Exogenous putrescine effect on cation concentration in leaf of Brassica juncea seedlings subjected to Cd and Pb along with salinity stress

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Abstract: The present study reports on cation levels in leaf tissue of the mustard seedlings subjected to metal and salinity stress (Cd/Pb 0.1-2mM and NaCl (50-100mM) for 7 days in controlled conditions. The leaf content of Na indicates the possibility of putrescine selective effect on ion flux in leaf es of plants under stress. It is shown that putrescine application caused considerable decline in Na content, while increased K level in leaf of the seedlings under low salinity and metal (Pb/Cd) stress. The considerable Na accumulation in leaf under high salinity and metal stress conditions were reversed up to 35% with putrescine. However, the declining trend in Ca++/Mg++ with increasing level of Pb or Cd along with salinity in external environment of the seedlings changed little but significant with putrescine. IAA application to the plant exposed to metal and salinity stress also checked the increase in Na level, but K level change was little. The IAA also had little effect on Ca++/Mg++ accumulation over control under severe stress. The experiments suggest the putrescine alleviation of growth in metal and salinity stressed plant through maintaining high K level and lowering Na in leaf, while little effect on divalent (Ca++ and Mg++) level. This indicates the possibility of putrescine selective effect on ion flux in leaf es of plants under stress.

Key words: Cations, Brassica juncea, Metals, Putrescine, Salinity stress.

Introduction
Salinity and Cd/Pb stress affects the plant metabolic processes directly or indirectly (Van-assche and Clijsters, 1990; Singh et al., 2000). Imbalance in nutrients and ions under metal and salinity stress affects osmotic status of cells thereby impairing plant growth (Cramer and Lauchli, 1986; Rubio et al., 1994). However, differential changes in cations are being noticed under stress conditions. For instance, either increase in Ca++ in stem (Patel et al., 1976) or decrease in Mn++, Fe++, Zn++ (Wallace et al., 1977) in Cd stressed plant is observed. Pb or Cd stress decreased the Fe content in shoot of Solanum melangena and in shoot/root of sugar beet (Greger and Lindberg, 1987) but not in Lycopersicum esculentum (Khan and Khan, 1983). Cd caused decrease in K+/Mg++- ATPase, even in the presence of K+ is observed in Beta vulgaris (Lindberg and Wingstrand, 1985), while activity was stimulated in rice at low Cd conc. (0.5mM), but those without any correlation with severe growth inhibition (Rose et al., 1992). Though, Cd at the range of 2-5 µM was found inhibitory for uptake of K+ in Betula pendula but the K+ level restored after long exposure of metal (Gussarsson and Jensen, 1992). On the other hand, it is propounded that the endogenous K+ level would affect Cd inhibition of K+ accumulation in plant (Trivedi and Erdei, 1992). The potassium availability is considered as a limiting factor for plant productivity (Maathuis et al., 1996). Hence, maintenance of adequate endogenous K+ level in the cells is thought to play a protective role against toxic effect of Na+. It is suggested that K+ <20mM in saline media could mitigate the Na+ toxicity (Cordovilla et al., 1995), but higher level of K+ had been found toxic as well (Lauter et al., 1998). There are a few earlier reports about either lower K+ content (Storey and Wynjones, 1978) or there is no change in K+ under high salinity (Munns, 1985). Recently, Ward et al. (2003) have raised some doubts of plant tolerance through exclusion of Na+ only. Cd or Pb caused disturbances in Ca++ level is observed (Benters et al., 1997 and references therein). The Ca++ caused reversal of salt induced growth inhibition is reported which is found to be partial in rapeseed (Porecelli et al., 1995; Elphick et al., 2001). However, it remains unexplained under multiple stresses. The cationic disturbance in plant is considered as a major mode of salinity effect (Greenway and Munns, 1980; Cramer et al., 1991) and that has been studied scarcely under metal stress (Asp et al., 1994). Moreover, it is assumed that knowledge of monovalent and divalent cation status information and its imbalance effect mitigation, especially under metal and salinity stress could give insight for better stress management in crops.

The plant growth regulators, especially GA3 have been implicated in plant growth improvement under stress (Ashraf et al., 2002). Putrescine (Put) a diamine and an obligate precursor for polyamines (PA) is considered to be a plant growth regulator and acts as an antistress agent (Galston and Yoneyama, 1991) and that has been studied scarcely under metal stress (Asp et al., 1994). Moreover, it is assumed that knowledge of monovalent and divalent cation status information and its imbalance effect mitigation, especially under metal and salinity stress could give insight for better stress management in crops.
disturbed under salt and metal stress. Moreover, putrescine effect on leaf cations level was evaluated under two type of stress assuming plant’s real field condition. The Put effect on cation level was compared with that of IAA, under similar conditions to probe the potential of two different growth regulators in mitigating ion imbalance in leaf of the plant exposed to Cd/Pb and salinity stress.

Materials and Methods

Seedlings (Brassica juncea cv RH-30) were raised in controlled condition (light 75 Wm$^{-2}$, 65%RH, temp 25±2 °C) for 7 days in half-strength Hoagland’s nutrient solution (pH 6.0) containing either Cd or Pb (0.1-2.0mM as their acetates) along with salinity (LH, 50mM, or HS, 100 mM NaCl) from the day of seed sowing. These seedlings were treated with either putrescine (Put 1.0 mM) or indole acetic- acid (IAA 0.01mM) included in the nutrient solution. The leaves of the seedlings of similar physiological age were taken for the measurement of cationic level and growth. Dry mass was measured after drying the sample at 65°C for 72 hr.

The acid digested dry powdered leaf (100mg) aliquot was used for estimation of Na$^+$ and K$^+$ by Digital flame photometer (Elico, Model CL-22D). The Ca$^{++}$ and Mg$^{++}$ were estimated in leaf sample by titration following the method of Cheng and Bray, (1951). For which, 1.0 ml aliquot was added in 3.0 ml ammonium buffer with 2 drops of 1% EBT (Eriochrome Black-T) followed by titration with N/100 EDTA. The development of green colour was taken as end point. And from this, the total content of Ca$^{++}$ plus Mg$^{++}$ was obtained by multiplying volume of EDTA into its normality into thousand and the product was divided by total volume of digested sample. For Ca$^{++}$ estimation, the N/100 EDTA was titrated with alkaline
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Putrescine and IAA effect on accumulation of Na⁺, K⁺ in leaf of the seedlings under Cd and salinity stress: The pattern of change in both ions in leaf of Cd stressed plant was similar to those of Pb. The Cd stressed plants showed decline about 50% in Na⁺ and 34% in K⁺ level in leaf (Fig. 2). Cd in combination of salinity elevated Na⁺ level and

The data presented are the mean of three replicates ± S.D. and significance level were determined using Student’s “t” test.

Results

Putrescine and IAA effect on Na⁺, K⁺ level in leaf of the seedlings under Pb and salinity stress: The level of Na⁺, K⁺ gradually decreased in leaf tissue on increase in external Pb conc. in the plant environment (Fig. 1). The decline in Na⁺ and K⁺ content was almost 40% at 2.0mM Pb over control; hence decline in Na⁺/K⁺ ratio. Put treatment to the seedling further decreased Na⁺ level with increasing level of Pb stress (2mM Pb) and similarly K⁺ level also. This caused reduction in ratio of Na⁺/K⁺ (from 0.16 to 0.05); which was prominent under high metal stress. Pb in combination of salinity elevated the Na⁺ level many fold and concomitantly decreased K⁺ level. But salinity and Pb stress induced Na⁺ accumulation was controlled and the K⁺ accumulation was elevated, even under severe stress in the presence of putrescine. IAA application also reduced level of Na⁺ under Pb plus salinity stress and elevated level of K⁺ to 30% under metal stress. However, IAA caused reduction in Na⁺ accumulation was little at 2mM metal. Moreover, IAA application to the plants under low (50mM) or high salinity (100mM NaCl) plus metal stress retarded the increasing level of the Na⁺ in leaf tissue, but K⁺ level was either low or remained almost same to that of control at both concentration of Pb in comparison to Put treatment.
Fig. 3: Putrescine and IAA effect on accumulation of Ca$^{2+}$, Mg$^{2+}$in leaf of the seedlings under Pb (A1 and A2), Cd (B1 and B2) and salinity stress. Rest legend is same as per Fig.1.
declined K⁺ level further over control (only metal). Put lowered the Na⁺ level and increased K⁺ level little in leaves of Cd stressed seedlings. But the high accumulation of Na⁺ in leaf of salinity and Cd-stressed seedlings was controlled remarkably by putrescine. This effect of put on Na⁺ accumulation was little but significant under severe stress (2mM Cd + 100mM NaCl). While K⁺ accumulation was maintained to a control level by Put, IAA application checked slightly the elevation of Na⁺ level under metal as well as metal plus salinity stress. However, under low metal stress, the IAA check was prominent. K⁺ level in leaves of seedlings under salinity and Cd stress changed little in presence of IAA. However, it caused little decline in K⁺ under high salinity and high metal stress.

**Ca²⁺ / Mg²⁺ content in leaf of Pb and salinity stressed plant with putrescine and IAA:** The Ca²⁺ content decreased little with increasing level of Pb. This decline was considerable under metal plus salinity (Fig. 3 A1). Mg²⁺ level declined remarkably (50%) at 2.0mM Pb and under salinity stress (Fig. 3 A2). This caused an increment in Ca²⁺/Mg²⁺ ratio. However, in the presence of Put, the Ca²⁺ content decrease was checked in plants under both metal plus salinity stress and Mg²⁺ content was either maintained to a control level (presence of only metal) or little increased over control and metal plus salinity. IAA application did not change the trend of Ca²⁺ accumulation under stresses but elevated the level under metal plus salinity over only metal. The change in Mg²⁺ level was not significant under metal plus high salinity over control except low salinity.

**Ca²⁺/Mg²⁺ content in leaf of Cd and salinity stressed plants treated with putrescine and IAA:** The trend of decline in both divalent cations in Cd stressed seedlings were same as Pb stressed seedlings (Fig 3 B1, B2). The declining trends in Ca²⁺ and Mg²⁺ further increased when subjected to salinity where Ca²⁺ decline was prominent. This led to overall reduction of divalent ratio. Putrescine application elevated Ca²⁺ and Mg²⁺
level little under low salinity plus metal. However, a similar response was observed with IAA under metal plus salinity stress.

Putrescine response on biomass accumulation under metal and salinity stress: Both Pb and Cd caused decline in biomass accumulation in seedlings which further declined when subjected to salt stress (Fig. 4). Decrease in biomass accumulation was reversed by Put as well as IAA application. A high level of biomass in seedlings even under twin stresses (metal + salinity) compared with that of metal stressed seedlings was maintained to the extent by Put as well as IAA.

Discussion

The role of ions in maintaining the cell osmoticum for growth in glycophytes under salinity has been well discussed (Greenway and Munns, 1980) but scarcely in metal stressed plants (Asp et al., 1994). The Na+ competition for K+ uptake system and binding sites in the cytoplasm might inhibit K+-dependent processes, therefore low Na+ and high K+ cytoplasmic content is suggested for plant growth (Rubio et al., 1994). Decrease in growth in metal stressed plant might be due to decreased content of Na+/K+ in leaf, which gradually increased with increase in Pb or Cd in the plant external medium (Fig. 1 and 2). Put induced growth might be due to selectively lowering of Na+ and elevating K+ content. Put response on plants under Cd/Pb along with salinity stress is corroborating the response with GAs in case of wheat under salt stress (Aldesuquy, 1995). Salinity and Cd suppression of K+ uptake has been observed in root of Betula pendula (Gussarsson and Jensen, 1992), which is suggested to be through suppressing K+ - ATPase system (Lindberg and Wingstrand, 1985; Rubio et al., 1994). Though, there are reports that prolong exposure of Cd did not inhibit either K+-ATPase (Rose et al., 1992) or K+ influx (Asp et al., 1994); however, Ward et al. (2003) have also suggested that simply exclusion of Na+ from cell may not essentially alleviate the K+ level in cell required for growth. Though, amongst physiological mechanisms underlying salt tolerance, ion exclusion specifically Na+ is considered more relevant characteristics for improving tolerance (Noble et al., 1992; Dionisio-seise and Tobita, 1998). Put or IAA controlled Na+ accumulation up to some extent under multiple stress condition (Fig. 1 and 2). The over all higher level of (Na+,K+) in leaves under severe condition (metal+salinity) in presence of Put suggests that it could be due to activation of Na+-K+ ATPase and/or alteration in inward and outward rectifiers on endomembrane responsible for efflux and influx respectively for Na+(Iwano,1995). Ashraf et al. (2002) also found GAs caused high titre of Na+ in two cultivars of wheat differing in salt sensitivity. Moreover, it has been suggested that decrease in K+ might be for mitigating entry of Na+ under altered permeability under stress (Murata et al., 1994). Bruggemann et al. (1998) have suggested that PA is likely to mediate a salinity induced decrease of ion flux across the vacular membrane by blocking FV channels. Similar response could be likely with Put under multiple stresses.

Under metal and salinity stress, divalent cations (Ca++ / Mg++) level decreased significantly. The Ca++ decline was considerable (Fig. 3). The Ca++ considered to be protective for plant growth under salinity (Colmer et al., 1994) was elevated little under moderate stress (0.1mM metal and 50mM NaCl) by Put. A similar response has been noticed in other cases also (Elphick et al., 2001). There was little effect of Put on Ca++ and Mg++ level in presence of both metals and high salinity. This shows that Put (divalent) might not be interacting with Ca++ or Ca++/Mg++ sites for eliciting responses and may have independent action. Moreover, it has been demonstrated that Ca++ didn’t replace the Put effect on ATPase activation (Reggiani et al., 1992). It is likely that little effect of Put on divalent cations might be due to its own positive bi-valent nature sufficient to mitigate the bivalent cations requirement for metabolic processes.

References


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