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Uptake and translocation of polyamines and inhibitors in hypocotyl segments of leafy spurge

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Introduction

Work continues in this laboratory on the role of the diamine, putrescence (*Put*), and the polyamines, spermidine (*Spd*) and spermine (*Spm*), in the development of leafy spurge. These compounds (which will be collectively called polyamines) are ubiquitous and are believed to be involved in the growth and development of plants; possibly at the level of cell division (Slocum and Flores 1991). Some scientists consider these polyamines as possible plant growth regulators. However, their role in plants has not been established with certainty. The objective of this research is to assess the role of the polyamines in the organogenesis of leafy spurge, and whether alteration of the polyamine pathway (Figure 1) may be a weak point for control of the weed. The metabolic relationship to ethylene, a known plant growth regulator, is shown in Figure 1.

These polyamines and several inhibitors of enzymes involved in the biosynthesis of the polyamines (Figure 1) were tested for their effects on organogenesis. The inhibitors used in this report were: DFMO (alpha-difluoromethylornithine), a specific inhibitor of ornithine decarboxylase (ODC) which catalyzes the conversion of ormithine to putrescine; DFMA (alpha-difluoromethyl arginine), a specific inhibitor of arginine decarboxylase (ADC) which catalyzes the conversion of arginine to agmatine, a precursor of putrescine; and MGBG (methylglyoxal-bisguanylhydrazone, a non-specific inhibitor of S-adenosylmethionine, which is a precursor to both spermidine and ethylene. The modifying effects of the natural auxin, IAA (indole-3-acetic acid), were included. The results include a brief summary of results reported at a USDA-ARS meeting in Minneapolis in 1991, but have not been presented to this symposium. Also included are the interactions of MGBG, *Spm* and IAA, as well as the rates of uptake and translocation of radio labeled polyamines and DFMO from the agar-solidified medium.

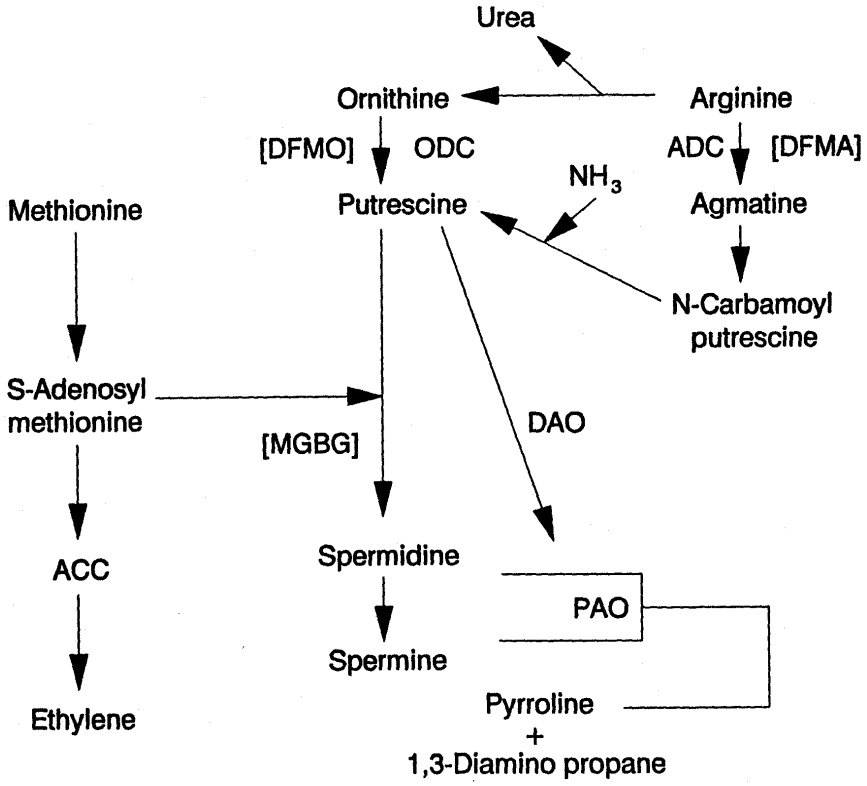

Figure 1. Polyamine biosynthesis and degradation.

Materials and methods

Hypocotyl segments (1 cm long) of aseptically-grown leafy spurge seedlings were placed on B5 nutrient medium (Gamborg *et al*. 1968) at full strength or 0.1 strength salts and vitamins. Sucrose was used at 2% (w/v) and 0.7 % (w/v) agar in all media. Eight hypocotyl segments were placed onto the agar-solidified medium. *Put*, *Spd*, DFMO, DFMA, MGBG, and IAA were also dissolved in the agar media. Radio labeled [^{14}C]-*Put*, [^{14}C]-*Spd*, [^{14}C]-*Spm* or [3H]-DFMO were used to determine uptake into hypocotyl segments laid horizontally or supported vertically in the agar. The hypocotyl segments were blotted, and weighed prior to being analyzed for [^{14}C] by combustion and [^{14}C]-CO_2 analysis.

Results

I. Brief summary of results reported at the USDA-ARS coordination planning meeting, Minneapolis, MN, April 23-25, 1991:

In full strength B5 medium, putrescine at 1 to 5 mM had no significant effect on root formation, while 0.5 mM appeared to stimulate roots. DFMO (0.5 mM) strongly inhibited root formation, while DFMA (0.5 mM) did not. The addition of *Put* (0.5 mM) only partially reversed the effects of DFMO.

IAA (1.1μM*)* greatly increased root formation; usually 3 to 5 times the number (per hypocotyl segment) as controls. When both DFMA and DFMO (0.5 mM) were applied together, root formation was inhibited. The inhibition was reversed by the addition of 1.1 M IAA, but not to the level achieved when IAA was used alone (without the inhibitors).

When the B5 salts and vitamins were diluted to one-tenth the normal concentration (sucrose remaining constant at 2% w/v) *Put* at 1 mM inhibited root formation. IAA (1.1 μM) reversed the inhibitory action of *Put*, so the number of roots per hypocotyl segment was similar to those treated with IAA alone.

II. The effects of MGBG:

In full strength B5 medium, MGBG (like *Put*) had no effect on root or shoot formation. However, as with *Put*, MGBG strongly inhibited organogenesis in hypocotyl segments grown on the diluted B5 medium. Both root and shoot formation were inhibited completely (Table 1). IAA did not reverse this inhibition, as it did with *Put*.

Table 1. Effects of MGBG and IAA on organogenesis in leafy spurge hypocotyl segments.[a,b]

Treatment	Concentration	Shoots	Roots
		No. organs/segment	
Controls	0	1.3	0.4
MGBG	0.2 mM	0	0
IAA	1.1 μM	0.3	2.0
IAA + MGBG	1.1 μM	0	0.03

[a]Methylglyoxal-bis-guanylhydrazone (MGBG) and indole-3-acetic acid (IAA).
[b]Hypocotyl segments were placed in 0.1 X B5 culture media for 28 days.

Because MGBG is an inhibitor of *Spd* biosynthesis, the presumption was that treatment of the hypocotyl segments with *Spd* would reverse the action of the inhibitor. However, the addition of 0.5 mM *Spd* did not reverse the inhibiton by MGBG (Table 2), but *Spd* inhibited both shoot and root formation in the 0.1 x B5 medium.

Table 2. Effects of MGBG and spermidine on organogenesis in leafy spurge hypocotyl segments.[a,b]

Treatment	Concentration	Shoots[c]	Roots[c]
	mM	No. organs/segment	
Controls	0	1.2 ± 0.1 [d]	0.4 ± 0.04 [d]
MGBG	0.1	0.6 ± 0.1 [d,e]	0.1 ± 0.02 [d,e]
Spermidine	0.5	0.05 ± 0.03[e]	0 [e]
MGBG + Spermidine	0.1 + 0.05	0 [e]	0.1 ± 0.03 [e]

[a] Methylglyoxal-bis-guanylhydrazone (MGBG).
[b] Hypocotyl segments were placed in 0.1 x B5 culture media for 28 days.
[c] Mean values and standard errors.
[d] Combined results of 3 experiments.
[e] Differs from controls ($P < 0.01$).

The results of one experiment on uptake and translocation of radio labeled *Put, Spd, Spm* and the ODC inhibitor, DFMO, from 0.1 x B5 medium are shown in Table 3. These results are from hypocotyls laid horizontally onto the medium.

Table 3. Uptake of (^{14}C)-polyamines and (^{3}H)-DFMO in leafy spurge hypocotyl segments. [a,b]

	4 hours	1 day	7 days	29 days
	nM/g			
Putrescine	–	3.1	7.7	14.1
Spermidine	3.5	5.2	4.5	–
Spermine	3.1	5.0	5.9	–
DFMO	–	0.6	14.6	37.6

[a] Values are expressed in nmoles per gram fresh weight calculated as equivalents of the parent compound. DFMO is α-difluoromethylornithine.
[b] Hypocotyl segments were placed in 0.1 X B5 culture media for 28 days.

Other experiments were done in which the hypocotyl segments were placed vertically into the agar and the upper half of the hypocotyls were separated from the portion that was in contact with the medium. This was done to avoid errors due to radioactive compounds adhering to the exterior of the hypocotyls, and to determine the extent of movement of radioactivity within the tissues. In media with one-tenth the normal salt concentration, at 28 days, uptake and translocation of [^{14}C]-polyamines and [^{3}H]-DFMO expressed as nanomoles of parent compound equivalents per mg fresh weight were 11 to 13 nmol of *Put* equivalents, 7.5 to 26 nmol of *Spd*, 2.5 to 10 nmol of *Spm*, and 15 to 26 nmol of DFMO.

Only one experiment has been completed with full strength B5 medium with the hypocotyls oriented vertically. In that experiment, lesser amounts of all four compounds were translocated than for hypocotyls oriented similarily in the diluted media. In that experiment, at 28 days, equivalents recovered for *Put*, *Spd*, *Spm* and DFMO were 2, 2.5, 3.5 and 6 nmol per mg fresh weight, respectively.

Discussion

Putrescine has been shown to stimulate cell division and growth of plant tissues (Slocum and Flores 1991). In leafy spurge, the application of exogenous putrescine as high as 5 mM concentration in B5 nutrient medium has no obvious effect on the formation of roots on isolated hypocotal segments when the B5 medium contains full strength salts and vitamins. The inhibition by *Put* in the diluted medium is difficult to rationalize. Putrescine has been shown to accumulate under potassium deficiency (T.A., Smith, chapter 1 in Slocum and Flores 1991). Preliminary results indicate that more *Put* may be taken up by hypocotyl segments grown in the dilute medium than in full strength medium, and may accumulate to a higher (phytotoxic?) level under those circumstances.

DFMO inhibits root formation strongly. Since DFMO inhibits the ODC pathway in the biosynthesis of putrescine from ornithine (Slocum and Flores 1991), the implication is that depletion of putrescine results in the failure of root formation. However, the addi-

tion of *Put* in the B5 medium did not reverse the inhibitory effects of DFMO in leafy spurge hypocotyl segments. The results of the uptake experiments indicate that *Put* did penetrate into the tissues, was translocated, and appeared to be present in sufficient quantities so as to overcome the inhibition by DFMO and/or DFMA.

DFMA did not inhibit root formation, in contrast to DFMO, implying that the ornithine pathway may be tied more closely to the control root formation than the arginine pathway.

IAA reversed (at least partially) the nearly total inhibition of root formation induced by treatment with both DFMO and DFMA applied together. It is tempting to speculate that IAA stimulates the formation of putrescine and bypasses the block of *Put* biosynthesis via the two major pathways (catalyzed by ADC and ODC). Preliminary results (not shown) indicate that *Put* levels are elevated during the times of organ formation, but roots are also formed in the absence of detectable *Put* (hypocotyl segments treated with DFMO and DFMA). Further work is underway to resolve these contradictions.

Because MGBG inhibits *Spd* biosynthesis (Slocum and Flores 1991) and MGBG inhibited both root and shoot formation in leafy spurge (grown in the diluted medium), it was presumed that the introduction of *Spd* to the medium should overcome this inhibition. However, this did not occur. In fact, *Spd* itself proved to be inhibitory to organogenesis (similar to *Put*). This contradiction remains under investigation.

Unlike treatment with *Put*, the addition of IAA did not reverse the inhibition induced by MGBG (as it did with hypocotyl segments treated with DFMO and DFMA). Therefore, the mechanism of IAA-*Spd* interaction differs from that for IAA-*Put* interactions.

All of the polyamines and DFMO were taken up readily by the hypocotyl segments. The quantities of radiolabeled equivalents recovered in the tissues were calculated as nmol per mg fresh weight. This is several fold greater than reported for the concentration of endogenous polyamines in other plant tissues (Slocum and Flores 1991) and in leafy spurge hypocotyl segments, as noted in preliminary determinations by this laboratory. Although it seems likely that the parent compounds are readily available within the hypocotyl tissues, polyamines are metabolized in plants (Slocum and Flores 1991). Their metabolism in leafy spurge is expected and they may form conjugates or become bound to plant constituents, as well as forming breakdown products, such as pyrroline and 1,3-diaminopropane.

Conclusions

The role of polyamines in leafy spurge organogenesis is not clear. Putrescine is not phytotoxic in media with a high salt concentration, but is phytotoxic in low salt medium. Spermidine is also phytotoxic in the low salt medium. Although organogenesis was inhibited by the inclusion of inhibitors of the enzymes involved in the biosynthesis of both compounds, the addition of exogenous *Put* and *Spd* did not reverse the action of these inhibitors. It is possible that neither *Put* nor *Spd* are required for organogenesis in leafy spurge. Exogenously applied *Put*, *Spd*, *Spm* and DFMO appear to be taken up readily by

hypocotyl segments. Further work on endogenous levels of polyamines, conjugates and bound forms is underway to aid in the clarification of their role in the control of plant growth.

Acknowledgements

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