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Profile of Polyamines During Sprouting and Growth of Saffron (*Crocus sativus* L.) Corms

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Abstract. The profile of free and conjugated polyamines putrescine, spermidine, and spermine was studied at the onset of sprouting and during various stages of vegetative growth in saffron (*Crocus sativus* L.) corms. Polyamines were extracted from the shoot meristems and estimated by high performance liquid chromatography. Free putrescine was not detected at the onset of sprouting, whereas free spermidine and spermine levels increased rapidly on sprouting and decreased during further stages of corm development. The levels of conjugated polyamines were several times higher than the free forms indicating their possible role in the developmental processes. A comparison of polyamine levels of vegetative and floral corms showed higher titers of free polyamines in vegetative and conjugated polyamines in floral corms.

The polyamines, putrescine (Put), spermidine (Spd), and spermine (Spm), have been associated with a variety of growth and developmental processes in higher plants (Evans and Malmberg 1989) and their endogenous levels are known to be indicators of such processes. These amines occur in free and conjugated forms (Torrigiani et al. 1987). Rapidly growing cells in plants are shown to be very rich in polyamines (Galston et al. 1990; Mikitzel and Knowles 1989). Increased polyamine titers have been noticed during sprouting in potato tubers (Kaur-Sawhney et al. 1982) and Jerusalem artichoke (Bagni and Serafini-Fracassini 1981). It is also known that polyamine titers increase during seed germination (Huang and Villanueva 1992; Gallardo et al. 1992), root and shoot formation (Chriqui et al. 1986), and in fruit development (Saftner and Baldi 1990). Amide conjugates of polyamines are known to be associated with the physiology of flowering, alkaloid biosynthesis, and response of the plant to viral infection (Slocum and Galston 1985). Recently, the morphogenetic role of polyamines has been demonstrated in in vitro potato tuber formation (Protacio and Flores 1992).

The highly priced spice, saffron is the dried stigma of *Crocus sativus* L. In India, its cultivation is restricted to the state of Jammu and Kashmir. It flowers during the months of October–November, and the corms remain photosynthetically active till March, enter a dormant phase, and remain so till July when they sprout again.

The objective of the present study was to examine the endogenous levels of free and conjugated polyamines at different stages of growth of saffron corms and compare the polyamine profiles of vegetative and florally comitted corms. This could possibly provide information for the type and quantity of polyamines to be used in vitro to exercise control over morphogenetic events.

Materials and Methods

Chemicals

Putrescine, spermidine, and spermine were obtained as their hydrochlorides from Sigma Chemical Co., and 1 mM stocks in 0.01 *N* HCl were standard solutions used for HPLC. All other chemicals were of analytical grade and solvents of HPLC grade.

Plant Material

Dormant corms of *C. sativus* were obtained from the Kishtwar region of Jammu (India) during the month of July for the first experiment involving sprouting and vegetative growth. To compare the differences in polyamine profiles of floral and vegetative corms, these were brought again during the month of September. The dormant corms brought from Kishtwar were planted in plas-

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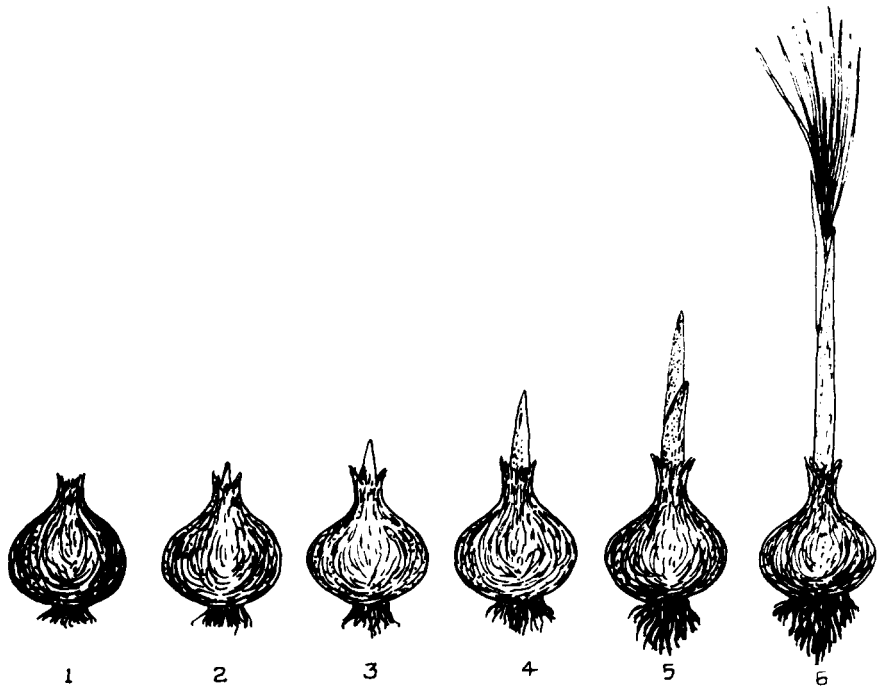

Fig. 1. Different stages of growth of saffron corms. **(Stage 1)** Dormant corm with sheath. **(Stage 2)** Initial sprouting with growing shoot tip. **(Stage 3)** Progressive sprouting. **(Stage 4)** Initial appearance of leaves. **(Stages 5 and 6)** Further growth of corms with profuse rooting.

tic pots (14 cm dia.) in a sand–soil mixture and maintained at 15°C under continuous light of 2000 lux. The vegetative and floral buds of the fresh corms were used for all analyses.

Extraction and Analysis of Polyamines

The polyamines were extracted by homogenizing 1 g (between six and 12 buds) of tissue in 10 ml of ice-cold 5% perchloric acid (PCA) in a prechilled mortar. The homogenates were placed in an ice bath for 1 h and centrifuged at 26,000 *g* for 15 min (Flores and Galston 1982). The supernatant (PCA soluble) fraction was used for free polyamine analysis. Conjugated polyamine levels were determined in both the PCA-soluble and PCA-insoluble fractions. Each experiment was repeated twice using three replicates for each stage of growth.

Acid hydrolysis of the PCA-soluble (1 ml) and PCA-insoluble extracts (500 mg) was performed in sealed glass vials with 11 *N* HCl at 110°C for 16 h. The hydrolysates were dried under vacuum in a water bath at 60°C, suspended in a known volume of 5% PCA, and subjected to HPLC.

High Performance Liquid Chromatography

Analysis of polyamines by benzoylation was performed by the method of Flores and Galston (1982).

Benzoylation

We mixed 50 µl of the standards and 500 µl of the sample extract with 1 ml of 2 *N* NaOH and 10 µl of benzoyl chloride, vortexed the solutions for 10 s, and incubated them at room temperature for 20 min. Then we added 2 ml of saturated NaCl. Benzoylated polyamines were extracted in 2 ml of diethyl ether and centrifuged at 5000 *g* for 10 min; 1 ml of the ether fraction was collected and evaporated to dryness. This was dissolved in 100 µl of methanol, and 5–10 µl were injected onto the HPLC column.

The benzoylated polyamines were separated on a $C_{18}\mu$ bondapak column (Waters, 10 µm) using an isocratic system of 60% methanol/water at a flow rate of 1 ml/min. Detection was at 254 nm. Each sample was replicated thrice for HPLC, and the values represent an average of the three. Values were expressed in nmol or µmol g^{-1} fresh wt. of tissue.

Results and Discussion

Polyamine Levels During Sprouting and Vegetative Growth of Corms

Free Polyamines. The stages in the ontogeny of the vegetative corms are presented diagrammatically in Fig. 1. All the three polyamines (Put, Spd, and Spm) were detected in dormant corms (Fig. 2). Putrescine was detected only in the dormant corms, whereas spermidine and spermine levels reached their peak values at stage 2 coinciding with the sprouting of corms. The increase in Spd content at stage 2 was about three- to fourfold and that of Spm was about fourfold the initial values at stage 1 (400–500 nmol/g FW tissue). There was a gradual decline in the levels of Spm and Spd during the subsequent stages of corm growth. Spm titers were higher than those of Spd during the first four stages of growth following at which point they declined. (Fig. 2). In Jerusalem artichoke (Bagni and Serafini-Fracassini

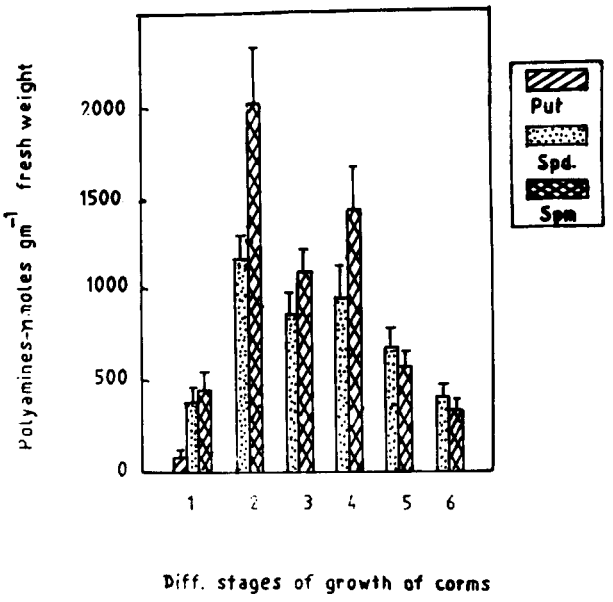

Fig. 2. Levels of free polyamines during sprouting and vegetative growth of saffron corms. Data were obtained from two independent experiments and represent mean values of six replicates. Vertical bars are ±SE.

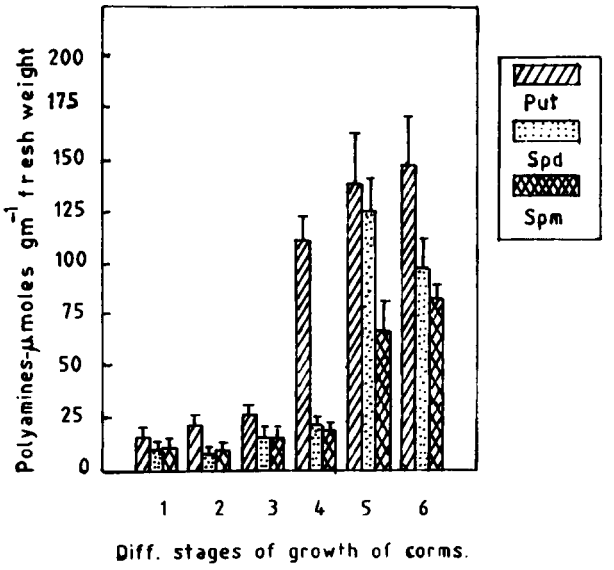

Fig. 3. Levels of total conjugated polyamines during sprouting and vegetative growth of saffron corms. Data were obtained from two independent experiments and represent mean values of six replicates. Vertical bars are ±SE.

1981) and potato tubers (Kaur-Sawhney et al. 1982), too, a sharp rise in polyamine levels was observed at the onset of sprouting. Contrary to other reports however, a complete disappearance of Put was observed after sprouting which could be due to its efficient conversion to Spd and Spm coinciding with the onset of sprouting. Also, Spd and Spm are known to be more involved in growth processes than their precursor Put (Slocum et al. 1985).

Conjugated Polyamines. In plants, two types of conjugated polyamines are considered PCA-soluble and PCA-insoluble ones (Martin-Tanguy 1985, Serafini-Fracassini and Mossetti 1985). In saffron corms, the conjugated polyamine titers (Figs. 2 and 3) were several fold higher than those of the free polyamines assayed during progressive stages of sprouting and growth. The levels of Put conjugates were the highest and those of Spm lowest. It has been speculated that the cellular levels of free polyamines are regulated through reversible conjugate formation (Slocum et al. 1985). The accumulation of these conjugates during progressive stages 1 to 6 when there is profuse rooting and sprouting of shoots indicates their possible role in these morphological events.

A comparison of the polyamine profiles of vegetative and floral corms showed marginally higher titers of free polyamines in the former (Fig. 4) and

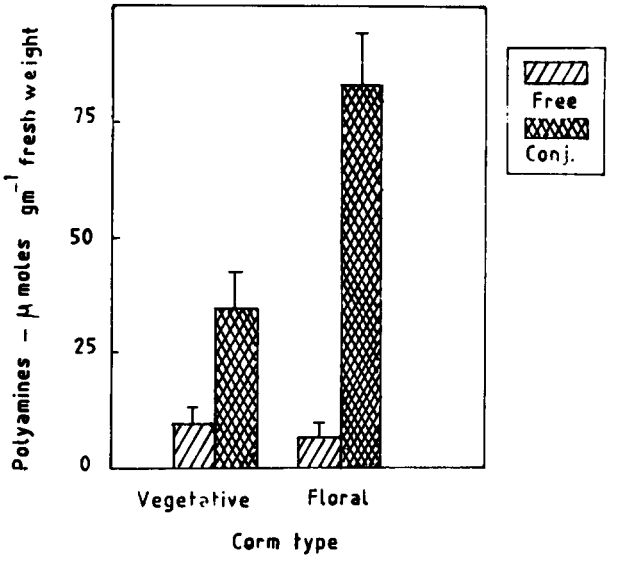

Fig. 4. Levels of free and conjugated polyamines in vegetative and floral corms of saffron. Data were obtained from two independent experiments and represent mean values of six replicates. Vertical bars are ±SE.

the levels of soluble and insoluble conjugates in the latter. This implies that the requirement of free endogenous polyamines may be higher for the vegetative phase of growth and that polyamine conjugates accumulate at the shoot meristems at the time of flowering. Similar results have been obtained in

tobacco (Smith 1985). Further work on the possible effects of exogenously applied polyamines on sprouting of dormant corms is in progress.

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