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BRIEF COMMUNICATION

Influence of cadmium and zinc on growth and photosynthesis of *Bacopa monniera* cultivated *in vitro*

G. ALI, P.S. SRIVASTAVA and M. IQBAL*

Department of Botany, Faculty of Science, Hamdard University, Hamdard Nagar, New Delhi - 110 062, Indi

Abstract

This study evaluates the impact of cadmium and zinc interaction on the amount of soluble proteins, CO_2 fixati stomatal conductance and intercellular CO_2 contents in regenerants of *B. monniera*. The regenerants were grown 16 weeks on MS medium containing cadmium and zinc in various concentrations. Cadmium decreased the stom conductance, photosynthetic rate and root growth but increased the protein content. Additional supply of zinc in medium reduced the adverse effects of cadmium on these parameters.

Additional key words: intercellular CO_2 concentration, net photosynthetic rate, protein content, root length, stomatal conductance.

Although certain metals like copper and zinc are essential for plants and are used as micronutrients, they inhibit plant growth and development when present above a critical level and behave like other heavy metals such as Cd, Hg and Pb which have no known function in plant metabolism (Ali *et al.* 1998a, 1999ab). Zinc becomes toxic at higher concentrations than cadmium (Van Assche and Clijsters 1986ab, Ali *et al.* 1999a) and its uptake and translocation in plants are greater than those of cadmium (Chakravarty and Srivastava 1997). We have used *Bacopa monniera* regenerants for analysing the effect of various combinations of cadmium and zinc on growth, soluble protein contents, stomatal conductance and net photosynthetic rate.

The cultures of *Bacopa monniera* (L.) Wettst. were established as described earlier (Ali *et al.* 1998ab). Four-week-old cultures with 500 mg fresh mass were transferred on Murashige and Skoog's (1962; MS) medium with 2 % sucrose, 0.2 mg dm^{-3} NAA, 0.5 mg dm^{-3} BAP and 50 mg dm^{-3} glutamine. This medium was also supplied with various combinations (25 + 25, 25 + 50, 50 + 25, 50 + 50 μM) of Cd (cadmium acetate) + Zn (zinc sulphate), respectively. The cultures were maintained at temperature of 25 ± 2 °C, relative humidity 55 ± 5 % and 14-h photoperiod (irradiance of 100 μmol m^{-2} s^{-1}). Root formation and root length were monitored after 4 weeks of growth. Total soluble protein content was estima following the method of Bradford (1976) using bov serum albumin as standard. *LI-6200 Portable Phc synthesis System* (*LI-COR*, Lincoln, USA) was used automatic measurement of stomatal conductance, inter CO_2 concentration and the net photosynthetic rate in samples.

Cadmium hampered development and growth of ro and shoots (Table 1). The stunted roots developed stun ends. Shoots became necrotic at the distal ends while l size and internode length got reduced. The effects w concentration dependent. Rooting ceased complet when cultures were kept on Cd-supplemented medium long duration (up to 16 weeks). These results corrobor the earlier findings on *Lupinus albus* (Costa and Sp 1997) and *Solanum melongena* (Mehindirata *et al.* 199 On the other hand, stimulatory effect of Zn has be demonstrated in many plant species (Chakravarty a Srivastava 1997, Ali *et al.* 1999a). Due to chemi similarity of Cd and Zn, an additional supply of reduces the toxicity of Cd. These results are in line w some earlier ones (Van Assche and Clijsters 1986a Chakravarty and Srivastava 1997) and suggest t additional Zn in the Cd-containing medium brings abo certain degree of recovery in plant growth.

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Abbreviations: BAP - 6-benzylaminopurine; NAA - 1-naphthaleneacetic acid.

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Cadmium treatment increased the content of soluble protein in *B. monniera* (Table 1). It is suggested that the presence of Cd stimulates mRNA synthesis leading to increase in the total protein (Hirt *et al.* 1989). Additional supply of $ZnSO_4$ caused minor reduction in the protein content and it was more than 3-fold higher in comparison to control.

Table 1. Effects of cadmium and zinc on root development, protein content, net photosynthetic rate (P_N), stomatal conductance (g_s), and internal CO_2 concentration (c_i) in 4-week-old regenerants of *B. monniera*. Mean ± SE based on 6 replicates; experiments were repeated twice.

Cd [μM]	Zn [μM]	Root formation [%]	Root length [cm]	Protein content [μg g^{-1}(f.m.)]	P_N [μmol(CO_2) $m^{-2}s^{-1}$]	g_s [mmol(H_2O) $m^{-2}s^{-1}$]	c_i [μmol mol^{-1}]
0	0	100	2.5 ± 0.03	6.2 ± 0.19	15.30 ± 0.16	0.384 ± 0.04	424 ± 12.5
25	0	94	1.8 ± 0.05	19.2 ± 0.13	12.20 ± 0.19	0.335 ± 0.02	342 ± 16.2
50	0	44	0.7 ± 0.08	24.2 ± 0.18	11.30 ± 0.14	0.312 ± 0.06	318 ± 13.2
0	25	100	2.7 ± 0.03	6.4 ± 0.14	15.30 ± 0.12	0.389 ± 0.03	330 ± 11.6
0	50	100	3.2 ± 0.07	6.8 ± 0.17	15.35 ± 0.17	0.393 ± 0.01	438 ± 12.2
25	25	100	2.0 ± 0.04	17.6 ± 0.19	12.70 ± 0.14	0.340 ± 0.09	350 ± 17.3
25	50	100	2.3 ± 0.09	15.3 ± 0.15	13.00 ± 0.19	0.345 ± 0.02	353 ± 16.3
50	25	70	1.2 ± 0.02	22.2 ± 0.12	11.60 ± 0.13	0.315 ± 0.05	323 ± 18.2
50	50	100	1.5 ± 0.06	20.7 ± 0.17	12.00 ± 0.11	0.319 ± 0.08	327 ± 11.6

Net photosynthetic rate, stomatal conductance and internal CO_2 concentration declined in the presence of Cd (similarly as they did due to excess of NaCl - Ali *et al.* 1999c), and they were improved by the additional supply of Zn. Photosynthesis might be inhibited by Cd due to disturbance in chlorophyll biosynthesis and electron transport (Bhardwaj and Mascarenhas 1989, Skorzynska-Polit and Baszynski 1997, Costa and Spitz 1997). Zn influences cell division and cell expansion besides having a role in chlorophyll formation (Davies *et al.* 1991, Hagemeyer and Breckle 1996). Zn can improve photosynthesis, stomatal conductance and translocation of photosynthates under Cd stress.

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