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Polyamines and Flower Development in the Male Sterile Stamenless-2 Mutant of Tomato (*Lycopersicon esculentum* Mill.)[1]

II. Effects of Polyamines and Their Biosynthetic Inhibitors on the Development of Normal and Mutant Floral Buds Cultured *in Vitro*

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ABSTRACT

The floral organs of the male sterile stamenless-2 (sl-2/sl-2) mutant of tomato (*Lycopersicon esculentum* Mill.) contain significantly higher level of polyamines than those of the normal (R Rastogi, VK Sawhney [1990] Plant Physiol 93: 439–445). The effects of putrescine, spermidine and spermine, and three different inhibitors of polyamine biosynthesis on the *in vitro* development of floral buds of the normal and sl-2/sl-2 mutant were studied. The polyamines were inhibitory to the *in vitro* growth and development of both the normal and mutant floral buds and they induced abnormal stamen development in normal flowers. The inhibitors of polyamine biosynthesis also inhibited the growth and development of floral organs of the two genotypes, but the normal flowers showed greater sensitivity than the mutant. The inhibitors also promoted the formation of normal-looking pollen in stamens of some mutant flowers. The effect of the inhibitors on polyamine levels was not determined. The polyamine-induced abnormal stamen development in the normal, and the inhibitor-induced production of normal-looking pollen in mutant flowers support the suggestion that the elevated polyamine levels contribute to abnormal stamen development in the sl-2/sl-2 mutant of tomato.

The polyamines are widely distributed in plants, and changes in their biosynthesis and metabolism have been correlated with several growth and developmental processes (7, 8, 24, 25). Recently, PAs[3] also have been implicated in plant reproductive processes such as the induction of flowering (3), floral organ differentiation (11, 12) and the regulation of male sterility (13).

In a companion paper (20), we reported that floral organs of a male sterile stamenless-2 (sl-2/sl-2) mutant of tomato (*Lycopersicon esculentum* Mill.) contain significantly higher levels of Put, Spd, and Spm, as well as ODC and SAMDC activity, than the normal. Further, the restoration of normal stamen development in the mutant by low temperatures (21) was associated with a decline in the levels of PAs and the activities of ODC and SAMDC (20), suggesting that the elevated levels of PAs in the sl-2/sl-2 mutant may have a role in abnormal stamen development.

In view of the above findings, it was considered important to determine whether an exogenous supply of PAs would induce stamen abnormalities in the normal flowers, and whether the inhibitors of PA biosynthesis would promote the formation of normal pollen in the mutant. Thus, the effects of Put, Spd, and Spm and three inhibitors of polyamine biosynthesis, *i.e.* MGBG, DFMO, and CHA (23, 24) on the *in vitro* growth and development of normal and mutant floral buds were investigated. The technique of *in vitro* culture of isolated floral buds was employed since it allows the assessment of the direct role of growth regulators by eliminating the interference of substances translocated from other plant parts. The floral buds of both the normal and sl-2/sl-2 mutant, excised at the sepal primordia stage, can be grown successfully to maturity *in vitro*, including the differentiation of micro- and megaspores (18, 19). A cytokinin (*i.e.* BAP) was essential for the growth and development of floral buds of both lines; however, the mutant buds also required GA_3.

MATERIALS AND METHODS

The seed source, maintenance of seed stock of the sl-2/sl-2 mutant and the normal (+/+) line of tomato (*Lycopersicon esculentum* Mill.), and plant cultivation methods were similar to those described previously (20). Plants were grown in a greenhouse with supplemental lighting provided by cool-white fluorescent tubes and incandescent bulbs for 16 h a day at an intensity of 180 μE s^{-1} m^{-2}; the temperature was 25 ± 3°C.

The inflorescences with young floral buds were detached from plants, surface sterilized in 15% 'Javex' (equivalent to a 0.9% sodium hypochlorite solution) for 15 min, and rinsed 3 to 4 times in sterile water. Floral buds with sepal primordia only and about 0.3 mm in length (18), were dissected asepti-

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[3] Abbreviations: PA(s), polyamine(s); sl-2/sl-2, stamenless-2; Put, putrescine; Spd, spermidine; Spm, spermine; ODC, ornithine decarboxylase; SAMDC, *S*-adenosylmethionine decarboxylase; MGBG, methylglyoxal-bis(guanylhydrazone); DFMO, difluoromethylornithine; CHA, Cyclohexylamine; BAP, benzylaminopurine.

cally and placed into 25 mL capacity glass jars, each containing 15 mL of the culture medium.

The basal medium consisted of Murashige and Skoog's mineral salts (15), White's vitamins and glycine (26), 100 mg/L inositol, 3% sucrose (w/v), and 10^{-6} M BAP. GA_3, at 10^{-6} M, was included in the medium for sl-2/sl-2 buds. All components were added to the medium and pH adjusted to 5.8 before autoclaving. The stock solutions of polyamines and inhibitors were filter sterilized and added to the cooled medium at concentrations of 10^{-6} to 10^{-3} M.

The cultures were reared in an incubator maintained at 25 ± 3°C, and illuminated by cool-white fluorescent tubes for 16 h/day at an intensity of 40 to 50 $\mu E\ s^{-1}\ m^{-2}$. The experiments were terminated after 5 weeks of incubation. The number of mature flowers, *i.e.* those with a full complement of well-developed floral organs and possessing ovules in the mutant (19), and ovules and normal-looking pollen in the normal (18), was recorded. The number of mutant flowers showing normal-looking pollen, and that of normal flowers showing abnormal stamens was also noted. The length of all floral organs was measured and an analysis of variance performed on the data (27). Differences in the means were tested by Duncan's multiple range test. All experiments reported are representative of three or more separate trials, each consisting of 10 replicates per treatment.

The microscopy procedures were similar to those described elsewhere (18).

Chemicals

Hydrochlorides of putrescine, spermidine, spermine, methylglyoxal-bis(guanylhydrazone), and benzylaminopurine, gibberellic acid, and cyclohexylamine were purchased from Sigma Chemical Co. Difluoromethylornithine was a gift from Merrell Dow Research Institute, Cincinnati.

RESULTS

Polyamines

The PAs alone, *i.e.* in the absence of other growth regulators, had no apparent effect on the *in vitro* growth of both the normal and mutant floral buds. However, they inhibited the BAP − and BAP + GA_3-promoted development of the normal and mutant floral buds, respectively. In the normal line, Put at 10^{-6} and 10^{-5} M did not affect the growth of various floral organs, but at 10^{-4} M, it significantly inhibited stamen growth without affecting the growth of other floral organs (Table I). At 10^{-3} M Put, however, the growth of all floral organs was reduced (Table I). In the mutant, Put at the various concentrations tested had no effect on the growth of sepals and petals. Stamen growth also was not affected by 10^{-6} M Put, but it was significantly reduced at 10^{-5} M and 10^{-4} M Put in comparison to the control (Table I). Gynoecium growth in the mutant buds was inhibited by 10^{-4} and 10^{-3} M Put.

Spd at 10^{-6} M inhibited the growth of stamens of normal buds, without affecting the growth of other floral organs (Table II). At 10^{-5} M Spd, the growth of gynoecium was also inhibited, and at 10^{-4} M the growth of sepals and stamens was affected (Table II). There was further growth inhibition of most organs of the normal buds at 10^{-3} M Spd. In the mutant buds, Spd at 10^{-6} M also reduced stamen growth only, without affecting other floral organs, and at 10^{-5} M, it also inhibited gynoecium growth (Table II). At 10^{-4} M Spd, the growth of sepals was inhibited, but petal growth was affected only by 10^{-3} M Spd (Table II).

Spm inhibited floral organ growth at relatively lower concentrations when compared to other PAs (*cf.* Table III with Tables I and II). In the normal buds, Spm at 10^{-6} M significantly inhibited the growth of stamens and gynoecium, but did not affect the growth of sepals and petals (Table III). At 10^{-5} M Spm, the growth of all organs was reduced, as compared to the control. There was no additional inhibition of growth at 10^{-4} M Spm, but at 10^{-3} M, the growth of all organs, except stamens, was further reduced (Table III). In the mutant

Table I. *Effect of Different Concentrations of Putrescine on the Lengths (in mm) of Various Organs of the Normal and sl-2/sl-2 Flowers of Tomato Grown in Vitro for 5 Weeks*

The medium for the normal floral buds contained 10^{-6} M BAP, and for the mutant 10^{-6} M BAP + 10^{-6} M GA_3. Values presented are the means.* $n = 25$.

Genotype	Concentration	Sepal	Petal	Stamen	Gynoecium
	M				
Normal	Control	7.1^a	9.5^a	5.2^a	5.4^a
	10^{-6}	7.6^a	10.3^a	5.1^a	5.3^a
	10^{-5}	7.5^a	10.0^a	5.6^{ab}	5.0^a
	10^{-4}	6.6^{ab}	9.6^a	4.0^{bc}	4.8^a
	10^{-3}	5.7^b	7.4^b	3.4^c	3.2^b
sl-2/sl-2	Control	13.2^a	15.2^a	7.3^a	5.4^a
	10^{-6}	14.0^a	16.9^a	6.6^{ab}	5.0^{ab}
	10^{-5}	13.8^a	16.4^a	6.2^b	4.8^{abc}
	10^{-4}	13.6^a	15.1^a	5.2^c	4.4^{bc}
	10^{-3}	12.2^a	14.4^a	5.1^c	4.0^c

* Values in a column followed by the same letter are not significantly different at P = 0.05.

Table II. *Effect of Different Concentrations of Spermidine on the Lengths (in mm) of Various Organs of the Normal and sl-2/sl-2 Flowers of Tomato Grown in Vitro for 5 Weeks*

The medium for the normal floral buds contained 10^{-6} M BAP, and for the mutant 10^{-6} M BAP + 10^{-6} M GA_3. Values presented are the means.* $n = 25$.

Genotype	Concentration	Sepal	Petal	Stamen	Gynoecium
	M				
Normal	Control	7.1^a	9.5^a	5.2^a	5.4^a
	10^{-6}	6.7^{ab}	10.7^a	4.3^b	4.6^{ab}
	10^{-5}	6.3^{abc}	10.4^a	4.4^b	4.0^b
	10^{-4}	6.1^{bc}	9.8^a	3.5^c	3.8^{bc}
	10^{-3}	5.6^c	7.7^b	3.3^c	3.0^c
sl-2/sl-2	Control	13.2^a	15.2^a	7.3^a	5.4^a
	10^{-6}	12.1^a	13.7^a	5.8^b	4.9^a
	10^{-5}	11.2^{ab}	13.4^a	5.1^{bc}	4.0^b
	10^{-4}	10.6^b	12.9^{ab}	4.4^c	3.5^{bc}
	10^{-3}	9.9^b	10.3^b	4.3^c	3.0^c

* Values in a column followed by the same letter are not significantly different at P = 0.05.

Table III. *Effect of Different Concentrations of Spermine on the Lengths (in mm) of Various Organs of the Normal and sl-2/sl-2 Flowers of Tomato Grown in Vitro for 5 Weeks*

The medium for the normal floral buds contained 10^{-6} M BAP, and for the mutant 10^{-6} M BAP + 10^{-6} M GA_3. Values presented are the means.* $n = 25$.

Genotype	Concentration	Sepal	Petal	Stamen	Gynoecium
	M				
Normal	Control	7.1[a]	9.5[a]	5.2[a]	5.4[a]
	10^{-6}	6.4[ab]	8.4[ab]	3.2[b]	3.6[b]
	10^{-5}	5.5[bc]	7.8[bc]	2.5[bc]	2.8[c]
	10^{-4}	4.9[c]	6.9[c]	2.1[c]	2.7[c]
	10^{-3}	3.7[d]	5.6[d]	1.7[c]	2.0[d]
sl-2/sl-2	Control	13.2[a]	15.2[a]	7.3[a]	5.4[a]
	10^{-6}	10.9[b]	15.8[a]	5.9[b]	4.5[b]
	10^{-5}	9.5[b]	12.6[b]	4.9[c]	3.4[c]
	10^{-4}	7.8[c]	9.5[c]	3.5[d]	3.2[c]
	10^{-3}	7.1[c]	8.9[c]	2.7[d]	2.3[d]

* Values in a column followed by the same letter are not significantly different at P = 0.05.

buds, Spm at 10^{-6} M inhibited the growth of sepals, stamens and gynoecium but did not affect petal growth (Table III). At higher concentrations, however, the growth of all floral organs was inhibited (Table III). It is important to note that in both lines, stamens showed greater sensitivity to Put and Spd, and stamens and gynoecium to Spm, than other floral organs (Tables I–III). Also, in both the genotypes, Spm was more inhibitory to floral organ growth than the other two PAs (Tables I–III).

Put, Spd, and Spm were also inhibitory to the *in vitro* maturation of floral buds. In the normal line, Put reduced the number of mature flowers at all concentrations tested, but was most inhibitory at 10^{-3} M (Fig. 1A). Similarly, Spd and Spm significantly reduced the number of floral buds reaching maturity, and their effects were also concentration dependent (Fig. 1, B and C). At 10^{-4} and 10^{-3} M Spm, no mature flowers were produced (Fig. 1C).

In the mutant, PAs also inhibited the maturation of floral buds, but the response was different from the normal. For example, at most Put concentrations, the number of mature flowers was not significantly different from the control (Fig. 2). Also, Spd at 10^{-6} and 10^{-5} M, and Spm at 10^{-6} M did not significantly affect the number of mature flowers, but they were inhibitory at higher concentrations (Fig. 2).

All three PAs also induced abnormal stamen development in the normal flowers (Fig. 1, A–C). The number of flowers with abnormal stamens increased with increasing PA concentrations, from 10^{-6} to 10^{-4} M, and there was no further increase at 10^{-3} M (Fig. 1, A–C). The stamens in such flowers were either elongated, filamentous structures (Fig. 3A) that did not differentiate sporogenous tissue, or were normal-looking, bilobed structures but contained shrunken pollen (Fig. 3B). In some stamens, both the normal and abnormal-looking pollen were observed in the same locule (Fig. 3C). The gynoecium in these flowers was normal looking and the ovary possessed numerous ovules.

Inhibitors of Polyamine Biosynthesis

The three inhibitors, MGBG, DFMO, and CHA selectively inhibit the activities of PA biosynthetic enzymes; MGBG that of SAMDC, DFMO that of ODC and CHA that of Spd synthase (23, 24).

In the normal buds, grown in a medium containing 10^{-6} M BAP, MGBG at 10^{-6} M significantly inhibited stamen and gynoecium growth without affecting the growth of sepals and petals (Table IV). In the mutant, 10^{-6} M MGBG did not affect the growth of any of the floral organs. Higher MGBG concentrations (10^{-5}–10^{-3} M) were inhibitory to the growth of all organs in both the genotypes (Table IV). DFMO was also inhibitory; in the normal line, it inhibited the growth of stamen and gynoecium at 10^{-6} M and that of all floral organs at 10^{-5} M (Table V). In the mutant, at 10^{-6} M, DFMO did not

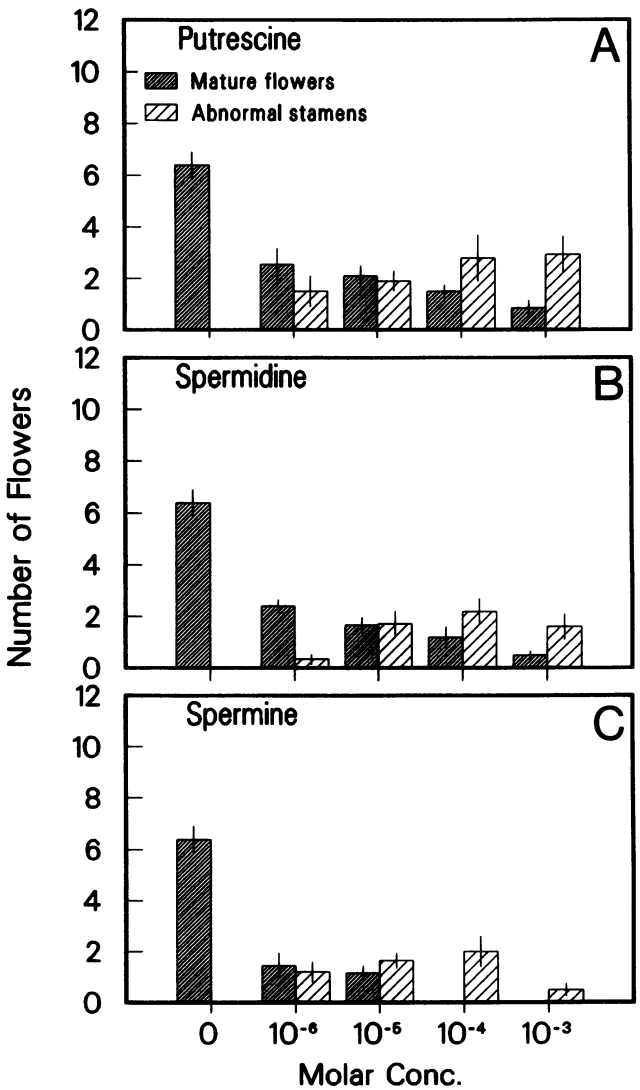

Figure 1. Effect of different concentrations of Put (A), Spd (B), and Spm (C) on the number of mature flowers, and those with abnormal stamen development, of the normal line produced *in vitro* after 5 weeks of culture. The medium contained 10^{-6} M BAP. $n = 30$ in each case. Vertical bars represent SE.

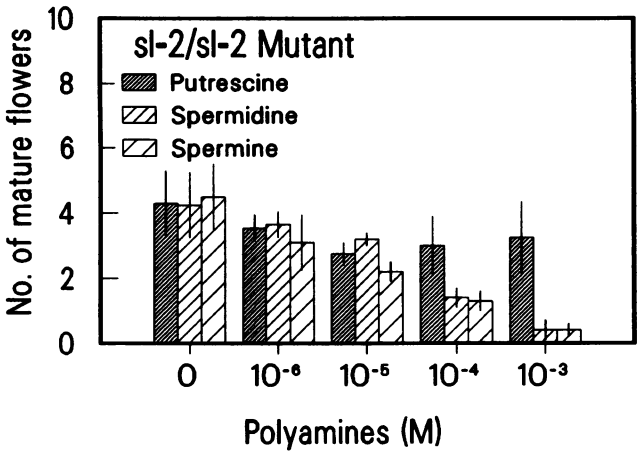

Figure 2. Effect of different concentrations of Put, Spd, and Spm on the number of mature flowers of the sl-2/sl-2 mutant produced *in vitro* after 5 weeks of culture. The medium contained 10^{-6} M BAP and 10^{-6} M GA_3. $n = 30$ for each treatment. Vertical bars represent SE.

affect floral organ growth and at 10^{-5} M, growth of stamen alone was affected (Table V). The growth of most floral organs in both the genotypes was inhibited at higher DFMO concentrations (Table V). CHA, at the various concentrations tested, inhibited the growth of all floral organs in the normal line (Table VI). In the mutant, CHA at 10^{-6} and 10^{-5} M affected the growth of stamens alone, but at 10^{-4} and 10^{-3} M it was inhibitory to the growth of most floral organs (Table VI).

The three inhibitors also affected the *in vitro* maturation of floral buds of both the genotypes. In the normal line, the number of mature flowers produced at 10^{-6} and 10^{-5} M MGBG was significantly lower than the control, and at 10^{-4} and 10^{-3} M, no mature flowers were produced (Fig. 4A). In addition, MGBG at 10^{-6} to 10^{-4} M induced abnormal stamen development in the normal flowers (Fig. 4A), *i.e.* stamens either did not differentiate sporogenous tissue or contained degenerated microspores/pollen grains. In the mutant, MGBG at 10^{-6} M did not significantly affect the maturation of floral buds but higher MGBG concentrations were inhibitory (Fig. 4B). At 10^{-4} M MGBG, some mutant flowers reached maturity as compared to none in the normal (*cf.* Fig. 4A with 4B). Further, despite the MGBG-induced inhibition of floral organ growth and maturation, some mutant flowers contained stamens with normal-looking pollen grains (Figs. 4B and 5A). Such pollen grains possessed well-developed exine and apertures (Fig. 5B). The response of the normal and mutant floral buds to DFMO and CHA, in terms of maturation, was essentially similar to that of MGBG (data not presented), *i.e.* there was greater inhibition of flower maturation in the normal line than the mutant. As well, DFMO and CHA, like MGBG, induced abnormal stamen development in normal flowers, but induced the production of normal pollen in some mutant flowers.

DISCUSSION

The experiments reported here show that the three PAs; Put, Spd, and Spm are inhibitory to the *in vitro* growth and development of floral buds of both the normal and sl-2/sl-2 mutant of tomato. The *in vitro* maturation of floral buds of the two genotypes, however, showed a different response to PAs. In addition, the stamen and gynoecium growth was more sensitive to PAs than the growth of other floral organs.

A comparison of the *in vitro* maturation of the normal and mutant floral buds showed that Put and Spm at all concentrations, and Spd at 10^{-6} and 10^{-5} M were more inhibitory to the normal than the mutant buds (*cf.* Fig. 1, A–C with Fig. 2). The normal buds, thus, showed greater sensitivity to PAs than the mutant. The different response of the normal and sl-2/sl-2 floral buds could well be related to the higher levels of endogenous PAs in the mutant than the normal flowers (20). To our knowledge, the role of PAs in the *in vitro* development of the excised floral buds, either directly or by the use of inhibitors, has not been investigated in any other system.

PAs are generally known to have a stimulatory effect on the growth of plant tissues. For example, exogenously supplied PAs promoted cell division in the dormant tubers (1, 2) and cultured tuber explants of *Helianthus tuberosus* (17). Similarly, Put stimulated root growth in *Phaseolus vulgaris* (16) and *Datura innoxia* (4), and cell division and colony formation in mesophyll protoplasts of *Alnus* (9). Also, the application of PAs increased fruit growth in apple trees (6). In view of the promotive effects of PAs on the growth of plant tissues, their inhibitory effect on the growth and development of tomato floral buds may seem surprising. However, it is quite possible that optimal level of PAs, necessary for the development of floral organs, are present in the normal and mutant buds. Thus, an exogenous supply of PAs, although variable

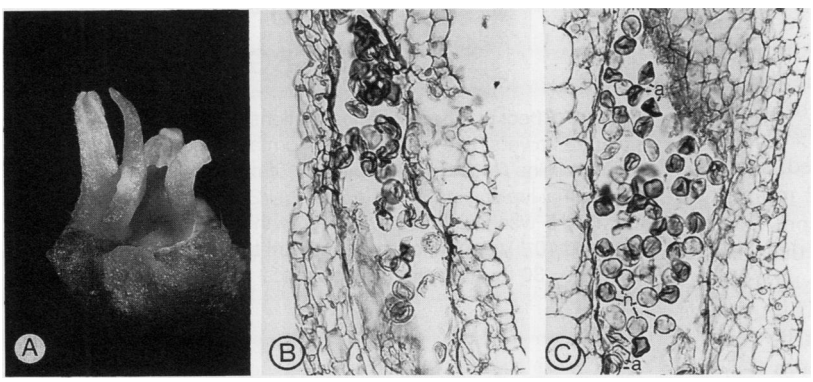

Figure 3. Stamens from a flower of the normal line grown *in vitro* for 5 weeks in a medium containing 10^{-5} M putrescine and 10^{-6} M BAP. (A) Filamentous stamens from putrescine-treated flowers. ×7; (B and C), longitudinal section through a stamen in A showing shrivelled pollen (B) ×304, and some normal (n) and abnormal (a) pollen in the same locule (C) ×305.

Table IV. *Effect of Different Concentrations of MGBG on the Lengths (in mm) of Various Organs of the Normal and sl-2/sl-2 Flowers of Tomato Grown in Vitro for 5 Weeks*

The medium for the normal floral buds contained 10^{-6} M BAP, and for the mutant 10^{-6} M BAP + 10^{-6} M GA_3. Values presented are the means.* $n = 25$.

Genotype	Concentration	Sepal	Petal	Stamen	Gynoecium
	M				
Normal	Control	7.1[a]	9.5[a]	5.2[a]	5.4[a]
	10^{-6}	7.3[a]	8.7[ab]	3.6[b]	4.5[b]
	10^{-5}	5.8[b]	7.7[bc]	2.3[c]	3.9[b]
	10^{-4}	4.2[c]	6.8[c]	2.2[c]	2.8[c]
	10^{-3}	2.7[d]	3.2[d]	1.3[d]	1.4[d]
sl-2/sl-2	Control	13.2[a]	15.2[a]	7.3[a]	5.4[a]
	10^{-6}	13.0[a]	14.6[a]	6.5[a]	5.1[ab]
	10^{-5}	10.7[b]	11.7[b]	5.4[b]	4.3[bc]
	10^{-4}	8.8[c]	9.4[c]	3.6[c]	3.5[c]
	10^{-3}	4.3[d]	4.9[d]	2.6[d]	2.0[d]

* Values in a column followed by the same letter are not significantly different at P = 0.05.

Table V. *Effect of Different Concentrations of DFMO on the Lengths (in mm) of Various Organs of the Normal and sl-2/sl-2 Flowers of Tomato Grown in Vitro for 5 Weeks*

The medium for the normal buds contained 10^{-6} M BAP, and for the mutant 10^{-6} M BAP + 10^{-6} M GA_3. Values presented are the means.* $n = 25$.

Genotype	Concentration	Sepal	Petal	Stamen	Gynoecium
	M				
Normal	Control	7.1[a]	9.5[a]	5.2[a]	5.4[a]
	10^{-6}	6.5[ab]	8.4[ab]	3.4[b]	3.8[b]
	10^{-5}	5.7[b]	7.9[b]	2.7[bc]	3.1[bc]
	10^{-4}	4.7[c]	6.3[c]	2.1[cd]	2.9[cd]
	10^{-3}	4.5[c]	6.2[c]	1.5[d]	2.2[d]
sl-2/sl-2	Control	13.2[a]	15.2[a]	7.3[a]	5.4[a]
	10^{-6}	12.9[a]	14.7[a]	6.7[ab]	5.2[a]
	10^{-5}	11.8[a]	14.0[a]	6.2[bc]	4.5[ab]
	10^{-4}	10.1[b]	13.7[a]	5.3[c]	4.1[b]
	10^{-3}	9.9[b]	10.8[b]	3.8[d]	2.8[c]

* Values in a column followed by the same letter are not significantly different at P = 0.05.

in its effect on the normal and mutant floral buds, is inhibitory to floral organ growth.

PAs also induced abnormal stamen development in some floral buds of the normal line (Figs. 1, A–C and 3). Although the level of PAs in such buds was not determined, the abnormalities in stamens appear to be specifically related to the exogenous supply of PAs as the growth and differentiation of other organs, including gynoecium, was not affected. Elsewhere, we reported that floral organs of the sl-2/sl-2 mutant possess significantly higher levels of Put, Spd, and Spm than those of the normal (20). Further, it was shown that the restoration of fertility in the sl-2/sl-2 mutant by low temperature was associated with a reduction in PA levels. Thus, the induction of abnormal stamen development in normal flowers by exogenous PAs supports the earlier suggestion that in sl-2/

Table VI. *Effect of Different Concentrations of CHA on the Lengths (in mm) of Various Organs of the Normal and sl-2/sl-2 Flowers of Tomato Grown in Vitro for 5 Weeks*

The medium for the normal buds contained 10^{-6} M BAP, and for the mutant 10^{-6} M BAP + 10^{-6} M GA_3. Values presented are the means.* $n = 25$.

Genotype	Concentration	Sepal	Petal	Stamen	Gynoecium
	M				
Normal	Control	7.1[a]	9.5[a]	5.2[a]	5.4[a]
	10^{-6}	6.0[b]	8.1[b]	4.0[b]	3.4[b]
	10^{-5}	4.9[c]	6.4[c]	3.0[c]	2.5[bc]
	10^{-4}	4.6[cd]	5.4[cd]	2.6[c]	2.1[c]
	10^{-3}	3.9[d]	4.6[d]	2.3[c]	1.9[c]
sl-2/sl-2	Control	13.2[a]	15.2[a]	7.3[a]	5.4[a]
	10^{-6}	13.6[a]	15.4[a]	6.0[b]	5.0[a]
	10^{-5}	12.6[ab]	14.9[a]	4.5[c]	4.9[a]
	10^{-4}	11.2[bc]	13.2[ab]	4.2[cd]	3.8[b]
	10^{-3}	9.6[c]	12.2[b]	3.5[d]	3.5[b]

* Values in a column followed by the same letter are not significantly different at P = 0.05.

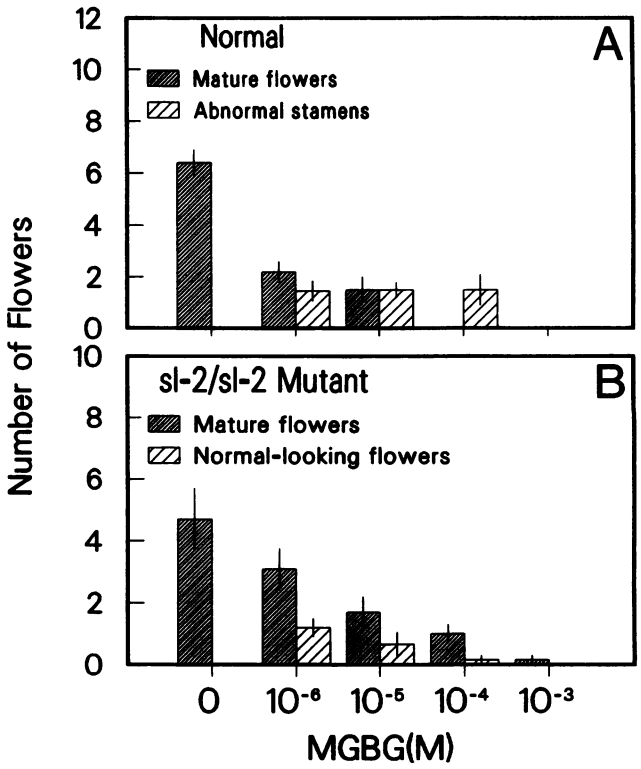

Figure 4. Effect of different concentrations of MGBG on the number of mature flowers, and those with abnormal stamen development, of the normal line (A) and on the number of mature flowers, and those with normal-looking pollen of the sl-2/sl-2 mutant (B) produced *in vitro* after 5 weeks of culture. The growth medium for normal buds contained 10^{-6} M BAP and that for mutant buds 10^{-6} M BAP and 10^{-6} M GA_3. $n = 30$ for each treatment. Vertical bars represent SE.

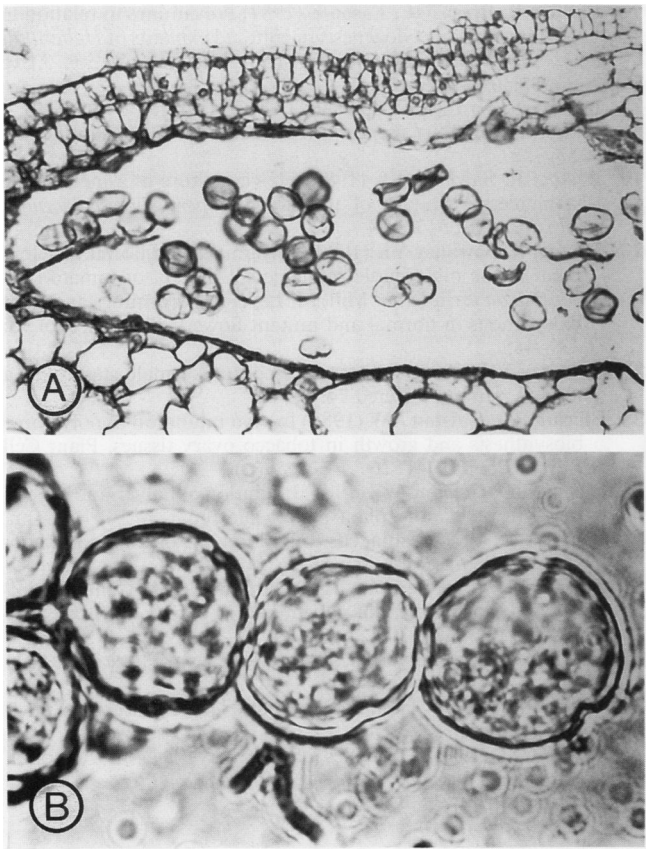

Figure 5. Normal-looking pollen grains produced in the stamens of sl-2/sl-2 flowers grown *in vitro* for 5 weeks. The medium contained 10^{-6} M MGBG, 10^{-6} M BAP and 10^{-6} M GA_3. (A), Longitudinal section through a mutant stamen showing normal-looking pollen grains in the anther locule. ×307. (B), Normal-looking pollen grains from an anther squash. ×1630.

sl-2 mutant flowers, the elevated levels of PAs may contribute to abnormal stamen development.

Polyamines have been implicated in the differentiation of floral organs and the regulation of male sterility in other systems as well. In tobacco, mutant lines selected for resistance to MGBG possessed altered PA levels in comparison to the wild type and showed male and female sterility and organ transformation (11, 12). In a cytoplasmic male sterile line of corn, the anthers contained lower levels of PA-conjugates than the wild type, and the restoration of fertility was correlated with an increase in PA-conjugates (13).

Another approach in investigating the role of PAs in flower development is the use of inhibitors of PA biosynthetic enzymes. In the normal flowers, MGBG and DFMO at 10^{-6} M inhibited the growth of stamens and gynoecium specifically, and that of all floral organs at higher concentrations (Tables IV and V). CHA, in contrast, was inhibitory at all concentrations (Table VI). The mutant floral organs, however, showed a lower sensitivity to the inhibitors than the normal. At 10^{-6} M, MGBG and DFMO did not affect the floral organ growth (Tables IV and V), and CHA at 10^{-6} and 10^{-5} M affected stamen growth only (Table VI). The *in vitro* maturation of normal and mutant floral buds was also affected by the inhibitors, but the inhibition was greater in the normal than the mutant (*cf.* Fig. 4A with 4B). The lower sensitivity of mutant floral organs to the inhibitors may be explained by higher endogenous levels of PAs in mutant flowers (20).

The inhibitory effect of MGBG, DFMO, and CHA on the normal and mutant suggests that PAs are required for the growth and development of tomato floral organs. This suggestion is based on the assumption that these inhibitors do affect PA levels in tomato flowers. Although the PA titers in inhibitor-treated flowers were not determined, it has been shown in other systems that these inhibitors reduce PA levels as well as affect the growth and differentiation of plant tissues (7, 24, 25). The inhibitor studies are particularly persuasive since in many cases, the inhibition by PA inhibitors is reversed by exogenous PAs. In tomato, fruit development was inhibited by DFMO and this inhibition was alleviated by exogenous Put (5). DFMO also inhibited the growth of tobacco ovaries as well as reduced Put levels, and this inhibition was partly overcome by Put (22). Similarly, in the embryogenic cell line of carrot, difluoromethylarginine, an inhibitor of arginine decarboxylase, inhibited embryogenesis as well as reduced the levels of Put and Spd, and the addition of PAs restored normal embryogenesis (14). As well, in thin layer explants of tobacco, CHA inhibited floral differentiation and the increase in Spd titers and its effects were partially reversed by exogenous Spd (10). Thus, based on these examples, it is not unreasonable to argue that the production of normal-looking pollen in sl-2/sl-2 mutant flowers (Fig. 5, A and B), grown in the presence of MGBG, DFMO, and CHA, was related to the reduction in PA levels.

In conclusion, the evidence from various accounts supports strongly the suggestion that the high level of PAs in flowers contributes to abnormal stamen development in the sl-2/sl-2 mutant of tomato.

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