Screening of Indian mustard genotypes for heavy metal accumulation tendency under in-vitro conditions

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Abstract

Indian mustard (*Brassica juncea* L.Czern. & Coss.) have been established as a potential phytoremediator for heavy metals. It may be due to its inherent metal tolerance genes, which is not yet been identified. Heavy metal contamination of soils is increasing nowadays. Cadmium (Cd) and nickel (Ni) are some of the examples for heavy metals found in the soil. The ability of Indian mustard genotypes to accumulate Cd and Ni was assessed under controlled conditions. The soils were artificially contaminated by 3 and 5mM metal concentrations of both cadmium acetate and nickel sulphate with respect to control. Shoot length, root length, fresh weight and dry weight of genotypes were analyzed at 60, 90 and 105 days after germination. Heavy metal accumulation (Both roots and shoots) and yield attributes at the time of harvesting were also analyzed. Our results showed significant difference in the case of root length, shoot length, fresh weight and dry weight between Cd and Ni treatments. Exogenous supply of 3mM Cd and Ni caused less reduction in root and shoot length in most of the genotypes whereas at higher doses (5mM) caused more reduction. At a stress dose of 5mM, the nickel accumulation in the shoots and roots was on average of 16.1, 0.75µgm/gm dry weight respectively. For the same stress dose, cadmium accumulation in shoots and roots was an average of 31.40µgm/gm and 12.71µgm/gm dry weight respectively. In both the cases shoots accumulated more amount of heavy metals compared to roots. It is, therefore, *Brassica juncea* offers better accumulation potential for Cd compared to Ni.

Key words: Indian mustard, phytoextraction, nickel, cadmium

Introduction

Metals are intrinsic components of earth's crust. Heavy metals are the stable metals or metalloids whose density is greater than 5g/cm ^{3}, namely lead (Pb), copper (Cu), nickel (Ni), cadmium (Cd) and mercury (Hg). The heavy metal contamination has increased in urban vicinities due to rapid industrialization and ill environmental management (Wagner, 1993). The presence of heavy metals in agricultural ecosystem has raised concern not only for crop quality but also for human health. There is an increasing concern about the accumulation of Cd and Ni in food chains. Commonly used methods dealing with heavy metal pollution are either the extremely costly process of excavation and burial or simply isolation of the contaminated sites. But in recent years, researchers have observed fast growing, biomass accumulating plants including agronomic crops for the ability to tolerate and accumulate metals in their roots and shoots as a detoxification mechanism (Mkansdawire et al., 2004; Chaney et al., 2005; Chang et al., 2005; Fayiga and Ma, 2006). *Brassica juncea* is a high biomass crop, which has a potential for bioremediation of heavy metals like Cd (Singh et al., 2001; Quadir et al., 2004) and Ni (Panwar et al., 2002; Kerked and Kramer, 2003). Recently transgenic Indian mustard over expressing gamma glutamine cysteine synthetase (Schafer et al., 1998; Zhu et al., 1999) have been reported as potential hyperacculator and can be used as a viable cost effective alternative to clean up metal contaminated soils. In this study the ability of Indian mustard (*Brassica juncea*) to uptake Cd and Ni was assessed under controlled conditions.

Materials and methods

Ten different varieties of Indian mustard (B-350, CSR-94, CSR-931, DWDR-486, EC-223389, IC-94280, NDR-873, PR-45, RWPC-10 and RW-2Z) were received from IARI, Pusa, New Delhi. These experiments were conducted at the controlled conditions. Initially about 25 seeds were planted in each pot of 12" height with 8" bottom. Each pot contained about 10 kg of soil and supplied initially with basal dose of N, P, K and S and second dose of N and S after 45-day of sowing. These pots were watered with 3.0 and 5.0mM Cd^{2+} salt and Ni^{2+} salt dissolved in half strength Hoagland solution, first as basal dose, and second after 7 days of sowing i.e., just after emergence of the seedlings from the pots. 100ml of half strength Hoagland solutions was used to irrigate all the pots after 30 days of sowing and thereafter on every 15 days interval to maintain the soil nutrient mineral ratios in pots. Thinning of all the pots was done after 15 days of sowing and 10 plants in each pot were retained. Growth of the plants was measured after 60, 90,105 days of sowing. The fresh and dry weight were recorded at 60 and 125 days after seedling emergence. At the maturity of crop the yield attributes of the genotypes were determined in terms of number of pods per plant, number of seeds per pod, weight of 1000 seeds and total yield per plant. The metal accumulation in different plant parts was also determined on atomic absorption spectrophotometer after digestion in a mixture of nitric-perchloric acid and expressed on the dry matter basis. Data was analyzed statistically using ANOVA.

Results

Exogenous supply of 3mM Cd and Ni caused less reduction in root and shoot length where as 5mM stress caused more inhibition in growth that is evident from the Figure 1. Decrease noticed in root length was in the range of 19.5 % for 5mM Cd and 38.47% at 5mM Ni for 105 days after exposure. Shoot length was inhibited by 10.67% and 28.39% compared to the control when analyzed after 105 days of exposure to 5mM stress doses of Cd and Ni respectively. Figure 2 shows the effect on fresh and dry weights of the *Brassica juncea* genotypes after 60 and 125 days after exposure of heavy metals. In comparison to control, all the genotypes exhibited sensitivity to Ni and showed highly significant ($p \leqslant 0.05$) reduction in fresh weight and dry weight in the late growth periods. Fresh weight and dry weight of the genotypes reduced by 54.64% and 46.12% respectively after 125 days of Ni stress dose. The heavy metal Cd at the lower concentrations increased the fresh weight (13.11%) and dry weight (18.85%) compared to the fresh and dry weight of the control when analyzed after 125 days of exposure. An increasing Cd supply markedly reduced the fresh and dry weight of genotypes 125 old *B.juncea* genotypes supplemented with 5mM Cd are capable of accumulating 12.71μgm/gm of their roots and between 31.40μgm/gm of their shoots on a dry weight basis. Metal accumulated under a stress of 5mM Ni was 0.75 μgm/gm in roots and 16.11μgm/gm in shoots respectively. All Brassica genotypes showed much higher Cd accumulation in both roots and shoots at both concentrations compared to Ni accumulation under similar conditions which is evident from the data (Table 1). The results indicate that *Brassica juncea* differ significantly in Ni and Cd shoot root ratio and showed higher phytoextraction potential for Ni compared to Cd. At the completion of crop growth we analyzed yield attributes of the genotypes in terms of number of pods per plant, number of seeds per pod, weight of 1000 seeds, yield per plant (Table 2).

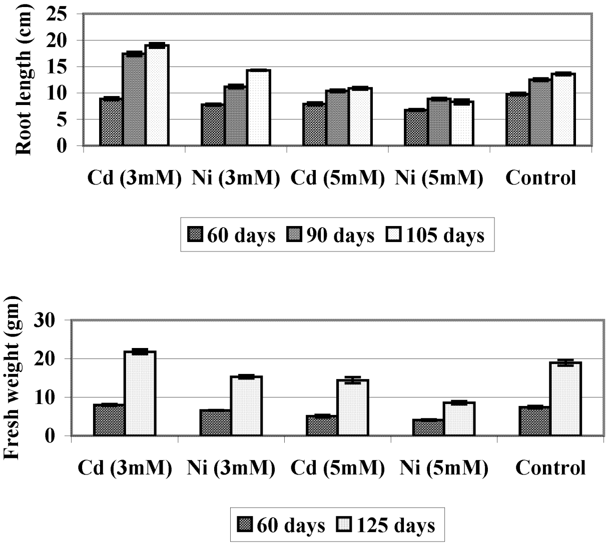

Fig. 1. The effect of Cd and Ni on root length and shoot length of *B.juncea* at different stress doses (3 &5mM) and at different days after exposure. The data represent means ± SE. Vertical bars represent SE.

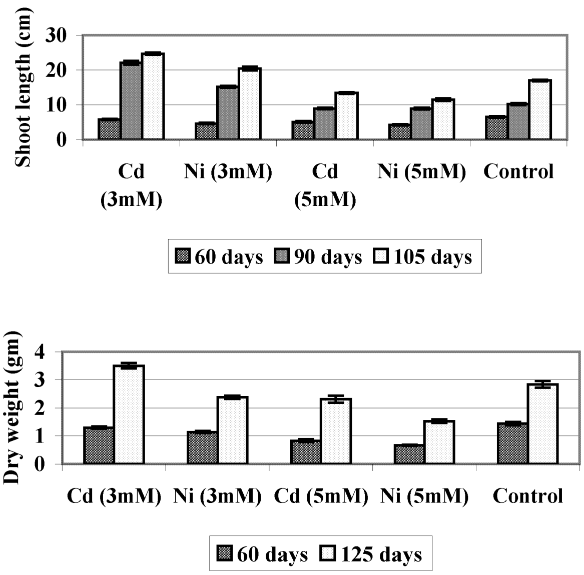

Fig. 2. The effect of Cd and Ni on fresh weight and dry weight of *B.juncea* at different stress doses (3 &5mM) and at different days after exposure. The data represent means ± SE. Vertical bars represent SE.

Table 1. Heavy metal accumulation and shoot / root ratio (S/R ratio) in Indian mustard genotypes grown in pots for 125 days with 3 and 5mM stress doses of cadmium acetate and nickel sulphate.

	Roots (µg/g) dry weight	Shoots (µg/g) dry weight	S/R ratio
3 mM Cd	5.93 (*0.2912*)	24.20 (*0.9163*)	4.06
5mM Cd	12.71 (*0.2762*)	31.40 (*0.6856*)	2.47
3mM Ni	0.27 (*0.0267*)	9.32 (*0.3021*)	35.86
5mM Ni	8.8 (*0.0240*)	16.11 (*0.3196*)	21.46

Mean of five replicates: in brackets: standard error ($p \leqslant 0.05$)

Table: 2 Yield Attributes of *B.juncea* genotypes after heavy metal exposure

	Control	Cadmium	Nickel
No of pods per plant [a]	72.88 (*4.700*)	68.53 (*2.017*)	59.77 (*0.7412*)
No of seeds per pod [b]	6.77 (*0.3239*)	5.88 (*0.9279*)	7.11 (*0.3093*)
Weight of 1000 seeds [c]	3.67 (*0.0837*)	3.28 (*0.0394*)	2.57 (*0.0570*)
Yield per plant [d]	1.77 (*0.1169*)	1.28 (*0.0768*)	1.08 (*0.0579*)

[a] Mean of 10 plant replicates [b]Mean of 1000 pods [c]Mean of 5 replicates [d]Mean of 10 plants
: in brackets standard error ($p \leqslant 0.05$)

Discussion

Heavy metal addition significantly reduced the root length and shoot length of *Brassica juncea* genotypes. A decrease in root and shoot length was observed in rice plants when grown for ten days in a medium containing these heavy metals (Rubio et al*.,* 1994). Ozturk et al., (2003) found similar affects using Cd in wheat cultivars. Recently Su et al., (2004) showed that Cd stress significantly reduced the root volume and length of oil seed rape and Indian mustard. It was found that heavy metals affect root growth more severely than shoot growth. The results of previous studies showed that Cd affects root growth more severely than shoot growth (Vaselov et al., 2003). Significant difference was found between the Cd and Ni exposure for the fresh and dry weight parameters. The Cd exposure significantly reduced the dry and fresh weight of roots and shoots of *B.campestris* (Zhu et al., 2004). The heavy metal concentration in the roots and shoots increased with increased heavy metal concentration in the soil and the relationship between two concentrations are similar to all genotypes. The accumulation of heavy metals like Cd (Singh and Brar, 2002) and Ni (Panwar et al., 2002) by *Brassica juncea* increased with increasing their levels in the soil. Wang and Su (2005) reported that the amount of Cd uptake increased with the increase of growth period of Indian mustard. Accumulation of Ni was found to be more in shoots than in roots. Plants that hyperaccumulate Ni exihibit an exceptional degree of Ni tolerance and the ability to translocate Ni in large amounts from root to shoot (Ingle et al*.,* 2005; Dan et al*.,* 2002). Increase in concentration of Ni (3mM to 5mM) did not change appreciably its content in roots but shoot accumulation showed considerable increase at higher dose of Ni in soil. All the genotypes accumulated Cd and Ni in their shoots more than their roots. Plants can extract Cd from the soil and transport it via the xylem in to shoots and leaves where it can accumulate (Blaylock et al., 2000). The ability of plants to accumulate metal in the shoots is important because the shoots represent the harvestable biomass. The higher relative shoot accumulation will result in a higher S/R ratio. The species able to accumulate relatively high metal concentration in above ground tissues would be the good candidate for phytoextraction (Dang et al., 2004). Plant potential for Cd extraction generally depends on shoot Cd concentration (Vassilev, 2002). Therefore, *B. juncea* genotypes exhibiting highest S/R ratio are the best candidates for phytoextraction. The genotypes respond in a direction of accumulating a good amount of heavy metal from the sink and the interesting among these genotypes is that accumulation of metal does not affect much growth and yield. However, number of pods per plant reduced in heavy metal exposure but the Ni metal has got positive effects in number of seeds per pod. Thus our study indicates that this heavy metal has positive effects on the growth of mustard genotypes even at moderately higher concentrations

Conclusions

The results presented show the ability of *Brassica juncea* genotypes for Cd and Ni uptake, accumulation and tolerance. Our results showed that there is significant difference in root length, shoot length, fresh weight and dry weight between Cd and Ni treatments. *Brassica juncea* offers better accumulation potential for Cd compared to Ni.

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