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REVIEW

Involvement of Polyamine in Floral and Fruit Development

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Abstract

Polyamines, including putrescine (Put), spermidine (Spd) and spermine (Spm), are polycationic compounds of low molecular weight that are widely distributed in living organisms. They are implicated in a variety of plant processes, such as floral and fruit development. In addition, correlation between polyamines and flower gender or fertility is reported in some plant species. Since polyamines, especially Spd and Spm share a common precursor, *S*-adenosylmethionine (SAM), with ethylene, they demonstrated competitive effects on functions in fruit development and ripening in many plants. Exogenous polyamines have been employed to regulate floral and fruit development and ripening in a lot of plants. Besides, polyamines have shown their potential for postharvest fruit management. Therefore, in this review, we focused on the involvement of polyamines in floral and fruit development along with perspectives of polyamine research.

Discipline: Horticulture
Additional key words: ethylene, postharvest, putrescine (Put), spermidine (Spd), spermine (Spm)

Introduction

Polyamines, mainly diamine putrescine (Put), triamine spermidine (Spd) and tetraamine spermine (Spm), are polycationic compounds of low molecular weight that are present in all of the living organisms. Polyamine biosynthesis in plants has been elucidated explicitly. Production of Put in plants is different from that in animals owing to the presence of the arginine decarboxylase (ADC) pathway through arginine in addition to the ornithine decarboxylase (ODC) pathway through ornithine. Spd is synthesized from Put via spermidine synthase (SPDS) with the addition of an aminopropyl moiety provided by decarboxylated *S*-adenosylmethionine (dcSAM), which is catalyzed by the enzyme of *S*-adenosylmethionine decarboxylase (SAMDC) using *S*-adenosylmethionine (SAM) as a substrate. Similarly, Spm is produced from Spd via spermine synthase (SPMS) with the same aminopropyl moiety rendered by dcSAM (Fig. 1). More detailed information on metabolism of polyamine (biosynthesis, degradation and transport) has been well documented elsewhere[44,45,89] and will not be repeated herein. The past decades have witnessed tremendous progress in polyamine research, such as successful establishment of quantification methods for polyamine titers in different tissues, isolation of genes responsible for polyamine biosynthesis, production of mutants, and genetic manipulation involving polyamine genes. Polyamines have been proposed to be a new category of plant growth regulators and are purported to be involved in a large spectrum of physiological processes, such as stress tolerance, embryogenesis, cell division, morphogenesis, and development[14,23,26,27,33,88]. The present paper will look at progress concerning the role of polyamines in reproductive growth and development, with emphasis on the interrelationship between polyamines and floral and fruit development.

Endogenous polyamine in relation to floral development

Involvement of polyamines in floral development has been reported in a wide range of crops based on sev-

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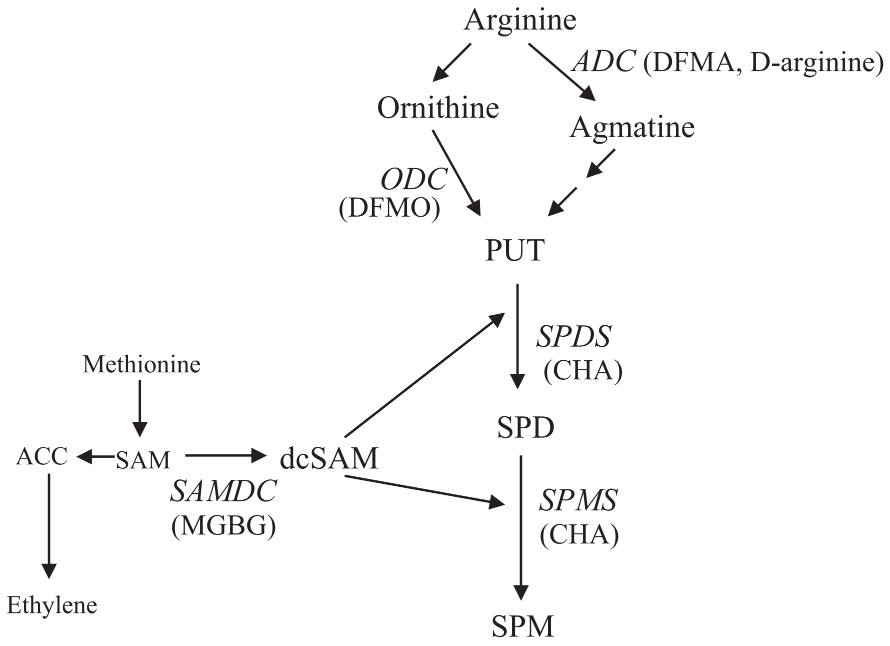

Fig. 1. Polyamine and ethylene biosynthetic pathway

Chemicals in the parentheses are specific inhibitors for the corresponding polyamine biosynthetic enzyme. ADC: Arginine decarboxylase, ODC: Ornithine decarboxylase, SAMDC: *S*-adenosylmethionine decarboxylase, SPDS: Spermidine synthase, SPMS: Spermine synthase, PUT: Putrescine, SPD: Spermidine, SPM: Spermine, ACC: 1-aminocyclopropane-1-carboxylic acid, SAM: *S*-adenosylmethionine, dcSAM: Decarboxylated SAM, DFMA: α-difluoromethylarginine, DFMO: α-difluoromethylornithine, MGBG: Methylglyoxal-*bis*(guanylhydrazone), CHA: Cyclohexylamine.

eral lines of evidence. Firstly, in some plants, an accordant change in total polyamine titers or a single polyamine type accompanies the floral development, as is revealed in *Pharbitis nil*[19], *Xanthium strumarium*[30], *Polianthes tuberosa*[31], strawberry[80], and tobacco[82]. Secondly, utilization of polyamine biosynthetic enzyme inhibitors prevented flowering, which was resumed when the inhibitors were absent or when polyamines were added exogenously. Much work has been carried out using inhibitors in order to probe the relationship between polyamines and floral development[2,3,20,34,77,82]. For instance, methylglyoxal bis(guanylhydrazone) (MGBG), an inhibitor of SAMDC, caused strong inhibition of flowering in *Spirodela punctata*, whereas the inhibitory effect was abolished by applying Spd exogenously[20]. The number of flower primodium of *Polianthes tuberosa* was significantly reduced by cyclohexylamine (CHA), a specific reversible inhibitor of SPDS/SPMS[31]. Exotic Spd can reverse the effects of the biosynthetic inhibitors, which possibly highlights the significance of Spd in the floral development[31]. In addition, the fact that use of polyamines promotes flowering of some plants under non-inductive conditions adds another dimension to the evidence[87]. Thirdly, acquisition of some lines of mutants plays an active role in identifying the implication of polyamine in floral development. So far, mutants have been obtained in several crops, such as *Arabidopsis*[2], tobacco[23,41] and *Petunia*[28]. For example, some of the tobacco mutants deficient in polyamine metabolism demonstrated aberrant morphology in anthers and ovules[41]. A mutant line, *Mgr 21*, which had incomplete and abnormal floral organs, exhibited significantly lower polyamines compared with the wild type[23]. Petunia mutants with abnormal polyamine titers demonstrated irregular development of the floral organs[28].

Apart from the implication in floral development, polyamines are related to flower genders or fertility. Flowers of different gender or of the same gender but from different species contain polyamines or their conjugates in a different manner. The male flowers have more neutral hydroxycinnamic amide than female ones, but the latter have more hydroxycinnamic amides than the former[43]. It has been shown in some work that different polyamine concentrations were found in the sterile and fertile organs. Correlation between polyamine and male sterility/fertility has been established in some plants, the sterile lines containing less polyamine than the fertile lines or the corresponding maintainer line, as is revealed in chrysanthemum[3], stem mustard[29], tobacco[42], maize[46,47], *Araceae* species[63], and tomato[65,66]. Therefore, exogenously applied polyamine can partially restore the fertility of the sterile line, whereas fertility of the maintaining line can be decreased by the inhibitor. However, Rastogi and Sawhney[65,66] reported that in tomato the male sterile mutant contained higher polyamines than normal plants and argued that the elevated levels of polyamines was possibly the reason for stamen sterility. Such discrepancy should be attributed to polyamine homeostasis in a given plant species. A threshold value is possibly present as to the polyamines required for a concerted action. When the gap between exact amount and the threshold is quite large the action will be discordant. It has been documented that an abnormal level of polyamines has effects on floral morphology[28,41]. The underlying function of polyamine for regulating fertility remains unknown and work to decipher this is scarce. Guo et al.[29] proposed that the lower level of free polyamines in cytoplasmic male sterile plants influenced cell division and enlargement, which then led to abnormal development and low viability of pollens.

Work on investigating the role of polyamine in pollen development has been carried out as well. Accumulating evidence has showed that endogenous polyamine is important for pollen germination and pollen tube growth[78,92]. Bagni et al.[6] reported that biosynthesis of polyamines took place before pollen tube emergence. MGBG reduced (at low concentration) or inhibited (at high concentration) pollen germination, which was promoted by Spd at low concentration[64].

It remains unknown in which way and in what matter polyamines affect floral development. Much more work is needed to unravel the mechanism involved. Caffaro et al.[11] proposed that polyamines might be part of a complex mechanism involved in flowering signal. An intimate connection between polyamines and floral development makes it possible to establish some physiological or biochemical markers using the individual polyamine, the ratios or the total polyamines[69,94]. High levels of Spd and Spm and low Put are associated with flowering of hazelnut trees, whereas the reverse trend is related to dormancy initiation[68]. We can monitor or regulate the floral development based on these parameters during the process.

Endogenous polyamine during fruit development and ripening

Investigation of polyamine titers during different stages of fruit development sheds light on the relationship between polyamine and fruit development[15]. In the early stage of fruit development, soon after full bloom, the polyamines are maintained at a high level, followed by decline with fruit development processing. The polyamines are normally at a low level when the fruit ripening starts. Such an evolution pattern has been reported in pumpkin[7], apple[8,9,93], pea[12], cherimoya[22], rambutan[36], peach[38], avocado[39,91], mango[40], *Phaseolus vulgaris*[56], tomato[72,81], pepper[73], grape[75], and pear[84]. In avocado, polyamines were at their maximal level during early fruit development, whereas they declined to 30% or less at full maturity[39]. The high polyamine concentrations may be related to the high growth rate[25] or active cell division[26]. In the initial stage of fruit development, active cell division occurs, which possibly needs sufficient polyamines. At the later stage of fruit development, cell division gives way to cell enlargement, in which polyamine synthesis is reduced. Decrease in polyamines at the late stage of fruit development has been regarded as a signal for fruit ripening[13]. However, during fruit maturation and ripening an increase in polyamine titers was also detected in citrus[54] and tomato[72].

Relationship between polyamine and ethylene

Ethylene, the only gaseous plant hormone, plays a crucial role in fruit ripening. Ethylene and polyamines, especially Spd and Spm, share a common precursor, SAM (Fig. 1). In one pathway, SAM evolves into ethylene through 1-aminocyclopropane-1-carboxylic acid (ACC), in the other SAM is decarboxylated into dcSAM, which serves as a donor of the aminopropyl group for production of Spd and Spm, respectively. Since they share this common precursor, it is normally accepted that they compete with each other during fruit development and ripening. Such a competitive relationship has been established in many plant species. The inverse relationship between ethylene and polyamine is primarily illustrated by their opposite synthesis patterns during fruit development and/or delay or inhibition of ethylene production by free polyamine. In long-keeping tomato fruits, less ethylene and more polyamines were produced in comparison with short-keeping ones[21]. In addition, blocking of ethylene production by an ethylene inhibitor, aminoethoxyvinylglycine (AVG), could promote polyamine production[4]. In this regard, it is proposed that they have different effects on regulation of fruit ripening, ethylene being stimulatory and polyamine inhibitory. Therefore, it is possible to regulate and control ethylene evolution by exogenous application of polyamine, which has been tried in apple[1], peach[10], litchi[32], apricot[49,55], plum[59], *Tradescantia*[79], nectarine[83], and avocado[91]. Exogenous application of polyamine delayed softening of peach[10] and apple[37] fruits on the tree as well. Suppression of ethylene synthesis by polyamines is attributed either to the suppression of protein synthesis[35], charge neutralization of membrane, conformation changes of the membrane targets leading to impaired functionality of the ethylene synthesizing system[1] or to the biased flux of SAM, the common precursor, to polyamine synthesis[24,52]. Kramer et al.[37] proposed that the effects of polyamine on delaying softening might ascribe to its role in improving rigidification of cell walls. This possibility can not be ruled out because it has been supposed that polyamine is of use for stabilizing cell walls and cell membranes[53].

Although the inverse relationship between polyamine and ethylene is present in many species, it is supposed that such a relationship is only true when the polyamine titers are high enough to affect ethylene production. Besides, it is possible that SAM is the limiting factor only when it is at a low level, which cannot satisfy the needs of the two biosynthetic processes. However, since it is reported that only 10% of total SAM is used for synthesis of ethylene and polyamine, the amount of SAM in plants should be sufficient[67,95]. Therefore, it is conceivable that the competition between both of them is not expected in some plant species. Over-expression of the yeast SAMDC gene in tomato gained more insight into the relationship between polyamine and ethylene[50]. These ripening transgenic tomato fruits showed enhanced accumulation of polyamine. Simultaneously, they produced more ethylene than the control, demonstrating that the polyamine and ethylene can function in harmony instead of mutual inhibition[50]. Similar phenomena have been observed in several other plants, such as cherimoya[22], apple[37,90], avocado[39], and eggplant[70]. In addition, exogenous supply of polyamines has been shown to stimulate, instead of inhibit, ethylene production in tobacco[57] and soybean[58]. There are two possible reasons for this unexpected phenomenon. Firstly, there is no competition, at least in some plant species, between ethylene production and polyamine biosynthesis. Secondly, exogenously applied polyamines increase the endogenous polyamines, which serve as a feedback inhibitor on the polyamine biosynthesis pathway. As a result, use of SAM in the pathway of polyamine is reduced or inhibited, leading to more contribution of SAM to ethylene production. Indeed, different tissue types and diverse experimental conditions may also account for such discrepancies.

Regulation of floral and fruit development by exogenous polyamines

Since polyamines are involved in flowering and fruit development, it is reasonable to modulate such processes by monitoring and orchestrating the polyamine biosynthesis. In this regard, approaches could be taken to promote or inhibit floral and fruit development. Promotion of flowering by exogenous polyamines has been demonstrated in *Spirodela punctata*[20], tobacco[34] and morning glory[87]. In addition, inhibition of flowering by polyamine inhibitors has been also attempted[20,80]. For instance, exogenous Spd caused more than 20% of floral buds to develop in tobacco thin-layer tissue cultures, whereas all of the buds were vegetative when the cultures were lacking in Spd, indicating the direct role of Spd in floral differentiation[34]. Application of 0.5 mM Put induced abundant flowering in morning glory, a short day plant, under continuous light condition[87]. CHA and MGBG inhibited flowering of *Spirodela punctata*, which can be reversed by Spd[20]. Tarenghi and Martin-Tanguy[80] reported that α-difluoromethylornithine (DFMO), an inhibitor of ODC, decreased the polyamine contents in strawberry and inhibited flowering, which could be restored by exogenous application of Put.

As is mentioned above, polyamines have been suggested to be associated with cell division. Therefore, they can be utilized to regulate fruit development. So far, exogenously applied polyamine has been reported to be effective for increasing fruit set and yield of apple[9,16], pear[17,18], litchi[51], and olive[71], fruit set and retention of mango[76], and improving embryo development of grape[62]. In addition, since polyamines (especially Spd and Spm) share the same precursor, SAM, with ethylene, polyamines have demonstrated their functions for delaying maturing and ripening in apricot[55] and grape[62], inhibiting abscission and reduced fruit drop[5,55], delaying flesh softening or increasing firmness in peach[10], strawberry[61] and nectarine[83]. However, it should be noted that the effect is pertinent to the polyamine type, time of application and concentration[5,10,83]. For example, Singh and Singh[76] reported that polyamines applied at full bloom caused higher fruit retention than those used before anthesis. Meanwhile, Spm was better for increasing fruit retention than Put and Spd. In apple, the effect of polyamines on increasing fruit set and yield was greater when they were applied during the first period of fruit growth than during the final stage of fruit growth[16].

The above-mentioned effects of polyamines have been ascribed to increased viability of the ovule and a prolonged pollination period[17], inhibition of enzymes involved in ripening or inhibition of ethylene synthesis[61], which may work independently or synergistically. However, there is no definite conclusion with respect to the mechanism of polyamine so far since the effects are at variance among different plant species, polyamine types and/or treatment conditions.

Use of exogenous polyamines in postharvest management

Polyamines, especially Spd and Spm, that compete with ethylene for the common substrate, SAM, make it plausible to modulate postharvest fruit development. Enormous work has demonstrated that exogenously applied polyamines have effects on fruit quality, with emphasis on fruit firmness, weight loss, ethylene evolution, soluble solutions and titratable acids. Spm or Spd treatment retarded softening of apple[37] and strawberry fruits[61]. Exogenously applied Put resulted in a reduction of mechanical damage and an increase in firmness of lemon[48], apricot[49] and plum[59]. Polyamines added exogenously reduced or delayed browning, peroxide level and ethylene production, coupled with elevated levels of polyamines in litchi fruits stored at 5ºC[32]. Plum fruits treated with 1 mM Put showed delayed and/or reduced ethylene production, together with higher fruit firmness, lower soluble solutions and titratable acids, reduced weight loss and delayed color change, leading to extended storage life[74,86]. Apricot fruits treated with Put showed significantly less weight loss relative to the untreated fruits[49]. In another report, Put treatment resulted in reduced or slow color change, ethylene emission and respiration in kiwifruit[60]. Similarly, Put applied by vacuum infiltration notably increased lemon fruit firmness and delayed the color change as compared to the control[85]. As is known, fruit softening is primarily due to breakdown of the cell wall. The above-mentioned effects of polyamines on fruit texture (fruit firmness and fruit softening) could be ascribed to their properties of inhibiting enzymes degrading pectic acids[49,85] and/or to their ability to bind to cell walls and membranes[61], leading to rigidification of cell walls and stabilized membranes. Inhibited ethylene production might also account for the enhanced firmness and delayed softening. The reasons for polyamines modifying soluble solutions and titratable acids remain unclear. However, a high concentration of polyamine may cause chemical injury to fruits, as is shown in apple. Spd or Spm higher than 1 mM led to development of small black spots in ‘Red Delicious’ and ‘McIntosh’ apples[37]. Therefore, use of polyamines at optimized concentration should be taken into consideration when they are used for postharvest management in terms of public acceptance of the treated fruits.

Concluding remarks

A lot of work on probing the relationship between polyamine and floral and/or fruit development has been carried out, which is of help for elucidating the possible mechanism of polyamine involved in these physiological processes. It has been clearly stated that polyamines are essential for cell division in the early fruit setting stage and that exogenously applied polyamine can interfere with ethylene production in fruits on the tree or after harvest. All of this will open a new avenue for employing polyamine to regulate or control flowering, fruit setting and ripening. However, some questions are still present concerning the exact part played by polyamine in the processes mentioned above. For example, it is still ambiguous with respect to the cause-and-effect relationship between polyamine titers and the physiological processes. We have the idea that at an early stage of fruit development the polyamines are at high levels, but it remains to be determined if such high levels promote cell division or it results from a physiological reaction in cell division. Similarly, the interrelationship between polyamine and ethylene is also in a dilemma since in certain species both of them function harmoniously, whereas in others they exhibit a competitive relationship. Their sharing of a common precursor is generally considered as the basis for their antagonism against each other in fruit development. However, how they compete for the substrate remains unclear if there is competition. To this end, more work is required to identify the exact role of polyamines. Utilization of mutants will help get more and deeper insight in revealing polyamine’s function in these processes. In addition, genetic transformation with sense and antisense genes of polyamine biosynthetic enzymes will provide a powerful alternative for investigating the correlation of polyamines with growth and development. We can expect that more information will be available in the near future with these sophisticated methodologies and techniques.

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