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BRIEF COMMUNICATION

# Putrescine effect on nitrate reductase activity, organic nitrogen, protein, and growth in heavy metal and salinity stressed mustard seedlings

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## Abstract

Putrescine effect on nitrate reductase activity, organic nitrogen and protein contents, and plant growth under Cd or Pb (0.1 - 2 mM) and salinity (5 and 100 mM NaCl) stresses was examined in Indian mustard (*Brassica juncea* L. cv. RH-30) seedlings. Cd or Pb and salinity inhibited nitrate reductase activity and decreased organic nitrogen and protein contents in leaf tissue. The increased nitrate reductase activity induced by putrescine was correlated with increased organic nitrogen and protein contents and growth of plants.

*Additional key words*: *Brassica juncea*, cadmium, lead, NaCl.

Reduction in growth and yield of crop plant under metal and salinity stress is due to impairment of growth related metabolic activities. Nitrate reductase activity (NRA) is considered to be a marker of nitrogen assimilation potential (Mishra and Srivastava 1983, Srivastava 1992). Heavy metal causes either inhibition in NRA (Burzynski and Grabowski 1984, Singh *et al.* 1988, Sinha *et al.* 1988a, Mishra *et al.* 1994) or induction at lower dose (Sinha *et al.* 1988b). Salinity also inhibited nitrogen assimilation, which was found to be unchecked even by exogenous supply of either $NO_3^-$ in *Nicotiana tobacco* (Sweby *et al.* 1994) or $NH_4^+$ in peanut and cotton (Leidi *et al.* 1992). Nitrogen containing compounds like amino acids, imino acids, amides including polyamines accumulation are considered to confer salt tolerance in plants (see Mansour 2000). Diamine putrescine (Put), is considered to be plant growth regulator (*e.g.* Smith 1985, Galston and Kaur-Sawhney 1990). It is suggested that polyamines including putrescine may alleviate stress responses of plants (Bouchereau *et al.* 1999, Kakkar *et al.* 2000). Moreover, polyamines, considered to be good nitrogen source for cultured tissues in general (Balint *et al.* 1987) and putrescine for stressed plants (Mishra and Sharma 1994). It may modulate stressed plant growth through stimulation of NRA even under twin stresses, a field condition often realized by plants. Therefore, study was performed to examine the putrescine effect on NRA, organic nitrogen and protein contents in leaf tissues of Indian mustard growing under heavy metal and salinity stress. The Put response was compared with ammonium nitrate as nitrogen source.

Indian mustard seeds (*Brassica juncea* L. cv. RH-30) were planted in Petri plates lined with filter paper after sterilization with 0.1% bleaching power followed by thorough washing with sterilized water. Seedlings were raised at controlled condition (photoperiod of 16 h, irradiance 73 W $m^{-2}$, temperature 25 ± 2 °C, and RH 60 - 70 %) in half strength Hoagland nutrient containing either Cd or Pb (0.1 - 2.0 mM) with or without putrescine (1 mM) or ammonium nitrate (10 mM). Salinity was created by adding NaCl (5 mM or 100 mM) in the nutrient solutions. For all measurements and estimation random sampling of morphologically and physiologically similar seedlings ($n$ = 50) were done after 7 d of sowing. Nitrate reductase activity was assayed either *in vivo* (Srivastava 1975) or *in vitro* (Stevens and Oaks 1973) in fresh leaves. Total organic nitrogen was estimated by Kjeldahl method (Lang 1958). Protein content was calculated by multiplying alcohol insoluble fraction of nitrogen with a factor of 6.25. Measurement of seedling growth in terms of fresh mass and dry mass was also done after 7 d of sowing. Dry mass was measured after

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*Abbreviations*: NRA - nitrate reductase activity; Put - putrescine.
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drying the sample at 65 °C in oven for 72 h.

Data represents the mean of three separate sets of experiments with ± S.D. *ANOVA* (two ways) was applied to test the significance of treatments.

Nitrate reductase activity measured *in vivo* decreased over 30 - 35 % at both the concentration of Cd or Pb as compare to control (Fig. 1). The decrease was further enhanced by salinity especially in 100 mM NaCl. The Pb (Sinha *et al.* 1988a,b), Cd (Singh *et al.* 1988, Mishra *et al.* 1994), and salinity (Leidi *et al.* 1992, Sweby *et al.* 1994) caused inhibition in NRA. Put supplementation protected the enzyme activity from both Pb or Cd inhibition as well as metal plus salinity inhibition. Putrescine elevation of enzyme activity was remarkable under severe stress (2 mM Pb or Cd + 100 mM NaCl). *In vitro* enzyme activity showed almost 50 % decrease with 0.1 or 2.0 mM Pb and Cd. This was protected in the presence of putrescine. The experiments performed here

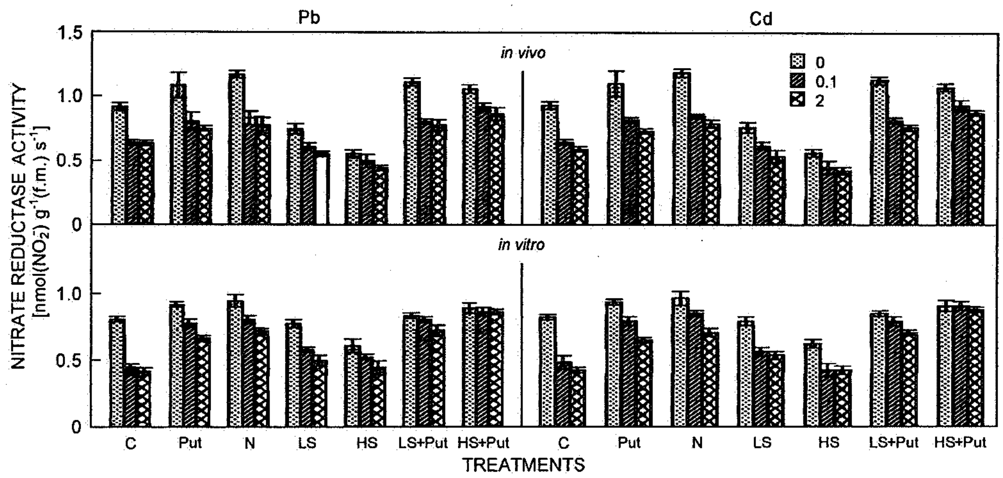


Fig. 1. Effect of Pb and Cd (0, 0.1 or 2 mM), putrescine, and NaCl on *in vivo* and *in vitro* measured nitrate reductase activity in leaves of the 7-d-old seedlings. Means of 3 separate sets of experiments ± S.D. Data significant at $P > 5$ % and 1 % using two way *ANOVA*. C - control, Put - putrescine (1 mM), N - $NH_4NO_3$ (10 mM), LS - low salinity (5 mM NaCl), HS - high salinity (100 mM NaCl).

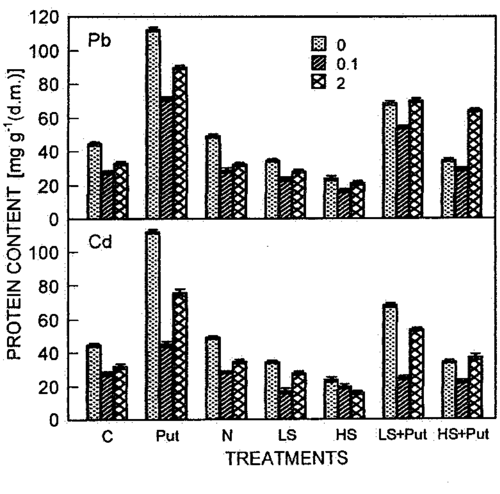


Fig. 2. Effect of putrescine on protein content in leaves of the seedlings growing under lead or cadmium and NaCl stress. Rest legend is the same as in Fig. 1.

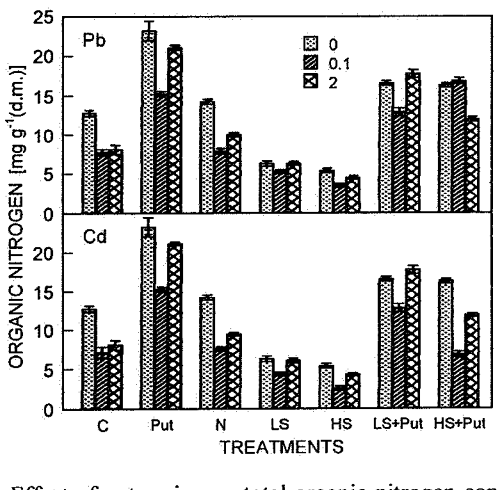


Fig. 3. Effect of putrescine on total organic nitrogen content in leaves of the seedlings growing under lead or cadmium and NaCl stress. Rest legend is the same as in Fig. 1.

demonstrated the Put stimulation of enzyme activity under multiple stresses. Unlike to Sweby *et al.* (1994) and Leidi *et al.* (1992), our experiments showed that $NH_4NO_3$ might also modulate enzyme activity inhibited due to metal stress, at least in *B. juncea* L. cv. RH-30. Putrescine effect on NR activity under different conditions has been observed in maize (Shankar *et al.* 2001).

Metal and salinity stress induced decline in protein (Fig. 2) and total organic nitrogen (Fig. 3) contents was also retarded by putrescine. The putrescine induced increment in organic nitrogen and protein contents was over two-fold. The data show that NR activity is correlated with organic nitrogen and protein content in the leaf of the seedling even under twin stresses. Salinity and metal stress adversely affected the seedling growth which was more pronounced at 2 mM Cd than Pb (Figs. 4, 5). Cd or Pb (2 mM) inhibited dry mass accumulation (20 % over control) which was further inhibited slightly under high NaCl concentration (Fig. 5). The seedling growth inhibition has been observed in *Brassica* under metal (Mishra *et al.* 1994) as well as salinity stress (Sharma *et al.* 1990). Enhanced dry mass production with Put supplementation indicates alleviation of negative effects of both stresses. The comparison with the effect of 10 mM $NH_4NO_3$ supports the hypothesis that Put may act not only as nitrogen source for plant growth under stress condition but also as a growth regulator, even under multiple stress condition.

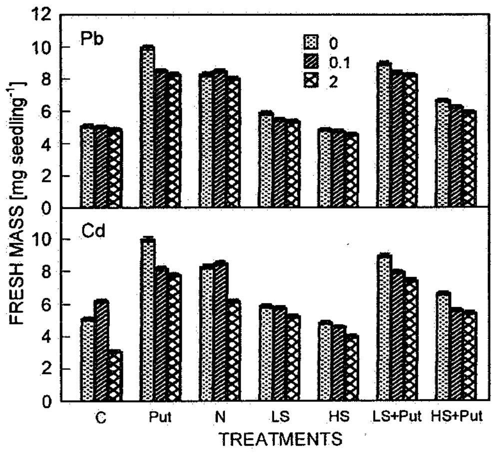


Fig. 4. Effect of putrescine, lead or cadmium and NaCl stress on fresh mass of the leaves of the 7-d old seedlings. Rest legend is the same as in Fig. 1.

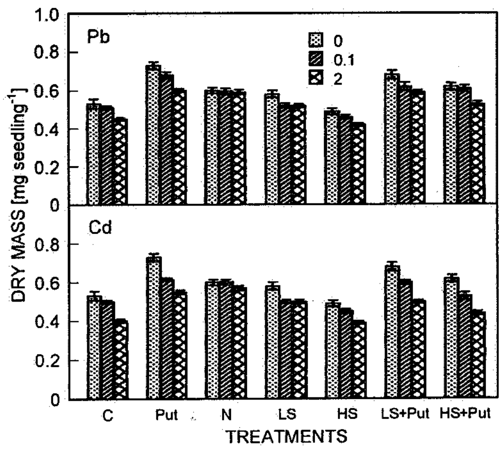


Fig. 5. Effect of putrescine, Pb or Cd and NaCl stress on dry mass of the 7-d old seedlings. Rest legend is the same as in Fig. 1.

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