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BRIEF COMMUNICATION

Stimulation of growth and nitrate assimilation in *Leucaena leucocephala* seedlings in response to spermidine supply

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Abstract

Supply of 100 μM spermidine (Spd) in the nutrient solution containing 10 mM nitrate as the sole nitrogen source, increased growth of roots and shoots, total nitrogen content and *in vivo* or *in vitro* nitrate reductase (NR) activity of leaves of 10-d old *Leucaena leucocephala* seedlings. Spd and the cytokinins benzyladenine or kinetin also increased growth, total nitrogen and *in vivo* NR activity of isolated cotyledons. The synergistic effects of nitrate, kinetin and Spd in increasing NR activity, indicate that the Spd acted at different level than the nitrate or cytokinin.

Key words: cytokinins, dry mass, fresh mass,nitrate reductase, nitrogen content

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Plant polyamines, spermine, spermidine (Spd), putrescine and cadaverine are considered to be important growth regulators and/or second messengers in hormone action (Galston 1983, Galston and Kaur-Sawhney 1987). High levels of endogenous polyamines are often correlated with rapid growth (Evans and Malmberg 1989). Exogenous supply of polyamines affects a variety of processes in growing as well as senescent organs (Smith 1985). The magnitude of the effect is obviously dependent upon the endogenous polyamine content. The effect of exogenous polyamine supply on nitrogen assimilation, however has not been examined, although the process is of fundamental importance to plant growth and development. Moreover, polyamine effect on nitrogen assimilation may be expected because of the observation that (a) nitrogen influences the endogenous polyamine content (Smith 1985, Tonin *et al.* 1991) and (b) polyamines can act as source of nitrogen, at least in cultured tissues (Bagni *et al.* 1978, Balint *et al.* 1987). The effect of Spd on growth and nitrate assimilation in cotyledons, roots and leaves of the seedlings of a tree species,

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Leucaena leucocephala was studied in the present investigation, to examine this possibility. Spermidine was used in our studies, as Spd supplied plants appeared to be taller and greener than the spermine supplied ones.

Seeds of *Leucaena leucocephala* L. cv. K-8 collected locally were surface sterilized with 0.1 % $CaOCl_2$ for about 10 min, washed thoroughly with distilled water, soaked in hot distilled water (about 80 °C) for 5 - 8 min and then imbibed at room temperature (27 ± 3 °C) for 2 d. Seeds were then planted in acid washed sand. Seedlings were raised in either 12 h irradiance (*ca.* 70 W m^{-2}) for 10 d or in darkness for 6 - 7 d, with half strength Hoagland's solution containing either no nitrogen or 10 mM nitrate as the sole nitrogen source. Spd was dissolved in the nutrient solution to make up the concentration of 100 μM, when required. This concentration was used following an evaluation of the data of a preliminary experiment with 10, 50, 100 and 200 μM Spd. Growth of the tissues was measured by weighing the selected group of plant organs. Nitrate reductase activity in the fresh tissues was measured by either *in vivo* (Srivastava 1974) or *in vitro* (Srivastava and Ormrod 1984) method.

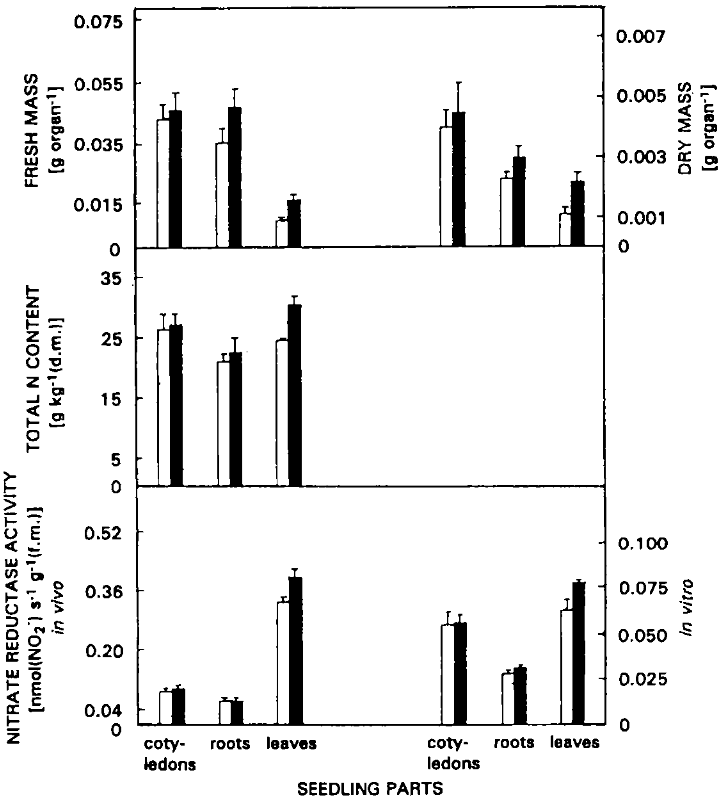

Fig. 1. Effect of spermidine on growth, total nitrogen content and nitrate reductase activity in *Leucaena leucocephala* seedlings. Seedlings were raised on half strength Hoagland's solution containing 10 mM nitrate as the sole nitrogen source with (*open columns*) or without (*full columns*) 100 μM Spd in light for 10 d.

Total nitrogen content of the dried tissues was measured by the micro-Kjeldahl method (Lang 1958). The data presented in this paper are average ± S.D. of three experiments in duplicate (n = 6).

The supply of 100 μM Spd in nutrient solution increased fresh and dry mass of the roots and leaves and total organic nitrogen content in leaves substantially (Fig. 1). There was little effect on cotyledons. NR activity, assayed by both *in vivo* and *in vitro* procedure, was increased by Spd supply only in leaves.

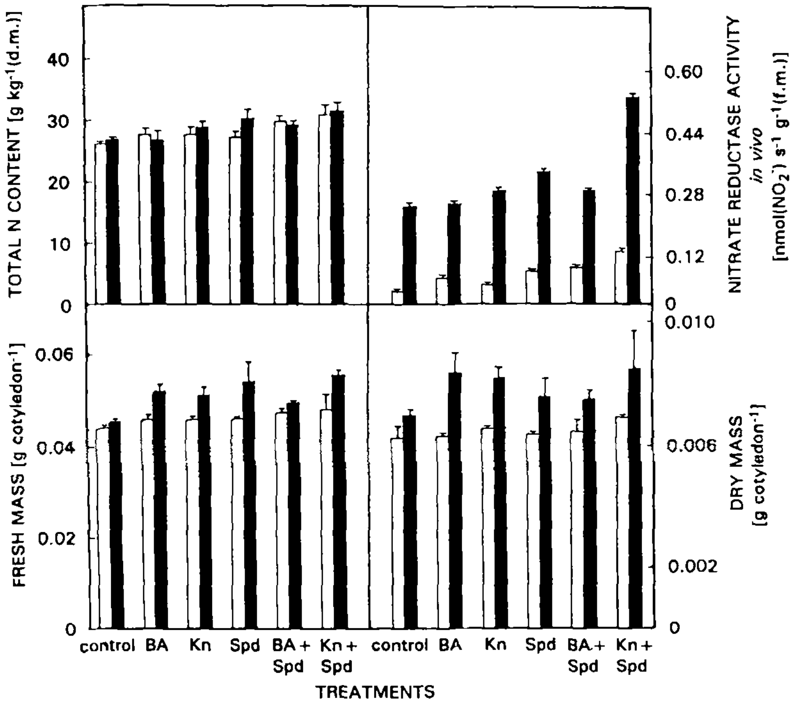

Fig. 2. Effect of cytokinins (50 μM) and spermidine (100 μM) on growth, total nitrogen content and nitrate reductase activity in isolated cotyledons in the absence or presence of nitrate. Cotyledons from 6 - 8 d old, dark grown *Leucaena leucocephala* seedlings were incubated in half strength Hoagland's solution containing either no nitrogen (*open columns*) or 10 mM nitrate (*full columns*) as nitrogen source for 24 h in light.

Growth of isolated cotyledons incubated in nutrient solution containing no nitrogen was little affected by benzyladenine (BA, 50 μM), kinetin (50 μM) or Spd (100 μM), although it increased appreciably with each of the growth regulator when nitrate was included in the incubation medium (Fig. 2). Total nitrogen content, however, increased in the absence of nutrient nitrogen in BA + Spd and in kinetin + Spd supplied cotyledons. Spd alone increased total nitrogen content of the cotyledons incubated in the nutrient solution containing nitrate. Each treatment increased *in vivo* NR activity in the cotyledons except for BA in the presence of nitrate. Kinetin and

Spd seemed to be having an additive effect in increasing NR activity; both in the absence as well as presence of nitrate in the incubation medium.

The effects of Spd on growth and nitrate assimilation in the seedlings of *Leucaena* is organ dependent; while there was stimulation of root and shoot growth, there was no effect on cotyledons. In intact seedlings, the cotyledons are known to export metabolites including polyamines and act as a source rather than the sink for metabolites and growth regulators (Chirst *et al.* 1989, Gallardo *et al.* 1992). The polyamine increased *in vivo* or *in vitro* NR activity, which was also reflected in the increased organic nitrogen in the leaves of intact seedlings and in isolated cotyledons. The non-responsiveness of the root enzyme, however, is not understood at present. As reported earlier for cotyledons from light grown seedlings (Pandey and Srivastava 1992), the cytokinins, BA and kinetin, increased *in vivo* NR activity in cotyledons from dark grown seedlings also. In the present study, the stimulatory effect of Spd on enzyme activity was synergistic to that of kinetin or nitrate (Fig. 2). Both of these, cytokinins (Lu *et al.* 1990) as well as nitrate (Metzger and Hazebrock 1989), are known to act at transcription level in NR induction. Apparently, the Spd acts probably at some other step of the enzyme synthesis/activation, and it is not only supplementary to the cytokinin, as is suggested for some other polyamine responses (Galston 1983). On the other 'hand, there is no synergism between BA and Spd supply. Among other reasons, it is likely that BA supply may increase endogenous polyamine content (Suresh *et al.* 1978, Walker *et al.* 1988) and the exogenous supply of Spd may elevate it further to a supraoptimum level, causing no apparent effect.

References

Bagni, N., Calzoni, G.L., Speranza, A.: Polyamines as sole nitrogen sources for *Helianthus tuberosus* explant *in vitro*. - New Phytol. **80**: 317-323, 1978.

Balint, R., Cooper, G., Stalbelt, M., Filner, P.: N-caffeoyl-4-amino n-butyric acid: a new flower specific metabolite in cultured tobacco cells and tobacco plants. - J. biol. Chem. **262**: 11062-11071, 1987.

Chirst, M., Harr, J., Felix, H.: Transport of polyamines in sugarbeet seedlings. - Z. Naturfoschung. **44 c**: 59-63, 1989.

Evans, P.T., Malmberg, R.L.: Do polyamines have roles in plant development ? - Annu. Rev. Plant Physiol. Plant mol. Biol. **40**: 235-269, 1989.

Gallardo, M., Matilla, A.J., Sanchez-Calle, I.M.: Effects of spermine, abscisic acid and temperature upon ethylene production in *Cicer arietinum* seeds. - Plant Physiol. Biochem. **30**: 19-27, 1992.

Galston, A.W.: Polyamines as modulators of plant development. - Bioscience **33**: 382-388, 1983.

Galston, A.W., Kaur-Sawhney, R.: Polyamines as endogenous growth regulators. - In: Davies, P.J. (ed.): Plant Hormones and Their Role in Plant Growth and Development. Pp. 280-295. Martinus Nijhoff, Boston 1987.

Lang, C.A.: Simple microdetermination of Kjeldahl nitrogen in biological materials. - Anal. Chem. **30**: 1692-1694, 1958.

Lu, J.-L., Ertl, J.R., Chen, C.M.: Cytokinin enhancement of the light induction of nitrate reductase transcript levels in etiolated barley leaves. - Plant mol. Biol. **14**: 585-594, 1990.

Metzger, J.D., Hazebrock, P.J.: Isolation of gibberellin precursors from heavily pigmented tissues. - Plant Physiol. **91**: 1488-1493, 1989.

Pandey, S., Srivastava, H.S.: Cytokinin effects on nitrate reductase activity in *Leucaena leucocephala* seedlings in the presence of different nitrogenous salts. - Indian J. Plant Physiol. **35**: 64-72, 1992.

Smith, T.A.: Polyamines. - Annu. Rev. Plant Physiol. **36**: 117-143, 1985.

Srivastava, H.S.: *In vivo* activity of nitrate reductase in maize seedlings. - Indian J. Biochem. Biophys. **11**: 230-232, 1974.

Srivastava, H.S., Ormrod, D.P.: Effects of nitrogen dioxide and nitrate nutrition on growth and nitrate assimilation in bean leaves. - Plant Physiol. **76**: 418-423, 1984.

Suresh, M.R., Ramakrishna, S., Adiga, P.R.: Regulation of arginine decarboxylase and putrescine levels in *Cucumus sativus* cotyledons. - Phytochemistry **17**: 57-63, 1978.

Tonin, G.S., Wheeler, C.T. Crozier, A.: Effect of changes in nitrogen nutrition on the polyamine content of *Alnus glutinosa*. - Plant Cell Environ. **14**: 415-421, 1991.

Walker, M.A., Roberts, D.R., Dumbroff, E.B.: Effect of cytokinin and light on polyamines during the greening response of cucumber cotyledons. - Plant Cell Physiol. **29**: 201-205, 1988.