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Varietal Differences in Spring and Autumn Sown Maize (*Zea mays*) for Tolerance against Cadmium Toxicity

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ABSTRACT

Contamination of agricultural soils with heavy metals, including cadmium (Cd), is global threat to agricultural productivity. Maize is sensitive to Cd toxicity, although varietal differences exist. However, the responses of maize varieties to Cd toxicity across the growing seasons are not reported. With the objective to find variability for Cd tolerance across the seasons, in this study, nine maize (*Zea mays* L.) varieties were investigated for their comparative responses against Cd toxicity in sand medium during spring and autumn growing seasons, 2009. Data indicated significant varietal differences in all the growth attributes i.e., length and dry weight of shoot and root, number and area of green leaves and number of roots per plant. Although, tolerance of maize varieties were relatively better during autumn season, Pak-Afgoi, EV-79 and SWL-2002 were better tolerant whilst EV-20 was more sensitive to Cd toxicity during both the seasons. Application of Cd resulted in the appearance of visual toxicity symptoms on the leaves (overall leaf chlorosis & necrosis) and roots (constriction & browning) in all the varieties during both the seasons, but with great varietal differences. Correlations of visual toxicity symptoms with shoot growth data revealed that reduced leaf chlorosis, not the leaf necrosis, was positively related to shoot dry weight in both the seasons, while this was also the case for leaf area in autumn season. However, reduced root constriction and browning were positively related to increased root dry weight. Overall the results suggested that great varietal differences exist in maize for Cd tolerance, which provides room for improving and cultivating this species in marginally Cd contaminated soils. Leaf chlorosis, root constriction and browning can be taken as important determinants of tolerance against Cd in maize.

Key Words: Cadmium toxicity; Growing seasons; Leaf chlorosis; Maize; Root growth

INTRODUCTION

Heavy metal contamination of soils with industrial effluents is great impediment to growth and productivity of plants especially in urban and peri-urban areas. After their discharge into the soils, the heavy metals are taken up by the plants and enter the food chain depending upon their concentration in the effluent (Jamali *et al*., 2007). Cadmium (Cd) is a non-essential but highly toxic elements and persistent environmental contaminant for living organisms (Di Toppi & Gabbrielli, 1999; Jinwal *et al*., 2009). The toxicity symptoms caused by excess of Cd include the reduced below- and above-ground growth (Wahid *et al*., 2009), chlorosis and necrosis of leaf and stem (Baryla *et al*., 2001; Ghani & Wahid, 2007). In maize, for instance, increased Cd levels inhibited the formation of lateral roots, while whatever roots formed were brown, rigid and twisted (Pál *et al*., 2006), while a reduction in coleoptiles extension was accompanied with their chlorosis and necrosis (Dong *et al*., 2005).

A number of studies show that physiological phenomena are more readily affected due to increased Cd toxicity. Reduced uptake and utilization of essential nutrients (Wahid *et al*., 2009), enhanced production of reactive oxygen species (Adhikari *et al*., 2006), inhibition of the chlorophyll biosynthesis and loss chlorophylls and carotenoids (Baryla *et al*., 2001; Wahid *et al.,* 2008) are amongst the important phenomena modulated by Cd stress.

Plant species display variable responses to increased levels of Cd, as evident from great inter- and intra-specific differences (Belimov *et al*., 2007; Wahid *et al*., 2009). There are a number of plant species which are hyperaccumulators of heavy metals including Cd (Milner & Kochian, 2008; Boyd, 2010), while crop plants usually lack the tendency of effective Cd partitioning and sequestration (Mazid *et al*., 2011). As of various growth stages, the Cd toxicity is more evident at juvenile, rather than advanced growth stages, which is used as index of heavy metal toxicity (Wu *et al*., 2006; Wahid *et al*., 2009).

Maize (*Zea mays* L.) is a commercially important crop all over the world. Being a short duration crop and well suited to agroclimatic conditions, it is normally grown in two seasons (spring & autumn) in Pakistan. It is well known that prevailing environmental conditions substantially modify the

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plant growth and ultimate yield in various plant species including maize (Anjum *et al.*, 2008; Lopes *et al.*, 2011). These responses are displayed by the appearance of symptoms on the above- and below-ground plant parts. Although response of maize grown in Cd-contaminated soils has been reported (Wang *et al.*, 2002; Zhang *et al.*, 2009), the studies are lacking that show the growth response of this important crop species to the growing seasons. Thus, we predict that in addition to varietal differences for Cd toxicity responses, appearance of Cd toxicity symptoms may also have marked impact on the maize performance during both the growing seasons. The objectives of this study were to find out the responses of maize varieties and relevance of visual toxicity symptoms to increased Cd levels in spring and autumn growing seasons, and to find out suitable varieties for cultivating in marginally Cd contaminated soils.

MATERIALS AND METHODS

General experimental details: Separate experiments were conducted in spring (February-March; temp 15–25°C, RH 55–70%) and autumn (July-September; temp 26–34°C, RH 60–70%) maize (*Zea mays* L.) growing seasons in 2009 and 2010 and all operations were similar in both the seasons. Seeds of nine maize varieties were obtained from Maize and Millets Research Institute (MMRI), Sahiwal, Pakistan. Selected 10 healthy seeds of all the varieties were sown in each of the plastic pots (30 cm long, 20 cm diameter) containing 5 kg of thoroughly washed sand. The pots were kept in a netting house under bright sunlight. Three days after germination, the seedlings were thinned to five per pot. The design of the experiment was completely randomized factorial with three replications.

Treatment application: The plants were grown supplemented with Hoagland nutrient solution after every seven days. Half of 30 days old plants of each variety were applied with increased Cd levels (0, 200, 400, 600, 800 & 1000 μM) using analytical grade (Merck) cadmium chloride ($CdCl_2.2.5H_2O$) dissolved in tap water, while the control plants received water only. Control and Cd-treated plants were grown for 20 more days.

Harvesting and data recording: Prior to uprooting the plants, the intact plants were measured for shoot length, number of green leaves and leaf area measured as maximum leaf length × maximum leaf width × 0.68 (correction factor) per plant. The number and length of roots were determined by uprooting the plants. For dry weight determination, the shoot and root parts were briefly washed with water, blotted dry, transferred to paper bags and kept in an oven set at 70°C for one week.

Symptomatic determinations: The visual toxicity symptoms such as leaf chlorosis, leaf necrosis, root constriction and root browning were visually recorded at harvest time in both the seasons. The chlorosis was scored based on the comparative yellowing of lamina and midrib. The necrosis was scored based on the necrotic spots on the leaf lamina. Likewise, root constriction and browning was scored by comparing the roots of all varieties.

Statistical analysis: In the absence of any significant difference between years, the data recorded from both the years were pooled to perform analysis of variance (ANOVA) separately for both the seasons using computer software MSTAT-C. To draw correlations amongst the plant growth and visual toxicity symptoms at the highest Cd level (1000 μM), the varieties were awarded scores; the lowest symptom given the highest score and vice versa.

RESULTS

Significance of variance sources: Data indicated significant difference in varieties ($P<0.01$), Cd treatments ($P<0.01$) along with significant ($P<0.01$) difference for all the growth attributes in this study.

Shoot and root elongation: In spring season, under control condition, all the varieties indicated greater shoot length, being the longest in Pak Pak-Afgoi had the longest and shortest in EV-20. All the Cd levels reduced this parameter significantly, although a minimum reduction was noted in Pak-Afgoi followed by EV-5098, while a maximum one in EV-20 followed by Sadaf (Fig. 1). In autumn season, data showed that control (untreated) plants of Pak-Afgoi and SWL-2002 had similar shoot length as compared to rest of the varieties. However, applied Cd levels substantially reduced this attribute although with differences among the varieties. EV-79 followed by Pak-Afgoi had the highest shoot length at 1000 μM Cd while it was the minimum in EV-20 followed by EV-77 and EV-5098. Rest of the varieties displayed intermediate behavior (Fig. 1).

For root length data in spring season, control plants of all the varieties indicated greater root length; Pak-Afgoi and SWL-2002 had the longest, while EV-20 displayed the shortest roots. All the Cd levels reduced this parameter significantly in the varieties, but lowest reduction was noted in Pak-Afgoi and SWL-2002 followed by EV-79 and a maximum one in EV-20 followed by EV-77 and Agaiti-2002 (Fig. 1). In autumn season, control plants of EV-78 had the longest, while EV-20 the shortest roots. However, application of all the Cd levels substantially reduced this attribute although great difference was discernible among the varieties. Sadaf followed by Pak-Afgoi had longest roots at 1000 μm, while it was the minimum in EV-20 followed by EV-5098. Remaining varieties displayed intermediate but quite variable root lengths (Fig. 1).

Shoot and root dry mass: It is manifested from the spring season data that in Cd-untreated (control) plants, Pak-Afgoi produced the highest shoot dry weight followed by SWL-2002, EV-78 and EV-5098, while it was the smallest in EV-20. Applied Cd significantly reduced in all the varieties, although Pak-Afgoi had the highest shoot dry weight at 1000 μM Cd while lowest one in EV-20 and EV-77 (Fig. 2). During autumn season, control plants of Agaiti-2002 and Pak-Afgoi had comparable shoot dry weight, while EV-20

Fig. 1: Changes in shoot and root length of maize varieties with the soil application of increased levels of Cd grown during spring and autumn seasons

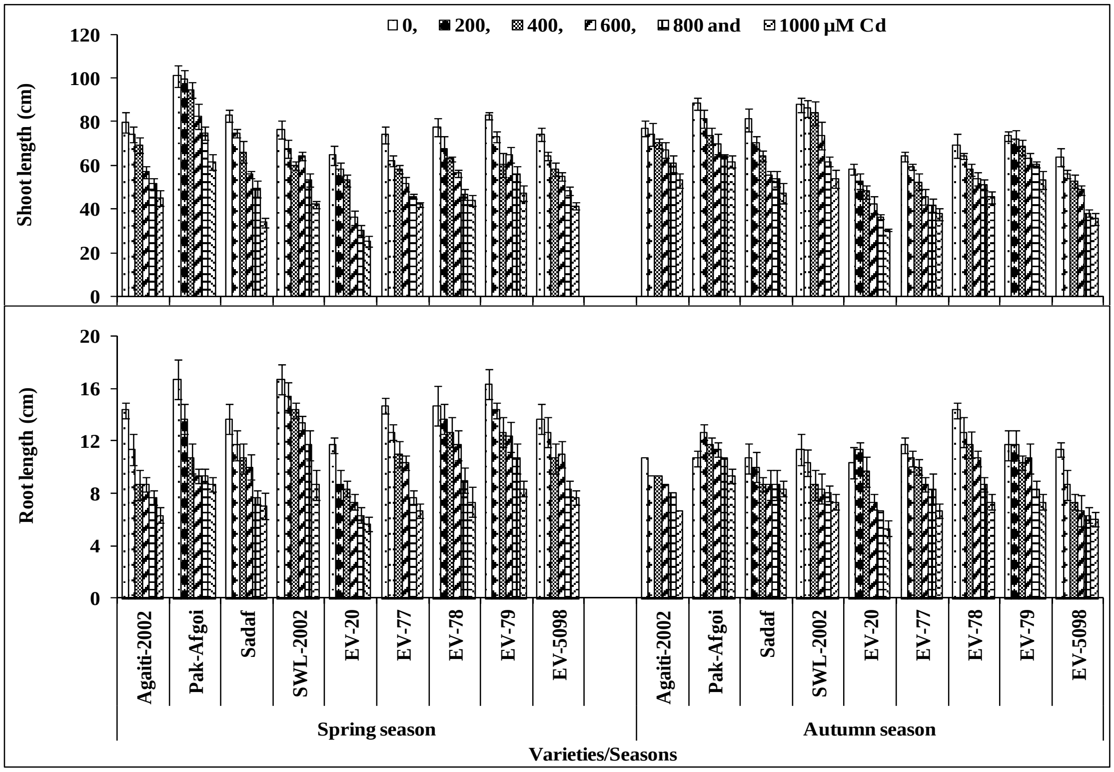


Fig. 2: Changes in shoot and root dry weight of maize varieties with the soil application of increased levels of Cd grown during spring and autumn seasons

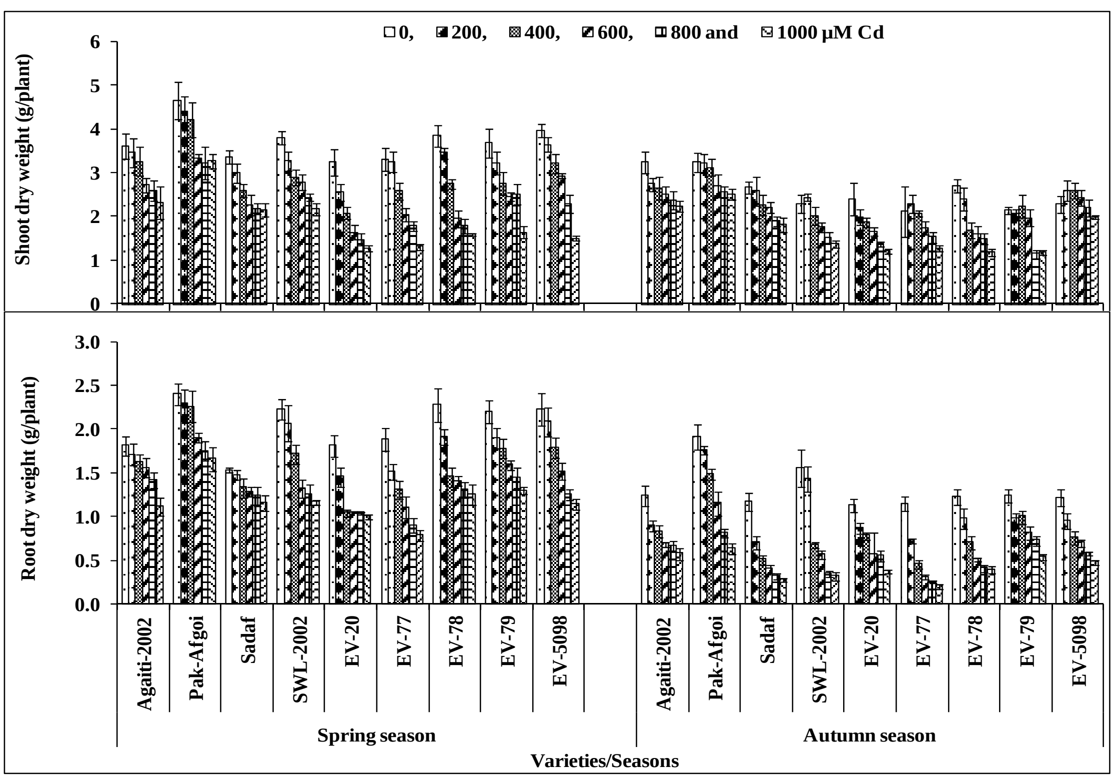
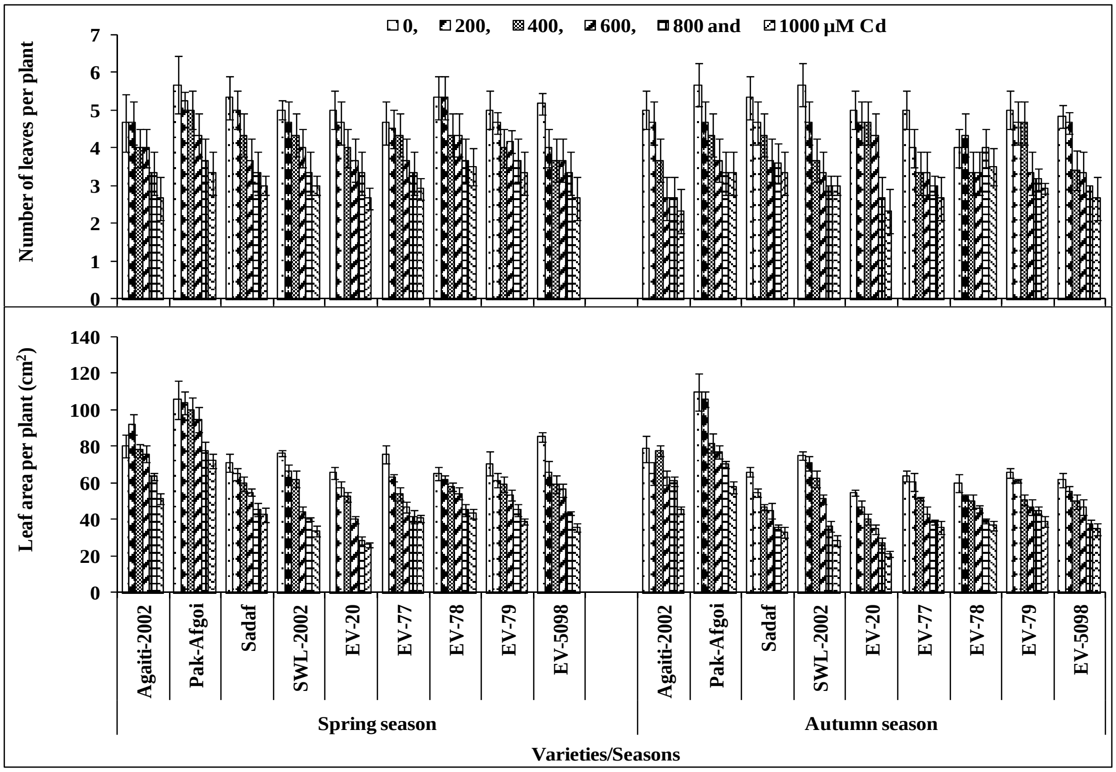


Fig. 3: Changes in number and area of green leaves of maize varieties with the soil application of increased levels of Cd grown during spring and autumn seasons



indicated the lowest value of this attribute. Although, there was concomitant reduction with increased Cd levels in all the varieties, a minimum loss of shoot dry weight was noted in Pak-Afgoi followed by Agaiti-2002, while it was the maximum in EV-79 followed by EV-78, EV-77 and EV-20, among the others (Fig. 2).

As regards root dry weight in spring season, control plants of most of the varieties had similar root dry weight but EV-20 manifested the lowest one. Although increased Cd concentrations decreased the value of this attribute in all the varieties, Pak-Afgoi exhibited a lowest, while EV-77 a highest reduction in this character (Fig. 2). During autumn season, control plants of Pak-Afgoi had greatest, while EV-20 had the smallest root dry weight, while EV-77, EV-78, EV-79 and EV-5098 had similar root dry weight. With an increase in Cd, noticeable decrease in this character was recorded in all the varieties. Nonetheless, Pak-Afgoi had lowest, while EV-77 the highest reduction at 1000 µM, while other varieties indicated intermediary trends (Fig. 2).

Foliage development: Data recorded in spring season revealed that in untreated set of plants, number of green leaves per plant was the highest in Pak-Afgoi but lowest in EV-20. With increase in root applied Cd levels, there was significant reduction in this attribute in all varieties. Nonetheless, a minimum reduction was recorded in Pak-Afgoi followed by EV-78, while a maximum one in EV-20 followed by EV-5098 and Agaiti-2002 (Fig. 3). In autumn season, control plants of Pak-Afgoi and SWL-2002 had similar number of green leaves as compared to rest of the varieties. Although applied Cd levels substantially reduced this parameter with significant differences in all the varieties. Pak-Afgoi and Sadaf followed by EV-78 had the highest number of leaves while it was the lowest in Agaiti-2002 followed by EV-20 (Fig. 3).

As for leaf area per plant, the spring season data revealed that control plants of Pak-Afgoi displayed a highest while EV-78 with lowest value of this parameter. Although Cd levels registered substantial adverse effect on leaf area per plant of all the varieties, Pak-Afgoi indicated minimum, while EV-20 a maximum reduction (Fig. 3). Data recorded during autumn season showed that Pak-Afgoi produced highest and EV-20 the lowest leaf area per plant amongst the varieties. Application of Cd treatments significantly reduced leaf area with great varietal difference. At 1000 µM Cd, Pak-Afgoi displayed highest leaf area as compared to rest of the varieties, while SWL-2002 indicated lowest followed by EV-20 (Fig. 3).

Number of roots per plant: During spring season, in control set of plants, highest number of roots was counted in EV-78 and EV-5098 followed by Pak-Afgoi while this number was the lowest in EV-20 and SWL-2002. Although, the application of Cd levels reduced this parameter significantly in all the varieties, a minimum reduction was manifested by Sadaf, while a maximum one by EV-20 (Fig.

Fig. 4: Changes in number of roots per plant of maize varieties with the soil application of increased levels of Cd grown during spring and autumn seasons

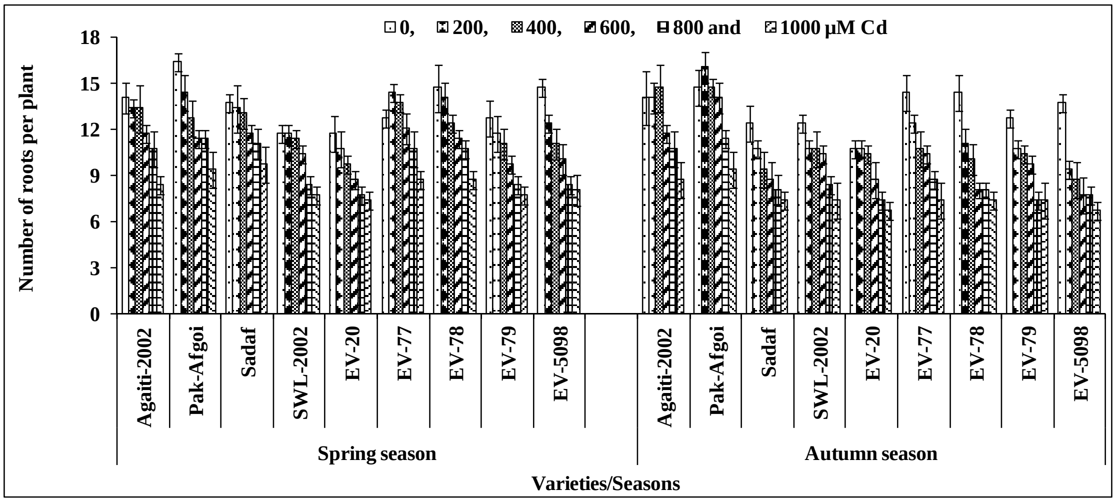


Table I: Visual symptoms and degree of Cd toxicity on shoot and root of maize varieties determined at 1000 µM Cd level

Degree of Cd toxicity	Spring season				Autumn season			
	Leaf chlorosis	Leaf necrosis	Root constriction	Root browning	Leaf chlorosis	Leaf necrosis	Root constriction	Root browning
Low	Pak-Afgoi	EV-79	Pak Afgoi	Pak-Afgoi	EV-79	Pak-Afgoi	Pak Afgoi	EV-78
↓	Sadaf	Pak-Afgoi	SWL-2002	EV-79	Pak-Afgoi	Sadaf	EV-78	Pak Afgoi
↓	SWL-2002	SWL-2002	EV-79	EV-5098	EV-5098	EV-78	Sadaf	EV-79
↓	EV-78	EV-5098	EV-5098	SWL-2002	Sadaf	EV-79	SWL-2002	SWL-2002
↓	EV-5098	EV-78	EV-78	Sadaf	SWL-2002	EV-5098	EV-79	EV-5098
↓	Agaiti-2002	Sadaf	Sadaf	EV-78	EV-77	SWL-2002	EV-5098	Sadaf
↓	EV-79	EV-77	EV-77	EV-77	EV-78	EV-77	Agaiti-2002	Agaiti-2002
↓	EV-77	Agaiti-2002	Agaiti-2002	EV-20	Agaiti-2002	Agaiti-2002	EV-77	EV-20
High	EV-20	EV-20	EV-20	Agaiti-2002	EV-20	EV-20	EV-20	EV-77

4). In autumn season, EV-77 and EV-78 displayed similar values followed by Pak-Afgoi, while this value was the lowest in EV-20. Application of increased levels of Cd reduced this attribute with varietal differences. Pak-Afgoi remarkably displayed highest value followed by Agaiti-2002 while lowest one in SWL 2002 followed by EV-20 (Fig. 4).

Visual toxicity symptoms: The varieties showed great differences for the Cd toxicity symptoms in both the seasons. A comparison of varieties revealed that in spring season, Pak Afgoi showed the lowest chlorosis of leaf, followed by EV-79 and SWL-2002 while the greatest chlorosis was manifested by EV-20 followed by EV-77 and Sadaf. The leaf necrosis was the lowest in EV-79 followed by Pak Afgoi and SWL-2002, whilst it was the highest in EV-20 followed Agaiti-2002 and EV-77. The leaf constriction was the least in Pak-Afgoi followed by SWL-2002 and EV-79, while the most in EV-20 followed by Agaiti-2002 and EV-77. The root browning was found to be nominal in Pak Afgoi and EV-79 but the highest in Agaiti-2002 followed by EV-20, EV-77 and EV-78 (Table I). In autumn season, on the other hand, the symptomatic data was relatively different than the spring season crop. Results showed that leaf chlorosis was much lower in EV-79 followed by Pak Afgoi and EV-5098 while quite enhanced in EV-20, Agaiti-2002, EV-77 and EV-78. Leaf necrosis was least visible in Pak-Afgoi, Sadaf and EV-5098 while it was the highest in EV-20, Agaiti-2002 and EV-77. Apparent root constriction was the lowest in Pak-Afgoi and EV-78, while the highest in EV-20, EV-78 and Agaiti-2002. Likewise, root browning was nominally noted in EV-78, Pak Afgoi and EV-79, while it was quite visible in EV-77, EV-20 and Agaiti-2002 (Table I).

Correlations of symptomatic and quantitative attributes: The Cd toxicity symptoms observed on the shoot and root of maize varieties were correlated with the shoot and root dry matter yield separately in both the seasons. These data revealed that in spring season, increased shoot dry matter yield was directly correlated to reduced leaf chlorosis, while shoot dry weight was not correlated with leaf necrosis and leaf area was not correlated to any of the foliar Cd toxicity symptoms.

Table II: Correlation coefficient (*r*) of growth attributes and visual toxicity symptoms of maize varieties at 1000 µM Cd level in spring and autumn seasons

Growth attributes	Toxicity symptoms	Spring season	Autumn season
Shoot dry weight	Leaf chlorosis	0.778**	0.687*
	Leaf necrosis	0.384ns	0.318ns
Leaf area	Leaf chlorosis	0.599ns	0.850**
	Leaf necrosis	0.327ns	0.542ns
Root dry weight	Root constriction	0.732*	0.267ns
	Root browning	0.715*	0.509ns
Number of roots	Root constriction	0.135ns	0.449ns
	Root browning	0.154ns	0.349ns

Significant at ** P<0.01, * P<0.05 and ns non-significant

Increased root dry weight was positively correlated to the least constriction and browning of roots (Table II). In autumn season, reduced leaf chlorosis, not leaf necrosis, was positively related to increased shoot dry weight and leaf area. For root, none of the Cd toxicity symptom was correlated with root dry weight or its number (Table II).

DISCUSSION

Although Cd toxicity is important to agricultural productivity in many ways, various plant species and varieties show variable responses to this particular toxic element (Mobin *et al.,* 2007; Gill *et al.,* 2010). Maize shows sensitivity to increased Cd levels thus shows hampered growth (Cao *et al.*, 2007). Results of experiments conducted on nine varieties of maize in spring and autumn seasons revealed that although there was generally a reduction in shoot an root elongation (Fig. 1), dry matter yield (Fig. 2), foliage development (Fig. 3) and proliferation of roots (Fig. 4), Pak-Afgoi was the most Cd tolerant while EV-20 the most Cd sensitive varieties for majority of the parameters (Fig. 1-4). These findings indicated the great varietal differences in maize for Cd toxicity response. These results further revealed that the behavior of varieties was quite differential during both the seasons, which further showed that seasonal changes have specific impact on growth and productivity of crop plants (Craufurd & Wheeler, 2009).

It is pertinent to mention that performance of maize varieties, especially Agaiti-2002, Pak-Afgoi and SWL-2002 was relatively better during autumn than spring season. This can be conveniently related to the prevailing climatic conditions when these varieties were cultivated. In fact maize, as a C_4 plant, has higher temperature optimum (~32^{o}C) for growth (Wahid, 2004). As mentioned above (M & M section), the temperature during spring season was rather lower for maize growth, which appeared to produce double stress; low temperature stress and Cd toxicity. Contrarily, the temperature range during autumn season was quite favorable, which helped the maize varieties to grow luxuriantly and presumably diluting the Cd stress.

Plants exposed to heavy metal stress often show some visual toxicity symptoms both on foliar parts and roots, which have great bearing on the potential of plants to withstand such adverse conditions (Bah *et al*., 2011). Such symptoms are also regarded as the indicators of stress tolerance (Ghani & Wahid, 2007; Wahid *et al*., 2009). From these experiments, we noted that the extent of the Cd toxicity symptoms noted as leaf chlorosis and necrosis and root constriction and browning was the least in Pak Afgoi, Sadaf and SWL-2002 while the highest in EV-20, Agaiti-2002 and EV-77 (Table I). Enhanced chlorosis leads to disruption of photosynthetic activities (Dong *et al.*, 2005) while deformation of roots results in hampered transport of water and nutrients (Page & Feller, 2005; Wahid *et al.,* 2009) In the present case, positive correlations between reduced leaf chlorosis and root constriction and browning with the plant dry matter yield in the spring season (Table II) further witnessed our assertion that prevailing conditions are really important in determining the growth and Cd toxicity in maize.

In conclusion, maize shows great varietal differences in Cd tolerance; however, prevailing environmental conditions are pivotal in this regard. Among the foliar toxicity symptoms, leaf chlorosis, root constriction and its browning are likely factors reducing the maize growth due to Cd toxicity. Correlations data suggested that Cd-toxicity was more damaging in spring than in autumn season. Nevertheless, the varietal differences for Cd tolerance suggest that maize improvement is possible for growing in Cd-polluted soils in both the seasons.

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