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SHORT COMMUNICATIONS

Xylem and Phloem Derived Polyamines during Flowering in Two Diverse Rose Species

Shweta Sood and Pramod Kumar Nagar

Division of Biotechnology, Institute of Himalayan Bioresource Technology, Palampur, 176 061 HP, India

ABSTRACT

Polyamine contents in xylem (root) and phloem (leaf) exudates in two diverse species of rose, viz. *Rosa damascena* Mill and *Rosa bourboniana* Desport, were analyzed before, during, and after flowering in the main flowering season, that is, April–May. Only free putrescine (Put) was detected in the xylem and phloem exudates at these time points, and it was high during the peak flowering period. In phloem, Put content was significantly higher in *R. bourboniana* than in *R. damascena* at all three stages; whereas in the xylem exudate it was relatively higher in *R. damascena* at the peak flowering period. A spray of α-difluoromethylornithine (DFMO), an irreversible inhibitor of the putrescine biosynthetic inhibitor ornithine decarboxylase (ODC), markedly decreased the flowering. This effect was reversed by application of Put alone or in combination with DFMO. The significance of this finding is discussed in light of polyamine translocation during flowering.

Key words: Flower development; Phloem; Polyamines; *Rosa bourboniana*; *Rosa damascena*; Translocation; Xylem.

INTRODUCTION

Polyamines (PAs) are low molecular weight cationic molecules implicated in various physiological and developmental processes in plants, animals, and microorganisms. In plants, di-amine putrescine (Put), tri-amine spermidine (Spd), and tetra-amine spermine (Spm) are frequently present in amounts varying from micromolar to more than millimolar (Kumar and others 1997). The various plant growth and developmental processes affected by PAs include stimulation of cell division, response to environmental stress, and regulation of rhizogenesis, embryogenesis, fruit and flower development (Galston and others 1997; Kakkar and others, 2000). In recent years new insights into the role of PAs in different plant developmental processes using transgenic approaches have also become available (Kakkar and Sawhney 2002).

The participation of PAs in the flowering process has been strongly suggested by certain studies

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*IHBT Communication: 0354
**Corresponding author; e-mail:* nagar_pk2001@yahoo.com

(Caffaro and Vicente 1995; Malmberg and McIndoo 1983), and supporting evidence in the control of floral transition has come from the use of inhibitors of Put, Spd, and Spm synthesis (Havelange and others 1996). Polyamines are translocated in the plant from the roots to the upper parts and vice-versa, leading to the hypothesis (Caffaro and others 1993) that long-distance translocation of these organic polycations may be both basipetal and acropetal. In young and adult plants of potato, basipetal transport of radioactivity was observed after feeding labeled PAs to cotyledons or mature leaves, respectively (Beraud and others 1992). This indicates that phloem sieve tubes are directly involved in the basipetal translocation of PAs. This translocation from leaves to axillary and apical buds in soybean (Caffaro and others 1994) has been considered part of the complex mechanism in flower signaling and the transition from vegetative to flowering buds.

Rosa damascena Mill, commonly known as scented rose, is an important essential oil yielding crop and the highly valued rose oil (0.045%) obtained from flowers after distillation is used in the perfume and cosmetic industries. This species flowers only once a year, that is, in April–May, and the flowering period lasts only around 25–30 days. *Rosa bourboniana* Desport, also an essential oil (0.015%) yielding crop, blooms three times a year (April–May, July-August, and October), but April–May is the main flowering period. In our previous studies (Sood and Nagar 2004), alterations in the levels of endogenous polyamines were studied in the petals of these two rose species during flower development in the main flowering season. Because PAs are known to be translocated in both xylem and phloem (Friedman and others 1986), we studied the polyamine levels in xylem and phloem during the main flowering season in both these diverse rose species.

Materials and Methods

Four-year-old bushes (10 each) of *R. damascena* (var. Himroz) and *R. bourboniana* growing in the Institute's experimental farm at Chandpur, Palampur (1290 m asl 32.6'N and 78.18'E) were used for the study. Cultural practices like pruning, thinning, and fertilization were performed according to standard methods. The study was conducted during the main flowering season (April–May) at three different time points: 20 days before flowering, during the peak flowering period and 20 days after flowering.

Xylem sap (root exudate) was collected under a mild vacuum during a 4 h period as described elsewhere (Lejeune and others 1988). In summary, exudate from 10 plants was obtained by fitting a 5 ml disposable syringe with silicon rubber tubing on the cut end of plants. The exudates were pooled and stored at −20°C until analysis.

The ethylene diamine tetraacetic acid (EDTA) method of King and Zeevaart (1974) modified by Lejeune and others (1988) was used for collection of phloem sap (leaf exudate). In brief, 20 adult leaves (the first 5 leaves below the half expanded leaf) of 10 plants were placed together in a 250 ml beaker containing 20 ml of 20 mM EDTA (pH 7.5) after the petioles were recut under distilled water. This was enclosed in an airtight vessel containing water to ensure maximum relative humidity and to prevent EDTA uptake by the leaves. The leaves were maintained in the EDTA solution for 8 h. From the leaf exudates, EDTA was eliminated by drying under a vacuum and redissolving in 1 ml of water followed by 20 ml of methanol. The EDTA that precipitated out in methanol was removed by centrifugation (8900 × *g*, 10 min, 5°C). Methanol was evaporated under a vacuum and the extract was suspended in 1 ml of distilled water and stored at −20°C until analysis.

Polyamines were extracted from leaf and root exudates with 10% chilled perchloric acid (PCA) by direct addition to each sample. Extraction and benzoylation of PAs were performed using a modification of the procedure described earlier (Sood and Nagar 2004). In brief, after the addition of PCA, the samples were vortexed, stored overnight at 4°C, and then centrifuged at 8000 × *g* for 30 min. The supernatant was the source of free polyamines as well as PCA-soluble polyamines conjugated to hydroxycinnamic acid. The pellet contained PCA-insoluble polyamines bound to macromolecules like proteins and nucleic acids. The PAs in the conjugated and bound fractions were released by acid (HCl) hydrolysis as described elsewhere (Sood and Nagar 2004). The original supernatant, the HCl hydrolyzed supernatant, and the HCl hydrolyzed pellet were separately treated with insoluble polyvinylpolypyrrolidone (PVPP) (50 mg ml^{-1}) and stirred for 1 h to remove phenolics and other interfering compounds. These were then filtered, and 0.5–0.6 ml of HCI (0.01 N) was used to clean the vials. Polyamines recovered from the hydrolyzed and non-hydrolyzed supernatant and from the pellet suspension were then benzoylated as described (Sood and Nagar 2004).

Reverse phase high performance liquid chromatography (HPLC) was carried out essentially as described previously (Sood and Nagar 2004) using a Lichrosorb RP−18 (5 μm) column (250 mm × 4 mm

Table 1. Putrescine Titer (free) in Leaf Exudates of *R. damascena* and *R. bourboniana*

	Putrescine n moles/ml leaf exudates		
Period	*Rosa damascena*	*Rosa bourboniana*	Mean
20 days before flowering	0.102^b	0.466^b	0.284^b
Peak flowering period	0.467^a	0.687^a	0.578^a
20 days after flowering	0.064^b	0.404^b	0.234^b
Mean	0.211^b	0.520^a	
CD ($p < 0.05$)			
Species (S)	0.042		
Period (P)	0.051		
S × P	0.073		

No conjugated putrescine was detected. Values are means of three replicates.
[a,b]Different letters on values show significant differences at $p < 0.05$.

i.d.) with a linear gradient of methanol:water at 22°C at a flow rate of 1 ml/min with UV detector (996 PDA detector) at 254 nm. Under these conditions, retention times for standard Put, Spd, and Spm were 4.26 ± 0.031, 6.237 ± 0.040, and 8.735 ± 0.079, respectively. The concentrations of PAs in the eluates were calculated from standard curve responses of the known polyamines (Put, Spd, and Spm, Sigma Chemical Co., st.Louis, Mo), which were benzoylated as described above.

α-difluoromethylornithine (DFMO) (5 mM) was dissolved in water with Tween −20 and sprayed on adaxial and abaxial surfaces of the leaves (50 ml per plant) 30 days before flowering. Putrescine (5 mM) was also sprayed like DFMO as an aqueous solution (50 ml per plant).

Statistical analysis of all the data was performed by two factor factorial (arrangement in) with three replications according to Gomez and Gomez (1984).

Results and Discussion

Free Put was the only polyamine in leaf exudate, whereas soluble conjugates bound to macromolecules could not be detected at all (Table 1). Similarly, neither Spd, Spm nor their conjugates could be detected in any of the PA fractions. In both the species, the Put level was significantly low 20 days before flowering but significantly high during the peak flowering period, with higher amounts in *R. bourboniana*. Twenty days after flowering the Put level was again very low and not significantly different than at 20 days before flowering. Interactions between species and periods showed that *R. bourboniana* had significantly higher Put content in leaf exudates during the peak flowering period compared to the rest of the periods (Table 1).

Just as in phloem sap, free Put was the only polyamine detected in xylem sap (Table 2), and its level was significantly lower 20 days after flowering compared to other periods in both species. The free put content increased during the peak flowering period and *R. damascena* showed higher levels than *R. bourboniana*. However, the interactions between species and periods showed a significantly higher Put content in *R. damascena* at the peak flowering period (Table 2).

DFMO is an irreversible inhibitor of putrescine synthesis mediated by ODC. When it was sprayed on leaves 30 days before flowering at three different concentrations (1, 5, and 10 mM), it inhibited flowering, with the most pronounced effect at 5 mM in both species; hence this concentration was used in the experiment. Furthermore, none of the concentrations used affected the growth or morphology of the plants. Attempts to reverse the inhibitory effect of DFMO on flowering by application of Put (5 mM) alone and together showed that it reversed the inhibition of flowering caused by DFMO (Figure 1).

Flower development is very sensitive to metabolic perturbations. It is possible that PAs have a specific role in this development because gradients of PAs are involved in determining the developmental fate of floral meristems. Malmberg and McIndoo (1983) were the first to suggest a role for PAs in flowering with abnormal flower development in a tobacco mutant with elevated PA levels. The present study and some earlier observations (Antognoni and others 1998) demonstrate that PAs can be transported by both xylem and phloem. Only free polyamines, especially, Put in its free form, were detected in both phloem and xylem sap. Put content was significantly higher in leaf exudates than in root exudates during the peak

Table 2. Putrescine Titer (free) in Root Exudates of *R. damascena* and *R. bourboniana*

	Putrescine n moles/ml root exudates		
Period	*Rosa damascena*	*Rosa bourboniana*	Mean
20 days before flowering	0.231^{b}	0.211^{b}	0.222^{b}
Peak flowering period	0.371^{a}	0.286^{a}	0.329^{a}
20 days after flowering	0.126^{c}	0.139^{c}	0.132^{c}
Mean	0.243^{a}	0.212^{b}	
CD ($p < 0.05$)			
Species (S)	0.023		
Period (P)	0.029		
S × P	0.040		

No conjugated putrescine was detected. Values are means of three replicates.
[a,b,c]Different letters upon values show significant differences at $p < 0.05$.

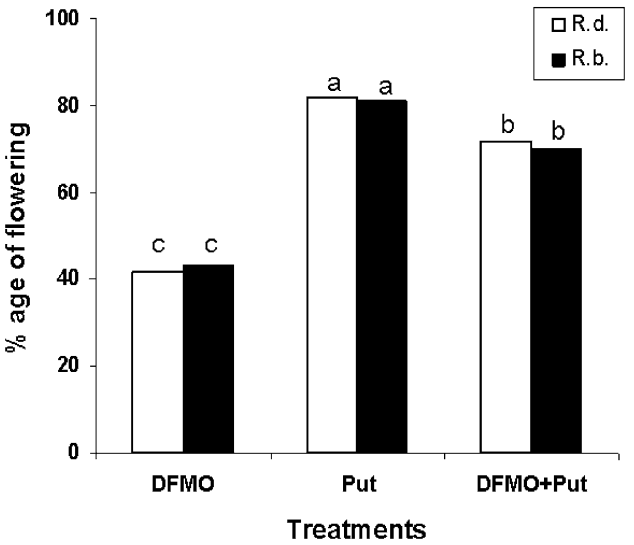

Figure 1. Effect of DFMO (5mM) and putrescine (5mM) alone and when combined on the flowering of *Rosa damascena* (R d.) and *Rosa bourboniana* (R b.). Different letters upon the bar show significant differences at $p < 0.05$.

flowering periods in both species studied (Tables 1 and 2). Because only free PAs (Put) were translocated in the present study, it supports the idea that polyamines conjugated to cinnamic acids are sequestered in the vacuoles and, therefore, are probably unable to enter the cytosolic fluid of the sieve tubes (Antognoni and others 1998). The presence of PAs in the xylem sap implies that they can be synthesized in the root system (Dumortier and others 1983) and exported to the shoots. This is not surprising in view of the extensive information on the abundance of inorganic and organic nitrogenous compounds present in xylem and phloem sap (Corbesier and others 2001). The content of Put in both the exudates varied considerably (Tables 1 and 2), suggesting that the physiological condition of the root system (where PAs can be synthesized) and of the shoot (where they are translocated and synthesized) affects the formation and partitioning of PAs. This consideration is well established for other nitrogenous constituents (Pate 1980) and may also apply to PAs, which are synthesized via the arginine-ornithine-citrulline cycle, with the incorporation of nitrogen from glutamate (Slocum and Flores 1991).

Free Put levels were significantly higher in xylem and phloem exudates during the peak flowering period, suggesting that Put may participate in the flowering process. In the phloem exudates Put content was significantly higher during this period in *R. bourboniana* than *R. damascena*, but in root exudates the opposite situation was observed. The participation of Put in controling the flowering process has been strongly suggested in other studies (Aribaud and Martin-Tanguy 1994; Caffaro and Vicente 1995). In *Sinapsis alba*, the Put titer did not increase appreciably in the root exudates (Havelange and others 1996), and one application of DFMO suppressed the early activities of both mitotic and DNA synthetic indices in the meristem. Higher Put content in both xylem and phloem during the peak flowering period and decrease thereafter (Tables 1 and 2), indicates that in both species the period during which Put is needed for flowering is short. Inhibition of flowering by spraying DFMO signifies that Put may qualify as a component of flowering, and because the inhibitory effect of DFMO was substantially reversed by an application of Put (Figure 1) suggests that Put is necessary for flowering (Wada and others 1994). Furthermore, different organs have different rates of PA synthesis, as well as a different PA content; therefore, PA

transport under physiological conditions may be necessary to regulate endogenous PA content and / or its activity.

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REFERENCES

Antognoni F, Fornale S, Grimma C, Komor E, Bagni N. 1998. Long distance translocation of polyamines in phloem and xylem of *Ricinus communis* L. plants. Planta 204:520–527.

Aribaud M, Martin-Tanguy J. 1994. Polyamine metabolism, floral initiation and floral development in chrysanthemum (Chrysanthemum morifolium R.). Plant Growth Regul 15:23–31.

Beraud J, Braun A, Feray A, Hourmant A, Pernot M. 1992. Long distance transport of 14 C putrescine in potato plantlets (Solanum tuberosum cv. Bintje). Biochem Physiol Pflanzen 188:169–176.

Caffaro SV, Antognoni F, Scaramagli S, Bagni N. 1994. Polyamine translocation following photoperiodic flowering induction in soybean. Physiol Plant 91:251–256.

Caffaro SV, Scaramagli S, Antognoni F, Bagni N. 1993. Polyamine content and translocation in soyabean plants. J Plant Physiol 41:563–568.

Caffaro SV, Vicente C. 1995. Early changes in the content of leaf polyamines during the photoperiodic flowering induction in soybean. J Plant Physiol 145:756–758.

Corbesier L, Havelange A, Lejeune P, Bernier G, Perilleux C. 2001. N content of phloem and xylem exudates during the transition to flowering in *Sinapis alba* and *Arabidopsis thaliana*. Plant Cell Environ 24:367–375.

Dumortier FM, Flores HE, Shekhawat NS, Galston AW. 1983. Gradients of polyamines and their biosynthetic enzymes in coleoptiles and roots of corn. Plant Physiol 72:915–918.

Friedman R, Levin N, Altman A. 1986. Presence and identification of polyamines in xylem and phloem exudates of plants. Plant Physiol 82:1154–1157.

Galston AW, Kaur-Sawhney R, Altabella T, Tiburcio AF. 1997. Plant polyamines in reproductive activity and response to abiotic stress. Bot Acta 110:198–207.

Gomez KA, Gomez AA. 1984. *Statistical procedures for agricultural research* New York: John Wiley and Sons. p 680.

Havelange A, Lejeune P, Bernier G, Kaur-Sawhney R, Galston AW. 1996. Putrescine export from leaves in relation to floral transition in *Sinapis alba*. Physiol Plant 96:59–65.

Kakkar RK, Nagar PK, Ahuja PS, Rai VK. 2000. Polyamines and plant morphogenesis. Biol Plant 43:1–11.

Kakkar RK, Sawhney VK. 2002. Polyamine research in plants—a changing perspective. Physiol Plant 116:281–292.

King RW, Zeevaart JAD. 1974. Enhancement of phloem exudation from cut petioles by cnelating agents. Plant Physiol 53:96–103.

Kumar A, Altabella T, Taylor MA, Tiburcio F. 1997. Recent advances in polyamine research. Trends Plant Sci 2:124–130.

Lejeune P, Kinet JM, Bernier G. 1988. Cytokinin fluxes during floral induction in the long day plant *Sinapis alba* L. Plant Physiol 86:1095–1098.

Malmberg L, McIndoo J. 1983. Abnormal floral development of a tobacco mutant with elevated polyamine levels. Nature 305:623–625.

Pate JS. 1980. Transport and partitioning of nitrogenous solutes. Annu Rev Plant Physiol 31:313–340.

Slocum RD, Flores HE. 1991. *Biochemistry and physiology of polyamines in plants* Boca Raton, FL: CRC Press. p 264.

Sood S, Nagar PK. 2004. Changes in endogenous polyamines during flower development in two diverse species of rose. Plant Growth Regul 44:117–123.

Wada N, Shinozaki M, Iwamura H. 1994. Flower induction by polyamines and related compounds in seedlings of Morning Glory (*Pharbitis nil*, cv. Kidachi). Plant Cell Physiol 35:469–472.