

Polyamines and abiotic stress: recent advances

Review Article

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Received October 1, 2006

Accepted January 31, 2007

Published online March 14, 2007; © Springer-Verlag 2007

Summary. In this review we will concentrate in the results published the last years regarding the involvement of polyamines in the plant responses to abiotic stresses, most remarkably on salt and drought stress. We will also turn to other types of abiotic stresses, less studied in relation to polyamine metabolism, such as mineral deficiencies, chilling, wounding, heavy metals, UV, ozone and paraquat, where polyamine metabolism is also modified.

There is a great amount of data demonstrating that under many types of abiotic stresses, an accumulation of the three main polyamines putrescine, spermidine and spermine does occur. However, there are still many doubts concerning the role that polyamines play in stress tolerance. Several environmental challenges (osmotic stress, salinity, ozone, UV) are shown to induce ADC activity more than ODC. The rise in Put is mainly attributed to the increase in ADC activity as a consequence of the activation of ADC genes and their mRNA levels. On the other hand, free radicals are now accepted as important mediators of tissue injury and cell death. The polycationic nature of polyamines, positively charged at physiological pH, has attracted the attention of researchers and has led to the hypothesis that polyamines could affect physiological systems by binding to anionic sites, such as those associated with nucleic acids and membrane phospholipids. These amines, involved with the control of numerous cellular functions, including free radical scavenger and antioxidant activity, have been found to confer protection from abiotic stresses but their mode of action is not fully understood yet. In this review, we will also summarize information about the involvement of polyamines as antioxidants against the potential abiotic stress-derived oxidative damage.

Keywords: Antioxidants – Polyamines – Abiotic stress – Stress tolerance

Abbreviations: ADC, Arginine decarboxylase; Agm, agmatine; DAO, diamine oxidase; ODC, ornithine decarboxylase; PA, polyamine; PAO, polyamine oxidase; Put, putrescine; Spd, spermidine; SPDS, Spd synthase; Spm, spermine; SPMS, Spm synthase

Introduction

The diamine putrescine (Put), the triamine spermidine (Spd) and the tetramine spermine (Spm) are the main poly-

amines (PAs) found in all living cells. They are aliphatic nitrogen compounds positively charged at physiological pH. This property allows PAs to interact with negatively charged macromolecules as DNA and RNA, proteins and phospholipids and in this way they are involved in the regulation of physical and chemical properties of membranes, nucleic acids structure and functions and modulation of enzyme activities (Galston and Kaur Sawhney, 1990). Polyamines are implicated in a wide range of regulatory processes such as promotion of growth, cell division, DNA replication and cell differentiation (Evans and Malmberg, 1989).

In plants, Put is synthesized by the decarboxylation of arginine or ornithine catalyzed by arginine decarboxylase (ADC; EC 4.1.1.19) or ornithine decarboxylase (ODC; EC 4.1.1.17), respectively. The following addition of two aminopropyl groups to Put in two reactions catalyzed by Spd synthase (SPDS; EC 2.5.1.16) and Spm synthase (SPMS; EC 2.5.1.22) leads to the formation of Spd and Spm, respectively. The aminopropyl moieties arise from the decarboxylation of S-adenosylmethionine by the enzyme S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50) (Slocum, 1991).

Free PAs level in plant cells depends not only on their synthesis but also on their transport, degradation and conjugation. Putrescine degradation is catalyzed by diamine oxidase (DAO; EC 1.4.3.6), a copper containing enzyme that oxidize the diamine at the primary amino group, whereas Spd and Spm are oxidized at their secondary amino groups by a flavin-containing polyamine oxidase (PAO; EC 1.5.3.3) (Flores and Filner, 1985). Moreover,

PAs can be conjugated to small molecules like proteins, antibiotics and phenolic acids, mostly hydroxycinnamic acid (Martin-Tanguy, 2001). For a deeper insight into PAs biosynthesis, catabolism and conjugation processes, see the revision of Bagni and Tassoni (2001).

In the last years, there has been a growing interest in the study of PAs participation in the defense reaction of plants against several environmental stresses (Kumer et al., 1997; Bouchereau et al., 1999; Kasukabe et al., 2004). Many genes for enzymes involved in PA metabolism have been cloned from several species, and their expression under several stress conditions have been analysed. It has been widely reported that in plant responses to abiotic stress, the increase in Put is generally due to the increase in ADC activity (Bouchereau et al., 1999). Two ADC genes (*ADC1* and *ADC2*) have been described in several plant species (Galloway et al., 1998). *ADC2* is induced by osmotic stress (Soyka and Heyer, 1999), wounding, jasmonates and abscisic acid (Perez-Amador et al., 2002). In the *Arabidopsis* genome, eight genes involved in PA biosynthesis have been recently identified (two genes for ADC, two genes for SAMDC, two genes for SPDS and two genes for SPMS) and their expression profiles were analysed in response to different abiotic stress conditions (Urano et al., 2003). In this review we will summarize data collected from the last years concerning the involvement of PA metabolism in the plant responses to several types of abiotic stresses as well as about the controversial role of PAs as antioxidants in plants.

Polyamines and oxidative stress

It has been widely reported that all types of abiotic stresses induce oxidative stress. In the last years, PAs have been tested as antioxidants under some environmental adverse conditions, though their precise role as antioxidants is still a matter of debate (Groppa et al., 2001; Chattopadhyay et al., 2002; Kakkar and Sawhney, 2003). Among the substances able to protect plant cells from oxidative attack, Løvaas (1997) reported that PAs could play a specific role in preventing photooxidative damage. Moreover, there is contradictory research data providing evidence for the lack of antioxidant activity and even pro-oxidant action of PAs. The antioxidative effect of PAs is due to a combination of their anion- and cation-binding properties involving a radical scavenging function (Bors et al., 1989), and a capability to inhibit both lipid peroxidation (Kitada et al., 1979) and metal-catalysed oxidative reactions (Tadolini, 1988). Spermine, Spd and Put all reduce level of superoxide radicals generated by senesc-

ing plant cells (Drolet et al., 1986). Alternatively, PA catabolism produces H_2O_2 , a signaling molecule that can enter the stress signal transduction chain promoting an activation of an antioxidative defence response, but can also act as a prooxidant agent.

Mineral nutrition

It is well known that PA metabolism is altered in response to an inadequate nutrition in K^+ , Mg^{2+} or PO_4^{3-} (Slocum et al., 1984; Flores, 1991). Polyamine metabolism under K^+ deficiency has been studied by several authors (Young and Galston, 1984; Watson and Malmberg, 1996) since the accumulation of Put in K^+ -deficient barley leaves was first reported by Richards and Coleman (1952). The increased free Put content found in K^+ -deficient plants has been related to a rise in ADC activity (Watson and Malmberg, 1996) and several studies have established a specific role of Put in maintaining a cation-anion balance in plant tissues (Bouchereau et al., 1999 and references therein). In *Arabidopsis* plants, K^+ deficiency induces an increase in ADC activity without apparent changes in mRNA or protein levels (Watson and Malmberg, 1996). The accumulation of the diamines Put and 1,3-diaminopropane was used by Sarjala and Kaunisto (2002) to define the critical levels of K^+ nutrition in birch leaves. Foliar K^+ concentrations below $7\text{--}8\text{ mg g}^{-1}\text{ DW}$ were found to correlate with the accumulation of diamines in *Betula pendula* and *Betula pubescens*. Recently, full genome microarrays were used to assess transcriptional responses of *Arabidopsis* seedlings to changing external supply of the essential macronutrient K^+ (Armengaud et al., 2004). The most prominent response was found for genes linked to the phytohormone jasmonic acid (JA). Moreover, JA responsive genes showed the same expression profile, including genes involved in PA biosynthesis (*ADC2*).

Respect to other mineral nutrients, in leaves of tobacco plants that suffered from boron deficiency, Camacho-Cristóbal et al. (2002) described an accumulation of caffeineylputrescine without a significant effect on the free PAs levels. In contrast to the results observed in leaves, free Put content of boron-deficient plants increased in roots while conjugated Put and Spd showed a higher level both in leaves and roots along the boron treatment (Camacho-Cristóbal et al., 2005). The authors mentioned that the effect of boron deficiency on the endogenous levels of Put cannot be attributed to a lower K^+ content.

In wheat and pepper plants, ammonium nutrition and, to a lesser extent, urea nutrition, were associated with

significant increases in Put content that were well correlated with a reduction in plant growth. These negative effects of ammonium and urea nutrition were repaired by the presence of nitrate in the nutrient solution, which also produced a significant decrease in the plant Put content (Houdusse et al., 2005).

Salinity, drought and osmotic stress

Salt and drought stress are the two major abiotic stresses in agriculture and reduced water potential is a common consequence of both of them. Salinity is a complex environmental constraint that presents two main components: an osmotic component due to the decrease in the external osmotic potential (ψ_s) of the soil solution, and the ionic component linked to the accumulation of ions which become toxic at high salt concentrations (mainly Na^+ and Cl^-). A high salt concentration disrupts the integrity of cellular membranes, the activity of various enzymes and the function of the photosynthetic apparatus. Plants respond to changes in this unfavourable environmental condition (i.e., alteration of the water status) accumulating low molecular-weight osmolytes such as proline (Kishore et al., 1995; Zhu et al., 1998) and PAs (Flores, 1991). Until now, it remains unclear which component of salt stress is responsible for PAs accumulation, despite numerous reports have tried to elucidate this essential clue for many years and differences in PAs content and in the activity of biosynthetic enzymes have been reported among and within many species (Flores, 1991; Tiburcio et al., 1997; Bouchereau et al., 1999; Capell et al., 2004; Zapata et al., 2004).

The measurement of PAs levels in different rice cultivars showed that salt-tolerant rice cultivars maintains a high level of higher PAs, e.g. Spd and Spm, whereas salt-sensitive rice cultivars maintains only a high level of Put (Basu and Ghosh, 1991; Krishnamurthy and Bhagwat, 1989). The salt tolerant cultivars AU1, Co43, and CSC1 were effective in maintaining high concentrations of Spd and Spm, while the content of Put was not significantly altered in the growth stages analysed when plants were exposed to salinity. The salt sensitivity in rice was associated with excessive accumulation of Put and with low levels of Spd and Spm in the shoot system of the salt-sensitive cultivars Co36, CSC2, GR3, IR20, TKM4, and TKM9 under saline conditions (Krishnamurthy and Bhagwat, 1989).

Root plasma membranes of rice salt-tolerant cultivars Nonabokra and Pokkali were rich in Spm/Spd, whereas the root plasma membranes of sensitive cultivars (M-1-48 and IR8) were rich in Put only (Roy et al., 2005). Free and

bound PAs content in root tonoplast vesicles were closely related to salt tolerance of barley plants (Zhao et al., 2000). Moreover, 200 mM NaCl induced reductions in the contents of phospholipids and PAs in tonoplast vesicles isolated from barley seedling roots, while exogenous Put or Spd (0.5 mM) application partially restored this effect, attenuating salt injury in barley seedlings (Zhao and Qin, 2004).

Polyamines accumulated in *Arabidopsis* wild-type plants (Col-0 and Ler-0) that were pre-treated with 100 mM NaCl before transfer to 125 mM NaCl, but not in plants that were directly transferred to 125 mM NaCl (Kasinathan and Wingler, 2004). On the other hand, the salt treatment that induced PA accumulation in wild-type plants did not lead to PA accumulation in the *spe1-1* and *spe2-1* mutants with reduced activity of ADC (Kasinathan and Wingler, 2004), demonstrating that decreased PA formation due to lower ADC activity lead to reduced salt tolerance. In accordance, Roy and Wu (2001) found that in rice plants transformed with an oat ADC cDNA, the accumulation of PAs as a consequence of a higher ADC activity enhanced plant biomass under salinity.

The potential role of PAs to overcome the adverse effect of salinity was also observed by Mutlu and Bozcuk (2005), who reported an increase of free, acid-soluble bound, and total Spm in leaf tissues of sunflower plants subjected to 50, 100, and 150 mM NaCl. Polyamine levels were measured by Zapata et al. (2004) in several plant species (spinach, lettuce, melon, pepper, broccoli, beetroot and tomato) subjected to salt stress (100 or 150 mM NaCl). With the exception of beetroot, Put concentration was lower in seedlings grown under saline conditions. However, salinity caused a significant increase in Spd and Spm in almost all the plant species studied. This meant that the pool of Put was directed to Spd and Spm synthesis. The (Spd + Spm)/Put ratio increased with salinity, which would be in agreement with the idea of a protective role of higher PAs (Spd and Spm) against salt stress. Moreover, Mansour and Al-Mutawa (1999) indicated that the cellular alterations induced by NaCl in wheat roots were alleviated by low concentrations of Spd or Spm, whereas Put was ineffective. Therefore, these results would indicate a clear role for Spd and Spm in protecting plasma membrane under salinity and, thus, enhancing salt tolerance. Accordingly, in rice, Spm and Spd significantly prevented the leakage of electrolytes and amino acids from roots and shoots of rice subjected to salinity and a positive correlation between salt tolerance and accumulation of higher levels of PAs was found (Chattopadhyay et al., 2002). El-Shintinawy (2000) also indicated that salinity

greatly enhanced the accumulation of Spm and Spd associated with a decrease in Put content in wheat cultivars. However, 200 mM NaCl did not significantly change the *OsSPDS2* (a SPDS gene) mRNA levels in rice plants (Imai et al., 2004).

Respect to the genes that encode ADC in *Arabidopsis*, *AtADC1* and *AtADC2*, Urano et al. (2004) showed that the induction of the *AtADC2* transcript correlates with the accumulation of free Put under salt and drought stress. When a *adc2-1* mutant that expressed *AtADC1* at the same level as the control plants but did not express *AtADC2* was used, free Put was reduced to 25% of that in control plants and did not increase under salt stress, whereas the levels of free Spd and Spm were normal. Furthermore, the *adc2-1* mutant was more sensitive to salt stress than the control plants. The stress sensitivity of *adc2-1* was recovered by the addition of exogenous Put. These results indicated that endogenous Put also plays an important role in salt tolerance in *Arabidopsis* (Urano et al., 2004). Soyka and Heyer (1999) using an *Arabidopsis thaliana* mutant line with an insertion that causes a knock-out of the *ADC2* gene demonstrated that *ADC2* is the gene responsible for the induction of the PA biosynthetic pathway by osmotic stress.

In order to differentiate the ionic and osmotic components of salt stress, Legocka and Kluk (2005) found that 260 mM NaCl and 360 mM sorbitol trigger organ-specific changes in PAs levels and in the activity of ADC in *Lupinus luteus*, a drought-tolerant plant. After a short-term exposure (4 h) of seedlings to both stresses, Put and Spd accumulated in roots and leaves. Long (24 h) salt and osmotic stress conditions produced a decline of Put and Spd in roots, and an increase in hypocotyls and leaves. During this period of time, the concentration of free Put significantly raised in the leaves without increasing ADC activity. This may indicate that during longer times of action for both stresses, Put synthesized in roots was carried through hypocotyl to leaves. Also, in roots and leaves of *Lupinus luteus* growing for 24 h on salt or sorbitol, a higher level of PAs bound to microsomal membranes was observed (Legocka and Kluk, 2005). Polyamines most likely rigidify microsomal membrane surfaces, stabilizing them against NaCl and osmotic stress damage. Moreover, the higher levels of PAs bound to microsomal membranes most likely minimize the damaging effect of NaCl and water deficit.

Short and long-term changes in free PAs induced by iso-osmotic concentrations of NaCl (100 and 200 mM) and mannitol (200 and 400 mM) were studied in *Fraxinus angustifolia* callus by Tonon et al. (2004). In a short-term

response (30 min), it was observed a very early and temporary increase in Put and Spm as a consequence of salt treatment, and a continuous accumulation of Spd and Spm in response to mannitol. Lefèvre et al. (2001) showed that in rice roots, Put accumulation under salt stress occurred in a time of hours, supporting a role of Put in short term salt stress response. The authors hypothesized that the ionic component alone may trigger short-term PA accumulation, independently of any osmotic component.

The expression of the *SAMDC1* gene in rice seedlings is dramatically induced by salinity and drought (Li and Chen, 2000). The transcript levels of *SAMDC1* in two rice varieties differing in salt tolerance was found to be higher in the salt-tolerant rice variety than in the salt-sensitive one, and occurred more quickly when both varieties were exposed to low concentrations of NaCl stress (0–150 mM).

In relation to the consequences of water deficiency, Capell et al. (1998) observed that when an oat *ADC* gene was overexpressed in rice, the plants showed improved drought tolerance in terms of chlorophyll loss. Wild-type plants of *Datura stramonium* responded to the onset of drought stress by increasing endogenous Put levels, but this was not enough to trigger the conversion of Put into Spd and Spm, (the agents that are believed to protect plants against water deficit (Capell et al., 2004). In contrast, transgenic plants expressing *Datura adc* produced much higher levels of Put under stress, promoting Spd and Spm synthesis and ultimately protecting the plants from drought. Accordingly, Spd significantly increased in water stressed chickpea plants (Nayyar and Chander, 2004). Liu et al. (2000) also reported that Spd was the major PA accumulated in water-stressed tissues, confirming its stress-specific role.

Endogenous levels of individual as well as total PAs in roots of 7-day-old seedlings subjected to –0.8 MPa water stress increased to a significantly greater extent in chickpea than in soybean plants and the stress injury was more evident as the PAs levels declined in both plant species (Nayyar et al., 2005). The reduced levels of PAs in soybean, especially Put and Spd, respect to chickpea, were related to the higher stress injury and decreased water content. Exogenous Put and Spd mitigated the stress-induced effects, particularly in soybean. The use of difluoromethylarginine (DFMA) and alpha-difluoromethylornithine (DFMO), the biosynthetic inhibitors of Put biosynthesis, as well as cyclohexylamine (CHA), biosynthetic inhibitor of Spd and Spm biosynthesis, supported the role of PAs in mediating the differential sensitivity of chickpea and soybean to water stress (Nayyar et al., 2005).

Barley seedlings treated with Spd prior to a water deficit period, reverted the increase in catalase and guaiacol peroxidase activities produced by this stress, suggesting that PAs are able to influence the activity of H_2O_2 -scavenging enzymes, moderating this signal molecule level (Kubis, 2003). In seedlings of Indian mustard treated with different concentrations of NaCl (50–200 mM), it was found that the exogenous addition of Put improved seedlings growth by preventing lipid peroxidation and denaturation of macromolecules through the induction of antioxidative enzymes (APOX, GR, CAT, SOD, POD) and the increase of glutathione and carotenoids (Verma and Mishra, 2005). These results are in accordance with Tang and Newton (2005), who reported that PAs (mainly Put) reduce salt-induced oxidative damage by increasing the activities of antioxidant enzymes and decreasing lipid peroxidation in callus and seedlings of Virginia pine.

Chilling

There are relatively few reports regarding PAs involvement in chilling stress (Bouchereau et al., 1999 and references therein, Shen et al., 2000; Kim et al., 2002). The effect of chilling treatment on PAs content in leaves is different in cold-tolerant and cold-sensitive cucumber cultivars (Shen et al., 2000). During chilling, Spd content in leaves markedly increased in cold-tolerant cucumber cultivars, but not in sensitive ones, while Put and Spm did not change. In addition, PAs pretreatment of the cold-sensitive cultivar alleviated chilling injury. The increase of Put, Spd and Spm, in the cold-tolerant cultivar during chilling might be the result of the increased ODC activity. Nayyar (2005) have reported that PAs levels increased six to nine times in chickpea (*Cicer arietinum* L.) subjected to chilling temperatures of field (12–15/4–6 °C; average maximum and minimum temperature, respectively). Relatively, Put elevation was the highest but short-lived and its decrease appeared to match with the onset of flower and pod abscission in stressed plants. Electrolyte leakage increased while cellular respiration declined in stressed plants and exogenous application of Put to stressed plants reversed these parameters (Nayyar et al., 2005). In mango fruit, chilling stress promoted Put accumulation at the early stages of ripening (Nair and Singh, 2004). However, Put increase did not avoid chilling injury. The depletion of endogenous Spd and Spm with chilling and the reduction of chilling injury with pre-storage application of these PAs indicate that chilling injury development in mango fruit seems to be associated with the biosynthesis of the higher PAs.

In cucumber, the addition of Spd to the growth medium previously to the cold treatment enhanced Spd content in all organs and resulted in higher cold tolerance, even if their PAs pools significantly decreased during chilling (He et al., 2002). Therefore, chilling damage might be prevented by PAs accumulation in response to cold, and also if PAs levels were high prior to chilling exposure and remain high during the whole stress treatment.

Polyamine levels in *Pringlea antiscorbutica* seedlings were generally either decreased or not significantly modified at 5 °C compared with 22 °C in the presence or absence of MS (Murashige and Skoog) salts (Hummel et al., 2004). Although *P. antiscorbutica* seedlings were not sensitive to low temperature, their PAs contents did not increase in response to cold, except in the case of Spm in seedlings grown on agar medium. High contents of Agm and Put in whole seedlings were associated with the cold tolerance of *P. antiscorbutica*, while low levels were associated with cold sensitivity in *A. thaliana* (Hummel et al., 2004).

Imai et al. (2004) reported that a novel SPDS gene, *OsSPDS2* from rice, was involved in chilling response in roots. This gene is closely related to *AtSPDS3* or *At5g53120*, a putative *Arabidopsis* SPDS gene. When seedlings grown at 25 °C were subjected to chilling stress at 12 °C, a prominent induction of *OsSPDS2* was detected in roots after 1 day of treatment and the mRNA levels steadily increased until day 10. When seedlings were returned to 25 °C, the *OsSPDS2* mRNA level rapidly decreased. In contrast, there was no significant change in *OsSPDS2* mRNA levels in shoots during the course of the chilling treatment.

In poplar seedlings grown at 4 °C, Put accumulation occurred during the beginning of the cold treatment while Spd and Spm accumulated after 4 or 7 days, respectively (Renaut et al., 2005). Put content usually reacts quickly to changes in the environment. However, its increase is often temporary when the stress is relatively mild. It has been proposed that Put may reflect the suboptimal growth conditions while Spd and Spm may help detoxifying free radicals or stabilizing macromolecules and membranes (Larher et al., 2003). On the other side, Spd and Spm contents are less and more slowly affected but seem to be better markers to evaluate plant stress levels (Hausman et al., 2000). Regarding PAs antioxidant role, it was observed that exogenous application of PAs reduced the level of H_2O_2 , malondialdehyde content and raised the level of antioxidants in 15-day-old chickpea plants subjected to water deficit and cold stress for 4 days (Nayyar and Chander, 2004).

Wounding

Arabidopsis plants responded to mechanical injury with an increased expression of the *ADC2* gene. However, no induction was observed for the *ADC1* gene and the genes coding for SPDS and SPMS, suggesting that *ADC2* is the only gene of polyamine biosynthesis involved in the wounding response mediated by jasmonate (Perez-Amador et al., 2002). The increase in the *ADC2* mRNA was followed by a rise in Put level and a decrease of Spm content. These results were confirmed by Cowley and Walters (2005) who found that mechanical wounding of the first leaves of oilseed rape led to significant increase of free Put levels in the wounded first leaf and in the unwounded second leaf. In this case, the raise in Put content seemed to be the result of an increased ADC activity and a reduced DAO activity. However, in chickpea plants, Rea et al. (2002) reported an increase of this catabolic enzyme in response to wounding, suggesting that the plant response against a mechanical injury might depend on the plant specie.

UV treatment

Increasing UV-B radiation (280–315 nm) in the biosphere may also induce an increase in reactive oxygen species (ROS) formation in plant tissues, leading to oxidative stress (Brosché and Strid, 2003) with changes in the antioxidant enzyme activities (Ait Barka, 2001; Baumbush et al., 1998) and PAs content (Gonzalez-Aguilar et al., 2001; Smith et al., 2001).

In *Phaseolus vulgaris* plants, total free PAs showed a marked decrease in response to UV-B radiation (primarily due to a reduction in Put content) which was correlated with UV-B induced chlorophyll loss (Smith et al., 2001). An increase in bound polyamines, whether cell wall bound or conjugated to phenolics, could account for a reduction in the levels of free PAs. In tobacco callus treated with UV-C, Zacchini and de Agazio (2004) found that Put content increased in upper layers of calli after 6 h of irradiation, but decreased after 24 and 48 h of irradiation, though remaining statistically higher than controls. No differences between control and UV-irradiated calli were detected in lower layers and Spd and Spm were not affected by UV treatment. In cucumber plants, UV-B radiation resulted in a dose-dependent decrease in leaf area, dry weight of foliage, and plant height as well as in a significantly increase in Put, Spd and Spm levels (An et al., 2004). However, the total PAs content declined slightly when electrolyte leakage increased dramatically

on the 18th day of treatment, especially under the higher radiation treatment. The authors suggested that PAs accumulation in cucumber leaves is an adaptive mechanism to the stress caused by UV-B radiation.

In tobacco cultivars Bel B and Bel W3 subjected to UV-B radiation in sun light simulators (GSF/Munich) with natural diurnal fluctuations of simulated UV-B, Lutz et al. (2005) described that an increase of PAs, especially Put in thylakoid membranes of the Bel B variety, comprises one of the primary protective mechanisms to the photosynthetic apparatus against UV-B radiation. Besides, the UV-B sensitivity of Bel W3 cultivar (which is sensitive to ozone) was attributed to its inability to enhance Put level in thylakoid membranes. After prolongation of UV-B exposure, when the plant was adapting to altered environmental conditions and developing other secondary responses (e.g., biosynthesis of carotenoids and of additional flavonoids), the PAs level declined.

Metal stress

In the last years, metal contamination of soils has considerably increased due to human activities. Metal toxicity in plants may result from the binding of metals to sulphydryl groups of proteins inhibiting enzymes activities or altering protein structure (Van Assche and Clijsters, 1990) and also stimulating the formation of ROS that lead to oxidation of macromolecules and oxidative stress (Gallego et al., 1996; Sandalio et al., 2001; Fornazier et al., 2002). It has been documented that metal excess also produced changes in PA metabolism (Weinstein et al., 1986; Geuns et al., 1997; Lin and Kao, 1999; Groppa et al., 2001, 2003).

Tobacco BY-2 cells exposed to 0.05 mM CdCl_2 produced a marked accumulation of total PAs (free PAs and their perchloric acid (PCA)-soluble and PCA-insoluble conjugates) during 3-day treatment (Kuthanová et al., 2004). The increase in total PAs content was primarily caused by the increase in Put concentration and correlated with the stimulation of DAO activity. However, the enzyme activity in Cd^{2+} -treated BY-2 cells declined on day 3, though the content of free Put did not change significantly (Kuthanová et al., 2004). Choudhary and Singh (2000) have shown that PAs accumulation in mungbean seedlings treated with 0.1–1.5 mM CdCl_2 did not correlate with the activity of DAO. Polyamine metabolism was differently affected in sunflower or wheat leaf discs treated either with Cd^{2+} or Cu^{2+} (Groppa et al., 2003) and both ADC and ODC were related to the increased Put levels found in 0.5 mM cadmium-treated wheat leaf discs. However, under copper treatment, ODC seemed to be the

main enzyme responsible for Put biosynthesis, in contrast to the result obtained by Lin and Kao (1999), who suggested that in copper-treated rice leaves, Put accumulation was linked to ADC activity. DAO activity in wheat leaf discs decreased with both metals and this fact might contribute to the higher Put content observed under Cd^{2+} treatment (Groppa et al., 2003). In sunflower leaf discs, the diminished Put content produced by Cd^{2+} was related to a decreased ADC and ODC activities, even though DAO activity was also significantly reduced. Copper also decreased ADC activity but did not modified ODC and DAO activities, thus the decrease in ADC activity might be responsible of the reduced Put levels observed (Groppa et al., 2003). In relation to the antioxidative behaviour of PAs in cadmium and copper-treated sunflower leaf discs, the exogenous addition of Spd or Spm completely reverted the metals-induced TBARS increase. The reduced glutathione reductase (GR) and superoxide dismutase (SOD) activities observed after Cd^{2+} or Cu^{2+} treatments were restored by 1 mM Spd or Spm added previously to the metals (Groppa et al., 2001).

The effects of foliar spraying with Spd, ranging from 0.25 to 0.50 mM, on the antioxidant system under Cd^{2+} stress was studied in *Typha latifolia* L. (Tang et al., 2005). The generation of superoxide anion ($\text{O}_2^{\bullet-}$), H_2O_2 and MDA content in both leaves and caudices of *Typha latifolia* L. decreased after spraying with Spd. The authors suggested that exogenous Spd elevated the tolerance of *T. latifolia* under Cd^{2+} stress primarily by increasing GR activity and the GSH level. In maize callus treated for 22 months with 0.5 mM lead, Zacchini et al. (2003) found an increase of free PAs content as well as the antioxidative capacity of the callus, being Put the polyamine that showed the higher rise.

Ozone stress

Ozone is the most phytotoxic gaseous pollutant in the atmosphere (Heagle, 1989). It enters the plant through stomata, inducing a variety of toxic effects like membrane dysfunction, generation of toxic compounds, inhibition of CO_2 assimilation, growth inhibition, early senescence and cell death (Schrauder et al., 1997; Miller et al., 1999; Rao et al., 2000). At the cellular level, in leaves of ozone-sensitive plants acute exposure to high ozone can induce chlorosis and necrotic lesions. As a mechanism for ozone-induced damage, the generation of ROS such as $\text{O}_2^{\bullet-}$ and H_2O_2 by ozone degradation in the apoplast has been proposed (Baier et al., 2005). Plants can respond against ozone toxicity also by increasing PAs levels (Langebartels

et al., 1991) and the contribution of plant PAs to the protection against ozone damage and ozone-derived oxidative damage has been well documented, though the precise mode of action remains a matter of debate (Ormrod and Beckerson, 1989; Scalet et al., 1995; Van Buuren et al., 2002). Studies on ozone-sensitive (Bel W3) and tolerant (Bel B) *Nicotiana tabacum* cultivars have shown an increase of Put, both free and conjugated to hydroxycinnamic acids and of Spd, in the ozone tolerant cultivar Bel B subjected to ozone application (Langebartels et al., 1991; Navakoudis et al., 2003). In the hypersensitive cultivar Bel W3, however, only a small increase or a decrease of Put was observed at a later stage when necrotic lesions have already been formed. The accumulation of free and conjugated Put was preceded by an increase of ADC activity in ozone-treated tobacco cultivar Bel B (Langebartels et al., 1991). Van Buuren et al. (2002) reported that in tobacco plants treated with ozone, free Put accumulated in undamaged tissue of Bel W3 and Bel B cultivars, whereas Put conjugated to soluble and cell-wall bound components accumulated predominantly in tissue undergoing cell death in Bel W3 plants. This accumulation was caused by a redirection of the conjugation pathway, as well as by a transient increase in ADC and ODC specific activities. Accumulation of conjugated Put is part of the ozone-induced programmed cell death response in Bel W3 plants. Ozone-induced synthesis of free Put was not correlated with ozone-resistance in Bel B plants, which are apparently impaired in signal transduction pathways that are necessary to control the cellular redox state (Van Buuren et al., 2002).

Paraquat

Paraquat (PQ) is a herbicide widely used in agriculture. Kurepa et al. (1998) analyzed the relationship between PQ toxicity (a well known oxidative stress inducer) and PA levels in *Arabidopsis* plants and found that PQ treatment led to an increase in Put, but not of Spd and Spm content. Additionally, PAs feeding offered high levels of protection against PQ toxicity with Spd being the most effective. Conversely, in sunflower leaf discs, 100 μM PQ produced a decrease of Put, Spd and Spm levels (Benavides et al., 2000). The decrease of Put content was due to the inhibition of both enzymes of the Put biosynthesis, ADC and ODC. Exogenous addition of PAs (1 mM) reversed PQ toxicity to different degrees according to the polyamine and/or the tested parameter. Spermidine was able to inhibit chlorophyll loss, while Spm reverted the effect of PQ at the level of TBARS and SOD activity almost complete-

ly. These results provide support for the argument that PAs are effective antioxidants, probably through their ability to act as radical scavengers (Benavides et al., 2000). In accordance, maize leaves pre-treated with 1 mM Spm or Put significantly prevented the losses in chlorophyll and carotenoid levels induced by PQ (Durmus and Kadioglu, 2005). Ascorbic acid content in the leaves pre-treated with both PAs was found to be higher than those of the leaves pre-treated with water and a similar result was observed with SOD activity. The authors suggested that PAs pre-treated plants may become more tolerant to oxidative stress due to increases in the antioxidative enzymes and antioxidants (Durmus and Kadioglu, 2005).

Remarkable hints and directions for future research

Along this review, it remains clear that PAs are natural compounds concerned with plant responses to several types of abiotic stresses though many holes stay behind the striking advances in the field reported in the last ten years. Environmental challenges from diverse sources “turn on” an unlimited number of responses regarding PAs metabolism. Nowadays, the availability of key genes involved in the biosynthetic pathways and catabolic processes have made possible to shed light on PA metabolism using diverse molecular strategies, specially through transgenic approaches and the use of mutants of PAs metabolism (Kumer et al., 1997; Urano et al., 2003, 2004; Kasukabe et al., 2004). However, PAs localization inside the cells, transport mechanisms, proteins involved in PAs distribution and the regulation of catabolic processes at the transcriptional, translational and post-transcriptional level are key points that need to be clarified. It should be kept in mind the need to relate PA metabolism and physiology with plant growth regulators from a functional point of view.

To mention a few examples that reflect the last advances in PAs research, Urano et al. (2003) reported the first genome-wide characterization of expression profiles of eight genes involved in the biosynthesis of PAs in *Arabidopsis*, which were classified as stress-inducible, constitutive or stress-repressive according to their differential expression patterns under diverse environmental stresses. Among them, *AtADC2* may play an important role in PAs biosynthesis under drought and NaCl stress. Two full-length SAMDC cDNAs, *MdSAMDC1* and *MdSAMDC2*, were isolated from apple *Malus sylvestris* (L.) Mill. by Hao et al. (2005). The results obtained suggest that *MdSAMDC1* is mainly involved in fruit development and cell growth while *MdSAMDC2* in stress

responses. Kasukabe et al. (2004) cloned a SPDS cDNA from *Cucurbita ficifolia* and introduced it in *Arabidopsis* under the control of the cauliflower mosaic virus 35S promoter. Transgenic plants exhibited a significant increase in SPDS activity and Spd content in leaves together with enhanced tolerance to various stresses including chilling, freezing, salinity, hyperosmosis, drought, and paraquat. During exposure to chilling stress (5 °C), the transgenic plants displayed a remarkable increase in ADC activity and conjugated Spd contents in leaves compared to the wild type. A cDNA microarray analysis revealed that several genes were more abundantly transcribed in the transgenics than in the wild type under chilling stress. These genes included those for stress-responsive transcription factors such as DREB and stress-protective proteins like rd29A. These results strongly suggest an important role for Spd as a signaling regulator in stress signaling pathways, leading to build-up of stress tolerance mechanisms in plants under stress conditions.

In relation to biotic stress responses and in order to identify regulatory components of the pathway involved in the hypersensitive response (HR) induced by tobacco mosaic virus (TMV) in tobacco plants, Uehara et al. (2005) found that the *ZFT1* gene, which encodes a Cys2/His2 type zinc-finger protein, is Spm-responsive and, interestingly, *ZFT1* overexpression rendered tobacco plants more tolerant to TMV. The authors proposed that *ZFT1* functions as a transcription repressor in a Spm signaling pathway, thereby accelerating necrotic local region formation in tobacco leaves. Another important observation regarding PAs implication in signaling pathway involved in biotic stress responses has been proposed. At least in some host-pathogen interactions, accumulation of H₂O₂ as a result of PAs catabolism could play a key role in triggering hypersensitive response (HR) and Spm increased levels could trigger PR proteins or caspases leading to HR (Walters, 2003). The role of H₂O₂ as a signaling molecule as well as the identification of regulatory components in the pathway that leads to plant responses to abiotic stresses are fundamental clues for the future. Mutants or transgenic plants exhibiting differential capabilities for ROS (O₂^{•−} and H₂O₂) formation and evolution could be useful to elucidate this fundamental point.

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