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Invited review:

Speculation: Polyamines are important in abiotic stress signaling

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ABSTRACT

The main role of polyamines was originally assumed to be as direct protective compounds important under stress conditions. Although in some cases a correlation was found between the endogenous polyamine content and stress tolerance, this relationship cannot be generalized. Polyamines should no longer be considered simply as protective molecules, but rather as compounds that are involved in a complex signaling system and have a key role in the regulation of stress tolerance. The major links in polyamine signaling may be H_2O_2 and NO, which are not only produced in the course of the polyamine metabolism, but also transmit signals that influence gene expression via an increase in the cytoplasmic Ca^{2+} level. Polyamines can also influence Ca^{2+} influx independently of the H_2O_2- and/or NO-mediated pathways. Furthermore, these pathways may converge. In addition, several protein kinases have been shown to be influenced at the transcriptional or post-translational level by polyamines. Individual polyamines can be converted into each other in the polyamine cycle. In addition, their metabolism is linked with other hormones or signaling molecules. However, as individual polyamines trigger different transcriptional responses, other mechanisms and the existence of polyamine-responsive elements and the corresponding transacting protein factors are also involved in polyamine-related signaling pathways.

Highlights:

- Polyamines are interconvertible in the polyamine cycle
- The statement "the higher the polyamine level the better" cannot be generalized
- In stress responses the ratio of signaling to direct protection is more important
- Polyamines are also involved in hormonal cross-talk
- H_2O_2 and NO are the major but not the only links in polyamine stress signaling

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Keywords: cross-talk, polyamine cycle, putrescine, spermidine, spermine

Contents

1. Introduction

Polyamines are aliphatic amines found in all living cells. The most abundant polyamines in plants are putrescine, spermidine and spermine, which can be found in relatively high amounts. However, specific roles have also been reported for less abundant polyamines, such as agmatine, cadaverine and thermospermine. Polyamines occur in free, conjugated (associated with small molecules such as phenolic acids) or bound forms (associated with various macromolecules). The total and individual polyamine contents vary markedly depending both on the plant species or organ and on the developmental stage, and are much higher in plants than those of endogenous phytohormones. The biosynthetic pathway and key enzymes of the polyamine metabolism are well documented [1]. Briefly, putrescine is synthesized by the decarboxylation of ornithine, catalysed by ornithine decarboxylase, or indirectly by the decarboxylation of arginine by arginine decarboxylase (ADC), via agmatine. Higher polyamines (spermidine and spermine or thermospermine) are produced by the sequential addition of aminopropyl moieties to the putrescine skeleton

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through enzymatic reactions catalyzed by the spermidine and spermine/thermospermine synthases (SPDS and SPMS/TSPMS). The donor of the aminopropyl groups is decarboxylated S-adenosyl-methionine, which is synthesized from S-adenosyl-methionine by S-adenosyl-methionine decarboxylase (SAMDC). Polyamines are catabolized by diamine oxidases (DAO) and polyamine oxidases (PAOs).

Polyamines were previously presumed to be simply direct protective compounds. Due to their cationic nature at physiological pH, they are able to interact with negatively charged macromolecules in a reversible way, thus stabilizing their structure, especially under stress conditions. They can bind to the phospholipid head groups of membranes influencing their permeability characteristics. They can also bind to various proteins non-specifically, stabilizing their structure and resulting in changes in their activity and/or function, as well as to chromatin, causing an alteration in the availability of genomic sites to DNA or RNA polymerases, leading to altered DNA and RNA synthesis [2]. There are several lines of evidence supporting the relationship between polyamines and photosynthesis. The conjugation of polyamines to photosynthetic complexes and proteins is catalyzed by transglutaminase [3] and leads to enhanced photosynthetic activity under stress conditions [4].

Besides their direct protective role, polyamines also regulate various fundamental cellular processes as signaling molecules. It has been increasingly shown that abiotic stress tolerance is chiefly influenced by the role of polyamines in signaling processes rather than by their accumulation. The present review focuses on this aspect of the mode of action of polyamines and attempts to find answers to the many open questions which have not yet been satisfactorily answered: 1. Is the accumulation of the individual polyamines essential for plant tolerance and abiotic stress responses? 2. How do they act in signaling? 3. What are their specific roles, and which of these is really necessary?

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2. Are polyamines markers of stress severity or indicators of stress tolerance?

The polyamine pool is dynamic, changing over time, and polyamines also undergo rapid interconvertion in the "polyamine cycle" (Fig. 1). Besides the PAOs, which catalyze the terminal catabolism of spermidine, spermine or thermospermine, five enzymes in Arabidopsis and three in rice were shown to be involved in the partial and/or full back-conversion of spermine/thermospermine to spermidine and of spermidine to putrescine [5-6]. Stress-responsive elements are found in the promoters of certain genes playing a role in polyamine synthesis (*ADC, SPDS, SPMS, SAMDC*), resulting in the early activation of polyamine biosynthesis in response to stress [7-8].

Several reviews [6, 9-10] have dealt with the relationship between tolerance and the capacity to enhance the synthesis of polyamines upon exposure to stress. Plants overexpressing genes encoding enzymes involved in polyamine biosynthesis accumulate higher levels of polyamines and show enhanced tolerance to various stresses [9-14]. This suggests that an increase in polyamine synthesis is effective against all types of stress. Most studies carried out so far have been focussed on the beneficial effects of polyamines, and emphasize that a correlation exists between stress tolerance and elevated polyamine contents. However, the real situation is more complicated, as in some cases the excess accumulation of polyamines due to the overexpression of these genes or to the absorbed exogenous polyamines is harmful to plant cells [3, 11].

Furthermore, while some plants accumulate polyamines, others have constant or even lower endogenous polyamine content when exposed to stress conditions, and individual plant species exhibit diverse responses in terms of polyamine levels. Salt tolerance was positively related with spermidine but negatively correlated with spermine levels in rice [15]. In another

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study on the same species, no correlation was found between either the initial levels or the stress-induced accumulation of polyamines (putrescine, spermidine or spermine) and drought tolerance [16]. The initial stress-induced accumulation of putrescine, spermidine and spermine was greater in drought-sensitive cultivated chickpea than in a tolerant wild species [17]. The amount of putrescine showed a substantial increase during cold hardening in winter wheat and a decrease in spring wheat, while the spermidine content increased in both, and the spermine level increased only in the spring wheat variety [18]. Low temperature also increased the putrescine level in cold-sensitive species, for example in maize [19]. Furthermore, higher freezing tolerance and a smaller pool of free spermine was found in *Thellungiella*, but not in related accessions of Arabidopsis [20].

These results show that it is difficult to establish a direct relationship between increased levels of polyamines, especially that of individual polyamines, and abiotic stress tolerance. Indeed, elevated polyamine content might be the cause of stress-induced injury and/or of some protective mechanisms, suggesting that the statement "the higher polyamine level the better" cannot be generalized. There is often an immediate increase in polyamine levels in response to stress, but after a while polyamine levels decrease and resemble those of non-stressed plants, even if the stress conditions persist [21].

Specifically, salt stress may lead to changed (spermidine+spermine)/putrescine ratio and salt-tolerant plant species were found to accumulate less putrescine [22]. In fact, the greater accumulation of putrescine, leading to a low (spermidine+spermine)/putrescine ratio, may even injure plants. It seems that the main factor responsible for stress tolerance is not so much an elevated level of putrescine as its enhanced turnover, and the ability to accumulate high spermidine and spermine levels. Although, it can be concluded that in many species polyamines are an indisputable part of acclimation to a given stress factor, their actual

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amounts may not play such a critical role as is generally assumed. The correlation between stress tolerance and polyamine levels is not general.

3. Possible action mechanisms of individual polyamines during abiotic stresses

So, which of the three most abundant polyamines plays the central role in plant stress responses? The possible polyamine action mechanism can be revealed in two ways. The application of exogenous polyamine treatment under normal and stressed growth conditions shows which other compounds or processes are influenced, while transgenic plants overexpressing genes responsible for polyamine biosynthesis or the use of loss-of-function mutants also help to identify polyamine-dependent stress responses. It should be taken into consideration, however, that: i. The exogenous polyamines absorbed or the elevated levels of endogenous polyamines can quickly be converted into each other. ii. The enzymes involved in biosynthesis and catabolism are also affected by polyamine treatment. iii. Polyamine production and/or transport mechanisms may be tissue-, compartment- and age-specific. iv. The effect of the treatment may also be genotype-dependent.

3.1. Putrescine

The beneficial, general stimulatory effect of putrescine has long been known. However, this effect is not obviously direct. Putrescine is also involved in the development of stress tolerance by regulating abscisic acid levels [23], activation of the antioxidant system, and induction of phenylalanine ammonia lyase, one of the key enzymes in the synthesis of flavonoids, but these changes were depending on the degree of stress tolerance of the plants and on growing – normal or stress – conditions [24-26]. Polyamines have been reported to promote protein synthesis [2], so they probably act at the transcription level rather than by

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direct activation. However, the induction of antioxidant enzymes via ROS production from polyamine oxidation was also occurred.

Putrescine acts as a buffer and osmolite, and induces increment in proline content leading to maintenance of leaf water status under stress conditions [27]. Exogenous putrescine increased the phospholipase D activity, which has a role in the mitigation of drought stress injury in the early stages of drought treatment [28]. Exogenous putrescine also enhanced the transcript levels of a heat shock protein gene, *HSP17*, during heat shock, and this response was found to be much more pronounced in thermotolerant than in susceptible cultivars [29] (Table 1).

Microarray analysis of arginine decarboxylase (*ADC2*) overexpression revealed both the up- and down-regulation of various stress-responsive, hormone- and signaling-related genes. These included genes encoding transcription factors belonging to the APETALA2/ethylene responsive factor domain family (e.g. DREB1C, DREB2A), genes involved in the biosynthesis of auxin, ethylene, abscisic acid, gibberellin and salicylic acid, genes for auxin transport, and genes coding for auxin-responsive proteins, ethylene- and abscisic acid-responsive transcriptional factors, and also jasmonate-induced proteins [30] (Table 1). These results confirm the dual role of putrescine (and polyamines in general): direct protection and participation in acclimation signaling pathways.

3.2. Spermidine

The protective effect of spermidine during salt or drought stress involved the higher transcription level of genes encoding antioxidant enzymes [31-32]. Thirty-four genes were up-regulated in spermidine-treated tomato fruits as compared with non-treated fruits. These genes are putatively involved in primary metabolism, signal transduction, hormone responses,

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transcription factors and stress responses, while 55 genes putatively involved in the energy metabolism, cell wall metabolism and photosynthesis were down-regulated [33]. The exogenous application of spermidine to the alga of the lichen *Xanthoria parietina* resulted in an increase in the transcript level of the gene *psbA* encoding the D1 protein in photosystem II [34]. Besides increased α-amylase and β-amylase activities, there was a reduction in the sugar, fructose and glucose contents and an elevation in the expression level of β-amylase gene after spermidine treatment [35] (Table 1).

Polyamines may modulate the up- or down-regulation of gene expression either directly or by stimulating the phosphorylation of regulatory proteins such as transcription factors. The overexpression of a spermidine synthase gene up-regulated the expression of various putative stress-regulated genes in chilled transgenic Arabidopsis compared to the corresponding wild type. These genes putatively encode transcription factors such as WRKY, MYB, B-box zinc finger proteins, DREB2B, sucrose non-fermenting protein 1 (SNF1)-related protein kinase 2 (SnRK2), calmodulin-related protein and stress-protective proteins, such as rd29A [11] (Table 1). SnRK2, characterized as OSPDK in rice, is Ca^{2+} independent and has a role in the activation of various stress regulatory molecules, such as the phosphorylation of OSBZ8, a bZIP class of ABRE-binding transcription factors. The gene expression of OSPDK is regulated both at the transcriptional and translational level both by spermidine and by abscisic acid [36].

Besides the transglutaminase reaction, which is of especial importance in the chloroplasts, another case of the covalent binding of polyamines to proteins is hypusine biosynthesis. The butylamino group of spermidine is used for the post-translational modification of the precursor of the eukaryotic translation initiation factor 5A (eIF5A), which

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modifies lysine to hypusine [37]. Hypusinated eIF5A is essential for the growth of all eukaryotic cells.

3.3. Spermine

Research in recent years has revealed the involvement of non-specific phospholipase C, a novel type of phospholipase, in plants root development, brassinosteroid signaling, abscisic acid sensing, and tolerance to abiotic stresses. Spermine treatment increased the phospholipase C activity, which demonstrated the interaction of spermine with the signal transduction cascade of phosphoinositide-Ca^{2+} [38].

Spermine treatment may also increase the expression levels of the stress-related genes that protect seedlings from stress damage (Table 1). Abscisic acid-responsive element binding factors (ABFs) are basic region/leucine zipper (bZIP) class transcription factors involved in the transcriptional regulation of abscisic acid- and/or stress-responsive genes via interaction with ABRE cis elements in their promoters. Trifoliate orange seedlings pre-treated with spermine had significantly higher expression of genes for heat shock proteins and ABFs than the control plants both under normal conditions and during combined drought and heat treatment [39]. Spermine has also been shown to induce certain enzymes, such as wound-induced protein kinase, and to be responsible for the post-translational activation of certain mitogen-activated protein kinases, such as salicylic acid-induced protein kinase. However, the activation of the latter also requires H_2O_2, and the inhibition of polyamine oxidase suppressed it [40]. This suggests that the generation of H_2O_2 via the oxidative degradation of polyamines plays a role in polyamine-related signaling processes. Spermine application also exerted an effect on mitochondrial functions since it transcriptionally activated the alternative oxidase pathway [40].

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Microarray analysis of *Atpao4* plants (in which the lack of *AtPAO4* induced a decrease in spermidine and an increase in spermine) detected several alterations in gene expression including the up-regulation of genes encoding drought stress response proteins (e.g. DRE-binding protein, LEA protein, LEA domain-containing protein, desiccation-responsive protein 29A and universal stress protein family protein) and genes involved in flavonoid and/or lignin biosynthesis, such as phenylalanine ammonia lyase 1 [41] (Table 1).

Arabidopsis plants with increased spermine levels caused by the overexpression of *SAMDC1* or *SPMS* differed in their transcriptome profiles. Nevertheless, many genes responded similarly in these genotypes, such as genes involved in the biosynthesis of jasmonic acid and abscisic acid, jasmonic acid- and salicylic acid-responsive genes, receptor-like kinases, mitogen-activated protein kinases, genes with a role in calcium regulation, or the genes of transcriptional factors [30]. As also observed for other polyamines, an elevated spermine level in Arabidopsis plants upregulated genes encoding heat shock transcription factors and heat shock proteins after exposure to high temperature [42]. Transcripts of antioxidant enzymes, such as ascorbate peroxidase, manganese superoxide dismutase and glutathione-S-transferase, were also induced more significantly by abiotic stressors, such as salt, cold or acidic stress, in tobacco plants overexpressing carnation *SAMDC* [43] (Table 1).

3.4. Thermospermine

Thermospermine is synthesized from spermidine by thermospermine synthase. The gene encoding this enzyme, ACAULIS5 (ACL5), is specifically expressed in xylem vessel elements. In the deficient Arabidopsis mutant, *acl5*, it was demonstrated that thermospermine modifies the expression of auxin-related genes [44]. Although the potential role of thermospermine in biotic stress protection has already been noted, its role in abiotic stress has

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not yet been established [45]. It should also be mentioned that, since thermospermine is usually indistinguishable from spermine in the standard analysis of polyamines, further improvements in analytical techniques may also shed some light on its role in other physiological processes in the future.

4. Signaling components providing linkage between polyamines and stress responses

Another important aspect of polyamines is their double-edged role, as being both sources of ROS and potential ROS scavengers and playing role as redox homeostasis regulators in plants [46]. Both the catabolism and back-conversion of polyamines by DAO and PAOs result in the production of H_2O_2 in the apoplast and peroxisomes [8]. H_2O_2 has long been known as a signal molecule. It is able to mediate various processes, such as stomatal closure, directly due to its ability to influence ion channels, while it can also activate specific stress response processes through the MAPK cascade [47]. Polyamines, especially spermidine, also induce superoxide anion ($O_2^{\cdot-}$) production by the activation of NADPH-oxidase. However, $O_2^{\cdot-}$ dismutates spontaneously or enzymatically to H_2O_2. The ratio of $O_2^{\cdot-}$ to H_2O_2 is an important signal in transcription [48], and might be the mediator of polyamines in plant adaptation to unfavorable conditions (Fig. 2).

Polyamines are related to the production not only of ROS, but also of NO. As a small, highly diffusible gaseous molecule, NO functions as an intra- and intercellular messenger, inducing various processes including stress responses in plants. Rapid NO accumulation was reported after spermidine and spermine treatments without a lag phase, whereas putrescine had little effect in Arabidopsis [49]. In contrast, exogenous polyamines, especially putrescine, induced NO generation in soybean [50]. Reduced NO release was observed in copper amino oxidase 1 knock-out Arabidopsis [51]. These results suggest that DAO might be involved in

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NO biosynthesis induced by polyamines. Polyamines also modulated the arginine-linked NO synthase and nitrate reductase pathways [52-53]. It seems that NO can fill the gap between several physiological effects of polyamines and the mitigation of stress [54].

In citrus plants 271 S-nitrosylated proteins were identified as being polyamine-regulated [55]. As Fe-superoxide-dismutase, dehydroascorbate reductase and monodehydroascorbate reductase are also polyamine-induced S-nitrosylation target proteins, NO-modulated S-nitrosylation is also a possible link between polyamines and stress responses [51]. The mode of action by which polyamines affect S-nitrosylation may differ for individual polyamines [55]. They influence the stress responses of plants through interconversion loops occurring in the polyamine cycle, since H_2O_2, as an upstream signaling molecule, also leads to NO production in plants. It should also be mentioned that NO acts as an intermediate signaling molecule in cytokinin, abscisic acid, auxin, cytokinin and ethylene signaling.

Stress adaptive responses are closely related to the ability of the plant to control ion transport and ion homeostasis. One of the best examples of the polyamine action mechanism in signaling is their influence on ion channels, which they exert both by direct binding and through polyamine-induced signaling molecules (ROS and NO). Polyamines may further regulate the activity of ion channels indirectly by membrane depolarization. The hyperpolarization-activated Ca^{2+} influx and the NO-induced release of intracellular Ca^{2+} result in a higher cytoplasmic Ca^{2+} concentration, which is a basic component in general stress responses such as stomatal movements; the cytosolic Ca^{2+} level also regulates several plasma membrane channels [56].

The overexpression of vacuolar cation/H^+ exchangers was observed in spermine synthesis deficient plants, suggesting that polyamines have a role in the regulation of the vacuolar Ca^{2+} level [57]. They are also able to block vacuolar cation channels in the sequence

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spermine 4^+ > spermidine 3^+ > putrescine 2^+. Thus, polyamines assist in vacuolar Na^+ sequestration during salt stress [6]. In contrast to the vacuolar cation channels, plasma membrane cation channels do not respond selectively to different types of polyamines. Polyamines inhibit the inward-rectifying K^+ channels in the guard cell membrane. This effect is concentration-dependent and correlated with stomatal closure. They also inhibit the inward-rectifying and outward-rectifying K^+ channels in root cells [6, 58].

Ion channels are the potential targets of ROS. Both H_2O_2 and hydroxyl radicals mediate multiple ion channels, including those responsible for Ca^+ influx and K^+ efflux [6, 58]. In addition, polyamine-induced NO generation also causes the inhibition of outward-rectifying K^+ channels by direct nitrosylation, and the activation of plasma membrane H^+-ATP-ase [6]. However, different polyamines have different effects on H+-ATPase, depending on their type, concentration, and tissue-specific expression [6]. These changes are related not only to their stimulatory effect on the interaction between 14-3-3 protein with H+ -ATPase [51], but also for example to polyamine-activated Ca^{2+} efflux [6].

5. Connection with hormones and other small hormone-like protective molecules

Since NO acts as an intermediate signaling molecule not only for polyamines, NO biosynthesis could be the key to the overlapping responses induced by plant hormones. In addition, microarray analysis revealed that increased polyamine contents caused modifications in the expression levels of genes involved in the synthesis of various plant hormones (see Section 3).

Putrescine and abscisic acid are integrated in a positive feedback loop in response to abiotic stress [5] (Fig. 3). Abscisic acid-responsive elements (ABRE) or ABRE-related motifs occur in the promoters of *ADC2, SPDS1* and *SPMS*, which are also highly up-regulated in

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response to drought stress [10]. Abscisic acid also influences the catabolism of polyamines as it induces the expression of PAO [59], while at the same time, polyamines influence abscisic acid synthesis [30]. The transcriptional regulation of the 9-cis-epoxycarotenoid dioxygenase gene (*NCED*), which encodes the key enzyme involved in abscisic acid biosynthesis via putrescine accumulation, has been reported in *ADC* overexpressing transgenic plants under stress conditions [60-61]. Conversely, the suppression of arginine decarboxylase (in both *adc1* and *adc2* plants) resulted in the reduced expression of *NCED3* and the down-regulation of abscisic acid-regulated genes. These plants had better tolerance of low temperature when exogenous putrescine was provided [23].

In contrast, polyamines and ethylene have antagonistic roles (Fig. 3.). Polyamines inhibit senescence, while ethylene promotes it [5]. Polyamines and ethylene compete for a common substrate, as S-adenosyl methionine is also a substrate for the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene. Polyamines, especially spermine, regulate ethylene biosynthesis by inhibiting the accumulation of ACC synthase transcripts [62], and ethylene is an effective inhibitor of ADC and SAMDC [5]. It was also reported that PAOs were involved in ethylene-induced H_2O_2 production in the guard cells of Arabidopsis due to increased transcript levels of the AtPAO2 and AtPAO4 genes and to the elevated activity of PAOs [63].

Gamma-aminobutyric acid (GABA) is involved in defense mechanisms, protecting plants from stress through the regulation of cellular pH, acting as an osmoregulator or signaling molecule. 4-aminobutanal that is produced by DAO and PAOs could be converted into GABA via Δ^1-pyrroline. Thus, an increase in polyamine level may be followed by an increase in GABA accumulation [64] (Fig. 3).

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Brassinosteroids are another group of phytohormones that regulate not only plant growth and development but also abiotic and biotic stress responses either alone or in interaction with other hormones, such as abscisic acid, auxin, cytokinins, ethylene, jasmonic acid, salicylic acid and gibberellins (GAs). A relationship between brassinosteroids and polyamines is suggested by the fact that epibrassinolide treatment was reported to influence the level of polyamines, which in turn alleviated copper stress [65] (Fig. 3). The application of a brassinosteroid analogue, which diminished the effect of salt stress, restored the polyamine level to that detected under non-stress conditions [66].

ADC2 overexpressing transgenic plants exhibited a reduction in both the contents of GA1, 4 and 9 contents, and in the expression levels of the *AtGA20ox1*, *AtGA3ox1* and *AtGA3ox3* transcripts [67], suggesting that putrescine accumulation represses GA synthesis. The loss-of-function mutant of *SAMDC4* resulted in hyposensitivity to exogenous auxin and hypersensitivity to cytokinin treatment. This finding suggests that polyamines increase the sensitivity of auxin perception, and repress cytokinin biosynthesis or signaling [68] (Fig. 3).

Salicylic acid has long been known as a signal molecule in the induction of defense mechanisms in plants; furthermore, it participates in the signaling of abiotic stresses. The parallel changes in salicylic acid and polyamine contents under stress conditions have only been described in a few studies, but recent results have suggested that salicylic acid treatment influences polyamine synthesis and/or catabolism (Fig. 3). It is also evident that different concentrations of salicylic acid had different effects on the polyamine metabolism [69]. In addition, seed pre-treatment with spermidine or spermine was very effective in enhancing the salicylic acid content of wheat under salt stress [70]. On the other hand, the MAP kinase kinase (MEK2)–salicylic acid-induced protein kinase/wound-induced protein kinase cascade is involved in regulating polyamine synthesis, especially putrescine synthesis, through the

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transcriptional regulation of biosynthesis genes in tobacco [71]. Based on these results, a relationship is thought to exist between the endogenous salicylic acid and polyamine contents, but the exact cross-talk mechanisms under abiotic stress conditions are still not clear.

6. Conclusions and future prospects

Most abiotic stress responses share common elements in their pathways, which are potential nodes for cross-talk. Several of these common elements may serve as major switching points and have a role in stress tolerance. It is becoming clear that polyamines are also switching points of this kind and that polyamine-induced responses are interconnected at many levels. The most abundant polyamines, namely putrescine, spermidine and spermine, are able to influence several physiological processes, including photosynthesis, the antioxidant system and ion channels, but not always with the same specificity and efficiency under stress conditions. The question arises of whether the accumulation of the individual polyamines is essential for plant tolerance and abiotic stress responses. The generation of transgenic plants overexpressing the genes involved in polyamine biosynthesis is promising tools to improve the stress tolerance of plants. However, examples have been reported where the polyamine levels do not correlate with stress tolerance, which also confirm that "the more the better" phrase cannot be true in all cases. Even if polyamines accumulate, this does not necessarily prove their unique involvement in direct stress protection. Polyamines should no longer be considered only as protective molecules, but rather as multifaceted compounds, which have a key role in the regulation of stress tolerance, are involved in direct interactions with other metabolic routes and hormonal cross-talk, and also activate the expression of stress-responsive genes. These mechanisms make it unnecessary or of secondary importance for polyamines to participate in direct protection. Indeed, small changes in polyamine

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concentration are need to fine tuning for the triple balance in polyamine signaling: i. balance in synthesis and catabolism. ii. balance between antioxidant function and ROS production. iii. balance with plant hormones.

So, how the polyamines act in signaling? They may have many modes of action. H_2O_2 and NO, the possible links between polyamines and stress responses, may act as two independent signaling molecules, but they are also interrelated in the polyamine-induced plant immune response, which could result in convergence. In addition, polyamines are able to influence Ca^{2+} influx independently of the H_2O_2- and/or NO-mediated pathways, and they are also reported to have a direct post-translational protein kinase activating effect. Recent gene expression studies have led to a greater understanding of the effect that polyamines and abiotic stress pathways have on each other. Nevertheless, as different polyamines may trigger differential transcriptional responses, other pathways must exist in addition to those mentioned above, where specific direct action on polyamine-responsive elements is responsible for the specific gene expression.

What, then, are the specific roles of polyamines, and which of these are really necessary? The polyamine metabolism is dynamic. The exogenous polyamines absorbed or the elevated levels of endogenous polyamines can be rapidly converted into each other in the polyamine cycle. In addition, the polyamine metabolism is also related to the synthesis of plant hormones and signaling molecules. The enzymes involved in biosynthesis and catabolism are also affected by polyamine treatment. Thus, only very complex experimental conditions, in which individual polyamine levels are influenced differently, could represent the real situation, in addition to which the effect of the treatment may be genotype-dependent. Polyamine analogs, which compete with polyamines for binding site for example on DNA,

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but metabolically stabile, do not enter the polyamine cycle, and/or inhibit PAO could be promising tools in these experiments.

The identification of polyamine-regulated downstream targets, such as polyamine-responsive elements and the corresponding transacting protein factors, and the discovery of connections between polyamines and other stress-responsive molecules have opened up new possibilities to investigate the function of individual polyamines at the transcriptional, translational and molecular levels.

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Table 1. Examples of changes in gene expression induced by gene modification or exogenous

polyamine treatment.

Overexpressed gene	Modified genes	Plant species	References
ADC2	APETALA2/ethylene-responsive factor domain family genes of AtGA20ox1, AtGA3ox1 and AtGA3ox3	Arabidopsis	[67]
ADC2	auxin, ethylene, abscisic acid and salicylic acid synthesis genes	Arabidopsis	[32]
	genes for auxin transport		
	genes for auxin-responsive proteins		
	ethylene- and abscisic acid-responsive transcriptional factor genes		
	genes for jasmonate-induced proteins		
ADC2	9-cis-epoxycarotenoid dioxygenase gene	*Lotus tenuis*	[60]
SPDS	genes of transcription factors (WRKY, MYB, B-box zinc finger proteins, DREB2B) rd29A	Arabidopsis	[11]
	gene for calmodulin-related protein		
	sucrose non-fermenting protein 1 (SNF1)-related protein kinase (SnRK) gene		
SPMS or SAMDC1	jasmonic acid and abscisic acid synthesis genes	Arabidopsis	[32]
	jasmonic acid- and salicylic acid-responsive genes		
	receptor-like kinases, mitogen-activated protein kinases		
	genes with a role in calcium regulation		
	genes for transcriptional factors		
SPMS or SAMDC1	heat shock proteins and heat shock transcription factors	Arabidopsis	[46]
SAMDC	genes for ascorbate peroxidase, manganese superoxide dismutase, and glutathione-S-transferase	tobacco	[44]
Mutant gene			
adc1 and adc2	9-cis-epoxycarotenoid dioxygenase gene abscisic acid-regulated genes	Arabidopsis	[23]
pao4	stress response proteins (e.g. DRE-binding protein, LEA protein, LEA domain-containing protein, desiccation-responsive protein 29A and universal stress protein family protein) genes involved in flavonoid and/or lignin biosynthesis, such as phenylalanine ammonia lyase 1	Arabidopsis	[42]
Exogenous polyamine			
putrescine	heat shock protein 17 gene	wheat	[31]
spermidine	photosystem II D1 protein gene	*Xanthoria parietina*	[36]
spermidine	genes involved in primary metabolism, signal transduction, hormone responses	tomato	[35]
	genes of transcription factors		
	genes involved in energy metabolism, cell wall metabolism, and photosynthesis		
spermidine	β-amylase gene	white clover	[37]
spermine	heat shock protein genes	trifoliate orange	[41]
	abscisic acid (ABA)-responsive element binding factor (ABF) gene		
	9-cis-epoxycarotenoid dioxygenase gene		

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Figure legends:

Fig. 1. Polyamine cycle. Higher polyamines, such as spermidine (SPD) and spermine (SPM) are synthesised from putrescine (PUT). However, the degradation of SPM or SPD may also lead to lower polyamines releasing hydrogen peroxide. (dcSAM: decarboxylated S-adenosyl methionine; SPDS: spermidine synthase; SPMS: spermine synthase; PAO: polyamine oxidase; $\Longrightarrow$: synthesis; $\longrightarrow$: catabolism).

Fig. 2. Schematic representation of signaling routes mediated by polyamines. (ABA: abscisic acid; GABA: γ-aminobutyric acid; PLC: phospholipase C; PLD: phospholipase D).

Fig. 3. Relationship existing between polyamines and plant hormones. (ABA: abscisic acid; BR: brassinosteroids; CK: cytokinin; ET: ethylene; SA: salicylic acid; GA: gibberellins; GABA: γ-aminobutyric acid).
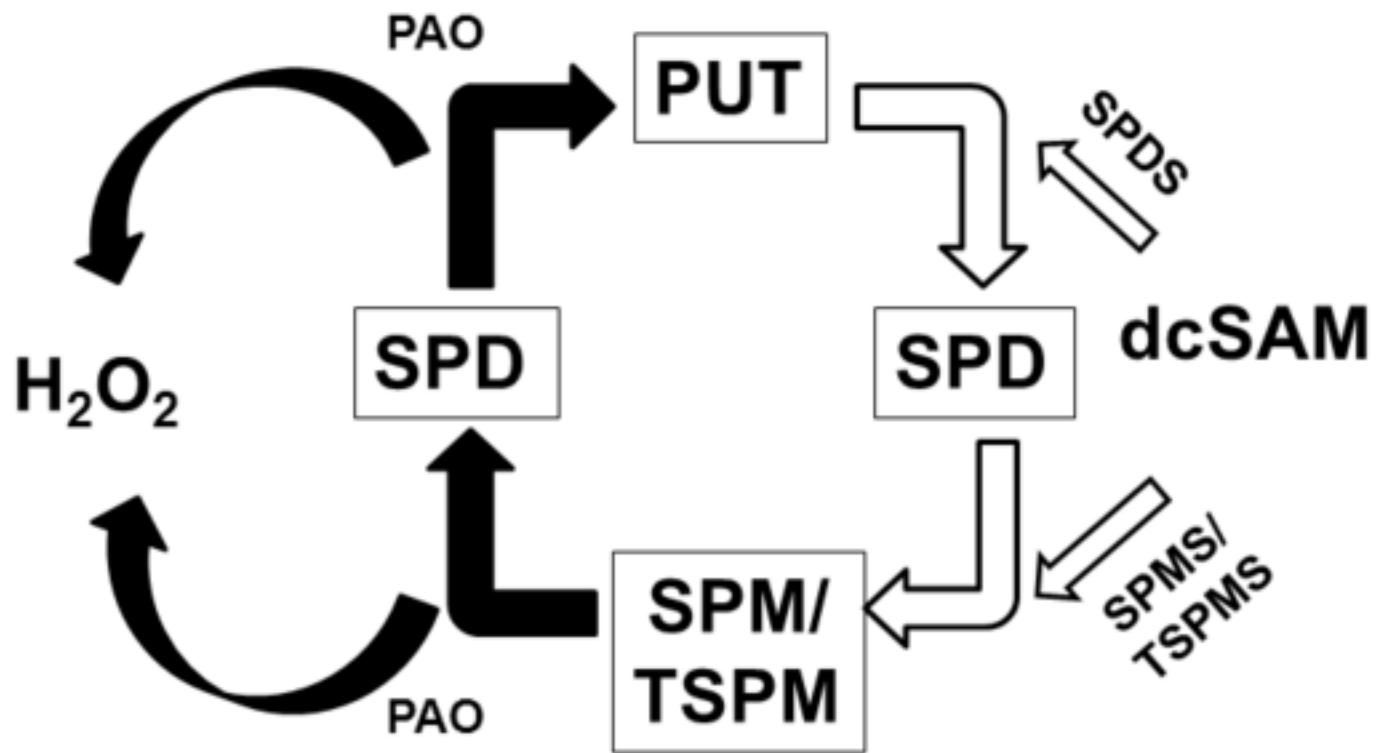
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Figure 1

Figure 2

Figure 3