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BRIEF COMMUNICATION

Effect of cadmium and copper on growth of *Bacopa monniera* regenerants

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Abstract

The effects of cadmium and copper on *in vitro* growth of *Bacopa monniera* (L.) Wettst. was monitored. Cadmium (25 and 50 μM $CdCl_2$) inhibited plantlet growth and addition of 50 or 100 μm $CuSO_4$ partially alleviated this negative effect. Cadmium increased both protein and proline contents, but to a lesser extent with the additional supply of $CuSO_4$.

Additional key words: proline, protein, regeneration.

Plants are often damaged due to the increasing pollution in the atmosphere by toxic chemicals. As it is often not feasible to modify the environment to suit the plants, efforts are now being made to modify the plants to suit the adverse environmental conditions. Metal-tolerant plants can help in reclaiming degraded land. Cadmium is one of the common industrial pollutants harmful to plants at low concentrations (*e.g.* Chakravarty and Srivastava 1992, Ali *et al.* 1998a). Copper is a micronutrient acting as a cofactor and/or as a part of prosthetic group of enzymes. Copper is toxic at higher concentrations than cadmium (Arduini *et al.* 1994) and the uptake and translocation of copper by plants are higher than those of cadmium (Gussarson *et al.* 1995). The association of cadmium and copper in environment and their chemical similarity can lead to interactions between these two ions (Gussarson *et al.* 1995), resulting in the lowering of cadmium toxicity (Van Assche *et al.* 1988, Wajda *et al.* 1989).

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Abbreviations: BAP - 6 - benzylaminopurine; MS - Murashige and Skoog's medium; NAA - 1-naphthaleneacetic acid.

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The present investigation was undertaken to *1*) regenerate plants on cadmium-containing medium, and *2*) ascertain the extent to which copper can alleviate the toxicity of cadmium during *in vitro* regeneration in *Bacopa*. The regeneration capability of plants grown on Cd and Cu was correlated to some stress-related markers such as proline (Chakravarty and Srivastava 1997a, Ali *et al.* 1998a,b,c,d) and protein (Liu and Li 1991, Chakravarty and Srivastava 1997a, Ali *et al.* 1998b,d) contents.

The cultures of *Bacopa monniera* (L.) Wettst. were established as described earlier (Ali *et al.* 1998 a,b,c,d). Four-week-old cultures with five, 20 mm long regenerants (each about 500 mg) were transferred on the maintenance medium (MM), *i.e.* MS + 2 % sucrose + 0.2 mg dm^{-3} NAA + 0.5 mg dm^{-3} BAP + 50 mg dm^{-3} glutamine. This medium was also supplied with various combinations (25 + 50, 25 + 100, 50 + 50, 50 + 100, 75 + 50, 75 + 100, 100 + 50, 100 + 100 μM) of Cd (cadmium chloride) and Cu (copper sulphate). All 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}). Fresh mass, number of shoots per vessel, shoot height and root differentiation were monitored at regular intervals. Proline content of the regenerants was determined following the method of Bates *et al.* (1973). Total-soluble protein content was estimated following the method of Bradford (1976) using bovine serum albumin as standard.

The regenerants of *Bacopa monniera* did not survive beyond 50 μM Cd but these could tolerate Cd up to 100 μM if grown with additional supply of Cu. On medium with Cd (without Cu), a decline was seen in all growth parameters, compared to the control (Table 1, 2). The decline was concentration and duration dependent (Table 1). Copper (50 and 100 μM $CuSO_4$) slightly stimulated growth of regenerants (Table 1). The addition of $CuSO_4$ alleviated negative effects of Cd (Table 1). The cultures were able to grow on Cd and Cu-containing media for the last three years. These results are in line with some earlier findings (Gussarson *et al.* 1995, Chaoui *et al.* 1997, Chakravarty and Srivastava 1997b, Ali *et al.* 1998a) and suggest that the additional supply of Cu in the Cd-containing medium causes certain degree of recovery in plant growth.

Addition of cadmium increased the proline content of the regenerants 2-fold (Table 2). The cultures grown on different combinations of Cd and Cu also had increased proline content, but to the lesser extent (Table 2). Since Cd ions are known to affect the integrity of membranes (Reddy and Prasad 1992), changes in the permeability can lead to water-stress like conditions resulting in an increase in proline levels (Pesci and Reggiani 1992). In *Bacopa* also, proline content increases in the presence of Cd in the medium, suggesting that permeability of membrane is being affected. Similar results were obtained in *B. monniera* cultures grown under salt stress (Ali *et al.* 1999).

The total protein content in *Bacopa* regenerants also increased with increasing concentration of cadmium in the medium. Similar results were obtained with linseed under cadmium stress (Chakravarty and Srivastava 1997a) and in *Bacopa* under aluminium stress (Ali *et al.* 1998b). Presence of cadmium might stimulate mRNA synthesis leading to a rise in the total protein content (Hirt *et al.* 1989). Additional

Table 1. Effect of $CdCl_2$ and $CuSO_4$ in different concentrations on growth of *Bacopa monniera*. Means ± SD based on 24 replicates; the experiment was repeated thrice. Treatments lasted for 4, 12 and 16 weeks. Five shoots of 1 - 1.5 cm height with 500 mg fresh mass were initially (every fourth week) inoculated in each culture vial.

Treatments		4 weeks			12 weeks			16 weeks		
$CdCl_2$ [µM]	$CuSO_4$ [µM]	shoot number [vessel^{-1}]	shoot height [cm]	fresh mass [g vessel^{-1}]	shoot number [vessel^{-1}]	shoot height [cm]	fresh mass [g vessel^{-1}]	shoot number [vessel^{-1}]	shoot height [cm]	fresh mass [g vessel^{-1}]
00	00	15 ± 2.81	5.2 ± 1.56	1.70 ± 0.39	17 ± 2.14	5.4 ± 1.62	1.73 ± 0.43	18 ± 2.11	5.2 ± 1.54	1.75 ± 0.47
25	00	11 ± 2.56	3.9 ± 1.15	1.20 ± 0.32	12 ± 2.11	4.1 ± 1.23	1.29 ± 0.29	12 ± 2.13	4.1 ± 1.15	1.31 ± 0.24
50	00	9 ± 2.23	2.0 ± 1.02	0.93 ± 0.16	5 ± 1.28	1.1 ± 0.78	0.55 ± 0.18	succumbed		
00	50	16 ± 2.12	4.5 ± 1.30	1.80 ± 0.26	17 ± 2.32	5.3 ± 1.26	1.85 ± 0.24	17 ± 2.16	5.5 ± 1.18	1.79 ± 0.32
00	100	18 ± 2.23	5.4 ± 1.24	1.90 ± 0.15	20 ± 2.19	5.7 ± 1.34	1.95 ± 0.14	22 ± 2.11	6.0 ± 1.23	2.10 ± 0.17
25	50	13 ± 2.67	4.0 ± 1.23	1.29 ± 0.23	14 ± 2.29	4.2 ± 1.18	1.37 ± 0.26	16 ± 2.23	4.4 ± 1.14	1.45 ± 0.23
25	100	15 ± 2.91	4.5 ± 1.50	1.42 ± 0.25	16 ± 2.76	4.8 ± 1.45	1.50 ± 0.26	17 ± 2.79	5.0 ± 1.36	1.68 ± 0.29
50	50	10 ± 2.83	3.0 ± 1.39	1.19 ± 0.27	12 ± 2.65	3.2 ± 1.43	1.28 ± 0.23	13 ± 2.54	3.6 ± 1.32	1.38 ± 0.26
50	100	13 ± 2.75	3.5 ± 1.12	1.28 ± 0.22	14 ± 2.82	3.7 ± 1.19	1.32 ± 0.18	15 ± 2.76	3.9 ± 1.23	1.45 ± 0.15
75	50	8 ± 2.56	2.0 ± 1.30	1.10 ± 0.19	10 ± 2.43	2.1 ± 1.41	1.18 ± 0.15	11 ± 2.42	2.3 ± 1.34	1.25 ± 0.18
75	100	10 ± 2.65	2.1 ± 1.21	1.20 ± 0.15	11 ± 2.56	2.5 ± 1.34	1.24 ± 0.16	12 ± 2.43	2.5 ± 1.29	1.32 ± 0.12
100	50	5 ± 1.99	1.7 ± 1.11	0.95 ± 0.11	6 ± 1.45	1.8 ± 1.23	1.11 ± 0.13	7 ± 1.60	2.0 ± 1.21	1.15 ± 0.16
100	100	7 ± 1.65	1.9 ± 1.29	1.12 ± 0.29	7 ± 1.34	2.0 ± 1.11	1.15 ± 0.18	9 ± 1.67	2.1 ± 1.17	1.20 ± 0.19

incorporation of Cu in the presence of Cd caused less increase in the protein content (Table 2).

Table 2. Effect of $CdCl_2$ + $CuSO_4$ on proline and protein contents in 16-week-old cultures of *Bacopa monniera*. Each treatment consisted of 3 replicates; the experiment was repeated thrice.

$CdCl_2$ [μM]	$CuSO_4$ [μM]	Proline content [μg g^{-1}(f.m.)]	Protein content [mg g^{-1}(f.m.)]	Rooting [%]
00	00	21.8 ± 0.12	6.2 ± 0.19	100
25	00	43.0 ± 0.19	19.2 ± 0.12	92 ± 2.11
50	00	48.8 ± 0.23	24.2 ± 0.15	42 ± 2.32
25	50	42.0 ± 0.43	17.5 ± 0.18	100
25	100	39.0 ± 0.33	15.1 ± 0.13	100
50	50	47.3 ± 0.28	22.5 ± 0.17	65 ± 2.16
50	100	46.0 ± 0.22	21.2 ± 0.11	82 ± 2.42
75	50	47.7 ± 0.38	23.7 ± 0.15	55 ± 2.18
75	100	46.5 ± 0.15	22.7 ± 0.12	60 ± 2.23
100	50	48.6 ± 0.23	26.2 ± 0.19	35 ± 2.35
100	100	47.5 ± 0.27	25.2 ± 0.11	47 ± 2.19

This study suggests that cadmium-tolerant plants can be regenerated on a medium containing cadmium and copper, and that the toxicity of cadmium can be circumvented by the addition of copper. The regenerants on such a medium show better growth and have a smaller proline content.

Owing to the dual role of copper, as an essential micro-element on one hand and as a toxic environmental pollutant on the other, complete exclusion during root uptake is not possible. Since copper is more readily taken up and translocated to different plant organs than cadmium (Brune *et al.* 1994), this may be the reason for the higher shoot regeneration in *Bacopa* in the presence of copper. Thus, by adding non toxic levels of Cu to the Cd-containing medium, the process of obtaining the cadmium-tolerant plants can be enhanced. Most of the cadmium in these regenerants accumulates in the cytoplasm of the root, whereas copper is readily translocated to the shoots and accumulates in cell wall as well as cytoplasm (Gussarson *et al.* 1995, Chakravarty and Srivastava 1997a). Our attempts to transfer Cd-tolerant plants to the soil are in progress. Whether the Cd-tolerant character is inheritable is yet to be ascertained.

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