu *et al*. 2005a; Mancuso and Marras 2006). Plants have evolved adaptation mechanisms to enhance their ability to survive long-term hypoxic stress (Liu *et al*. 2005a). The capacity to accumulate Put under low oxygen condition may be one of the adaptive mechanisms, which has been evidenced in several *Gramineae* species (reviewed by Bouchereau *et al*. 1999). A relationship between ADC activity and the ability to tolerate anoxia was observed in seedlings of rice, barnyard grass, maize, rye, barley and wheat (Reggiani *et al*. 1990). The application of exogenous Put partially increased the survival of wheat roots under oxygen-deficit stress. Three possible underlying mechanisms for the elevation of Put and ADC activity were put forward (Reggiani 1994). First, the decarboxylation reaction under anaerobic conditions is beneficial as a result of the consumption of hydrogen ions, thereby allowing attenuation of anoxia-induced acidification. Second, the biosynthesis and accumulation of Put would serve as a homeostatic buffering mechanism to maintain cellular ionic balance. Last, Put synthesized via ADC pathway during the anaerobic germination of rice is considered to be involved in the elongation of the coleoptile (Reggiani *et al*. 1989). Elevated Put could also function as a free radical scavenger to remove anoxia-induced ROS.

Under submergence for *Scirpus*, the Put levels and the activities of ADC and ODC increased, whereas Spd and Spm level and SAMDC activity decreased (Lee *et al*. 1996). The reduced Spd and Spm level may be also account for the Put accumulation, which may favor the ethylene biosynthesis due to the shifted SAM to ethylene biosynthesis pathway. This possibility could not ignore since ethylene plays an important role in alcohol dehydrogenase induction during later stages of hypoxia and in long-term structural adaptation mechanisms (Liu *et al*. 2005a).

Paraquat

Paraquat (methyl viologen, 1,1'-dimethyl-4,4'-bipyrydinium dichloride) is a contact foliar herbicide, which can cause rapid membrane damage by producing highly toxic superoxide radicals (Calderbank 1968). Put and paraquat are similar in the distribution of their positively charged amine groups. This commonness stimulated an idea that Put and paraquat might share the same transport system, which is supported by evidence from *E. coli* (Kashiwagi *et al*. 1990) and plants (Hart *et al*. 1992). Hart *et al*. (1992) showed that paraquat is taken up by maize root epidermal and cortical cells via a system that also functions in the absorption of diamines. This result indicates the importance of Put in paraquat resistance in plants.

A rapid increase in free and bound PAs and was observed at the first hour after herbicide atrazine spraying in pea, whereas the conjugated PAs in trichloroacetic acid-soluble fraction decreased (Kurepa *et al*. 1993, 1998). It was further shown that atrazine increases cell senescence by lipid peroxidation and loss of unsaturated fatty acids from thylakoid membranes of pea plant chloroplasts, which was partially relived by the Spm supplement (Stoynova *et al*. 1999). It was reported that some paraquat-resistant weed species have increased levels of PAs (Szigeti *et al*. 1996; Ye *et al*. 1997; Rácz 2000; Soar *et al*. 2003).

In resistant *Conyza bonariensis* biotypes, the constitutively elevated Put levels coincided with the increased ADC and ODC activities, and constitutively elevated antioxidant enzyme activities, as well as paraquat resistance (Ye *et al*. 1997). Exogenous Put application (0.1 mM) completely prevented paraquat-induced damage in the resistant biotype, however, the addition of Put failed to enhance paraquat resistance in the sensitive biotype (Ye *et al*. 1997), which may exclude the possibility that Put functions as a direct quencher of oxidant caused by paraquat. The authors postulated that Put might function in concert with other mechanisms conferring resistance, which is supported by the results from horseweed (Rácz *et al*. 2000). Recently, Soar *et al*. (2003) showed that paraquat translocation is correlated with paraquat-induced injury and is reduced in paraquat-resistant biotype in *Arctotheca calendula*. The resistant *A. calendula* plants contain higher constitutive Put and Spd levels than susceptible plants. Pre-treatment with either Cad or Spd marked reduced paraquat translocation in the susceptible biotype after paraquat application; however, pre-treatment with Put had no effect (Soar *et al*. 2004).

The resistant horseweed plants have higher constitutive Put and Spd levels than susceptible ones, whereas Spm does not differ between them (Rácz *et al*. 2000). A marked increase of PAs content was detected after paraquat treatment in each biotype. Exogenously applied Put (0.1 mM) combined with paraquat exerted little or no protective effect on detached leaves for both biotypes. However, it cannot rule out the possibility that this failure to confirm the protective effect against paraquat of exogenous Put may be due to the low concentration of Put applied when compared to other successful cases.

Contrary to the above examples, there were no obvious differences in PA levels between the wild type and mutant *gi-3* of *Arabidopsis*, which has an increased tolerance to paraquat. Exogenous addition of Put, Spd, and Spm effectively counteracted the toxic effects of paraquat in both wild type and the *gi-3i*, with Spd being the most effective (Kurepa *et al*. 1998). Unlike other reports, a general decrease in the contents of the three main PAs was observed in paraquat-treated sunflower leaf discs (Benavides *et al*. 2000). Exogenous application of PAs (1 mM) could reduce the damage produced by 100 μM paraquat to different degrees. Spm was the most effective in affording protection, followed by Spd and Put.

Paraquat treatment resulted in a higher Put and lower Spd and Spm levels in rice leaves (Chang and Kao 1997). Pretreatment with Spd and Spm (5 mM) caused dramatically increased endogenous Spd and Spm levels and was demonstrated to be able to reduce paraquat toxicity possibly due to increased activities of catalase and peroxidase induced by the pretreatment.

Considering the idea that Put and paraquat share same transport system, the toxic effect of paraquat would be most efficiently reversed by Put (Kurepa *et al*. 1998); however, from above-mentioned cases, we can see that Spd or Spm, rather than Put, is the most effective to counteract the paraquat toxicity. One possible explanation is that "in plants paraquat is taken up by cells via a PA transporter whose function is under stringent negative regulation by Spd (Kurepa *et al*. 1998). This hypothesis needs further evidence to support it.

ENVIRONMENTAL POLLUTANTS

Sulfur dioxide

Sulfur dioxide (SO_2) is one of the air pollutants that can affect basic plant growth and development processes. Decades ago, Priebe *et al*. (1978) revealed that free and bound Put and Spd in peas increased in response to SO_2 fumigation. The supplement of ammonium nutrition resulted in similar results. As both SO_2 fumigation and ammonium supplement increase the H^+ ion concentration of the cells, the authors postulated that PAs are synthesized to bind these H^+ ions, thus maintaining an ion homeostasis in the cells. Contrarily, the accumulation of Put was greatly inhibited after SO_2 treatment in coldstored loquat (*Eriobotrya japonica* Lindl. cv. 'Dahongpao') fruits, whereas no significant changes were observed for Spd and Spm levels (Zheng *et al*. 2000).

Ozone

Ozone (O_3) is considered as one of the most phytotoxic pollutants, having serious effects on vegetation (Krupa and Manning 1988). Exogenous PAs were found to result in a significant suppression of O_3-induced leaf injury in tomato and tobacco, indicating a role of PAs in ozone stress response (Ormrod and Beckerson 1986). Increase of PA contents (mainly Put and Spd) in response to acute and chronic ozone stress was consistently observed in wheat (Drolet *et al*. 1986), tolerant tobacco cultivar (Langebartels *et al*. 1991; van Buuren *et al*. 2002; Navakoudis *et al*. 2003), potato (Reddy *et al*. 1993), and lentil (*Lens culinaris*) seedlings (Maccarrone *et al*. 1997). *In vitro* experiment suggested that free PAs are scavengers of oxygen radicals (Drolet *et al*. 1986), while Bors *et al*. (1989) presented PA conjugates with HCAAs as the probable main protection mechanism from ozone-triggered ROS accumulation. In ozone-treated potato plants, the levels of mRNA transcripts for the large and small subunits of ribulose 1,5-bisphosphate carboxylase/oxygenase (rbcL and rbcS, respectively) decreased, which at least partially accounts for the accelerated loss of ribulose 1,5-bisphosphate carboxylase/oxygenase protein due to ozone-induced accelerated senescence (Reddy *et al*. 1993). The increase in ethylene emission was observed in stressed potato plants, meanwhile increased PA biosynthesis and PA titers were also observed (van Buuren *et al*. 2002).

Navakoudis *et al*. (2003) showed that exogenous Put (1 mM) applied to sensitive tobacco cultivar 'Bel W3' resulted in tolerant phenotype through reversing ozone effects on thylakoid-bound PAs, and especially Put. While application of the Put inhibitor, 1,4-diamino-butanone in ozone-tolerant Bel B plants impeded the increase of Put bound to thylakoid membranes, resulting in a sensitive phenotype.

Ultraviolet (UV)-B radiation

In the past 50 years, the concentration of ozone has decreased by about 5%, mainly due to the release of anthropogenic pollutants (Pyle 1996). Increased UV-B (290–320 nm) radiation resulting from ozone depletion is one of global environmental problems, which has deleterious effects to the plant on the earth (Tevini and Teramura 1989; Ries *et al*. 2000; Frohnmeyer and Staiger 2003). PAs have been reported to play an important role in the protection of plants against UV-B damage. High photosynthetically active radiation level could decrease UV-B-dependent damage, and Kramer *et al*. (1992) showed that PAs were involved in the photoprotective effect of high photosynthetically active radiation. The Put and Spd accumulated in cotyledon and leaf tissues in response to UV-B radiation in both sensitive and insensitive cucumber cultivars, but levels were not correlated with sensitivity to UV-B (Kramer *et al*. 1991). Later, An *et al*. (2004) also demonstrated that UV-B radiation resulted in a dose-dependent accumulation of PAs in UV-B treated cucumber.

The sensitive legume, *Phaseolus vulgaris* L. cv. 'Top crop' exposed to UV-B radiation showed a marked decrease in total free-PA levels, primarily due to a decrease in Put, which was correlated with UV-B induced damage such as chlorophyll loss (Smith *et al*. 2001). An increase of PAs, and especially of Put level in thylakoid membranes upon elevated UV-B exposure was observed in the tobacco variety Bel B, while the cultivar Bel W3, sensitive to UV-B, was incapable to enhance Put level in thylakoid membranes, which may attribute to its sensitivity (Lutz *et al*. 2005). It was also observed that the PA levels reduced after prolonged exposure to UV-B, which was in agreement with the similar phenomena noticed by An *et al*. (2004).

Lin *et al*. (2002) revealed that the PA titers were enhanced after exposure to UV-B radiation for 7-14 d in different rice cultivars, however the Spd and Spm levels were lower than that in the control after prolonged treatment to 21-28 d, while Put remained at a higher level, which was in accordance with the fluctuation in PA metabolic enzymes' activity: For example, enzymes activities of ADC, ODC and SAMDC increased by 165.7%, 104.6% and 89.6%, respectively in the leaves of cultivar 'Shan You 63' at 7-14 d after treatment; the activities for ADC and ODC increased by 89.7% and 3.7%, respectively, while SAMDC was reduced by 40.1% at 21-28 d after treatment. The activity for PAO was much lower at 7-14 d after treatment, whereas it was markedly higher at 21-28 d after treatment. These results by Lin *et al*. (2002) presented good evidence to the adaptation change of PA metabolism after prolonged UV-B exposure (An *et al*. 2004; Lutz *et al*. 2005). The accumulation of PAs upon UV-B radiation is in favor of the hypothesis that PAs could play an important role in the development of the structure and function of the photosynthetic apparatus, mainly based on the capability of PAs to stabilize chlorophyll protein complexes (Besford *et al.* 1993; Andreadakis and Kotzabasis 1996; Sfichi *et al.* 2004).

Heavy metals

Heavy metals are important environmental pollutants, which can cause serious problems to all organisms when present in atmosphere, soil and water, even in trace concentrations (Gratao *et al.* 2005). Heavy metal stress often triggers oxidative damage by producing highly ROS such as OH^- and $O_2^{\cdot-}$, which in turn leads to disintegration of biomembranes by lipid peroxidation (Panda *et al.* 2003). Plants exposed to metals often synthesize a set of diverse metabolites including PAs to fend off the damage (Sharma and Dietz 2006). PAs may function as signalling molecules and antioxidants in response to heavy metal stress (Groppa *et al.* 2001; Wang *et al.* 2004; Sharma and Dietz 2006).

Treatment of oat seedlings and detached oat leaves with cadmium chloride ($CdCl_2$) caused an up to 10-fold increase in Put content, which resulted from the induction of raised ADC activities by Cd treatment, however had little or no effect on Spd and Spm (Weinstein *et al.* 1986). Cd^{2+}-induced accumulation of Put in the free and soluble conjugated fractions was also observed in bean leaves, whereas Spd was slightly reduced. Cd or lead (Pb) stress combined with salinity inhibited nitrate reductase activity and decreased organic nitrogen and protein contents in leaf tissue of Indian mustard (*Brassica juncea* L.), the administration of Put reverted the nitrate reductase activity and partially recovered the reduced organic nitrogen and protein contents, indicating a role for Put to mitigate the adverse effect of the two stresses (Singh *et al.* 2002).

Copper (Cu) is a heavy metal that is an essential microelement for plant growth since it is constituent of many enzymes and proteins involved in different metabolic processes. However, it is highly phytotoxic at high concentrations, since it is implicated in numerous physiological processes (Fernandes and Henriques 1991). Cu^{2+} treatment increased the Put levels in detached rice leaves (Lin and Kao 1999). In European pear, $CuSO_4$ stress induced the changes of free PAs in the wild type and *MdSPDS1* transgenic lines (Wen *et al.* 2007). Put titers of all tested lines and wild type decreased at the 15th day after treatment. Spd and Spm titers differed among the lines, and (Spd+Spm)/Put ratios increased variously among the tested lines and wild type. Groppa *et al.* (2001, 2003) reported that Cd or Cu at 0.5 mM reduced Put and Spd content, without affecting Spm in sunflower leaf disks. They further demonstrated that Spm or Spd completely restored the activity of glutathione reductase, an antioxidant enzymes, that had been inhibited by Cu or Cd stress. However, Put content increase was observed in both cadmium- and copper-treated wheat leaves using the same experimental design by these authors, where Spd titers had no change and Spm was reduced about a half (Groppa *et al.* 2007b). The enhanced Put levels were accordant with the induced ADC and ODC activities and reduced DAO activity. This data from the same group gives a clearly support to the idea that PA metabolism in response to stress is sometimes dependent on plant species (Groppa *et al.* 2007b). The authors continually showed that Put and Spd levels were increased marked when higher intensity of stress (1 mM Cd or Cu) was used, indicating the dosage dependent feature of PA metabolism in response to stress (Groppa *et al.* 2007a). Wang *et al.* (2007) reported that Put content rose markedly in Cu^{2+} treated leaves of *Nymphoides peltatum*, while Spd and Spm titers reduced significantly. Exogenous application of both Spd and Spm effectively alleviated damages caused by Cu stress, but the effect of Spd was more significant than that of Spm.

Elevated Put and Dap after mercury (Hg) treatment were observed in the green alga *Chlorogonium elongatum* (Agrawal *et al.* 1992). The Spd and Spm content dropped significantly under Hg^{2+} stress (3 mg/L), while that of Put was increased markedly in *N. peltatum*. Exogenous Spd increased the Spd and Spm content and enhanced the antioxidant enzymes activities, and thereby ameliorate Hg^{2+} damage (Wang *et al.* 2004). Put levels were significantly increased after in both barley and rape, following stress with chromium (Cr) (III) and Cr (VI) (Hauschild 1993).

WOUNDING

PAs play an essential role in the wound healing responses in animals (Banan *et al.* 1998); however, little is known about the functions of PAs in wounding response. Exogenous PAs inhibited the wound-induced RNase and the rise in RNase activity in excised potato tuber discs, which may result from senescence-linked events (Altman 1982). It was showed that DAO activity increases in plant cell walls in response to wounding (Scalet *et al.* 1991). Perez-Amador *et al.* (2002) demonstrated an increase in the *ADC2* expression levels in response to mechanical wounding and methyl jasmonate treatment in *Arabidopsis*, providing the first experimental evidence for the involvement of PAs in plant wound responses. Transcript of *ODC1* gene accumulated strikingly at the 3 h and was continued until 12 h after wounding treatment in hot pepper (*Capsicum annuum* L.) (Yoo *et al.* 2004). Furthermore, the authors showed that the wounding signal does not seem to be transferred to the untreated region of the plant. *ADC2* is transiently induced upon wounding, followed by a transient increase in free Put levels concurrent with a transient decrease in the free Spm levels (Perez-Amador *et al.* 2002). Cowley and Walters (2005) also observed similar increased free-Put in wounded oilseed rape, which may result from both the increased ADC activity and the reduced Put catabolism. Further work needs to be done to determine the precise role of Put and/or Spd in wounding response.

BIOLOGICAL STRESS

Work on PAs and plants responses to microorganisms relatively lags behind other areas of plant PA research (Walters 2000). Nevertheless, much progress has been achieved regarding PAs and plant disease, extensively reviewed by Walters (Walters 2000, 2003). Greenland and Lewis (1984) were the first to show that PA levels are altered by pathogen infection. Later, PAs have been found involved in the response of plant infected by fungal pathogens (Greenland and Lewis 1984; Asthir *et al.* 2004) and viruses (Torrigiani *et al.* 1997), and in the interaction with mycorrhizal (Walters 2000). Like the behaviors under abiotic stresses, PA metabolism change after infected by pathogen also varies from decrease to increase.

In barley leaves infected with the fungal pathogen *Puccinia hordei*, "Green islands" will be formed, which surround the infection sites of rust and powdery mildew fungi. Spd levels in this tissue were enhanced 6 to 7-fold when compared to healthy control. Elevated PAs were also observed in barley infected by the powdery mildew fungus *Blumeria graminis* f. sp. *Hordei* (Walters *et al.* 1985), where the change of PA contents was concomitant with increased activities of ADC, ODC and SAMDC, and in wheat leaves infected with black stem rust (Foster and Walters 1992). Increased free and conjugated Put and Spd levels, as well as enhanced ADC and ODC activities, were revealed in tobacco leaves exhibiting hypersensitive response (HR) to *Tobacco mosaic virus* (TMV) (Torrigiani *et al.* 1997). Negrel *et al.* (1984) showed that ODC activity was 20-fold enhanced in tobacco leaves exhibiting HR to TMV infection.

In the response to abiotic stress, *ODC* induction is not so often to be seen; however *ODC* seems to be tightly involved in biotic stress. Indeed, the *ODC* gene was found up-regulated during the HR process (Yoda *et al.* 2006). A gene encoding putative ODC was identified by a differential screening cDNA library of the resistant hot pepper inoculated with avirulent tobacco mosaic virus (Yoo *et al.* 2004). Like hot pepper acidic pathogenesis related (PR) gene, *CaPR-1*, transcripts of the *CaODC1* gene started to accumulate at the 24 h post-inoculation of TMV-P0 and the signal was spread systemically. *CaODC1* showed an HR-specific expression pattern. Furthermore, the authors showed that *CaODC1* could be involved in a salicylic acid-independent, jasmonate acid- and/or ethylene-dependent plant defense against a broad range of pathogens including viruses and bacteria.

By contrast, infection of tobacco leaves with four different pathogens brought about a decrease of Put and Spd (Edreva 1997). The extent of decrease was not related to the type of pathogens (fungi, bacterial and virus) but to the severity of damage symptoms. The authors suggested that the reduction in PA levels in these interactions was a non-specific response to tissue damage. A similar response in PAs was observed in tomato infected with the fungus *Rhizopus stolonifer* (Bakanashvili *et al.* 1987), in tomato and *Gynura aurantiaca* infected by *Citrus exocortis viroid* (Belles *et al.* 1991). Belles *et al.* (1993) demonstrated that the reduction in Put was due to a decreased ODC activity in tomato.

When sugarcane (*Saccharum officinarum* L. cv. 'Barbados 42231') infected with the smut fungus *Ustilago scitaminea*, there was a decrease in the free and conjugated forms of Put and Spd titers, and an increase in free and conjugated forms of Spm (Legaz *et al.* 1998). Yamakawa *et al.* (1998) showed that TMV-infected necrotic lesion-forming tobacco leaves contained 20-fold higher free-Spm content in intercellular spaces than mock-inoculated leaves. After evaluating the effect of PAs on *PR-1* gene expression, it was found that Spm was most effective, followed by Spd whereas free Put and Cad had no effect. The authors further demonstrated that Spm induced various PR proteins accumulation and conferred TMV resistance to host plants via a salicylic acid-independent signaling pathway, providing a first evidence to link Spm to plant defense response against viral infection. This work was taken a stage further by another group from Japan (Takahashi *et al.* 2003, 2004a, 2004b) who identified two MAPKs, SIPK and WIPK and a subset of HR marker genes including *HIN1*, *HIN9*, *HIN18*, *HSR203J*, *HMGR*, *HSR201*, and *HSR515* as Spm responsive. After in-depth studies on these genes, they proposed a model for Spm signal transduction pathways in tobacco (Takahashi *et al.* 2004b). During HR triggered by TMV infection, free-Spm is accumulated in intercellular spaces, which is oxidized by DAO and/or PAO to generate H_2O_2 and to enhance Ca^{2+} flux into the cytoplasm. These series of events cause mitochondrial malfunction via an unknown mechanism, activating of a subset of HR marker genes as motioned above, which may contribute to defense against secondary pathogen attack and/or lead to programmed cell death during HR. Later, two Cys_2/His_2-type zinc-finger proteins were also revealed to be involved in this Spm signalling pathway (Uehara *et al.* 2005; Mitsuya *et al.* 2007). The model seems fit when infected with powdery mildew in barley, as evidenced by work from Cowley and Walters (2002).

PAs conjugated to phenolic compounds, HCAAs, have been shown to accumulate in incompatible interactions between plants and a variety of pathogens (reviewed in Walters 2003). In tobacco ('Xanthi' n.c.), there was an accumulation of numerous HCAAs, including feruloylputrescine and feruloyltyramine during the HR to TMV infection (Martin-Tanguy *et al.* 1973, 1976) as reviewed by Walters (2003). It was shown that treatment of tobacco leaf discs with coumaroyl and caffeoylputrescine reduced local lesion formation by 90% following TMV inoculation (Martin-Tanguy *et al.* 1976; Walters 2003). Lloyd and Naidoo (1983) proposed a linear correlation between the production of phenolics and resistance acquisition against smut infection in sugarcane. Torrigiani *et al.* (1997) suggested that high levels of PA conjugates might be required for the necrotic lesion to develop in tobacco leaves exhibiting HR. Therefore, conjugation of PAs to phenolics has been described as a defense mechanism against infection of pathogens (Legaz *et al.* 1998).

Little is known about the interaction between plants and mycorrhiza. The role of PAs in the mycorrhizal symbiosis was firstly demonstrated by El Ghachtouli *et al.* (1995, 1996), who showed that exogenously applied PAs (0.5 mM) increased *Glomus intraradices* colonization frequency of pea roots. Kytoviita and Sarjala (1997) showed that ecto-mycorrhizal symbiosis increased free Put levels in mycorrhizal roots of Scots pine. The application of an isolate of *Streptomyces griseoluteus*, a bean rhizosphere, to soil amended with arginine significantly promoted the growth of bean plants, and resulted in a significant increase in the levels of PAs compared with control plants (Nassar *et al.* 2003). A higher content of total free PAs in arbuscular mycorrhizal mycorrhized *Lotus glaber* plants was detected compared to non-mycorrhized ones (Sannazzaro *et al.* 2007). Furthermore, mycorrhization also increased (Spd+ Spm)/Put ratios in *L. glaber* roots. The authors proposed that modulation of PA pools might be one of the mechanisms used by arbuscular mycorrhizal to improve *L. glaber* adaptation to saline soils. Such hypothesis and roles of PAs in plant-mycorrhizal interactions await further research to test.

GENETIC MANIPULATION TO MODULATE PA METABLOLIC FLUXES AND STRESS RESPONSE

A wide range of plant developmental and stress-related processes correlates with changes in PA levels, however, it remains to be determined if alterations in PA content are a direct cause of these effects (Panicot *et al.* 2002a). Since all the genes in the PA biosynthetic pathway have been cloned, genetic manipulation of these genes offers a convenient strategy to elucidate the regulatory functions of PAs in plant systems and a very effective and potential tool to improve the tolerance against adverse environmental conditions as well (Panicot *et al.* 2002a; Alcázar *et al.* 2006a, 2006b). Kakkar and Sawhney (2002) and Alcázar *et al.* (2006a, 2006b) present a good review on this topic. Herein studies on PA pathway manipulation related to stress tolerance will be briefly introduced.

Masgrau *et al.* (1997) obtained transgenic tobacco plants containing oat *ADC* fused with a tetracycline-inducible promoter. Transgenic tobacco lines, induced during vegetative stage, displayed different degrees of an altered phenotype, the severity of which was correlated with Put content. The detrimental effects caused by overexpression of *ADC* gene was similar to those observed by *ADC* activation induced by osmotic stress in the oat leaf system (Borrell *et al.* 1996). The authors postulated that high levels of endogenous Put in cells are toxic to the plant (Masgrau *et al.* 1997). Over-expression an oat *ADC* gene in rice resulted in improved drought tolerance; however, constitutive over-expression of this gene severely affected the development of the transgenic plants (Capell *et al.* 1998). Capell *et al.* (2004) reported transgenic plants over-expressing the *Datura ADC* gene under the control of maize Ubi-1 promoter. Under drought stress, transgenic plants expressing *Datura ADC* produced much higher levels of Put that extends beyond the critical threshold required to initiate the conversion of excess Put to Spd and Spm, promoting Spd and Spm synthesis and ultimately protecting the plants from drought, whereas wild-type plants were incapable to produce sufficient Put to trigger the Put conversion. Expression of the *ADC* transgene under the control of an ABA-inducible promoter led to stress-induced up-regulation of ADC activity and PA accumulation in transgenic rice plants (Roy and Wu 2001), suggesting enhanced tolerance to salinity stress for transgenic plants.

It failed to produce sense *SAMDC* transgenic plant with the 35S *SAMDC* gene construct without *SAMDC* uORF, which might be resulted in toxicity by excessive cellular PA in potato transformants (Kumar *et al.* 1996). Over-expression of the carnation *SAMDC* gene construct containing a short uORF in the 5′-untranslated region in transgenic tobacco (*N. tabacum* L. cv. 'Wisconsin 38') plants led to constitutive increases in PA content and enhanced tolerance to multiple abiotic environmental stresses with inducing of the genes for anti-oxidative enzymes (Wi *et al.* 2006). The transgenic plants were healthy and did not show any difference in organ phenotype compared to the wild-type. Furthermore, the transgenic plants had increased number and weight of seeds and elevated net photosynthetic rate. This study demonstrated the feasibility of engineering plant for increased tolerance of abiotic stress and enhanced productivity through overproduction of PAs achieved by over-expression of *SAMDC* gene with short uORF. Over-expression of *Arabidopsis SAMDC* in tobacco plants resulted in increased SAMDC activity, accumulation of dcSAM and perturbation of PA levels (Franceschetti *et al.* 2004). A comparative proteomics analysis between transgenic plants and wild type plants revealed that chloroplast ribonucleoproteins and defense protein PR-1, a known marker for systemic acquired resistance, were induced in transgenic lines compared with wild type (Franceschetti *et al.* 2004). Ripening induced over-expression of *SAMDC* in tomato led to PA compositional changes by increasing the flux of Put into Spd and Spm, resulted in tomato with higher levels of Spd and Spm, which have a longer vine life and better quality as well (Mehta *et al.* 2002). Furthermore, ethylene production in transgenic tomato was consistently higher than those in non-transgenic control fruit, suggesting that PA and ethylene biosynthesis pathway can act simultaneously in ripening tomato fruit. Waie and Rajam (2003) reported that transgenic tobacco lines with over-expresion of *SAMDC* exhibited tolerance to salinity and drought as well as to fungal wilts caused by *Verticillium dahliae* and *Fusarium oxysporum*. This is the first report in which the genetic manipulation of PA metabolism resulted in enhanced tolerance to both abiotic and biotic stresses in transgenic plants.

A. thaliana over-expressing *SPDS* transgenes from *Cucurbita ficifolia* (*FSPDS1*) showed a 5- to 6-fold increase in *SPDS* activity, resulted in a 1.3- to 2-fold and 1.6- to 1.8-fold increase in the free-Spd and free-Spm titers, respectively. Several transgenic lines also exhibited higher free-Put contents. These transgenic plants were highly tolerant to multiple environmental stresses including chilling and freezing temperatures, salinity, drought, hyperosmosis, and paraquat toxicity (Kasukabe *et al.* 2004). A comparison analysis by means of cDNA microarray between the chilled leaves of transgenic line and wild type revealed that genes encoding transcription factors such as WRKY, B-box zinc finger proteins, NAM proteins, *DREB2B*, MYB, and NAC domain proteins were up-regulated in the transgenes. The *FSPDS1*-transgenic sweet potato (*Ipomoea batatas*, cv. ‘Kokei 14’) plants were also found with improved tolerance to salt, drought, chilling, heat and paraquat stresses (Kasukabe *et al.* 2006). An apple *SPDS* gene (*MdSPDS1*) was transformed into European pear (*Pyrus communis* L. cv. ‘Ballad’), and a total of 21 transgenic lines showing various Spd titers and *MdSPDS1* expression levels were obtained (Wen *et al.* 2007). It was proved that the overexpression of *MdSPDS1* gene substantially increased the tolerance to multiple stresses by altering the PA titers in pear.

High level of PAs may be cytotoxic, thus it is a good alternative to use the stress-inducible promoters to regulate the expression of PA biosynthetic genes as evidenced by successful examples in rice (Capell *et al.* 2004), in tomato (Mehta *et al.* 2002), etc. A total of 16 transgenic rice cell lines expressing the oat *adc* cDNA accumulates increased level of Put in callus but not in vegetative tissue or seeds with the exception of only one line, which showed very significant increases in Put preferentially in seeds. Therefore, in order to identify transgenic plants which express a desired phenotype or genotype, it is generally necessary to screen adequate numbers of independently derived transgenic plants (Noury *et al.* 2000).

CONCLUDING REMARKS

Tons of papers have been produced on the relationships between PA and environmental stresses; however, there is still paucity of direct and unequivocal evidence on the involvement of PA in stress tolerance. Many existing important findings came from the genetic and molecular analysis of mutants with either altered concentrations of PAs or altered sensitivity to PAs from model plants like *Arabidopsis* and tobacco, thus more efforts should be devoted to the study using mutant plants. Genetic manipulation of PA pathway genes has provided some valuables information concerning their roles in stress response. Nevertheless, it is necessary to better understand the mechanism of PA homeostasis in plant cells in order to devise proper genetic manipulation strategy. The combined application of transcriptomic and proteomic approaches will help to elucidate the PA roles in signaling network under environment stresses. Recently, Spm has been demonstrated to have important role in various stress response. Genetic manipulation of *SPMS* gene may be also a good strategy to confer multiple stresses. Few reports have been published on the genetic manipulation to confer biotic stress, which may be a direction deserving more attention. Regarding the enhanced tolerance of transgenic plants with perturbed PA titers, the evidence from the field trial is still lacking. Some successful evidences of PA genetic manipulation to counteract environment stresses are expected to promote its extended application to other crops.

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REFERENCES

Agrawal SB, Agrawal M, Lee EH, Kramer GF, Pillai P (1992) Changes in polyamine and glutathione contents of a green alga, *Chlorogonum elongatum* (Dang) France exposed to mercury. *Environmental and Experimental Botany* **32**, 145-151

Alcázar R, Cuevas JC, Patron M, Altabella T, Tiburcio AF (2006a) Abscisic acid modulates polyamine metabolism under water stress in *Arabidopsis thaliana*. *Physiologia Plantarum* **128**, 448-455

Alcázar R, Marco F, Cuevas JC, Patron M, Ferrando A, Carrasco P, Tiburcio AF, Altabella T (2006b) Involvement of polyamines in plant response to abiotic stress. *Biotechnology Letters* **28**, 1867-1876

Allen KD (2002) Assaying gene content in *Arabidopsis*. *Proceedings of the National Academy of Sciences USA* **99**, 9568-9572

Altman A (1982) Polyamines and wounded storage tissues – Inhibition of RNase activity and solute leakage. *Physiologia Plantarum* **54**, 194-198

An LZ, Liu GX, Zhang MX, Chen T, Liu YH, Feng HY, xu SJ, Qiang WY, Wang XL (2004) Effect of enhanced UV-B radiation on polyamine content and membrane permeability in cucumber leaves. *Russian Journal of Plant Physiology* **51**, 658-662

Andreadakis A, Kotzabasis K (1996) Changes in the biosynthesis and catabolism of polyamines in isolated plastids during the chloroplast photo development. *Journal of Photochemistry and Photobiology B* **33**, 163-170

Anisul Islam M, Blake TJ, Kocacinar F, Lada R (2003) Ambiol, spermine, and aminoethoxyvinylglycine prevent water stress and protect membranes in *Pinus strobus* L. under drought. *Trees* **17**, 278-284

Antognoni F, Fornale S, Grimma C, Komor E, Bagni N (1998) Long distance translocation of polyamines in phloem and xylem of *Ricinus communis* L. plants. *Planta* **204**, 520-527

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. *Plant Science* **166**, 3-16

Asthir B Spoor W, Duffus CM (2004) Involvement of polyamines, diamine oxidase and polyamine oxidase in resistance of barley to *Blumeria graminis* f. sp. *Hordei*. *Euphytica* **136**, 307-312

Aziz A, Larher F (1995) Changes in polyamine titers associated with the proline response and osmotic adjustment of rape leaf discs submitted to osmotic stresses. *Plant Science* **112**, 175-186

Aziz A, Martin-Tanguy J, Larher F (1997) Plasticity of polyamine metabolism associated with high osmotic stress in rape leaf discs and with ethylene treatment. *Plant Growth Regulation* **21**, 153-163

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

Bagni N, Tassoni A (2006) The role of polyamines in relation to flowering senescence. In: Teixeira da Silva JA (Ed) *Floricltre, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1^{st} Edn, Vol I), Global Science Books, London, pp 88-95

Bailey-Serres J, Mittler R (2006) The roles of reactive oxygen species in plant cells. *Plant Physiology* **141**, 311

Bakanashvili M, Barkai-Golan R, Kopeliovitch E, Apelbaum A (1987) Polyamine biosynthesis in *Rhizopus*-infected tomato fruits: possible interaction with ethylene. *Physiological and Molecular Plant Pathology* **31**, 41-50

Banan A, McCormack SA, Johnson LR (1998) Polyamines are required for microtubule formation during gastric mucosal healing. *American Journal of Physiology* **274**, G879-G885

Basra RK, Basra AS, Malik CP, Grover IS (1997) Are polyamines involved in the heat shock protection of mung bean seedlings? *Botanical Bulletin of Academia Sinica* **38**, 165-169

Basu HS, Schwietert HCA, Feuerstein BG Marton LJ (1990) Effect of variation in the structure of spermine on the association with DNA and the induction of DNA conformational changes. *Biochemical Journal* **269**, 329-334

Basu R, Ghosh B (1991) Polyamines in various rice (*Oryza sativa* L.) genotypes with respect to sodium chloride salinity. *Plant Physiology* **82**, 575-581

Belles JM, Perez-Amador MA, Carbonell J, Conejero V (1991) Polyamines in plants infected by citrus exocortis viroid or treated with silver ions and ethephon. *Plant Physiology* **96**, 1053-1059

Belles JM, Perez-Amador MA, Carbonell J, Conejero V (1993) Correlation between ornithine decarboxylase and putrescine in tomato plants infected by citrus exocortis viroid or treated with ethephon. *Plant Physiology* **102**, 933-937

Benavides MP, Gallego SM, Comba ME, Tomaro ML (2000) Relationship between polyamines and paraquat toxicity in sunflower leaf discs. *Plant Growth Regulation* **31**, 215-224

Besford RD, Richardson CM, Campos JL, Tiburcio AF (1993) Effect of polyamines on stabilization complexes in thylakoid membranes of osmotically stressed oat leaves. *Planta* **189**, 201-206

Borrell A, Bestford T, Altabella T, Masgrau C, Tiburcio AF (1996) Regulation of arginine decarboxylase by spermine in osmotically-stressed oat leaves. *Physiologia Plantarum* **98**, 105-110

Borrell A, Culianez-Macia FA, Altabella T, Besford RT, Flores D, Tiburcio AF (1995) Arginine decarboxylase is localized in chloroplasts. *Plant Physiology* **109,** 771-776

Bors W, Langebartels C, Michel C, Sandermann H (1989) Polyamines as radical scavengers and protectants against ozone damage. *Phytochemistry* **28**, 1589-1595

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

Calderbank A (1968) The bipyrydinium herbicides. *Advanced Pest Control Research* **8**, 127-235

Capell T, Bassie L, Christou P (2004) Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. *Proceedings of the National Academy of Sciences USA* **101**, 9909-9914

Capell T, Escobar H, Liu H, Burtin D, Lepri O, Christou P (1998) Overexpression of the oat arginine decarboxylase cDNA in transgenic rice (*Oryza sativa* L.) affects normal development patterns *in vitro* and results in putrescine accumulation in transgenic plants. *Theoretical and Applied Genetics* **97**, 246-254

Chang CJ, Kao CH (1997) Paraquat toxicity is reduced by polyamines in rice leaves. *Plant Growth Regulation* **22**, 163-168

Chen S, Wang S, Huettermann A, Altman A (2002) Xylem abscisic acid accelerates leaf abscission by modulating polyamine and ethylene synthesis in water-stressed intact poplar. *Trees* **16**, 16-22

Coghlan SE, Walters DR (1990) Polyamine metabolism in 'green-islands' on powdery mildew-infected barley leaves: possible interactions with senescence. *New Phytologist* **116**, 417-424

Cohen SS (1971) *Introduction to the Polyamines*, Prentice-Hall, Englewood Cliffs, NJ, 179 pp

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

Cowley T, Walters DR (2002) Polyamine metabolism in barley reacting hypersensitively to the powdery mildew fungus *Blumeria graminis* f. sp. *hordei*. *Plant, Cell and Environment* **25**, 461-468

Cowley T, Walters DR (2005) Local and systemic changes in arginine decarboxylase activity, putrescine levels and putrescine catabolism in wounded oilseed rape. *New Phytologist* **165**, 807-811

Delis C, Dimou M, Efrose RC, Flemetakis E, Aivalakis G, Katinakis P (2005) Ornithine decarboxylase and arginine decarboxylase gene transcripts are co-localized in developing tissues of *Glycine max* etiolated seedlings. *Plant Physiology and Biochemistry* **43**, 19-25

Desiderio MA, Dansi P, Tacchini L, Bernelli-Zazzera A (1999) Influence of polyamines on DNA binding of heat shock and activator protein 1 transcription factors induced by heat shock. *FEBS Letters* **455**, 149-153

Dondini L, Bonazzi S, Serafini-Fracassini D (2000) Recovery of growth capacity by polyamines and of chloroplast transglutaminase activity in a polyamine-deficient variant strain of *Dunaliella salina*. *Journal of Plant Physiology* **157**, 473-480

Dondini L, Bonazzi S, Del Duca S, Bregoli AM, Serafini-Fracassini D (2001) Acclimation of chloroplast transglutaminase to high NaCl concentration in a polyamine-deficient variant strain of Dunaliella salina and in its wild type. *Journal of Plant Physiology* **158**, 185-197

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

Edreva A (1997) Tobacco polyamines as affected by stresses induced by different pathogens. *Biologia Plantarum* **40**, 317-320

El Ghachtouli N, Martin-Tanguy J, Paynot M, Gianinazzi S (1996) First report of the inhibition of arbuscular mycorrhizal infection of *Pisum sativum* by specific and irreversible inhibition of polyamine biosynthesis or by gibberellic acid treatment. *FEBS Letters* **385**, 189-192

El Ghachtouli N, Paynot M, Morandi D, Martin-Tanguy J, Gianinazzi S (1995) The effect of polyamines on endomycorrhizal infection of wildtype *Pisum sativum*, cv. Frisson (nod+myc+) and two mutants (nodmyc+and nodmyc-). *Mycorrhiza* **5**, 189-192

Feirer RP, Hocking KL, Woods PJ (1998) Involvement of arginine decarboxylase in the response of *Arabidopsis thaliana* to osmotic stress. *Journal of Plant Physiology* **153**, 733-738

Fernandes JC, Henriques FS (1991) Biochemical, physiological, and structural effects of excess copper in plants. *Botanical Review* **57**, 246-273

Flores HE, Filner P (1985) Metabolic relationships of putrescine, GABA and alkaloids in cell and root cultures. In: Newman KH, Barz W, Reinhard E (Eds) *Primary and Secondary Metabolism of Plant Cell Cultures*, Springer, New York, USA, pp 37-42

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

Foster SA, Walters DR (1991) Polyamine concentrations and arginine decarboxylase activity in wheat exposed to osmotic stress. *Physiologia Plantarum* **82**, 185-190

Foster SA, Walters DR (1992) Polyamine concentrations and activities of ornithine and arginine decarboxylase in wheat infected with the stem rust fungus. *Journal of Plant Physiology* **140**, 134-136

Franceschetti M, Hanfrey C, Scaramagli S, Torrigiani P, Bagni N, Burtin D, Michael AJ (2001) Characterization of monocot and dicot plant *S*-adenosylmethionine decarboxylase gene families including identification in the mRNA of a highly conserved pair of upstream overlapping open reading flames. *Biochemical Journal* **353**, 403-409

Franceschetti M, Perry B, Thompson B, Hanfrey C, Michael AJ (2004) Expression proteomics identifies biochemical adaptations and defense responses in transgenic plants with perturbed polyamine metabolism. *FEBS Letters* **576**, 477-480

Friedman R, Altman A, Levin N (1989) The effect of salt stress on polyamine biosynthesis and content in mung bean plants and in halophyte. *Physiologia Plantarum* **76**, 295-302

Frohnmeyer H, Staiger D (2003) Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. *Plant Physiology* **133**, 1420-1428

Fukao T, Bailey-Serres J (2004) Plant responses to hypoxia – is survival a balancing act? *Trends in Plant Science* **9**, 449-456

Galston AW, Kaur-Sawhney R (1990) Polyamines in plant physiology. *Plant Physiology* **94**, 406-410

Geny L, Broquedis M, Soyer JP, Bouard J (1997) Effect of potassium nutrition on polyamine content of various organs of fruiting cuttings of *Vitis vinifera* L. cv Cabernet Sauvignon. *American Journal of Enology and Viticulture* **48**, 85-92

Gratao PL, Polle A, Lea PJ, Azevedo RA (2005) Making the life of heavy metal-stressed plants a little easier. *Functional Plant Biology* **32**, 481-494

Greenland AJ and Lewis DH (1984) Amines in barley leaves infected with brown rust and their possible relevance to formation of 'green islands'. *New Phytologist* **96**, 283-291

Groppa MD, Benavides MP, Tomaro ML (2003) Polyamine metabolism in sunflower and wheat leaf discs under cadmium and copper stress. *Plant Science* **164**, 293-299

Groppa MD, Ianuzzo MP, Tomaro ML, Benavides MP (2007a) Polyamine metabolism in sunflower plants under long-term cadmium or copper stress. *Amino Acids* **32**, 265-275

Groppa MD, Tomaro ML, Benavides MP (2001) Polyamines as protectors against cadmium or copper-induced oxidative damage in sunflower leaf discs. *Plant Science* **161**, 481-488

Groppa MD, Tomaro ML, Benavides MP (2007b) Polyamines and heavy metal stress: the antioxidant behavior of spermine in cadmium- and copper-treated wheat leaves. *BioMetals* **20**, 185-195

Ha HC, Sirisoma NS, Kuppusamy P, Zweier JL, Woster PM, Casero RA (1998) The natural polyamine spermine functions directly as a free radical scavenger. *Proceedings of the National Academy of Sciences USA* **95**, 11140-11145

Hamana K, Matsuzaki S (1992) Polyamines as a chemotaxonomic marker in bacterial systematics. *Critical Reviews in Microbiology* **18**, 261-283

Hanfrey C, Sommer S, Mayer MJ, Burtin D, Michael AJ (2001) *Arabidopsis thaliana* polyamine biosynthesis: absence of ornithine decarboxylase and the mechanism of arginine decarboxylase activity, *The Plant Journal* **27**, 551-560

Hanzawa Y, Takahashi T, Michael AJ, Burtin D, Long D, Piñeiro M, Coupland G, Komeda Y (2000) *ACAULIS5*, an *Arabidopsis* gene required for stem elongation, encodes a spermine synthase. *The EMBO Journal* **19**, 4248-

4256

Hart JJ, di Tomaso JM, Linscott DL, Koehian LV (1992) Transport interactions between paraquat and polyamines in roots of intact maize seedlings. *Plant Physiology* **99**, 1400-1405

Hashimoto T, Tamaki K, Suzuki K, Yamada Y (1998) Molecular cloning of plant spermidine synthases. *Plant and Cell Physiology* **39**, 73-79

Hauschild MZ (1993) Putrescine (1,4-diaminobutane) as an indicator of pollution-induced stress in higher plants: barley and rape stressed with Cr(III) or Cr(VI). *Ecotoxicology and Environmental Safety* **26**, 228-247

He L, Nada K, Kasukabe Y, Tachibana S (2002) Enhanced susceptibility of photosynthesis to low-temperature photoinhibition due to interruption of chill-induced increase of S-adenosylmethionine decarboxylase activity in leaves of spinach (*Spinacia oleracea* L.). *Plant and Cell Physiology* **43**, 196-206

Heino P, Palva ET (2003) Signal transduction in plant cold acclimation. In: Hirt H, Shinozaki K (Eds) *Plant Responses to Abiotic Stress, Topics in Current Genetics* (Vol 4), Springer-Verlag, New York, USA, pp 151-185

Hiatt A, Malmberg RL (1988) Utilization of putrescine in tobacco cell lines resistant to inhibitors of polyamine synthesis. *Plant Physiology* **86**, 441-446

Hou MH, Lin SB, Yuann JM, Lin WC, Wang AHJ, Kan LS (2001) Effects of polyamines on the thermal stability and formation kinetics of DNA duplexes with abnormal sequence. *Nucleic Acids Research* **29**, 5121-5128

Illingworth C, Mayer MJ, Elliott K, Hanfrey C, Walton NJ, Michael AJ (2003) The diverse bacterial origins of the *Arabidopsis* polyamine biosynthetic pathway. *FEBS Letters* **549**, 26-30

Imai R, Ali A, Pramanik HR, Nakaminami K, Sentoku N, Kato H (2004) A distinctive class of spermidine synthase is involved in chilling response in rice. *Journal of Plant Physiology* **161**, 883-886

Janowitz T, Kneifel H, Piotrowski M (2003) Identification and characterization of plant agmatine iminohydrolase, the last missing link in polyamine biosynthesis of plants. *FEBS Letters* **544**, 258-261

Kakkar PK, Rai VK, Nagar PK (1998) Polyamine uptake and translocation in plants. *Biologia Plantarum* **40**, 481-491

Kakkar RK, Sawhney VK (2002) Polyamine research in plants – a changing perspective. *Physiologia Plantarum* **116**, 281-292

Kashiwagi K, Hosokawa N, Furuchi T, Kobayashi H, Sasakawa C, Yoshikawa M, Igarashi K (1990) Isolation of polyamine transport-deficient mutants of *Escherichia coli* and cloning of the genes for polyamine transport proteins. *The Journal of Biological Chemistry* **265**, 20893-20897

Kasinathan V, Wingler A (2004) Effect of reduced arginine decarboxylase activity on salt tolerance and on polyamine formation during salt stress in *Arabidopsis thaliana*. *Physiologia Plantarum* **121**, 101-107

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

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

Kaur-Sawhney R, Tiburcio AF, Altabella T, Galston A (2003) Polyamines in plants: an overview. *Journal of Cell and Molecular Biology* **2**, 1-12

Kim TE, Kim SK, Han TJ, Lee JS, Chang SC (2002) ABA and polyamines act independently in primary leaves of cold-stressed tomato (*Lycopersicon esculentum*). *Physiologia Plantarum* **115**, 370-376

Kinnersley AM, Turano FJ (2000) Gamma aminobutyric acid (GABA) and plant responses to stress. *Critical Reviews in Plant Sciences* **19**, 479-509

Kitashiba H, Honda C, Moriguchi T (2006) Identification of polyamine oxidase genes from apple and expression analysis during fruit development and cell growth. *Plant Biotechnology* **23**, 425-429

Knott JM, Romen P, Sumper M (2007) Putative spermine synthases from *Thalassiosira pseudonana* and *Arabidopsis thaliana* synthesize thermospermine rather than spermine. *FEBS Letters* **581**, 3081-3086

Königshofer H, Lechner S (2002) Are polyamines involved in the synthesis of heat-shock proteins in cell suspension cultures of tobacco and alfalfa in response to high-temperature stress? *Plant Physiology and Biochemistry* **40**, 51-59

Kramer GF, Krizek DT, Mirecki RM (1992) Influence of photosynthetically active radiation and spectral quality on UV-B induced polyamine accumulation in soybean. *Phytochemistry* **31**, 1119-1125

Kramer GF, Norman HA, Krizek DT, Mirecki RM (1991) Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. *Phytochemistry* **30**, 2101-2108

Krishnamurthy R, Bhagwat KA (1989) Polyamines as modulators of salt tolerance in rice cultivars. *Plant Physiology* **91**, 500-504

Krupa SV, Manning WJ (1988) Atmospheric ozone formation and effects on vegetation. *Environmental Pollution* **50**, 101-137

Kumar A, Taylor MA, Mad Arif SA, Davies HV (1996) Potato plants expressing antisense and sense *S*-adenosylmethionine decarboxylase (SAMDC) transgenes show altered levels of polyamines and ethylene: antisense plants display abnormal phenotypes. *The Plant Journal* **9**, 147-158

Kurepa J, Smalle J, van Montagu M, Inzé D (1998) Oxidative stress tolerance and longevity in *Arabidopsis*: the late-flowering mutant *gigantea* is tolerant to paraquat. *The Plant Journal* **14**, 759-764

Kurepa J, Smalle J, van Montagu M, Inzé D (1993) Polyamines and paraquat toxicity in *Arabidopsis thaliana*. *Plant and Cell Physiology* **39**, 987-992

Kytoviita MM, Sarjala T (1997) Effects of defoliation and symbiosis on polyamine levels in pine and birch. *Mycorrhiza* **7**, 107-111

Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 251-275

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

Larher F, Aziz A, Deleu C, Lemesle P, Ghaffar A, Bouchard F, Plasman M (1998) Suppression of the osmoinduced proline response of rapeseed leaf discs by polyamines. *Physiologia Plantarum* **102**, 139-145

Lee TM, Lur HS, Chu C (1997) Role of abscisic acid in chilling tolerance of rice (*Oryza sativa* L.) seedlings: II. Modulation of free polyamine levels. *Plant Science* **126**, 1-10

Lee TM, Shieh YJ, Chou CH (1996) Role of putrescine in enhancing shoot elongation in *Scirpus mucronatus* under submergence. *Physiologia Plantarum* **96**, 419-424

Legaz ME, de Armas R, Pinon D, Vincente C (1998) Relationships between phenolics-conjugated polyamines and sensitivity of sugarcane to smut (*Ustilago scitaminea*). *Journal of Experimental Botany* **49**, 1723-1728

Legocka J, Kluk A (2005) Effect of salt and osmotic stress on changes in polyamine content and arginine decarboxylase activity in *Lupinus luteus* seedlings. *Journal of Plant Physiology* **162**, 662-668

Li CZ, Jiao J, Wang GX (2004) The important roles of reactive oxygen species in the relationship between ethylene and polyamines in leaves of spring wheat seedlings under root osmotic stress. *Plant Science* **166**, 303-315

Lin CC, Kao CH (1999) Excess copper induces an accumulation of putrescine in rice leaves. *Botanical Bulletin of Academia Sinica* **40**, 213-218

Lin WX, Wu XC, Liang J, Guo YC, He HQ, Chen FY, Liang YY (2002) Effect of enhanced UV2B radiation on polyamine metabolism and endogenous hormone contents in rice (*Oryza sativa* L.). *Chinese Journal of Applied Ecology* **13**, 807-813 (In Chinese with English abstract)

Liu F, VanToai T, Moy LP, Bock G, Linford LD, Quackenbush J (2005a) Global transcription profiling reveals comprehensive insights into hypoxic response in *Arabidopsis*. *Plant Physiology* **137**, 1115-1129

Liu HP, Dong BH, Zhang YY, Liu ZP, Liu YL (2004) Relationship between osmotic stress and the levels of free, conjugated, and bound polyamines in leaves of wheat seedlings. *Plant Science* **166**, 1261-1267

Liu HP, Yu BJ, Zhang WH, Liu YL (2005b) Effect of osmotic stress on the activity of H+-ATPase and the levels of covalently and noncovalently conjugated polyamines in plasma membrane preparation from wheat seedling roots. *Plant Science* **168**, 1599-1607

Liu J, Yu BJ, Liu YL (2006a) Effects of spermidine and spermine levels on salt tolerance associated with tonoplast H^+-ATPase and H^+-PPase activities in barley roots. *Plant Growth Regulation* **49**, 119-126

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

Liu JH, Nada K, Honda C, Kitashiba H, Wen XP, Pang XM, Moriguchi T (2006b) Polyamine biosynthesis of apple callus under salt stress: importance of arginine decarboxylase pathway in stress response. *Journal of Experimental Botany* **57**, 2589-2599

Lloyd HL, Naidoo G (1983) Chemical array potentially suitable for determination of smut resistance of sugarcane cultivars. *Plant Disease* **67**, 1103-1105

Lutz C, Navakoudis E, Seidlitz HK, Kotzabasis K (2005) Simulated solar irradiation with enhanced UV-B adjust plastid- and thylakoid-associated polyamine changes for UV-B protection. *Biochimica et Biophysica Acta* **1710**, 24-33

Maccarrone M, Veldink GA, Vliegenthart JFG, Finazzi Agrò A (1997) Ozone stress modulates amine oxidase and lipoxygenase expression in lentil (*Lens culinaris*) seedlings. *FEBS Letters* **408**, 241-244

Maestri E, Klueva N, Perrotta C, Gulli M, Nguyen HT, Marmiroli N (2002) Molecular genetics of heat tolerance and heat shock proteins in cereals. *Plant Molecular Biology* **48**, 667-681

Maiale S, Sánchez DH, Guirado A, Vidal A, Ruiz OA (2004) Spermine accumulation under salt stress. *Journal of Plant Physiology* **161**, 35-42

Mancuso S, Marras AM (2006) Adaptative response of *Vitis* root to anoxia. *Plant and Cell Physiology* **47**, 401-409

Margosiak SA, Dharma A, Bruce-Carver MR, Gonzales AP, Louie D, Kuehn GD (1990) Identification of the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase as a substrate for transglutaminase in *Medicago sativa* L. (alfalfa). *Plant Physiology* **92**, 88-92

Martínez-Romero D, Serrano M, Valero D (2003) Physiological changes in pepino (*Solanum muricatum* Ait.) fruit stored at chilling and non-chilling temperatures. *Postharvest Biology and Technology* **30**, 177-186

Martin-Tanguy J (1997) Conjugated polyamines and reproductive development: biochemical, molecular and physiological approaches. *Physiologia Plantarum* **100**, 675-688

Martin-Tanguy J (2001) Metabolism and function of polyamines in plants: recent development (new approaches). *Plant Growth Regulation* **34**, 135-148

Martin-Tanguy J, Martin C, Gallet M (1973) Presence de composes aromatiques lies a la putrescine dans divers *Nicotiana* viruses. *Comptes Rendus des Sciences de l'Academie des Sciences. Paris, Serie D* **276**, 1433-1435

Martin-Tanguy J, Martin C, Gallet M, Vernoy R (1976) Sur le puissants inhibitors de multiplication du virus de la mosaique de tabae. *Comptes Rendus des Sciences de l'Academie des Sciences. Paris, Serie D* **282**, 2231-2234

Marton L, Morris D (1987) Molecular and cellular functions of the polyamines. In: McCann PP, Pegg A, Sjoerdsma A (Eds) *Inhibition of Polyamine Metabolism*, Academic Press, San Diego, USA, pp 79-105

Masgrau C, Altabella T, Farras R, Flores D, Thompson AJ, Besford RT, Tiburcio AF (1997) Inducible over-expression of oat arginine decarboxylase in transgenic tobacco plants. *The Plant Journal* **11**, 465-473

Mehta RA, Cassol T, Li N, Ali N, Handa AK, Mattoo AK (2002) Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. *Nature Biotechnology* **20**, 613-618

Messiaen J, Cambier P, van Cutsem P (1997) Polyamines and pectins. I. Ion exchange and selectivity. *Plant Physiology* **113**, 387-395

Minocha SC, Minocha R (1995) Role of polyamines in somatic embryogenesis. In: Bajaj YPS (Ed) *Biotechnology in Agriculture and Forestry (Vol 30) Somatic Embryogenesis and Synthetic Seed I*, Springer-Verlag, Heidelberg, Germany, pp 53-70

Mitsuya Y, Takahashi Y, Uehara Y, Berberich T, Miyazaki A, Takahashi H, Kusano T (2007) Identification of a novel Cys2/His2-type zinc-finger protein as a component of a spermine-signaling pathway in tobacco. *Journal of Plant Physiology* **164**, 785-793

Nadeau P, Delaney S, Chouinard L (1987) Effect of cold hardening on the regulation of polyamine levels in wheat (*Triticum aestivum* L.) and alfalfa (*Medicago sativa* L.). *Plant Physiology* **84**, 73-77

Nam KH, Lee SH, Lee J (1997) Differential expression of ADC mRNA during development and upon acid stress in soybean (*Glycine max*) hypocotyls. *Plant and Cell Physiology* **38**, 1156-1166

Nassar AH, El-Tarabily KA, Sivasithamparam K (2003) Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine-producing isolate of *Streptomyces griseoluteus*. *Plant Growth Regulation* **40**, 97-106

Navakoudis E, Lütz C, Langebartels C, Lütz-Meindl U, Kotzabasis K (2003) Ozone impact on the photosynthetic apparatus and the protective role of polyamines. *Biochimica et Biophysica Acta* **1621**, 160-169

Nayyar H, Kaur S, Smita, Kumar S, Singh KJ, Dhir KK (2005) Involvement of polyamines in the contrasting sensitivity of chickpea (*Cicer arietinum* L.) and soybean (*Glycine max* (L.) Merrill.) to water deficit stress. *Botanical Bulletin of Academia Sinica* **46**, 333-338

Negrel J, Vallee JC, Martin C (1984) Ornithine decarboxylase activity and the hypersensitive reaction of tobacco to tobacco mosaic virus in *Nicotiana tabacum*. *Phytochemistry* **23**, 2747-2751

Noury M, Bassie L, Lepri O, Kurek I, Christou P, Capell T (2000) A transgenic rice cell lineage expressing the oat arginine decarboxylase (*adc*) cDNA constitutively accumulates putrescine in callus and seeds but not in vegetative tissues. *Plant Molecular Biology* **43**, 537-544

Oliver D, Baukrowitz T, Fakler B (2000) Polyamines as gating molecules of inward-rectifier K^+ channels. *European Journal of Biochemistry* **267**, 5824-5829

Ormrod DP, Beckerson DW (1986) Polyamines as antioxidants for tomato. *HortScience* **21**, 1070-1071

Panda SK, Chaudhury I, Khan MH (2003) Heavy metal induced lipid peroxidation affects antioxidants in wheat leaves. *Biologia Plantarum* **46**, 289-294

Panicot M, Masgrau C, Borrell A, Cordeiro A, Tiburcio AF, Altabella T (2002a) Effects of putrescine accumulation in tobacco transgenic plants with different expression levels of oat arginine decarboxylase. *Physiologia Plantarum* **114**, 281-287

Panicot M, Minguet EG, Ferrando A, Alcázar R, Blázquez MA, Carbonell J, Altabella T, Koncz C, Tiburcio AF (2002b) A polyamine metabolon involving aminopropyl transferase complexes in Arabidopsis. *Plant Cell* **14**, 2539-2551

Papadakis AK, Roubelakis-Angelakis KA (2005) Polyamines inhibit NADPH oxidase-mediated superoxides generation and putrescine prevents programmed cell death syndrome induced by the polyamine oxidase generated hydrogen peroxide. *Planta* **220**, 826-837

Pennazio S, Roggero P (1990) Exogenous polyamines stimulate ethylene synthesis by soybean leaf tissues. *Annals of Botany* **65**, 45-50

Pennell RI, Lamb C (1997) Programmed cell death in plants. *Plant Cell* **9**, 1157-1168

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

Piotrowski M, Janowitz T, Kneifel H (2003) Plant C-N hydrolases and the identification of a plant *N*-carbamoylputrescine amidohydrolase involved in polyamine biosynthesis. *The Journal of Biological Chemistry* **278**, 1708-1712

Preston, C, Holtum JAM, Powles SB (1992) Do polyamines contribute to paraquat resistance in *Hordeum glaucum*? In: Miura N (Ed) *Research in Photosynthesis* (Vol 3), Kluwer Academic Publisher, Dordrecht, Germany, pp 571-574

Priebe A, Klein H, Jager HJ (1978) Role of polyamines in SO_2-polluted pea plants. *Journal of Experimental Botany* **29**, 1045-1050

Pyle JA (1996) Global ozone depletion: observation and theory. In: Lumbsden PJ (Ed) *Plants and UV-B. Responses to Environmental Change*, Cambridge University Press, Cambridge, UK, pp 3-12

Rácz I, Lásztity D, Darkó É, Hidvégi E, Szigeti Z (2000) Paraquat resistance of horseweed (*Erigeron canadensis* L.) is not caused by polyamines. *Pesticide Biochemistry and Physiology* **68**, 1-10

Rajasekaran LR, Blake TJ (1999) New plant growth regulators protect photosynthesis and enhance growth under drought of Jack Pine seedlings. *Journal of Plant Growth Regulation* **18**, 175-181

Rea G, Metoui O, Infantino A, Federico R, Angelini R (2002) Copper amine oxidase exoression in defense responses to wounding and *Ascochyta rabiei* invasion. *Plant Physiology* **128**, 865-875

Reddy GN, Arteca RN, Dai YR, Negm FB, Pell EJ (1993) Changes in ethylene and polyamines in relation to mRNA levels of the large and small subunits of ribulose biphosphate carboxylase/oxygenase in ozone-stressed potato foliage. *Plant, Cell and Environment* **16**, 819-826

Reggiani R (1994) Purification and synthesis under anaerobic conditions of rice arginine decarboxylase. *Plant and Cell Physiology* **35**, 1245-1249

Reggiani R, Aurisano N, Mattana M, Bertani A (1993) Influence of K^+ ions on polyamine leve1 in wheat seedlings. *Journal of Plant Physiology* **141**, 136-140

Reggiani R, Giussani P, Bertani A (1990) Relationship between the accumulation of putrescine and the tolerance to oxygen-deficit stress in Gramineae seedlings. *Plant and Cell Physiology* **31**, 489-494

Reggiani R, Hochkoeppler A, Bertani A (1989) Polyamines and anaerobic elongation of rice coleoptile. *Plant and Cell Physiology* **30**, 893-898

Richards FJ, Coleman RG (1952) Occurrence of putrescine in potassium-deficient barley. *Nature* **170**, 460-464

Ries G, Heller W, Puchta H, Sandermann H, Seidlitz HK, Hohn B (2000) Elevated UV-B radiation reduces genome stability in plants. *Nature* **406**, 98-101

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

Roy M, Gosh B (1996) Polyamines, both common and uncommon, under heat stress in rice (*Oryza sativa*) callus. *Physiologia Plantarum* **98**, 196-200

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

Roy P, Niyogi K, Sengupta DN, Ghosh B (2005) Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H^+-ATPase in salt-tolerant and salt-sensitive rice cultivars. *Plant Science* **168**, 583-591

Sanchez DH, Cuevas JC, Chiesa MA, Ruiz OA (2005) Free spermidine and spermine content in *Lotus glaber* under long-term salt stress. *Plant Science* **168**, 541-546

Sannazzaro AI, Echeverría M, Albertó EO, Ruiz OA, Menéndez AB (2007) Modulation of polyamine balance in *Lotus glaber* by salinity and arbuscular mycorrhiza. *Plant Physiology and Biochemistry* **45**, 39-46

Santa-Cruz A, Acosta M, Pérez-Alfocea F, Bolarin MC (1997) Changes in free polyamine levels induced by salt stress in leaves of cultivated and wild tomato species. *Physiologia Plantarum* **101**, 341-346

Santa-Cruz A, Perez-Alfocea F, Caro M, Acosta M (1998) Polyamines as short-term salt tolerance traits in tomato. *Plant Science* **138**, 9-16

Sarjala T, Taulavuori K, Savonen EM, Edfast AB (1997) Does availability of potassium affect cold hardening of Scots pine through polyamine metabolism? *Physiologia Plantarum* **99**, 56-62

Scalet M, Federico R, Angelini R (1991) Time courses of diamine oxidase and peroxidase activities and polyamine changes after mechanical injury of chickpea seedlings. *Journal of Plant Physiology* **137**, 571-575

Sfichi L, Ioannidis N, Kotzabasis K (2004) Thylakoid-associated polyamines adjust the UV-B sensitivity of the photosynthetic apparatus by means of light-harvesting complex II changes. *Photochemistry and Photobiology* **80**, 499-506

Shabala S, Cuin TA, Pottosin I (2007) Polyamines prevent NaCl-induced K^+ efflux from pea mesophyll by blocking non-selective cation channels. *FEBS Letters* **581**, 1993-1999

Sharma SS, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of Experimental Botany* **57**, 711-726

Sharp RE, Poroyko V, Hejlek LG, Spollen WG, Springer GK, Bohnert HJ, Nguyen HT (2004) Root growth maintenance during water deficits: physiology to functional genomics. *Journal of Experimental Botany* **55**, 2343-2351

Shen HJ, Xie YF, Li RT (1994) Effect of acid stress on polyamine levels, ion efflux, protective enzymes and macro-molecular synthesis in cereal leaves. *Plant Growth Regulation* **14**, 1-5

Shen W, Nada K, Tachibana S (2000) Involvement of polyamines in the chilling tolerance of cucumber cultivars. *Plant Physiology* **124**, 431-439

Shih CY, Kao CH (1996) Growth inhibition in suspension-cultured rice cells under phosphate deprivation is mediated through putrescine accumulation. *Plant Physiology* **111**, 721-724

Singh DB, Verma S, Mishra SN (2002) Putrescine effect on nitrate reductase activity, organic nitrogen, protein, and growth in heavy metal and salinity stressed mustard seedlings. *Biologia Plantarum* **45**, 605-608

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

Slocum RD, Kaur-Sawhney R, Galston AW (1984) The physiology and biochemistry of polyamines in plants. *Archives of Biochemistry and Biophysics* **235**, 283-303

Smith JL, Burritt DJ, Bannister P (2001) Ultraviolet-B radiation leads to a reduction in free polyamines in *Phaseolus vulgaris* L. *Plant Growth Regulation* **35**, 289-234

Smith TA (1971) The occurrence, metabolism and functions of amines in plants. *Biological Reviews of the Cambridge Philosophical Society* **46**, 201-241

Smith TA (1973) Amine levels in mineral deficient *Hordeum vulgare* leaves. *Phytochemistry* **12**, 2093-2100

Smith TA, Negrel J, Bird CR (1983) The cinnamic acid amides of the di- and polyamines. In: Bachrach U (Ed) *Advances in Polyamine Research*, Raven Press, New York, USA, pp 347-370

Smith TA, Wilshire G (1975) Distribution of cadaverine and other amines in higher plants. *Phytochemistry* **14**, 2341-2346

Soar CJ, Karotam J, Preston C, Powles SB (2003) Paraquat translocation in paraquat resistant *Arctotheca calendula* (L.) Levyns is a consequence of the primary resistance mechanism, not the cause. *Pesticide Biochemistry and Physiology* **76**, 91-98

Soar CJ, Preston C, Karotam J, Powles SB (2004) Polyamines can inhibit paraquat toxicity and translocation in the broadleaf weed *Arctotheca calendula*. *Pesticide Biochemistry and Physiology* **80**, 94-105

Soyka S, Heyer AG (1999) *Arabidopsis* knockout mutation of *ADC2* gene reveals inducibility by osmotic stress. *FEBS Letters* **458**, 219-223

Stoynova EZh., Karanov EN, Alexieva V (1999) Subcellular aspects of the protective effect of spermine against atrazine in pea plants. *Plant Growth Regulation* **29**, 175-180

Szigeti Z, Rácz I, Darkó É, Lásztity D, Lehoczki E (1996) Are either SOD and catalase or the polyamines involved in the paraquat resistance of *Conyza canadensis*? *Journal of Environmental Science and Health* **31B**, 599-604

Takahashi Y, Berberich T, Miyazaki A, Seo S, Ohashi Y, Kusano T (2003) Spermine signalling in tobacco: activation of mitogen-activated protein kinases by spermine is mediated through mitochondrial dysfunction. *The Plant Journal* **36**, 820-829

Takahashi Y, Berberich T, Yamashita K, Uehara Y, Miyazaki A, Kusano T (2004a) Identification of tobacco HIN1 and two closely related genes as spermine-responsive genes and their differential expression during the Tobacco mosaic virus-induced hypersensitive response and during leaf- and flower-senescence. *Plant Molecular Biology* **54**, 613-622

Takahashi Y, Uehara Y, Berberich T, Ito A, Saitoh H, Miyazaki A, Terauchi R, Kusano T (2004b) A subset of hypersensitive response marker genes, including HSR203J, is the downstream target of a spermine signal transduction pathway in tobacco. *The Plant Journal* **40**, 586-595

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

Tassoni A, Antognoni F, Battistini ML, Sanvido O, Bagni N (1998) Characterization of spermidine binding to solubilized plasma membrane proteins from zucchini hypocotyls. *Plant Physiology* **117**, 971-977

Tassoni A, Fornale S, Bagni N (2003) Putative ornithine decarboxylase activity in *Arabidopsis thaliana*: inhibition and intracellular localization. *Plant Physiology and Biochemistry* **41**, 871-877

Tavladoraki P, Schinina ME, Cecconi F, di Agostino S, Manera F, Rea G, Mariottini P, Federico R, Angelini R (1998) Maize polyamine oxidase: primary structure from protein and cDNA sequencing. *FEBS Letters* **426**, 62-66

Tevini M, Teramura AH (1989) UV-B effects on terrestrial plants. *Journal of Photochemistry and Photobiology* **50**, 479-487

Tiburcio AF, Kaur-Sawhney R, Galston AW (1986a) Polyamine metabolism and osmotic stress. II. Improvement of oat protoplasts by an inhibitor of arginine decarboxylase. *Plant Physiology* **82**, 375-378

Tiburcio AF, Masdeu MA, Dumortier FM, Galston AW (1986b) Polyamine metabolism and osmotic stress. I. Relation to protoplast viability. *Plant Physiology* **82**, 369-374

Torrigiani P, Altamura MM, Pasqua G, Monacelli B, Serafini-Fracassini D, Bagni N (1987) Free and conjugated polyamines during *de novo* floral and vegetative bud formation in thin cell layers of tobacco. *Physiologia Plantarum* **70**, 453-460

Torrigiani P, Rabiti AL, Bortolotti C, Betti L, Marani F, Canova A, Bagni N (1997) Polyamine synthesis and accumulation in the hypersensitive response to TMV in *Nicotiana tabacum*. *New Phytologist* **135**, 467-473

Uehara Y, Takahashi Y, Berberich T, Miyazaki A, Takahashi H, Matsui K, Ohme-Takagi M, Saitoh H, Terauchi R, Kusano T (2005) Tobacco ZFT1, a transcriptional repressor with a Cys_2/His_2 type zinc finger motif that functions in spermine-signaling pathway. *Plant Molecular Biology* **59**, 435-448

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

Urano K, Yoshiba Y, Nanjo T, Ito Y, Seki M, Yamaguchi-Shinozaki K, Shinozaki K (2004) *Arabidopsis* stress-inducible gene for arginine decarboxylase *AtADC2* is required for accumulation of putrescine in salt tolerance. *Biochemical and Biophysical Research Communications* **313**, 369-375

van Buuren ML, Guidi L, Fornalè S, Ghetti F, Francescheti M, Soldatini GF, Bagni N (2002) Ozone-response mechanisms in tobacco: implications of polyamine metabolism. *New Phytologist* **156**, 389-398

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Science* **151**, 59-66

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

Walters DR (2000) Polyamines in plant-microbe interactions. *Physiological and Molecular Plant Pathology* **57**, 137-146

Walters DR (2003) Polyamines and plant disease. *Phytochemistry* **64**, 97-107

Walters DR, Wilson PWF, Shuttleton MA (1985) Relative changes in levels of polyamines and activities of biosynthetic enzymes in barley infected with the powdery mildew fungus, *Erysiphe graminis* DC. Ex Merat f.*sp. hordei* Marchal. *New Phytologist* **101**, 695-705

Wang CY (1987) Changes of polyamines and ethylene in cucumber seedlings in response to chilling stress. *Physiologia Plantarum* **69**, 253-257

Wang X, Shi G, Xu, Q, Hu J (2007) Exogenous polyamines enhance copper tolerance of *Nymphoides peltatum*. *Journal of Plant Physiology* **164**, 1062-1070

Wang X, Shi GX, Ma GY, Xu QS, Khaled R, Hu JZ (2004) Effects of exogenous spermidine on resistance of *Nymphoides peltatum* to Hg^{2+} stress. *Journal of Plant Physiology and Molecular Biology* **30**, 69-74 (In Chinese with English abstract)

Watson MB, Emory KK, Piatak RM, Malmberg RL (1998) Arginine decarboxylase (polyamine synthesis) mutants of *Arabidopsis thaliana* exhibit altered root growth. *Plant Journal* **13**, 231-239

Watson MB, Malmberg RL (1996) Regulation of *Arabidopsis thaliana* (L.) Heynh arginine decarboxylase by potassium deficiency stress. *Plant Physiology* **111**, 1077-1083

Weinstein LH, Kaur-Sawhney R, Rajam MV, Wettlaufer SH, Galston AW (1986) Cadmium-induced accumulation of putrescine in oat and bean leaves. *Plant Physiology* **82**, 641-645

Wen XP, Pang XM, Matsuda N, Kita M, Inoue M, Hao YJ, Honda C, Moriguchi T (2007) Over-expression of the apple spermidine synthase gene in pear confers multiple abiotic stress tolerance by altering polyamine titers. *Transgenic Research* in press

Wi SJ, Kim WT, Park KY (2006) Overexpression of carnation *S*-adenosylmethionine decarboxylase gene generates a broad-spectrum tolerance to abiotic stresses in transgenic tobacco plants. *Plant Cell Reports* **25**, 1111-1121

Zheng YH, Su XG, Li SY, Xi YF (2000) Quality, active oxygen and polyamines metabolic changes in coldstored loquat fruits as affected by postharvest SO_2 treatment. *Acta Phytophysiologica Sinica* **26**, 397-401 (in Chinese with English abstract)

Xie YF, Shen HJ, Li MZ (1999) Effects of acid stress on endogenous polyamine and active oxygen metabolism of woody plants. *Scientia Silvae Sinicae* **35**, 117-121 (in Chinese with English abstract)

Xiong L, Zhu JK (2002) Salt tolerance. In: Meyerowitz EM, Somerville CR (Eds) *The Arabidopsis Book*, American Society of Plant Biologists, Rockville, MD, www.aspb.org/publications/arabidopsis/, pp 1-22

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

Yamaguchi K, Takahashi Y, Berberich T, Imai A, Takahashi T, Michael A, Kusano T (2007) A protective role for the polyamine spermine against drought stress in *Arabidopsis*. *Biochemical and Biophysical Research Communications* **352**, 486-490

Yamakawa H, Kamada H, Satoh M, Ohashi Y (1998) Spermine is a Salicylate-independent endogenous inducer for both tobacco acidic pathogenesis-related proteins and resistance against tobacco mosaic virus infection. *Plant Physiology* **118**, 1213-1222

Yan SP, Zhang QY, Tang ZC, Su WA, Sun WN (2006) Comparative proteomic analyses provide new insights into chilling stress responses in rice. *Molecular Cell Proteomics* **5**, 484-496

Yang JC, Zhang JH, Liu K, Wang ZQ, Liu LJ (2007) Involvement of polyamines in the drought resistance of rice. *Journal of Experimental Botany* **58**, 1545-1555

Ye B, Miller HH, Zhang J, Gressel J (1997) Constitutively elevated levels of putrescine and putrescine-generating enzymes correlated with oxidant stress resistance in *Conyza bonariensis* and wheat. *Plant Physiology* **115**, 1443-1451

Yeo AR (1999) Predicting the interaction between the effects of salinity and climate change on crop plants. *Scientia Horticulturae* **78**, 159-174

Yoda H, Hiroi Y, Sano H (2006) Polyamine oxidase is one of the key elements for oxidative burst to induce programmed cell death in tobacco cultured cells. *Plant Physiology* **142**, 193-206

Yohannes E, Thurber AE, Wilks JC, Tate DP, Slonczewski JL (2005) Polyamine stress at high pH in *Escherichia coli* K-12. *BMC Microbiology* **5**, 59

Yoo TH, Park CJ, Ham BK, Kim KJ, Paek KH (2004) Ornithine decarboxylase gene (*CaODC1*) is specifically induced during TMV-mediated but salicylate-independent resistant response in hot pepper. *Plant and Cell Physiology* **45**, 1537-1542

Young ND, Galston AW (1983) Putrescine and acid stress: Induction of arginine decarboxylase activity and putrescine accumulation by low pH. *Plant Physiology* **71**, 767-771

Young ND, Galston AW (1984) Physiological control of arginine decarboxylase activity in potassium deficient oat shoots. *Plant Physiology* **76**, 331-335

Zapata PJ, Serrano M, Pretel MT, Amorós A, Botella MA (2003) Changes in ethylene evolution and polyamine profiles of seedlings of nine cultivars of *Lactuca sativa* L. in. response to salt stress during germination. *Plant Science* **164**, 557-563

Zhao FG, Qin P (2004) Protective effect of exogenous polyamines on root tonoplast function against salt stress in barley seedlings. *Plant Growth Regulation* **42**, 97-103