Current Topics in Peptide & Protein Research
Vol. 19, 2018

Mini-Review

Polyamines are central molecules in nitrogen metabolism

María D. Groppa[1,2], Laura Recalde[1] and María P. Benavides[1,2,*]

[1]Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina; [2]IQUIFIB-CONICET, Junín 956, Buenos Aires (C1113AAC), Argentina.

ABSTRACT

Nitrogen (N) is one of the main nutrients for plants and is a constituent of many stable compounds such as amino acids, proteins and many other molecules such as polyamines (PAs). Plant growth is mostly dependent on tissue N concentration, but frequently, N availability from the environment is scarce, and consequently, plant productivity can be compromised. Polyamines are simple molecules essential for survival. They are able to influence and regulate various developmental processes as well as plant responses to environmental stress. This review summarizes recent advances in the study of the multifaceted behaviour of PAs during the lifespan of the plants, and the study of their metabolism as a part of a network of highly interdependent pathways associated to N metabolism involving the relationship among their precursors, like glutamate, arginine and ornitine, intermediates and catabolic products like nitric oxide and γ-aminobutyric acid (GABA).

KEYWORDS: polyamines, nitrogen, growth, glutamate, nitric oxide, amino acids.

INTRODUCTION

Polyamines are simple small, positively charged, organic molecules that exert a multifunctional role in plant development and stress responses [1-3]. The three most common PAs in plants are putrescine (Put), spermidine (Spd) and spermine (Spm), with some plants also having thermospermine (tSpm) in place of or in addition to Spm [4, 5]. Polyamine metabolic pathways including the biosynthesis, catabolism, turnover and conjugation with various organic molecules have been widely studied in all kingdoms. As much as their cellular functions are multiple, and sometimes contradictory, so are their roles in plant metabolism. The cellular concentrations of PAs usually fluctuate according to the stage of plant development, growth phase, the type of nitrogen (N) nutrition as well as environmental conditions during the growth cycle [6]. Their accumulation in large amounts in the cell could presumably sequester extra N thus reducing ammonia toxicity and also balance the total N distribution into multiple pathways in plants [7-9]. So, the relevance of the metabolic interactions of PAs with a huge variety of metabolic pathways in the context of N metabolism deserves to be studied to assess their biological significance in plants.

Nitrogen: a crucial nutrient for plant growth and development

Nitrogen is a constituent of many stable compounds, including inorganic ones such as nitric oxide (NO), dinitrogen gas (N_2), ammonium (NH_4^+) and nitrate (NO_3^-) salts, and organic compounds like amino acids and nucleotides, the building blocks of proteins and nucleic acids, respectively [10]. Also, it is an essential constituent of several important molecules such as PAs, hormones, coenzymes, and various secondary metabolites including alkaloids and chlorophyll. Plant growth is mostly dependent on tissue N concentration, but frequently, N availability from the environment is scarce which limits the productivity of natural and managed ecosystems. Nitrogen deficit produces

*Corresponding author: mbenavi@ffyb.uba.ar

chlorosis, growth decline, and subsequently plant cell death. However, it has been suggested that high N supply could even be deleterious to plant development [11] causing oxidative stress along with lipid peroxidation [12].

Nitrogen is taken up from the soil solution by plant roots and is mostly acquired as nitrate or ammonium, which are converted into the amino component of amino acids and proteins. The available form and concentration of N in the soil can vary greatly from μM to mM ranges and can depend largely not only on an ample range of soil physico-chemical properties and soil microbial activity, but also on environmental conditions such as water supply and temperature [13, 14].

The uptake, translocation and storage of nitrate and ammonium are regulated by the presence of three families of transporters (NRT1, NRT2 and CLC) whereas NH_4^+ uptake is carried out by plasma membrane-located AMT/MEP/Rh transporters [15, 16]. Once inside the plant, NO_3^- could be translocated to the leaves, stored in vacuoles or reduced to NO_2^- by cytosolic nitrate reductase (NAD(P)H) (NR, EC 1.7.1.1), and further plastidial nitrite reductase (NADH) (NiR, EC 1.7.1.15) reduces nitrite to ammonium. Ammonium is taken up by roots and transported into plastids/chloroplasts. The first step in the assimilation of ammonia is the adenosine triphosphate (ATP)-dependent reaction with glutamate (Glu) to form glutamine (Gln), catalysed by glutamine synthetase (GS, EC 6.3.1.2). The second enzyme involved in ammonia assimilation is glutamate synthase, also known as glutamine:2-oxoglutarate amidotransferase (GOGAT, EC 1.4.1.13), in a reaction that yields two molecules of glutamate [13, 14, 17-19]. This pathway is known as GS/GOGAT cycle. Glutamate is the precursor, among others, of three major N-rich metabolites namely proline (Pro), the non-protein amino acid γ-aminobutyric acid (GABA), and the diamine putrescine (Put) [20-22, 9], and other nitrogen compounds that are precursors of a large network of N-containing metabolites, including arginine (Arg), ornithine (Orn) and NO. All these compounds play important functions during plant growth and development, as signalling molecules, and also in the response to different kind of stresses.

Ammonium or nitrate as a source for polyamine biosynthesis

Polyamines are carbon-nitrogenous molecules discovered in 1678 by Antonie van Leeuwenhoek, who described the presence of crystalline substances in human semen, but it was Rosenheim in 1924 who synthesized the PAs putrescine (Put), spermine (Spm) and the related base spermidine (Spd) for the first time [23]. Polyamines are present ubiquitously in cells of all living organisms, except some archaeal methanogens and halophiles [24, 25, 2, 8]. They are part of the overall N metabolism, though they were not considered among the compounds that function in nitrogen nutrition, i.e., amino acid and protein synthesis [7].

Many years ago, the pioneer works of Le Rudulier and Goas [26, 27] and Smith [28] pointed out the special relevance of these metabolites to the mineral nutrition of plants, through N sequestration in the forms of ammonium and nitrate, or the regulation of cell homeostasis. It was reported that ammonium produced a higher Put accumulation than nitrate as a nitrogen source [28, 7], whereas a huge increase in Put content, and a more moderate increase in other PAs, was found in soybean seedlings grown with ammonium as compared with nitrate [26]. Further studies established that Orn was converted to Put more rapidly in ammonium-fed seedlings [27]. The detoxifying role of Put avoiding NH_4^+ accumulation was also reported by other authors [29-31]. Wang *et al*. [32] found that low levels of nitrate are related to Put accumulation in broccoli, carrots and beets. According to Serapiglia *et al*. [31], a reduction of nitrate availability in the growth medium of red spruce cells inhibits GS activity and reduces total soluble proteins and PAs.

Glutamate, arginine and ornithine as precursors in polyamine biosynthesis

The involvement of PAs in plant growth and development, through N acquisition or regulation of its cell homeostasis, has been well documented [28, 26, 27, 7], but their specific cellular functions related to nitrogen nutrition in plants still remain largely undeciphered. The metabolism of these compounds is part of a complex network of highly interdependent pathways that are vital to N overall metabolism. The alteration of cellular PA levels,

either through exogenous application of PAs or precursor metabolites like Orn or Arg, using genetic manipulation, or inhibitors of the activity of the enzymes responsible for Put biosynthesis, arginine decarboxylase (ADC, EC 4.1.1.19) and ornithine decarboxylase (ODC, EC 4.1.1.17), can be very useful to explore the network that connects the interactive Arg-Orn-PAs metabolic pathways to the regulation of growth and development [33-37, 9, 38]. In addition, Arg, Pro, GABA and Put levels reflect several responses of the plants to different types of stress, and hence, the control of their metabolic status is necessary to maintain plant cell homeostasis [34, 39, 40, 37, 9, 41-43].

In plants, Glu can be considered the initial precursor for the biosynthesis of PAs, because Orn is formed from Glu through pyrroline-5-carboxylate (P5C) in a reaction catalyzed by ornithine aminotransferase (OAT, EC 2.6.1.13), and Arg is synthetized from Orn, and both amino acids are the source for Put, the diamine that triggers PA biosynthesis [24, 25]. Glutamate is also the source of the amide group needed for the biosynthesis of most other amino acids like Gln, Pro, as well as other nitrogenous compounds such as GABA [44]. Because Pro and GABA levels are frequently altered by many biotic or abiotic stresses, it is crucial to establish whether upon a plant response to stress Glu is used by glutamate decarboxylase (GAD, EC 4.1.1.15) (depending on substrate affinity and/or localization) to form GABA, by Δ1-pyrroline-5-carboxylate synthetase (P5CS, EC 1.2.1.41) to give rise to P5C, precursor of Pro, or to form Orn and Arg, the primary precursors for polyamines [45, 41, 43]. Moreover, Glu is also the precursor for glutathione (GSH) synthesis, a key step to obtain a non-enzymatic compound essential to the antioxidant plant machinery [46, 47].

Arginine could be considered as a suitable storage form of organic N in plants, because it has the highest nitrogen-to-carbon ratio among the proteinogenic amino acids [41]. Although several steps of Arg biosynthesis still remain poorly characterized in plants, its synthesis seems to be localised predominantly in plastids, with some ambiguous localisation prediction of enzymes in the cytosol and is tightly regulated by various feedback mechanisms, in accordance with the overall nutritional status [41]. The role of Arg as an important amino acid for nitrogen storage in plants is complemented by Arg catabolism. Arginine degradation to Orn and urea mediated by arginase (EC 3.5.3.1) in mitochondria is followed by urea exportation to the cytosol where it is further degraded to ammonia by urease (EC 3.5.1.5) [48], recycling N for the adjustment of the production of NO, PAs and Pro. Orn produced by Arg catabolism inside the mitochondria was controversially proposed as a precursor for Pro biosynthesis, alternative to Glu [49]. Although elevated levels of Pro resulted from supplying Arg [50], the biochemical pathway of the conversion of Arg to Pro in plants and its physiological relevance is still unclear. One hypothesis says that Orn, derived from Arg catabolism, is converted by OAT to Glu-γ-semialdehyde (GSA)/P5C and then to Pro by Δ1-pyrroline-5-carboxylatereductase (P5CR, EC 1.5.1.2), but this route is now questioned [34, 51].

Polyamine homeostasis is regulated by a dynamic balance between PAs biosynthesis and catabolism, conjugation and transport. The diamine Put can be formed either from Orn, in a reaction catalyzed by ODC, or from Arg by ADC, *via* two additional steps [52, 53, 5]. The triamine Spd and tetraamine Spm are synthesized from Put and decarboxylated S-adenosylmethionine (dcSAM) by the enzymes spermidine synthase (SPDS, EC 2.5.1.16) and spermine synthases (SPMS, EC 2.5.1.22), respectively; dcSAM is produced from SAM by SAM decarboxylase (SAMDC, EC 4.1.1.50) [24]. The production of Put through ADC is considered the key step for limiting PA biosynthesis in plants whereas in animals this role is performed by ODC [8].

Although Orn is an intermediate in the Glu to Arg pathway in plants and in most plants the cellular concentration of Orn is much lower compared with Arg or Glu, its significance in the regulation of Put production has rarely been discussed [54, 37, 9]. Majumdar *et al*. [37], using transgenic plants carrying an inducible mouse ODC gene (mODC), revealed unique aspects in the regulation of PA metabolism, showing that the cellular Orn concentration plays a key role not only in Put but also in Arg, Pro and GABA production, and

its own biosynthesis is engaged to its demand in the PA biosynthetic pathway, suggesting the occurrence of an Orn-sensing (monitoring) and a complex signal transduction mechanism. In this model, Spd and Spm showed slight changes.

The co-existence of ADC and ODC in the Put biosynthetic pathway may be related to their differential contribution to developmental processes and tissue specificity [55, 38]. The increased metabolic conversion of Arg or Orn into Put may considerably affect the pool of other amino acids and metabolites in the cell [9, 43]. It has been implied that ODC is particularly active in cell proliferation, whereas ADC is involved in embryo and organ differentiation and stress response [56-60, 55, 8, 61, 38]. However, regulation of the flux of Glu into Orn/Arg/Put and Pro under conditions of increased need for the biosynthesis of Put (e.g., due to abiotic stress response or experimental up-regulation of Put production *via* transgenic approaches) is still enigmatic [9]. In two *Araucaria angustifolia* cell lines with different embryogenic potential, cellular PA metabolism was modulated by using Arg or Orn at two time points during cell growth, showing an increase in citrulline content two days after subculturing with Arg, followed by a higher expression of genes related to PA catabolism in the responsive cell line; whereas, in the blocked cell line, only an accumulation of PAs was observed. Under certain conditions, Orn levels could pass over the control of PA biosynthetic enzymes. The other substrate of importance for PA biosynthesis is methionine (Met), which is the primary source of SAM biosynthesis; SAM is also required for numerous methylation reactions in the cell [43].

GABA and proline as nitrogen products of polyamine catabolism

During their catabolism, PAs are oxidized by either copper-containing amine oxidases (CuAO, EC1.4.3.6), homodimers in which each subunit contains a copper ion, or by polyamine oxidases (PAO, EC 1.5.3.11) which bear a non-covalently bound molecule of flavin adenine dinucletide (FAD) as a cofactor [25, 62-64]. CuAOs from microbes, animals and plants oxidise the diamines Put and cadaverine (Cad) at the primary amino groups releasing the corresponding aminoaldehydes and ammonium [65, 66]. The terminal catabolism of tri and tetramines is exerted mainly by apoplastic PAOs; however, the substrate specificity and reaction products of PAO vary depending on the organisms and isoenzymes involved [67]. It has been reported that plant PAO oxidizes the carbon on the endo side of the N4 nitrogens of spermidine and spermine and thereby produces 4-aminobutanal and 1-(3-aminopropyl)-4-aminobutanal, respectively [68, 62]. Both reactions also produce 1,3-diaminopropane (DAP) and H_2O_2. These reactions are known as PA degradation reactions. Some of the intracellular PAOs are also able to oxidize Therm-Spm and norspermine (Nor-Spm) with the production of Spd and norspermidine (Nor-Spd), respectively [69-71, 67].

Along with the terminal degradation of PAs to recycle C and N, it has now become clear that PA catabolism has a vital role in the regulation of plant growth and development [3], mainly because the catabolic products contain N in their molecules, essential for the nutrient status of the plant [72]. Among the most important nitrogen-containing catabolic products are GABA, a four carbon nonprotein amino acid very important in numerous physiological functions in all organisms [73, 74], and Pro, a nitrogenous osmolyte involved in stress responses [39]. In addition, oxidation of PAs results in the formation of H_2O_2, a signaling molecule that is perhaps a major player in PA-induced regulation of various biological processes [75].

Both, GABA and Pro are known to be involved in stress responses [76]. GABA can be synthesized from Put, which can be converted into GABA by the actions of CuAO and aldehyde dehydrogenase (ALDH, EC 1.2.1.3) [77, 9, 36]. An alternative pathway for GABA biosynthesis, that has been well characterized by Shelp's group [22], is through the so-called GABA shunt, *via* a direct decarboxylation of Glu in a reaction catalyzed by GAD [73]. In plants, increases in GABA levels have been reported in response to different stresses. However, in this condition, the exact role of GABA still needs to be defined [78]. Its metabolism in plants is complex, since various associated enzymes are spatially compartmentalized in the cell [22]. Moreover, it is not known whether

GABA biosynthesis and catabolism are regulated at the transcriptional or post-transcriptional level [9]. In tomato fruits, it was suggested that low temperature stimulated protein hydrolysis during early storage, thereby temporarily increasing the pools of amino acids available for various metabolic processes and, elevated Glu, particularly, influences the relative level of GABA much more than that of polyamines [42]. A notable accumulation of GABA following NH_4^+ feeding was observed in tobacco plants in response to *Pseudomona syringae* attack [76], indicating a diversion of Put metabolism away from higher PA biosynthesis. Wang *et al*. [79] have demonstrated that exogenous application of GABA to melon roots under hypoxia, accelerates PA biosynthesis and conversion and prevents its degradation, thus alleviating damage.

The proteinogenic amino acid Pro functions as an osmolyte, radical scavenger, electron sink, stabilizer of macromolecules, and a cell wall component [80]. Under salinity, proline accumulates to whole tissue concentrations up to 1 M [81]. In addition to Orn, Pro is mainly synthesized from Glu through successive enzymatic steps, namely the kinase and deshydrogenase activities of P5CS to form GSA that subsequently cyclizes to P5C, and the activity of P5CR, that converts P5C to proline [82]. Alternatively, Pro is generated from Orn by OAT, which transaminates GSA to produce P5C, and then Pro by the action of P5CR [83]. Equally as Glu-Pro pathway, Pro-Orn pathway is also reversible, using OAT. On the other hand, Pro can be oxidized back to Glu by the action of Pro dehydrogenase (PRODH, EC 1.5.5.2) and P5C dehydrogenase (P5CDH, EC 1.2.1.88) [84]. Legocka *et al*. [85] found a negative correlation between Pro accumulation and Put content. They observed that Put degradation by diamine oxidase (DAO) leads to a greater production of GABA which indirectly supports the pool of Pro.

Polyamines: a new way towards NO

Polyamines and nitric oxide are actively involved in physiological processes and in the response against different types of stress. The fact that both nitrogen metabolites share similar functions could be related to a possible interrelation between them where the NO would act as a mediator of PA actions. However, there are still very few studies regarding this metabolic relationship and the potential link between PAs and NO needs to be verified [86, 87].

Nitric oxide is an important signalling molecule involved in many physiological processes during plant development and nutrient assimilation, having a crucial role in the nitrate-sensing pathway [88]. It is produced *via* a variety of pathways in plants [89]. Briefly, these pathways can be classified into two groups according to nitrogen-containing precursors: the L-arginine-dependent pathway (oxidative pathway), and the NO_2^- dependent pathway (reductive pathway). NO_2-dependent NO synthesis involves NR, one of the key enzymes of nitrogen assimilation, which reduces NO_2^- to NO both *in vitro* and *in vivo* in specific physiological contexts [90]. Alternatively, formation of NO through the reduction of NO_2^- by the mitochondrial respiratory chain can also be observed, particularly in roots [91, 92]. Finally, NO can be produced by an apoplastic non-enzymatic conversion of NO_2^- to NO at acidic pH, in the presence of reductants such as ascorbic acid [93].

In the oxidative pathway, NO formation is related to nitrate, nitrite, L-arginine and polyamines, showing an interconnection between these nitrogen metabolites. Thalineau *et al*. [94], using transformed roots of *Medicago truncatula*, demonstrated that a crosstalk between NO and N metabolism is evident since NO appears to regulate nitrate transport and assimilation, and in return NO can be modulated by N supply. Nitric oxide also regulates gene expression and activities of nitrogen assimilation enzymes in *Triticum aestivum* plants, and the level of N supply and the N form are also involved in this response [95]. Inhibition of NR by NO in wheat leaves was reported by Rosales *et al*. [96]; however, an activation of the enzyme activity by NO was observed in cabbage [97]. In tomato roots the effect of NO on NR activity (activation or inhibition) depended on nitrate concentration [98]. The adaptation of rice plants to variations in nitrogen supply involves an increased lateral root development and N-uptake rate in response to the NO produced by NR [99].

As it was mentioned before, biosynthetic pathways of PAs and NO are overlapping in such a way that

NO can be formed by PAs; however there is no known enzyme that catalyzes this biosynthesis. Tun *et al*. [100] observed that mainly Spm and Spd induced NO biosynthesis in the elongation zone of the Arabidopsis root tip and in primary leaves but not in cotyledons suggesting that this induction is tissue specific. Similar results were obtained in wheat roots where exogenous addition of Spm enhanced NO production and the addition of the NO donor SNP increased polyamine content of roots suggesting that a complex interaction among PAs-NO was taking place [58, 87]. A NO-induced rise in PA content in response to abiotic stress was also observed in *Prosopis farcta* and *Scrophularia striata* plants [101, 102]. During PA-induced stomatal closure of *Arabidospis thaliana* guard cells, a significant increase in NO levels was observed [103]. Although no nitric oxide synthase (NOS) enzyme has been identified in plants until now, the authors suggested the involvement of a NOS-like enzyme in NO raise because L-NAME completely reversed the stomatal closure caused by PAs. In addition, the increase in NO during stress could be related to PA catabolism by DAO and PAO. Wimalasekera *et al*. [104] reported that *A. thaliana* plants lacking a copper amine oxidase gene CuAO1 showed a lower NO formation compared to wild-type plants confirming the involvement of DAO in NO biosynthesis.

CONCLUSION

In the past few years, several studies have demonstrated that PAs metabolism is closely linked to almost all aspects of plant growth, development and responses to stresses. The interconnection among the precursors of PA biosynthesis and the products of PA catabolism is extremely complex and the elucidation of this intricate relationship is just beginning. PAs interact with a broad network of cellular metabolic pathways, like the GABA shunt, N uptake and assimilation, the TCA cycle, signaling pathways involving H_2O_2 or NO, stress and senescence-related metabolism, and oxidative stress pathways to regulate directly or indirectly various biological functions in which they play essential roles. However, due to their holistic behaviour in the overall plant metabolism, PAs also display potential damaging effects, acting as growth inhibitors or oxidative agents.

At present, many questions remain regarding the contribution of PAs to many developmental processes, such as what is the role(s) of the additional nitrogenous molecules produced by PA oxidation or degradation (like GABA, Pro or NO), which is the precise function of PAs in the regulation of N:C balance and whether PAs are intermediary compounds in the stress protection, or have a role themselves. A detailed metabolic and signaling investigation addressing these and other fundamental questions are required to provide a broader view of the roles and mechanisms of PAs during plant development and will provide scientists with many challenging questions for many years to come.

ACKNOWLEDGEMENTS

This work was supported by grants from Universidad de Buenos Aires, Argentina (UBACYT 20020130100178BA), from Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 266, IQUIFIB-CONICET, Argentina) and ANPCyT (Agencia Nacional de Promoción Científica y Tecnológica, Argentina). L. R is a Universidad de Buenos Aires fellow, M. D. G and M. P. B are career investigators from CONICET.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

REFERENCES

1. Yamaguchi, K., Takahashi, Y., Berberich, T., Imai, A., Miyazaki, A., Takahashi, T., Michael, A. and Kusano, T. 2006, FEBS Lett., 22, 6783-6788.
2. Alcazar, R., Altabella, T., Marco, F., Bortolotti, C., Reymond, M., Koncz, C., Carrasco, P. and Tiburcio, A. F. 2010, Planta, 231, 1237-1249.
3. Tiburcio, A. F., Altabella, T., Bitrián, M. and Alcázar, R. 2014, Planta, 240, 1-18.
4. Kusano, T., Berberich, T., Tateda, C. and Takahashi, Y. 2008, Planta, 228, 367-381.
5. Slocum, R. D. 2005, Plant Physiol. Biochem., 43, 729-745.

6. Moschou, P. N., Wu, J., Cona, A., Tavladoraki, P., Angelini, R. and Roubelakis-Angelakis, K. A. 2012, J. Exp. Bot., 63, 5003-5015.
7. Altman, A. and Levin, N. 1993, Physiol. Plant., 89, 653-658.
8. Minocha, R., Majumdar, R. and Minocha, S. C. 2014, Front. Plant Sci., 5, 175.
9. Majumdar, R., Barchi, B., Turlapati, S. A., Gagne, M., Minocha, R., Long, S. and Minocha, S. C. 2016, Front. Plant Sci., 7, 78.
10. Bloom, A. J. 2015, Curr. Op. Plant Biol., 25, 10-16.
11. Valentinuz, O. R. and Tollenaar, M. 2006, Agron. J., 98, 94-99.
12. Zhang, Y-M., Chen, H., He, C-L. and Wang, Q. 2013, PLoS One, 8(7), e69225.
13. Miller, A. J., Fan, X., Orsel, M., Smith, S. J. and Wells, D. M. 2007, J. Exp. Bot., 58, 2297-2306.
14. Gent, L. and Forde, B. G. 2017, J. Exp. Bot., 68(10), 2531-2540.
15. Khademi, S., O'Connell, J., Remis, J., Robles-Colmenares, Y., Miercke, L. J. W. and Stroud, R. M. 2004, Science, 10(305), 1587-1594.
16. Mur, L. A. J., Simpson, C., Kumari, A., Gupta, A. K. and Gupta, K. J. 2016, Ann. Bot., 119, 703-709.
17. Lea, P. J. and Miflin, B. J. 1974, Nature, 251, 614-616.
18. Crawford, N. M. and Forde, B. G. 2002, Molecular and developmental biology of inorganic nitrogen nutrition, The Arabidopsis Book 1, e0011.
19. Lea, P. J. and Miflin, B. J. 2011, Ann. Plant Rev., 42, 1-40.
20. Larosa, P. C., Rhodes, D., Rhodes, J. C., Bressan, R. A. and Csonka, L. N. 1991, Plant Physiol., 96, 245-250.
21. Mattoo, A. K., Minocha, S. C., Minocha, R. and Handa, A. K. 2010, Amino Acids, 38, 405-413.
22. Shelp, B. J., Bozzo, G. G., Trobacher, C. P., Zarei, A., Deyman, K. L. and Brikis, C. J. 2012, Plant Science, 193, 130-135.
23. Bachrach, U. 2010, Plant Physiol. Biochem., 48, 490-495.
24. Cohen, S. S. 1998, A guide to the polyamines. Oxford University Press, New York.
25. Bagni, N. and Tassoni, A. 2001, Amino Acids, 20, 301-317.
26. LeRudulier, D. and Goas, G. 1975, Physiol. Veg., 13, 125-136.
27. LeRudulier, D. and Goas, G. 1977, Phytochemistry, 16, 509-512.
28. Smith, T. A. 1983, Recent Adv. Phytochem., 18, 7-54.
29. Primikirios, N. I. and Roubelakis-Angelakis, K. A. 2001, Plant Mol. Biol., 45, 669-678.
30. Skopelitis, D. S., Paranychianakis, N. V., Paschalidis, K. A., Pliakonis, E. D., Delis, I. D., Yakoumakis, D. I., Papadakis, A. K., Stephanou, E. G. and Roubelakis-Angelakis, K. A. 2006, Plant Cell, 18, 2767-2781.
31. Serapiglia, M. J., Minocha, R. and Minocha, S. C. 2008, Tree Physiol., 28, 1793-1803.
32. Wang, X. H., Wang, S. J., Chen, Z., Gong, B., Wang, X. F., Win, M., Shi, Q. H., Li, Y. and Yang, F. J. 2016, Russ. J. Plant Physiol., 63(4), 549-557.
33. Minocha, R., Lee, J. S., Long, S., Bhatnagar, P. and Minocha, S. C. 2004, Tree Physiol., 24, 551-560.
34. Funck, D., Stadelhofer, B. and Koch, W. 2008, BMC Plant Biol., 8, 40.
35. Mohapatra, S., Minocha, R., Long, S. and Minocha, S. C. 2010, Amino Acids, 38, 1117-1129.
36. Page, A. F., Minocha, R. and Minocha, S. C. 2012, Amino Acids, 42, 295-308.
37. Majumdar, R., Shao, L., Minocha, R., Long, S. and Minocha, S. C. 2013, Plant Cell Physiol., 54(6), 990-1004.
38. de Oliveira, L. F., Navarro, B. V., Cerruti, G. B., Elbl, P., Minocha, R., Minocha, S. C., dos Santos, A. L. W. and Floh, E. I. S. 2018, Plant Cell Physiol., 59(5), 1084-1098.
39. Signorelli, S., Coitiño, E. L., Borsani, O. and Monza, J. 2014, J. Phys. Chem. B, 118, 37-47.
40. Signorelli, S., Dans, P. D., Coitiño, E. L., Borsani, O. and Monza, J. 2015, PLoS One, 10(3), e0115349.
41. Winter, G., Todd, C. D., Trovato, M., Forlani, G. and Funck, D. 2015, Front. Plant Sci., 6, 534.

42. Brikis, C. J., Zarei, A., Chiu, G. Z., Deyman, K. L., Liu, J., Trobacher, C. P., Hoover, G. J., Subedi, S., DeEll, J. R., Bozzo, G. G. and Shelp, B. J. 2018, Hort. Res., 5, 61.
43. Wuddineh, W., Minocha, R. and Minocha, S. C. 2018, Polyamines in the Context of Metabolic Networks. In: R. Alcázar and A. Tiburcio (Eds.), Polyamines. Methods in Molecular Biology, Humana Press, New York, NY, Vol. 1694, 1-23.
44. Forde, B. G. and Lea, P. J. 2007, J. Exp. Bot., 58(9), 2339-2358.
45. Scott-Taggart, C. P., van Cauwenberghe, O. R., McLean, M. D. and Shelp, B. J. 1999, Physiol. Plant., 106, 363-369.
46. Foyer, C. H. and Noctor, G. 2005, Plant Cell, 17, 1866-1875.
47. Diaz-Vivancos, P., de Simone, A., Kiddle, G., and Foyer, C. H. 2015, Free Rad. Biol. Med., 89, 1154-1164.
48. Witte, C. P. 2011, Plant Sci., 180, 431-438.
49. Delauney, A. J., Hu, C. A., Kishor, P. B. and Verma, D. P. 1993, J. Biol. Chem. 268(25), 18673-18678.
50. da Rocha, I. M., Vitorello, V. A., Silva, J. S., Ferreira-Silva, S. L., Viégas, R. A., Silva, E. N. and Silveira, J. A. 2012, J. Plant Physiol., 169, 41-49.
51. Funck, D., Winter, G., Baumgarten, L. and Forlani, G. 2012, BMC Plant Biol., 12, 191.
52. Tiburcio A. F., Kaur-Sawhney, R. and Galston, A. W. 1990, Polyamine Metabolism, In: P. K. Stumpf and E. E. Conn (Eds.). The biochemistry of plants. New York: Academic Press, 283-325.
53. Walden, R., Cordeiro, A. and Tiburcio, A. F. 1997, Plant Physiol., 113, 1009-1013.
54. Mayer, M. J. and Michael, A. J. 2003, J. Biochem., 134, 765-772.
55. Vuosku, J., Jokela, A., Läärä, E., Sääskilahti, M., Muilu, R., Sutela, S., Altabella, T., Sarjala, T. and Häggman, H. 2006, Plant Physiol., 142, 1027-1038.
56. Kevers, C., Le Gal, N., Monteiro, M., Dommes, J. and Gaspar, T. 2000, Plant Growth Regul., 31, 209-214.
57. Groppa, M. D., Rosales, E. P., Iannone, M. F. and Benavides, M. P. 2007, Phytochemistry, 69, 2609-2615.
58. Groppa, M. D. and Benavides, M. P. 2008, Amino Acids, 34, 35-45.
59. Alcázar, R., Garcia-Martinez, J. L., Cuevas, J. C., Tiburcio, A. F. and Altabella, T. 2005, Plant J., 43, 425-436.
60. Alcázar, R., Cuevas, J. C., Planas, J., Zarza, X., Bortolotti, C., Carrasco, P., Salinas, J., Tiburcio, A. F. and Altabella, T. 2011, Plant Sci., 180, 31-38.
61. Alcázar, R. and Tiburcio, A. F. 2016, Polyamines in stress protection: applications in agriculture. In Wiley-VCH Verlag GmbH & Co (Eds). Abiotic stress response in plants, 411-422.
62. Cona, A., Rea, G., Angelini, R., Federico, R. and Tavladoraki, P. 2006, Trends Plant Sci., 11, 80-88.
63. Moschou, P. N., Paschalidis, K. A. and Roubelakis-Angelakis, K. A. 2008, Plant Signal. Behav., 3, 1061-1066.
64. Tavladoraki, P., Cona, A., Federico, R., Tempera, G., Viceconte, N., Saccoccio, S., Battaglia, V., Toninello, A. and Agostinelli, E. 2012, Amino Acids, 42, 411-426.
65. Alcázar, R., Marco, F., Cuevas, J. C., Patron, M., Ferrando, A., Carrasco, P., Tiburcio, A. F. and Altabella, T. 2006, Biotechnol. Lett., 28, 1867e1876.
66. Angelini, R., Cona, A., Federico, R., Fincato, P., Tavladoraki, P. and Tisi, A. 2010, Plant Physiol. Biochem., 48, 560-564.
67. Liu, T., Kim, D. W., Niitsu, M., Maeda, S., Watanabe, M., Kamio, Y., Berberich, T. and Kusano, T. 2014, Plant Cell Physiol., 55, 1110-1122.
68. Federico, R. and Angelini, R. 1991, Polyamine catabolism in plants. In: R. D. Slocum and H. E. Flores (Eds.), Biochemistry and physiology of polyamines in plants, Boca Raton, FL: CRC Press, 41-56.
69. Tavladoraki, P., Rossi, M. N., Saccuti, G., Perez-Amador, M. A., Polticelli, F., Angelini, R. and Ferderico, R. 2006, Plant Physiol., 41, 1519e1532.
70. Fincato, P., Moschou, P. N., Spedaletti, V., Tavazza, R., Angelini, R., Federico, R., Roubelakis-Angelakis, K. A. and Tavladoraki, P. 2011, J. Exp. Bot., 62, 1155-1168.

71. Kim, D. W., Watanabe, K., Murayama, C., Izawa, S., Niitsu, M., Michael, A. J., Berberich, T. and Kusano, T. 2014, Plant Physiol., 165, 1575-1590.
72. Tisi, A., Federico, R., Moreno, S., Lucretti, S., Moschou, P. N., Roubelakis-Angelakis, K. A., Angelini, R. and Cona, A. 2011, Plant Physiol. Biochem., 157, 200-215.
73. Fait, A., Fromm, H., Walter, D., Galili, G. and Fernie, A. R. 2008, Trends Plant Sci., 13(1), 14-19.
74. Podlešáková, K., Ugena, L., Spíchal, L., Doležal, K. and De Diego, N. 2019, Biotech., 48, 53-65.
75. Gupta, K., Sengupta, A., Chakraborty, M. and Gupta, B. 2016, Front Plant Sci., 7, 1343.
76. Gupta, K., Dey, A. and Gupta, B. 2013, Acta Physiol. Plant., 35(7), 2015-2036.
77. Cheng, W. H., Wang, F. L., Cheng, X. Q., Zhu, Q. H., Sun, Y. Q., Zhu, H. G. and Sun, J. 2015, Front. Plant Sci., 6, 1063.
78. Michaeli, S. and Fromm, H. 2015, Front. Plant Sci., 6, 419.
79. Wang, C., Fan, L., Gao, H., Wu, X., Li, J., Lv, G. and Gong, B. 2014, Plant Physiol. Biochem., 82, 17-26.
80. Matysik, J., Alia Bhalu, B. and Mohanty, P. 2002, Curr. Sci., 82, 525-532.
81. Anwar, A., She, M., Wang, K., Riaz, B. and Ye, X. 2018, Int. J. Mol. Sci., 19, 3681.
82. Sharma, S. S. and Dietz, K. J. 2006, J. Exp. Bot., 57(4), 711-726.
83. Szabados, L. and Savouré, A. 2010, Trends Plant Sci., 15, 89-97.
84. Ribarits, A., Abdullaev, A., Tashpulatov, A., Richter, A., Heberle-Bors, E. and Touraev, A. 2007, Planta, 225, 1313-1324.
85. Legocka, J., Sobieszczuk-Nowicka, E., Ludwicki, D. and Lehmann, T. 2017, Acta Soc. Bot. Pol., 86(3), 3549.
86. Yamasaki, H. and Cohen, M. F. 2006, Trends Plant Sci., 11, 522-524.
87. Recalde, L., Vázquez, A., Groppa, M. D. and Benavides, M. P. 2018, Protoplasma, 255, 1295-1307.
88. Manoli, A. Begheldo, M., Genre, A., Lanfranco, L., Trevisan, S. and Quaggiotti, S. 2014, J. Exp. Bot., 65, 185-200.
89. Gupta, K. J., Fernie, A. R., Kaiser, W. M. and van Dongen, J. T. 2011, Trends Plant Sci., 16(3), 160-168.
90. Yamasaki, H. and Sakihama, Y. 2000, FEBS Lett., 468, 89-92.
91. Stöhr, C., Strube, F., Marx, G., Ullrich, W. R. and Rockel, P. 2001, Planta, 212(5-6), 835-841.
92. Horchani, F., Prevot, M., Boscari, A., Evangelisti, E., Meilhoc, E., Bruand, C., Raymond, P., Boncompagni, E., Aschi-Smiti, S., Puppo, A. and Brouquisse, R. 2011, Plant Physiol., 155(2), 1023-1036.
93. Bethke, P. C., Badger, M. R. and Jones, R. L. 2004, Plant Cell, 16, 332-341.
94. Thalineau, E., Truong, H-N., Berger, A., Fournier, C., Boscari, A., Wendehenne, D. and Jeandroz, S. 2016, Front. Plant Sci., 7, 472.
95. Balotf, S., Islam, S., Kavoosi, G., Kholdebarin, B., Juhasz, A. and Ma, W. 2018, PLoS One, 13(1), e0190269.
96. Rosales, E. P., Iannone, M. F., Groppa, M. D. and Benavides, M. P. 2010, Plant Physiol. Biochem., 49, 124-130.
97. Du, S., Zhang, Y., Lin, X., Wang, Y. and Tang, C. 2008, Plant Cell Environ., 31, 195-204.
98. Jin, C. W., Du, S. T., Zhang, Y. S., Lin, X. Y. and Tang, C. X. 2009, Ann. Bot., 104, 9-17.
99. Sun, H., Li, J., Song, W., Tao, J., Huang, S., Chen, S., Hou, M., Xu, G. and Zhang, Y. 2015, J. Exp. Bot., 66, 2449-2459.
100. Tun, N. N., Santa-Catarina, C., Begum, T., Silveira, V., Handro, W., Floh, E. I. and Scherer, G. F. 2006, Plant Cell Physiol., 47(3), 346-354.
101. Zafari, S., Sharifi, M., Mur, L. A. J. and Chashmi, N. A. 2017, Ecotoxicol. Environ. Saf., 142, 293-302.
102. Falahi, H., Sharifi, M., Ahmadian Chashmi, N. and Zare Maivan, H. 2018, Plant Physiol. Biochem., 130, 139-147.
103. Agurla, S., Gayatri, G. and Raghavendra, A. S. 2017, Protoplasma, 255(1), 153-162.
104. Wimalasekera, R., Villar, C., Begum, T. and Scherer, G. F. E. 2011, Mol. Plant, 4(4), 663-678.