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Putrescine and Silver Nitrate Influences Shoot Multiplication, *In Vitro* Flowering and Endogenous Titers of Polyamines in *Cichorium intybus* L. cv. Lucknow Local

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ABSTRACT

The influence of putrescine (Put) and $AgNO_3$ on shoot multiplication, *in vitro* flowering and endogenous titers of polyamines in *Cichorium intybus* L. cv. Lucknow local was investigated. Exogenous administration of Put at a concentration of 40 mM resulted in maximum tissue response in terms of shoot numbers (34.6 ± 2.61) and shoot lengths (7.6 ± 0.57 cm) on MS media supplemented with 2-iP (2.0 mg L^{-1}) and GA_3 (0.5 mg L^{-1}) as observed on the 35th day. Exogenous application of 40 µM $AgNO_3$ resulted in maximum shoot number (36.8 ± 2.63) and shoot lengths (7.9 ± 0.76 cm) on day 35 on the same media. Endogenous titers of conjugated spermidine decreased sharply from day 7–21, whereas endogenous conjugated spermine levels peaked on day 28 (1265 ± 94.9 nmoles g^{-1} FW), after treatment with 40 mM Put. Whereas, $AgNO_3$ (40 µM) fed samples resulted in higher titers of endogenous conjugated spermine (1405 ± 105.6 nmoles g^{-1} FW, 3.62 fold over control) on day 14. All other treatments showed decreasing endogenous levels during the whole culture period. Both Put (40 mM) and $AgNO_3$ (40 µM) resulted in floral initiation and floral development on day 28 and 14 (3.76 ± 0.16, 4.2 ± 0.21 flowers per shoot apices), respectively. To investigate the role of Put (40 mM) and $AgNO_3$ (40 µM) on morphogenetic response and endogenous conjugated polyamine titers in shoots of chicory, polyamine inhibitors (DFMA and DFMO) were used. The morphogenetic response and the endogenous conjugated pool of polyamines were diminished in DFMA and DFMO treatments, but could be restored by addition of Put (40 mM) and $AgNO_3$ (40 µM). Under exogenous Put feeding, ethylene production was reduced in shoot cultures of chicory. This study shows for the first time the influence of polyamines on multiple shoot initiation from axillary buds of *C. intybus* L. cv. Lucknow local and also indicates the promotive effect of Put and $AgNO_3$ on autoregulation of polyamine biosynthesis, thereby regulating *in vitro* flowering, the endogenous pool of polyamines and shoot multiplication.

Key words: PAs; Putrescine; Spermidine; Spermine; *In vitro* flowering; Shoot multiplication

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Introduction

Polyamines (PAs) play an important role in cell growth and division in both prokaryotes and eukaryotes (Evans and Malmberg 1989). In PA biosynthesis, ornithine and arginine form putrescine (Put) via ornithine decarboxylase and arginine decarboxylase. Put is used as a precursor, together with the aminopropyl moeity derived from S-adenosyl methionine (SAM) after decarboxylation to synthesize spermidine (Spd) and spermine (Spm) (Evans and Malmberg 1989; Smith 1990). In plants the diamine Put, triamine Spd, and tetramine Spm are always present in amounts varying from micromolar to more than millimolar (Galston and Kaur-Sawhney 1990). Changes in PAs have been related to environmental stresses (Flores 1990) and various physiologic processes including rhizogenesis (Jarvis and others 1985) and development of flowers (Gerats and others 1988). Several studies have indicated that PAs may be important for cell growth and somatic embryogenesis of *Daucus carota* (Montague and others 1979) and formation of floral buds from thin layer explants of *Nicotiana tabacum* (Torrigiani and others 1987). However, the role of PAs in plant morphogenesis is not yet clear. Endogenous levels of PAs such as Put, Spd, and Spm are known to influence a variety of growth and development processes in higher plants (Evans and Malmberg 1989). They occur in free and conjugated forms and titers depend on the external conditions of light and temperature (Galston and Kaur-Sawhney 1990). Increasing PA titers have been noted during sprouting in potato tubers (Kaur-Sawhney and others 1982) and Jerusalem artichoke (Bagni and Serafini-Fracassini 1985). PA titers also increase during seed germination (Gallardo and others 1992; Huang and Villeneuva 1992) root and shoot formation (Chriqui and others 1986). Conjugated endogenous PAs are associated with the physiology of flowering, metabolite synthesis, and responses to viral infections (Slocum and Galston 1985). PA biosynthesis is closely associated with ethylene biosynthesis as both compete for the same precursor SAM (Evans and Malmberg 1989). Ethylene is a gaseous hormone involved in regulation of a wide range of physiologic responses in plants (Reid 1987). A synergistic effect between PAs and ethylene inhibitors on exogenous addition of the same, resulting in enhanced shoot regeneration as seen in *Brassica campestris* has been reported (Chi and Pua 1989).

Many of the studies demonstrating PA function have been made possible through the use of inhibitors of PA biosynthesis, such as α-DL-difluromethylornithine (DFMO) and α-DL-difluromethylarginine (DFMA), which selectively inhibit ornithine decarboxylase and arginine decarboxylase, respectively (Kallio and McCann 1981). Inhibition of PA biosynthesis blocks differentiation in plants, but the effect can be removed by addition of PAs (Fierer and others 1984). Unlike animals, plants synthesize Put through ODC and arginine through an alternative pathway involving ADC (Fierer and others 1984).

Cichorium intybus L. cv. Lucknow local is an important crop finding its commercial use as a coffee additive. The work reported here studies the effect of exogenously administered Put and silver nitrate on shoot multiplication from axillary buds and *in vitro* flowering in *C. intybus* L. cv. Lucknow local and investigates its effect on titers of conjugated endogenous PAs.

Materials and Methods

Plant Material

Seeds from 10 different plants of the same cultivar of *C. intybus* L. cv. Lucknow local were obtained from Banthara Research Station, National Botanical Research Institute (N.B.R.I.), Lucknow.

Culture Conditions

Seeds were washed in running tap water and were surface sterilized by rinsing them in 70% ethanol for 10 s followed by surface sterilization in an aq. solution of 0.1% (w/v) $HgCl_2$ for 3–5 min in sterile deionized water. The MS basal medium (Murashige and Skoog 1962) was supplemented with 3% sucrose (Hi medium, India). The pH of the media was

Table 1. Effect of Exogenously Fed Putrescine on Phenotypical Response in Shoot Cultures of *Cichorium intybus* L. cv. Lucknow Local

Sl. No.	Media + putrescine (mM)	Number of Shoots	Length of Shoots (cm)	Number of Flowers (per Shoot Apex)
a	Control	19.2 a	5.4 a	Nd
b	10	21.6 b	5.2 a	Nd
c	20	22 b	6.2 b	Nd
d	30	25 c	6.6 c	Nd
e	40	34.6 d	7.68 d	3.76 d
f	50	16 e	2.6 e	Nd
SEM	SEM	±0.33 (24 df)	±0.10 (24 df)	±0.16 (24 df)

Means of the same row followed by different letters differ significantly according to Duncan's multiple range test ($p < 0.05$, $n = 5$).

Table 2. Effect of Exogenously Fed Putrescine and Polyamine Inhibitors on Phenotypical Response in Shoot Cultures of *Cichorium intybus* L. cv. Lucknow Local

Sl. No.	Media + Putrescine (mM) + Polyamine Inhibitors (mM)	Number of Shoots	Length of Shoots (cm)	Number of Flowers (per Shoot Apex)
a	Control	19.2 a	5.4 a	Nd
b	40 Put	34.6 a	7.68 b	3.76 a
c	1 DFMA	12 c	2.6 c	Nd
d	1 DFMO	12 c	2.06 d	Nd
e	1 (DFMA + DFMO) each	8 d	1.2 e	Nd
f	1 DFMA + 40 Put	22.8 e	4.4 f	1.2 e
g	1 DFMO + 40 Put	24.2 e	5.3 a	1.61 e
h	1 (DFMA + DFMO) + 40 Put	20.2 a	4.18 g	Nd
SEM	SEM	±0.48 (32 df)	±0.09 (32 df)	±0.18 (32 df)

Means of the same row followed by different letters differ significantly according to Duncan's multiple range test ($p < 0.05$, $n = 5$).

adjusted to 5.8 ± 0.1 before gelling with 0.8% agar (Hi medium, India). The gelled medium was autoclaved at 1.06 kg cm^{-2} pressure and 121°C for 15 min. The seeds were inoculated onto MS basal medium and incubated at 25 ± 2°C under cool light (4.41 Jm^{-2} s^{-1} 18 h day^{-1}). Axillary buds were excised after germination from the seedlings and inoculated onto a MS media supplemented with 2′-3′-*O*-isopropylidene adenosine (2-iP) (2 mg L^{-1}) and gibberellic acid (GA_3) (0.5 mg L^{-1}) with various concentrations of Put and $AgNO_3$ and were incubated under similar conditions.

Chemicals

Putrescine was obtained as its hydrochloride from Sigma Co. (St. Louis, MO, USA); DFMO and DFMA were obtained from Marion Merrell Research Co. Cincinnati, OH, and were incorporated into the media after filter sterilization using a 0.22-µM filter (Sartorius Ltd.) to obtain the desired concentration range of 10–50 mM and 1 mM each, respectively. $AgNO_3$ was obtained from Qualigens Co. Ltd., India, and was administered to the media after filter sterilization using a 0.22-µM filter (Sartorius Co. Ltd.) to obtain the desired final concentration. All other chemicals were of analytical grade and solvents were of high pressure liquid chromatography (HPLC) grade.

Extraction of Endogenous PAs

Extraction of endogenous conjugated PAs was carried out by acid hydrolysis of perchloric acid (PCA) soluble and insoluble extract. PAs were analyzed by

Figure 1. *In vitro* flowering in *Cichorium intybus* L. cv. Lucknow local, when treated with 40 mM putrescine.

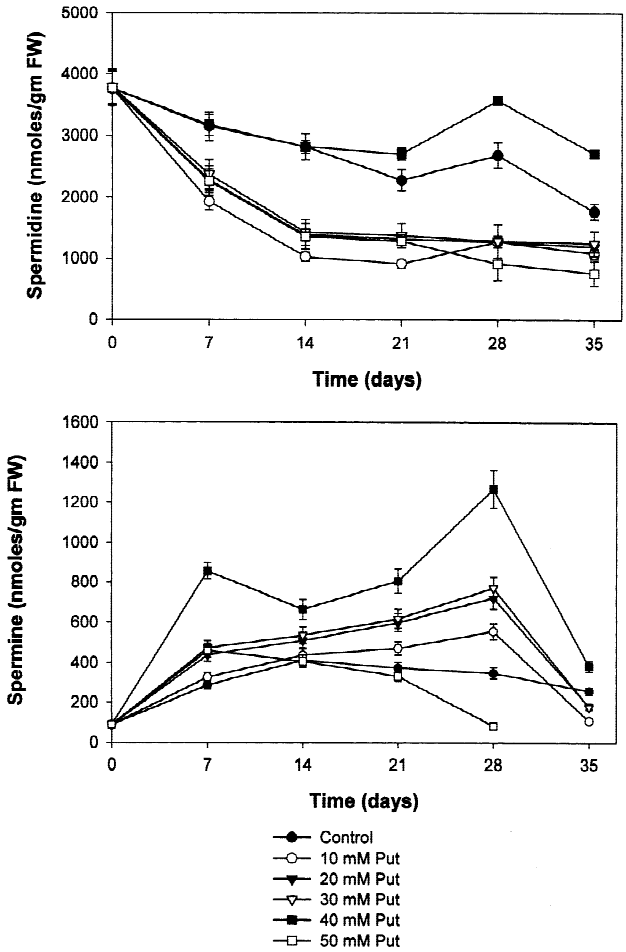

Figure 2. Effect of exogenously administered putrescine on endogenous conjugated titers of polyamines in *C. intybus* (Lines are Mean ± S.D., n = 5).

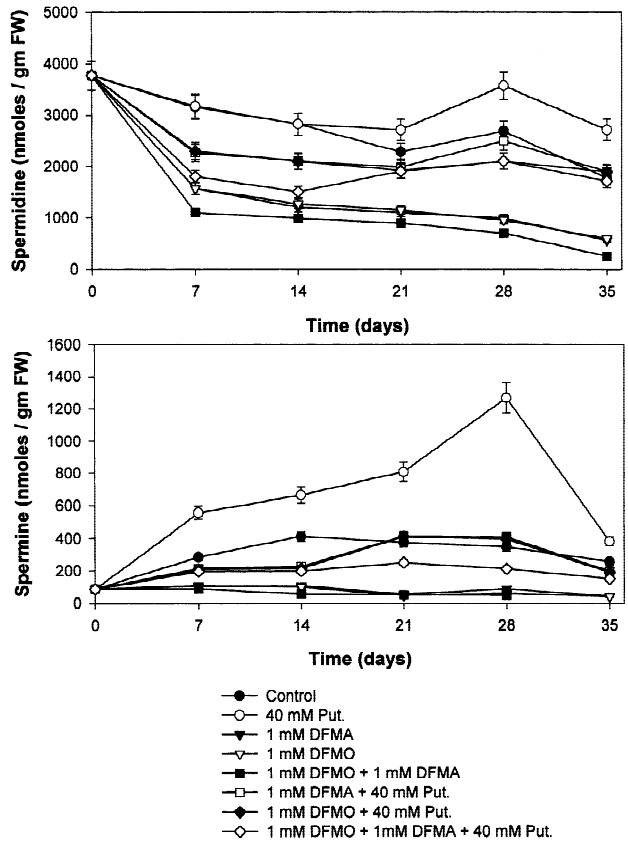

Figure 3. Effect of exogenously administered putrescine and polyamine inhibitors on endogenous conjugated titers of polyamines in *C. intybus* (Lines are Mean ± S.D., n = 5).

benzoylation according to the method adapted by Flores and Galston (1982). Averages of five values were expressed in nmoles g^{-1} FW of tissue.

Ethylene Measurements

Ethylene release was measured from explants grown on medium with Put and $AgNO_3$. The serum stopper of each flask in which explants were grown was removed. The flasks were allowed to stand for 1 h in a laminar flow hood under dim light with a constant airflow (0.48 m s^{-1}); flasks were resealed with the serum stopper. Cultures were allowed to stand in the hood and at the end of 3 h, a 5-mL gas sample was drawn using a pressure lock syringe (Merck India Ltd.) and assayed by gas chromatograph (GC) (Shimadzu Ltd. Japan). The conditions for GC were as described by Chi and others (1994).

Statistical Analyses

Data were subjected to analyses of variance appropriate to a completely randomized design and means were separated using Duncan's new multiple range test (Steel and Torrie 1980).

Results

Effect of Exogenously Fed Put and PA Inhibitors on Shoot Multiplication, *In Vitro* Flowering and Endogenous Conjugated Levels of PAs

Put was added exogenously at various concentrations ranging from 10–50 mM to MS (Murashige and Skoog 1962) media containing 2-iP (2′-3′-*O*-isopropylidene adenosine) (2 mg L^{-1}) and GA_3 (0.5 mg L^{-1}). Of the various treatments (Table 1), Put at 40 mM level gave the maximum number of shoots (34.6 ± 2.61) as well as increase in length of shoots (7.69 ± 0.57 cm) compared with the control (shoot number, 19.2 ± 1.44; shoot length, 5.4 ± 0.42 cm). Other treatments (Table 1) with Put below 40 mM also showed significant increases over controls in terms of tissue response. Put treatment at the 50 mM level inhibited shoot multiplication both in

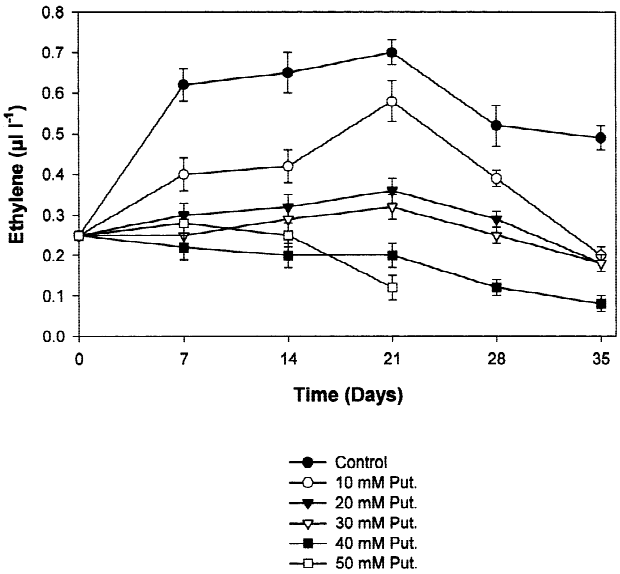

Figure 4. Influence of putrescine on ethylene production in *C. intybus* (Lines are Mean + S.D., n = 5).

shoot number (16.0 ± 1.23) and length of shoot (2.6 ± 0.25 cm). The PA inhibitors DFMA and DFMO (1 mM each) were added exogenously to the chicory shoot cultures to study the promotive effect of Put (40 mM). Combined treatment with DFMA and DFMO (1 mM each) resulted in minimum tissue response in terms of number of shoots and growth of shoots (8.0 ± 0.54 shoots per culture; 1.2 ± 0.09 cm) (Table 2) in chicory plants. Put (40 mM) treatment supplemented with 1 mM DFMA restored the morphogenetic response in terms of tissue response in shoot cultures (22.8 ± 1.67 shoots per culture; 4.2 ± 0.35 cm) of chicory plants compared with respective controls (19.2 ± 1.44 shoots per culture; 5.4 ± 0.42 cm) (Tables 1 and 2).

Put at the 40 mM level influenced floral initiation and floral development in chicory shoot cultures (3.26 ± 0.16 per shoot apices) (Table 1) (Figure 1); other treatments did not induce *in vitro* flowering. Treatment with PA inhibitors and the combined (DFMA and DFMO) treatment (1 mM level) resulted in lack of flowering response, whereas floral induction (1.2 ± 0.18; 1.61 ± 0.18 flowers per shoot apex) occurred under treatment with Put (40 mM) with DFMA or DFMO supplementation at 1 mM levels in chicory shoot cultures, respectively (Table 2).

Titers of endogenous conjugated PAs were studied after feeding with Put; 40 mM Put treatment resulted in peak endogenous conjugated Spm (1265 ± 94.9 nmoles g^{-1} FW) titers on day 28 (floral initiation day) compared with the control (298.6 ± 54.6 nmoles g^{-1} FW) (Figure 2). Other concentrations (10–40 mM) showed marginal increases of endogenous conjugated Spm titers compared with controls (Figure 2). Treatment within 50 mM Put inhibited both morphogenesis (Table 1) and total endogenous PA levels (Figure 2). To check the promotive effect of Put (40 mM), titers of endogenous conjugated PAs were measured after PA inhibitor treatment (Figure 3). Treatment with DFMA and DFMO at 1 mM level each produced very low conjugated endogenous Spm titers in chicory shoot cultures (48.2 ± 3.6 nmoles g^{-1} FW) (Figure 3). Spm titers increased after supplementation with Put (40 mM) to the media containing 1 mM DFMA or 1 mM DFMO in chicory (408 ± 30.6 nmoles g^{-1} FW; 396 ± 29.77 nmoles g^{-1} FW, respectively) plants on day 28 (Figure 3).

Ethylene production under exogenous Put (10–50 mM) administration in chicory plants was monitored (Figure 4). Under Put (40 mM) treatment ethylene production was minimal in chicory (0.08 µL L^{-1}) compared with contro1s (0.49 µL L^{-1}) on day 35 (Figure 4).

Effect of Exogenously Fed $AgNO_3$ and PA Inhibitors on Shoot Multiplication, *In Vitro* Flowering and Endogenous Conjugated Levels of PAs

Axillary buds of *C. intybus* were cultured in MS (Murashige and Skoog 1962) medium containing 2-iP (2 mg L^{-1}), GA_3 (0.5 mg L^{-1}) and various levels of $AgNO_3$ in the range of 10–50 µM. Of the various treatments, $AgNO_3$ at the 40-µM level influenced maximum shoot multiplication (36.8 ± 2.63) and increased length of shoots (7.9 ± 0.76 cm) (Table 3). Lower levels of $AgNO_3$ (10–30 µM) were promotive, whereas a concentration of 50 µM inhibited tissue response (Table 3).

PA inhibitors DFMA and DFMO were administered to the media containing shoot cultures of chicory. Treatment with DFMA and DFMO at 1 mM levels each showed minimum tissue response in terms of shoot multiplication and length of chicory shoots (8.9 ± 0.67 shoots per culture; 1.6 ± 0.14 cm) in plants (Table 4). $AgNO_3$ (40 µM) treatment supplemented to the media containing 1 mM DFMA restored the morphogenetic response in terms of shoot multiplication and shoot length (25.2 ± 1.8 shoots per culture; 6.1 ± 0.44 cm) compared with controls (19.2 ± 1.86 shoots per culture; 5.4 ± 0.76 cm) (Table 3 and Table 4). Similarly, DFMO (1 mM) treatment administered with $AgNO_3$ (40 µM) resulted in the same restoration as observed earlier with DFMA and $AgNO_3$ 40 µM treatment (Tables 3 and 4). $AgNO_3$ (40 µM) also induced floral initiation

Table 3. Effect of Exogenously Fed $AgNO_3$ on Phenotypical Response in Shoot Cultures of *Cichorium intybus* L. cv. Lucknow Local

Sl. No.	Media + $AgNO_3$(μM)	Number of Shoots	Length of Shoots (cm)	Number of Flowers (per Shoot Apex)
a	Control	19.2 a	5.4 a	Nd
b	10	22.2 b	5.4 a	Nd
c	20	24.8 c	6.7 b	Nd
d	30	28.4 d	6.7 b	Nd
e	40	36.8 e	7.9 c	4.2 e
f	50	12.6 f	3.1 d	Nd
SEM	SEM	±0.48 (24 df)	±0.10 (24 df)	±0.21 (24 df)

Means of the same row followed by different letters differ significantly according to Duncan's multiple range test ($p < 0.05$, $n = 5$*).*

Table 4. Effect of Exogenously Fed $AgNO_3$ and Polyamine Inhibitors on Phenotypical Response in Shoot Cultures of *Cichorium intybus* L. cv. Lucknow Local

Sl. No.	Media + $AgNO_3$ (μM) + Polyamine Inhibitors (mM)	Number of Shoots	Length of Shoots (cm)	Number of Flowers (per Shoot Apex)
a	Control	19.2 a	5.4 a	Nd
b	40 $AgNO_3$	36.8 b	7.9 b	4.2 b
c	1 DFMA	14.4 c	3.0 c	Nd
d	1 DFMO	13.4 c	3.0 c	Nd
e	1 (DFMA + DFMO) each	9.4 d	1.68 d	Nd
f	1 DFMA + 40 $AgNO_3$	25.2 e	6.1 e	1.3 e
g	1 DFMO + 40 $AgNO_3$	25.4 e	6.08 e	1.2 e
h	1 (DFMA + DFMO) + 40 $AgNO_3$	20.2 a	5.02 f	Nd
SEM	SEM	±0.34 (32 df)	±0.08 (32 df)	±0.07 (32 df)

Means of the same row followed by different letters differ significantly according to Duncan's multiple range test ($p < 0.05$, $n = 5$*).*

and floral development in shoot cultures (4.2 ± 0.21 flowers per shoot apex) (Table 3). Other treatments failed to elicit a floral response (Table 3). No *in vitro* flowering was observed in chicory plants under PA inhibitor treatment (Table 4), whereas *in vitro* flowering was restored by treatment with $AgNO_3$ (40 μM) and combined DFMA and DFMO at 1 mM levels in chicory plants (1.3 ± 0.07; 1.2 ± 0.07 flowers per shoot apex respectively) (Table 4).

Influence of $AgNO_3$ was studied to check its effect on titers of endogenous conjugated PAs. $AgNO_3$ treatment (10–40 μM) showed higher titers of Spm in chicory plants (Figure 5) compared with controls. Put and Spd titers decreased with the increase in Spm titers in all treatments over the culture period (Figure 5). Treatment with DFMO and DFMA (1 mM each) administered to shoot cultures of chicory resulted in a minimum accumulation of endogenous conjugated PAs (Figure 6). DFMA (1 mM) and DFMO (1 mM) treatment with $AgNO_3$ (40 μM) resulted in increased endogenous conjugated pools of PAs in shoot cultures of chicory (Figure 6). Ethylene production was monitored after exogenous administration of $AgNO_3$ (10–50 μM) in chicory plants (Figure 7). Increasing $AgNO_3$ concentrations coincided with an increase in ethylene production in shoot cultures (Figure 7).

Discussion

The addition of Put (40 mM) and $AgNO_3$ (40 μM) resulted in increased shoot proliferation, *in vitro* flowering and increased endogenous levels of PAs. Similar effects of $AgNO_3$ on *in vitro* shoot multiplication (Chi and Pua 1989; Pua and Chi 1993) and

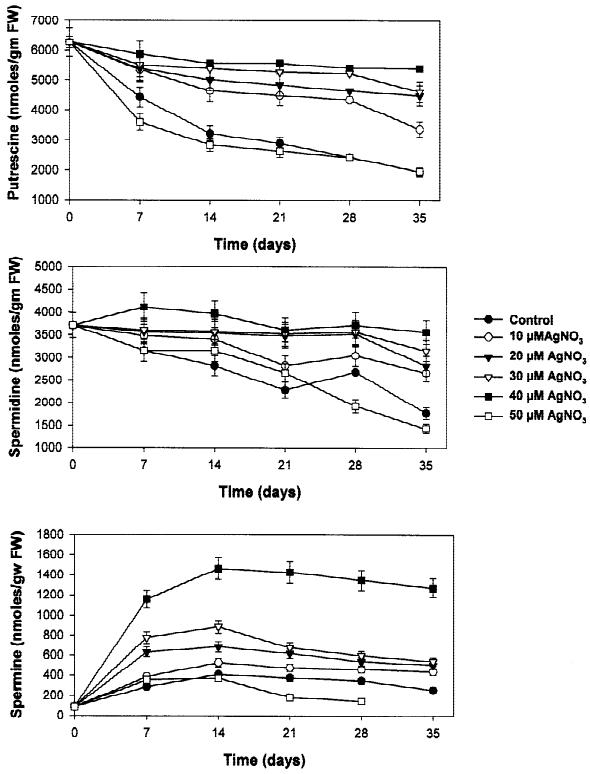

Figure 5. Effect of exogenously fed $AgNO_3$ on endogenous conjugated titers of polyamines in *C. intybus* (Lines are Mean ± S.D., n = 5).

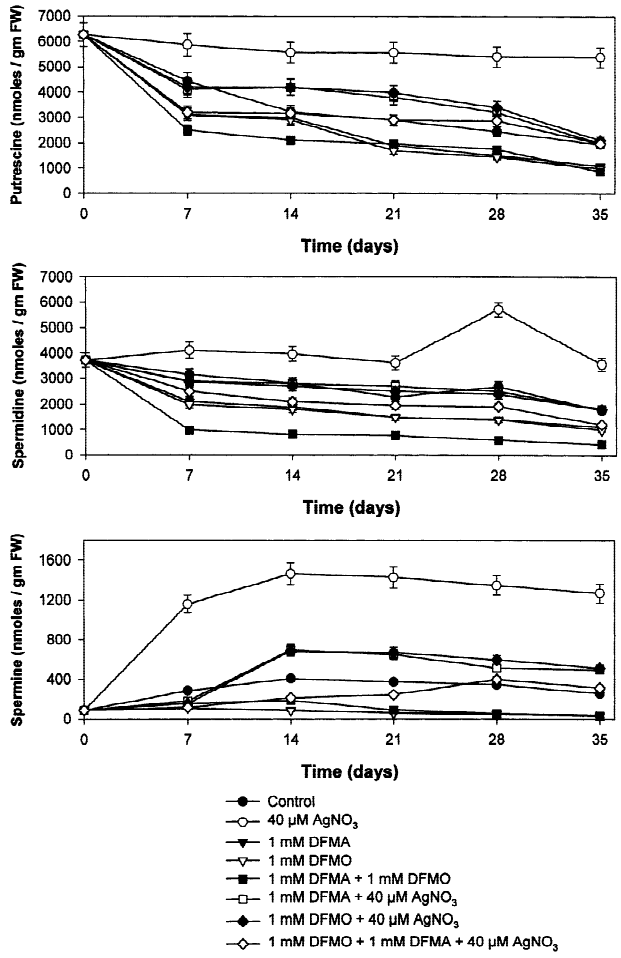

Figure 6. Effect of exogenously fed $AgNO_3$ and polyamine inhibitors on endogenous conjugated titers of polyamines in *C. intybus* (Lines are Mean ± S.D., n = 5).

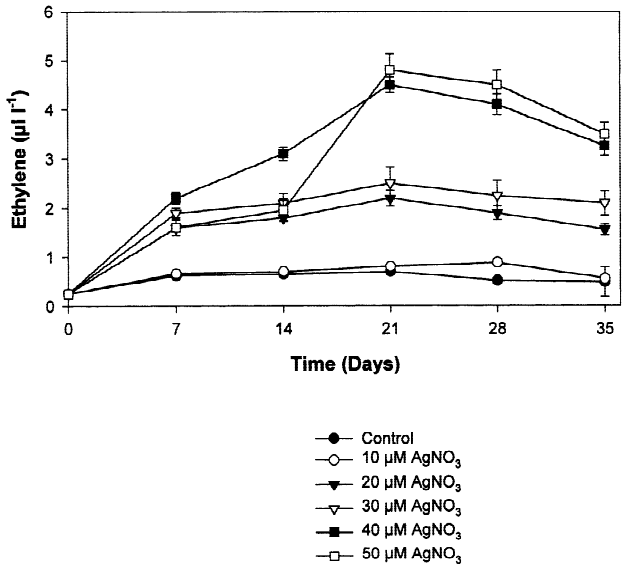

Figure 7. Influence of $AgNO_3$ on ethylene production in *Cichorium intybus.* (Lines are Mean ± S.D., n = 5).

floral bud differentiation (Gerats and others 1988) have been reported. Other workers reported shoot organogenesis in carrot (Fierer and others 1984), alfalfa (Meijer and Simmonds 1988), and tobacco (Torrigiani and others 1987) on administration of $AgNO_3$. Furthermore, treatments of Put in several systems have promoted shoot multiplication (Chi and Pua 1989). Bagni and Serafini Fracassin (1985) have reported that exogenous PAs serve as a mere nitrogen source for the plants but in contrast in our earlier communication we have found that exogenous feeding of these PAs does have a physiological role to play in growth and production of secondary metabolites in hairy root cultures of *Beta vulgaris* and *Tagetes patula* (Bais and others 2000). In both Put (40 mM) and $AgNO_3$ (40 µM) treated cultures, endogenous conjugated spermine levels were higher in shoot cultures of chicory during the whole culture period, whereas Put and Spd levels were lower. Significantly the period of maximum accumulation of spermine (40 mM Put treatment on day 28) coincided with the day of *in vitro* flowering. This observation agrees with the report of floral initiation in tobacco by Torrigiani and others (1987). Smulders and others (1990) reported that ethylene at higher

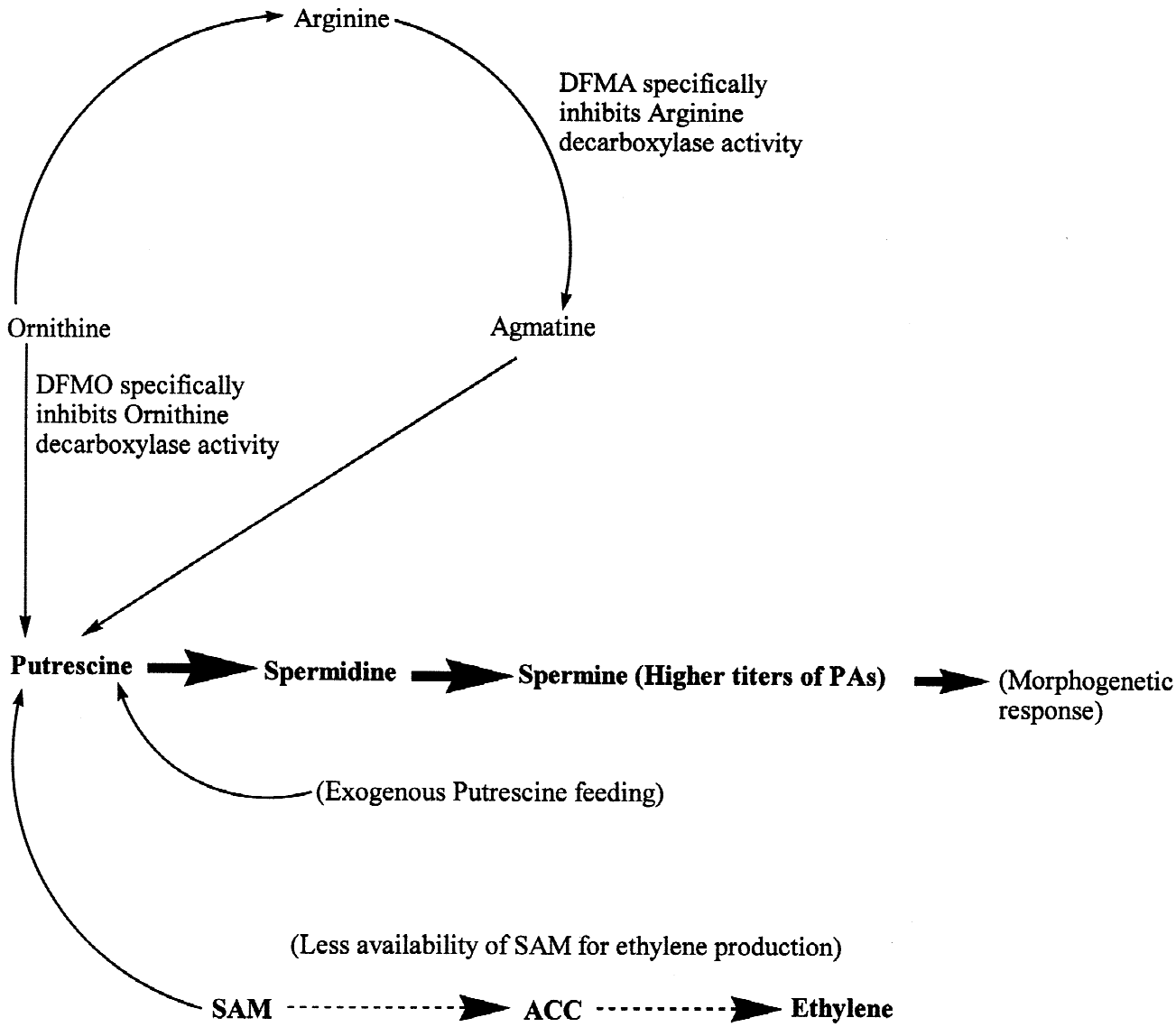

Figure 8. Proposed interplay of ethylene biosynthesis and PAs under the influence of exogenous putrescine feeding.

concentrations inhibited floral bud formation in tobacco. This effect was negated by the use of $AgNO_3$, which was correlated to ethylene action inhibition Beyer (1976). Kaur-Sawhney and others (1988) found that total endogenous levels of Spd played an important role in floral bud differentiation in the thin layer explants of tobacco. Similarly, in chicory shoot cultures, exogenous Put/$AgNO_3$ feeding led to floral differentiation and seed setting (data not shown), which was correlated with increased endogenous polyamine pools under these treatments and further evidenced by the use of biosynthetic inhibitors of PA metabolism, leading to morphogenesis (Figures 8, 9).

The promotive role of Put (40 mM) and $AgNO_3$ (40 µM) was confirmed by the use of biosynthetic PA inhibitors. Treatment with DFMA and DFMO (1 mM each) showed a hindered and inhibited morphogenetic response in chicory plants that was reversed by administration of Put (40 mM) and $AgNO_3$ (40 µM) (Tables 1–4). These results may also suggest the possible involvement of both ODC and ADC in shoot morphogenesis and *in vitro* flowering, as already reported by Tiburcio and others (1989). Similarly, Berta and others (1997) found that thinning of cell walls of tobacco thin cell layer lines after DFMA and DFMO administration could be reversed by PAs.

This study also led to the development of floral induction in plants of *C. intybus*. The chicory plants, which flower biennially, can be forced to flower experimentally for studies on *in vitro* pollination and seed development. Miyazaki and Yang (1987) have

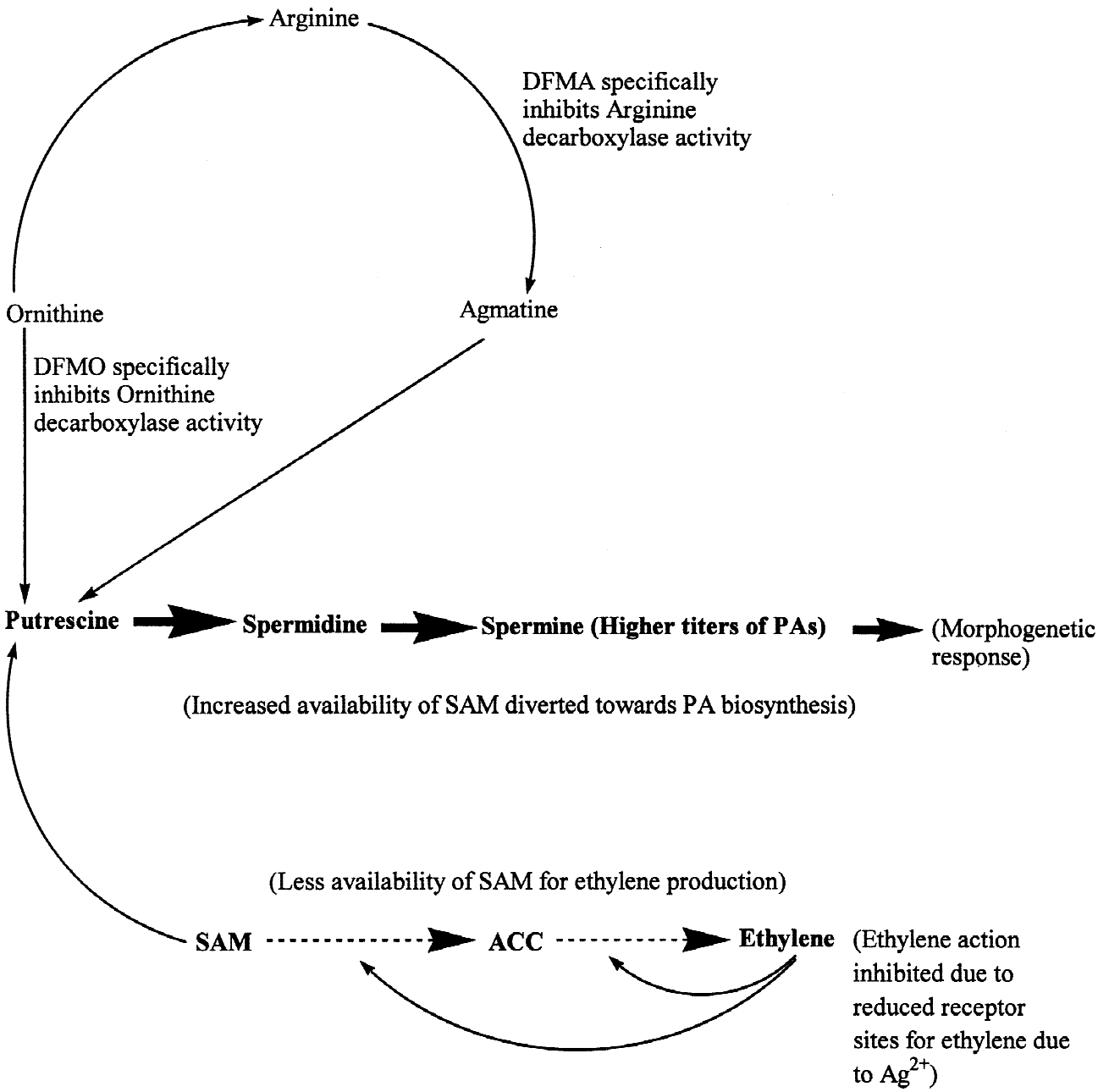

Figure 9. Proposed interplay of ethylene biosynthesis and PAs under the influence of exogenous $AgNO_3$ feeding.

reported the influence of Put and $AgNO_3$ on the competitive use of SAM. From this study we postulate that the use of SAM by Put for its conversion to Spd would possibly result in a lower availability of SAM for ethylene biosynthesis. As observed under all Put (10–50 mM) treatments, ethylene production was on the lower side with increased titers of endogenous conjugated PAs (Figure 2) in chicory plants, which confirms the phenomenon presented in Figure 8. Whereas $AgNO_3$, which inhibits ethylene action through Ag^{2+} ions by reducing the receptor capacity to bind ethylene (Yang 1985), would result in higher titers of ethylene in the tissues. Higher ethylene titers in turn may feed back and inhibit earlier steps of its own pathway, making SAM available for PA biosynthesis (Figure 9), as evidenced by higher titers of endogenous conjugated PAs and ethylene production under $AgNO_3$ (10–40 µM) treatments (Figure 5). Pua and Chi (1993) have reported the same stimulatory effect of $AgNO_3$ feeding on ethylene production and PAs titers in *Brassica juncea*. The mechanism by which ethylene produc-

tion is stimulated in response to $AgNO_3$ is unknown, although ethylene overproduction as a result of Ag^{2+} treatment has also been reported in tomato fruits (Penarrubia and others 1992) and in an ethylene overproducing *Arabidopsis* mutant (Guzman and Ecker 1990). According to Theologis (1992), $AgNO_3$-stimulated ethylene production can be explained by receptor interference by Ag^{2+} that triggers cells to overproduce ethylene. Reports on somatic embryogenesis in carrot (Roustan and others 1990) showed that the potent ethylene action inhibitor $AgNO_3$ helps in increasing the ADC activity, which in turn increases endogenous PAs in carrot embryogenic cultures. This helps to clarify the role of $AgNO_3$, which promotes ADC activity through the feedback inhibition, which in turn would have influenced increased PA biosynthesis and hence the morphogenetic response in shoot cultures of chicory. As reported earlier by Fierer and others (1984), use of DFMA inhibited ADC activity in carrot cell cultures. In our work, treatments with DFMA and DFMO resulted in lower levels of endogenous conjugated PAs. PA titers were restored after $AgNO_3$ (40 µM) supplementation, possibly because of increased ADC activity, which further contributed to the morphogenetic response in shoots of chicory. Roustan and others (1990) showed that ethylene may block PA metabolism in plants, which in turn hinders the morphogenetic response. Nevertheless, we cannot rule out the possibility that ethylene interacts with other biochemical processes required for cellular differentiation during shoot morphogenesis in chicory shoot cultures.

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