

Changes in free polyamines and gene expression during peach flower development

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Abstract

Free polyamine contents and expressions of five genes encoding for polyamine biosynthetic enzymes were investigated at four different stages during peach (*Prunus persica* L. Batsch cv. Akatsuki) flower development. Fresh mass of peach flowers increased, accompanied by reduction in contents of total polyamines and putrescine/spermidine ratio due to decrease in putrescine content. Spermidine, the largest fraction, and spermine, the least part, underwent minor change. Expressions of the five key genes involved in polyamine biosynthesis during flower development did not parallel the changes in free polyamines.

Additional key words: HPLC, *Prunus persica*, putrescine, spermidine, spermine.

Polyamines, especially spermidine (Spd), spermine (Spm) and their diamine precursor, putrescine (Put), are low molecular mass compounds that are universally distributed in living systems, such as animals, plants and microorganisms. They have been suggested to be implicated in response to biotic and abiotic stresses, fruit development, *in vitro* embryogenesis, morphogenesis and other cellular processes like RNA and DNA replication and protein synthesis (Kakkar *et al.* 1997/98, Martin-Tanguy 2001, Lin and Kao 2002, Gemperlová *et al.* 2005, Liu *et al.* 2006a,b,c, Jiménez-Bremont *et al.* 2006). It has also been proposed that polyamines are one of indispensable members of several internal components required for flower initiation and floral organ morphogenesis (Evans and Malmberg 1989, Galston *et al.* 1997). Several lines of evidence have revealed the possible correlation of polyamines with flower induction, initiation and floral organ development (Tiburcio *et al.* 1988, Applewhite *et al.* 2000, Huang *et al.* 2004). Furthermore, utilization of some special mutants adds new avenues for probing interrelationship between polyamine biosynthesis and floral development. In this

regard, understanding of the polyamine biosynthesis during flower development is of benefit for regulating and/or modifying this process through application of exogenous polyamines. However, comprehensive information with reference to free polyamine contents and gene expression during peach flower development is lacking so far. Thus, changes in polyamine biosynthesis during floral development, based on free polyamine contents and gene expression, were investigated in this study.

Flowers at four development stages, designated as initial stage (small buds), red stage (buds with red point at the top), balloon stage and full bloom stage were collected from a peach (*Prunus persica* L. Batsch) cultivar Akatsuki. At each stage more than 30 flowers were sampled. After fresh mass determination the samples were frozen in liquid nitrogen and stored at -80 °C for polyamine analysis and RNA extraction. To extract free polyamines, 0.1 g of samples was homogenized and extracted in 1 cm³ of cold 5 % perchloric acid. Free polyamines were quantified by high performance liquid chromatography (HPLC, Shimadzu,

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Abbreviations: ADC - arginine decarboxylase; DFMA - α -difluoromethylarginine; DFMO - α -difluoromethylornithine; ODC - ornithine decarboxylase; Put - putrescine; SAMDC - S-adenosylmethionine decarboxylase; Spd - spermidine; SPDS - spermidine synthase; Spm - spermine; SPMS - spermine synthase.

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Kyoto, Japan) according to the method as reported previously (Zhang *et al.* 2003). Polyamines were analyzed in triplicate. Total RNA (10 µg) that was isolated from flowers at the four stages according to the procedure described by Wan and Wilkins (1994) was electrophoresed in 1.2 % form-aldehyde denatured agarose gel. RNA blotting, prehybridization and hybridization were carried out according to Zhang *et al.* (2003). DIG-labeled probes for ADC, ODC, SAMDC, SPDS and SPMS that have been cloned from peach and designated as *pPpADC*, *pPpODC*, *pPpSAMDC*, *pPpSPDS* and *pPpSPMS*, respectively, were used herein (Liu *et al.* 2006c). Addition of antibody, membrane washing, equilibration and treatment by chemi-luminescent substrate (CSPD) were performed according to manufacturer instructions (Roche, Mannheim, Germany).

Average fresh mass of the peach flowers at initial stage, red stage, balloon stage and full bloom stage was 0.0366, 0.1244, 0.1569 and 0.2747 g, respectively. Three main free polyamines, putrescine (Put), spermidine (Spd)

and spermine (Spm) were detected at each stage (Table 1). The content of Spd was the highest at any given stage, followed by Put and Spm, respectively. Notable change was observed in Put content, whereas minor fluctuations occurred in Spd and Spm contents. Put was at its peak value at initial stage, decreased sharply at red stage, continued with a slight decrease to balloon stage, and maintained constant until full bloom stage. Variation in total polyamine content was similar to that of Put. The ratio of Put/Spd decreased from initial stage to balloon stage, followed by a negligible rise at full bloom stage. The increase in fresh mass of peach flowers was accompanied by decrease in both total polyamines and ratio of Put/Spd due to unambiguous decline in Put. It has been described that decrease in polyamines may be associated with increase in fresh mass due to cell enlargement (Biasi *et al.* 1988). A high ratio of Put/Spd has been proposed to indicate active cell division, whereas the decrease in the ratio indicated cell enlargement (Kushad *et al.* 1990). Therefore, it seems that

Table 1. Changes in contents of free polyamines [nmol g⁻¹(f.m.)] and Put/Spd ratio during peach flower development. Means ± SE, n = 3.

Developmental stages	Put	Spd	Spm	Total	Put/Spd
Initial stage	247.8 ± 35.7	256.5 ± 14.6	96.2 ± 13.0	600.5 ± 40.4	0.97
Red stage	117.1 ± 7.7	263.9 ± 3.0	71.4 ± 5.5	452.4 ± 10.9	0.44
Balloon stage	85.5 ± 5.4	252.2 ± 6.7	63.8 ± 2.5	401.5 ± 8.7	0.34
Full bloom stage	98.9 ± 21.1	237.7 ± 8.7	100.8 ± 5.5	437.4 ± 20.9	0.42

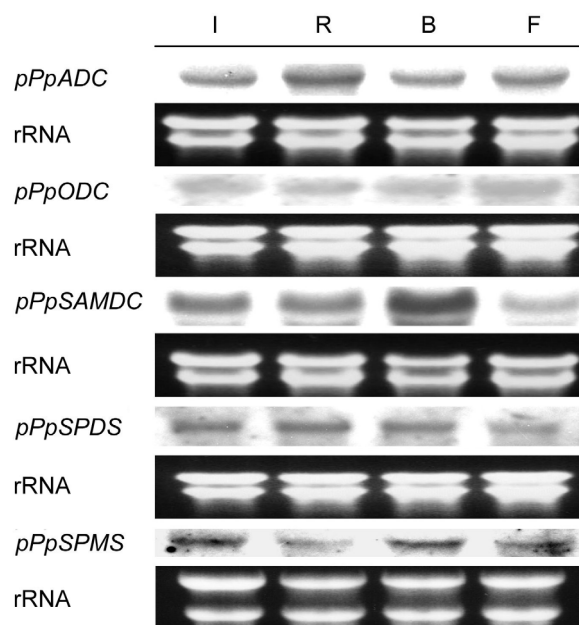


Fig. 1. Expression patterns of five key genes involved in polyamine biosynthesis at the four stages during peach flower development. I - initial stage, R - red stage, B - balloon stage, F - full bloom stage, *pPp* - partial *Prunus persica*.

reduction of Put content may be related to flower development in peach. In the future, using suicide inhibitor of Put biosynthesis, such as DFMA and DFMO, will add new dimensions for ascertaining the important role of Put in peach flower development. A slight increase in both total polyamines and the ratio of Put/Spd at full bloom stage may be related to fruit setting at early stage of fruit development (Kushad 1998).

Transcriptional levels of five polyamine biosynthetic genes, *pPpADC*, *pPpODC*, *pPpSAMDC*, *pPpSPDS* and *pPpSPMS*, were monitored at the four developmental stages of peach flowers (Fig. 1). Steady-state mRNA of *pPpADC* began to accumulate at red stage, when it reached the highest level during the development. It reduced to the lowest level at balloon stage, followed by a rise at full bloom stage. The expression level of *pPpODC* was quite low at all tested stages, especially at the balloon stage. *pPpSAMDC* expression exhibited minor change at the first two stages, showed a peak at the balloon stage, and then decreased to a very low level at the last stage. Expression of *pPpSPDS* showed a pattern similar to that of *pPpSAMDC*, but the difference in the expression level between red stage and balloon stage was less obvious in *pPpSPDS* than in *pPpSAMDC*. The expression level of *pPpSPMS* was the highest at initial stage and balloon

stage in comparison to other stages, and the lowest level was detected at red stage. It seems that expression of the five genes did not parallel the change in the free polyamine contents. Inconsistency in polyamine contents and gene expression has been reported in many cases (Zhang *et al.* 2003), which should be explained by the regulation of polyamines at the post-transcriptional and/or translational levels, as delineated elsewhere (Hummel *et al.* 2004). In addition, polyamine conjugation and/or oxidation may as well be responsible for the inconsistency. Conjugated polyamines have been shown to play a key role in floral development (Martin-Tanguy 1997) and are of particular significance for regulation of polyamine concentration (Bagni and Tassoni 2001). Previous work demonstrated that ornithine decarboxylase

(ODC) and arginine decarboxylase (ADC) are two important polyamine biosynthetic enzymes for plant development (Smith 1985). However, their relative contribution to Put synthesis is still poorly understood, which is affected by the type of tissues and specific developmental stages (Aribaud and Martin-Tanguy 1994). In the present research, the expression level of ADC gene was always higher than that of ODC gene during peach flower development. This compels us to consider that ADC gene may be more active during peach flower development, at least in the cultivar used in this study. However, extra work is needed to investigate the ADC and ODC enzyme activities to validate this hypothesis.

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