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Polyamines, polyamine oxidases and nitric oxide in development, abiotic and biotic stresses

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ABSTRACT

Nitric oxide (NO), polyamines (PAs), diamine oxidases (DAO) and polyamine oxidases (PAO) play important roles in wide spectrum of physiological processes such as germination, root development, flowering and senescence and in defence responses against abiotic and biotic stress conditions. This functional overlapping suggests interaction of NO and PA in signalling cascades. Exogenous application of PAs putrescine, spermidine and spermine to *Arabidopsis* seedlings induced NO production as observed by fluorimetry and fluorescence microscopy using the NO-binding fluorophores DAF-2 and DAR-4M. The observed NO release induced by 1 mM spermine treatment in the *Arabidopsis* seedlings was very rapid without apparent lag phase. These observations pave a new insight into PA-mediated signalling and NO as a potential mediator of PA actions. When comparing the functions of NO and PA in plant development and abiotic and biotic stresses common to both signalling components it can be speculated that NO may be a link between PA-mediated stress responses filing a gap between many known physiological effects of PAs and amelioration of stresses. NO production indicated by PAs could be mediated either by H_2O_2, one reaction product of oxidation of PAs by DAO and PAO, or by unknown mechanisms involving PAs, DAO and PAO.

1. Introduction

Nitric oxide (NO) is a key signalling molecule that mediates variety of physiological functions and defence responses against abiotic and biotic stresses in plants. These functions include regulation of seed germination [1–3], root development [4,5], flowering [6,7], fruit ripening [8,9], senescence [10,11], and adaptive responses to abiotic stresses like drought, heat, chilling and high salinity [12–17] and in defence responses against biotic stresses [12,13,17–24]. NO is also known as an intermediate signalling molecule in cytokinin [25–28], abscisic acid [29,30], auxin [4,5,31], ethylene [8] and polyamine [32] signalling.

Polyamines (PAs) are low molecular weight, polycationic, nitrogenous growth regulators present ubiquitously in all living cells. The most common free PAs in plants are di-amine putrescine (put), tri-amine spermidine (spd) and tetra-amine spermine (spm) with rare accumulation of long chain PAs under special conditions [33,34]. PAs also occur as conjugated forms bound mainly to cinnamic acids. These conjugated PAs are the main phenolic components of reproductive organs [34]. PAs are required for normal growth and development of prokaryotes and eukaryotes. In plants, PAs play critical roles in a range of developmental and physiological processes such as regulation of cell proliferation, somatic embryogenesis, differentiation and morphogenesis [35,36], dormancy breaking of tubers and in seed germination, development of flowers and fruits [35–37] and senescence [38,39]. PAs are also known to enhance the tolerance to environmental stresses such as salinity, chilling, drought, potassium deficiency [34–42] and defence signalling against pathogens [43].

PA catabolism is crucial in regulating PA levels in cells. PAs are oxidatively catabolised by amine oxidases which include copper-binding diamine oxidases (CuAO)/diamine oxidases (DAO) and FAD-binding polyamine oxidases (PAO) and they are widespread in all living organisms [44,45]. In plants, diversified roles of DAO and PAO have been reported in cell growth and development [45–47], abiotic stresses such as drought and salt stress tolerance [48,49] and defence responses leading to disease resistance [45,50–52].

When considering the common functions of NO and PA in plant development and abiotic and biotic stresses it can be speculated that NO may be a link between PA-mediated stress responses. The findings on PA-induced NO biosynthesis in *Arabidopsis* seedlings provide new insight into PA-mediated signalling and NO as a potential mediator of PA actions [32]. NO biosynthesis as a result of catabolism of PA by DAO and PAO is one hypothesis to explain many functions on PA-mediated stress responses.

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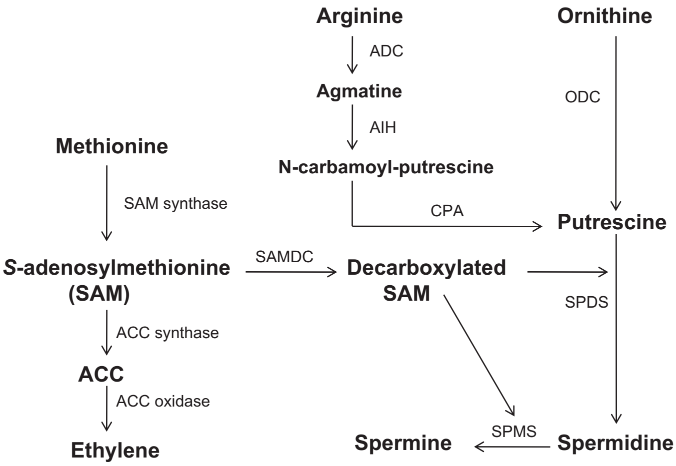

Fig. 1. Schematic representation of pathways of biosynthesis of the major plant polyamines in plants. ADC: arginine decarboxylase; AIH: agmatine iminohydrolase; CPA: *N*-carbamoylputrescine aminohydrolase; ODC: ornithine decarboxylase; SPDS: spermidine synthase; SPMS: spermine synthase; SAM synthase: *S*-adenosylmethionine synthase; SAMDC: *S*-adenosylmethionine decarboxylase; ACC synthase: 1-amino-cyclopropane-1-carboxylic-acid synthase; ACC oxidase: 1-amino-cyclopropane-1-carboxylic-acid oxidase.

2. Polyamine biosynthesis

The biosynthesis of polyamines is initiated with the formation of diamine put. Put biosynthesis in plants occurs through two distinct pathways either from decarboxylation of ornithine or arginine by ornithine decarboxylase (ODC) or arginine decarboxylase (ADC) (Fig. 1) [40,41,53]. Put formation through ADC pathway is followed by two successive steps catalyzed by agmatine iminohydrolase (AIH) and *N*-carbamoylputrescine aminohydrolase (CPA). The triamine spd and tetramine spm are formed by successive addition of aminopropyl groups to put and spd respectively in reactions catalyzed by spermidine synthase (SPDS) and spermine synthase (SPMS) [53]. The aminopropyl groups are produced from decarboxylation of *S*-adenosylmethionine (SAM) catalyzed by SAM decarboxylase (SAMDC) [53]. The ODC pathway has been detected in many plant species and genes coding for ODC have been characterized [54]. Since *Arabidopsis* lacks ODC pathway, put is produced exclusively through the ADC pathway [55]. Two genes encoding ADC, *ADC1* and *ADC2* have been identified in *Arabidopsis* and they are expressed in a tissue specific manner [56]. The expression of genes involved in polyamine biosynthesis is strongly regulated by an array of abiotic and biotic stress responses and polyamine metabolism interacts largely with other metabolic pathways [40–42,56,57].

3. Polyamine (PA)-induced NO biosynthesis

Recent experimental evidences on PA-induced NO biosynthesis proposes NO as a signalling intermediate in PA action. A fluorimetric assay using cell impermeable NO-binding dye diaminorhodamine-4 M (DAR-4M) showed that NO released into the reaction medium was significantly induced above the endogenous levels when *Arabidopsis* seedlings as well as tobacco BY-2 cells were treated with 1 mM of different PAs namely spm, spd and put [32]. The observed NO release by 1 mM spm treatment in *Arabidopsis* seedlings was very rapid without apparent lag phase [32]. This response was quenched when the seedlings were treated with 2-aminoethyl-2-thiopseudourea (AET), an inhibitor of an animal nitric oxide synthase (NOS), and by 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-1-oxy-3-oxide (cPTIO), a NO scavenger [32]. PA-induced NO production was observed in *Arabidopsis* nitrate reductase impaired double mutant *nia1nia2*, suggesting nitrate reductase is not the only contributor of PA-induced NO production (unpublished results).

When *Arabidopsis* seedlings were treated with spm, spd and put an increase in tissue specific distribution of NO-dependent fluorescence was observed as investigated by using cell-permeable NO-sensitive fluorophore diaminorhodamine-4M-acetoxymethyl (DAR-4M-AM) [32]. As compared with endogenous levels, spm and spd treatments showed enhanced NO-dependent fluorescence in the elongation zone of the root tip and primary leaves. A clear decrease in fluorescence was seen in *Arabidopsis* seedlings treated with cPTIO prior to PA treatment, indicating specificity of NO attributed fluorescence [32]. In comparison to *Arabidopsis* wild type (WT), T-DNA insertional knockouts lacking a copper amine oxidase gene *CuAO1* showed impaired PA-induced NO release [58]. Moreover, compared to PA-treated WT, knockouts of *CuAO1* showed significantly reduced NO-fluorescence in the primary root tips [58]. The altered PA response of the knockouts suggests a function of *CuAO1* in PA-induced NO production. It can be speculated that yet unknown enzyme/s or DAO and PAO are responsible for NO synthesis. Rapid induction of NO release by spm without an apparent lag phase favours the presence of an enzyme which directly converts PA to NO.

4. Nitric oxide (NO) and polyamines (PAs) in plant growth and development

Since the discovery of NO in plants, it became increasingly evident that it is an essential signalling molecule that mediates diverse growth and development processes such as seed germination, root development, flowering and senescence [1,3,6–8,10]. Like NO, PAs are also known to play key regulatory roles in many physiological processes such as organogenesis, embryogenesis, floral initiation and development, fruit development and senescence [35–39]. The level of PAs throughout a plant life cycle depends on their biosynthesis, transport, degradation and conjugation. Recent studies indicate that PAs may act as signals in intricate cross talks with hormonal pathways. PA-induced NO biosynthesis might be a potential linking signal in many of the developmental functions in plants.

4.1. Embryogenesis, germination and growth

PAs are crucial components in embryogenesis [37,38,59,60]. Effect of PA on embryoid formation in higher plants has first been identified in cell cultures of *Daucus carota* [61] and embryoid formation was shown to be reduced by chemical inhibitors of spd synthesis [62]. Exogenous application of PAs to suspension cultures of *Ocotea catharinensis*, *Araucaria angustifolia* and *Pinus taeda* altered the endogenous NO in somatic embryos suggesting PA-induced NO may play an important role in embryogenesis [63–66].

Recent experimental findings illustrate NO as one of the signalling components in promoting seed germination and breaking seed dormancy. Treatment with NO donors has shown to reduce seed dormancy in *Arabidopsis*, warm-season grasses and barley [1–3,67]. In *Arabidopsis*, rapid accumulation of NO leading to a rapid decrease in ABA enhanced the braking of seed dormancy [68]. *nia1nia2noa1-2*, a mutant impaired in nitrate reductase (NR) and nitric oxide-associated1 mediated NO biosynthetic pathway showed reduced seed germination potential [69]. Increased production of reactive oxygen species (ROS) including H_2O_2 was reported promoting seed germination in germinating radish seeds [70] and in pea seeds [71]. Expression of genes related to ROS production including amine oxidases is differentially affected