


# Photosynthesis and growth response of maize (*Zea mays* L.) hybrids exposed to cadmium stress

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**Abstract** Cadmium (Cd) is a biologically non-essential heavy metal while the cultivation of Cd-tolerant varieties/hybrids (V) seems the most promising strategy for remediation of Cd-contaminated soils. For this, 24-day-old seedlings of seven maize hybrids, DKC 65-25, DKC 61-25, DKC 919, 23-T-16, 32-B-33, 31-P-41, and Syn hybrid, were grown in hydroponic conditions for 21 additional days in various Cd concentrations (0, 5, 10, and 15  $\mu\text{M}$ ). Effects of variety, Cd, and their interaction were highly significant ( $p \leq 0.05$ ) for studied plant agronomic and physiological traits except the V  $\times$  Cd interaction for leaf chlorophyll content, root-shoot length, and root dry weight. The Cd accumulation in root and shoot increased gradually with increasing Cd treatments while copper (Cu), zinc (Zn), and manganese (Mn) uptake was decreased in all hybrids. The reduction in root and shoot biomass and Cd uptake was lower in 32-B-33 and 23-T-16 compared to other hybrids. The highest accumulation of Cu, Zn, and Mn was observed in 32-B-33, DK C65-25, and 31-P-41, respectively. The differential uptake and accumulation of

Cd by maize hybrids may be useful in selection and breeding for Cd-tolerant genotypes.

**Keywords** Cadmium · Chlorophyll contents · Gas exchange parameters · Growth · Micronutrients · Maize hybrids

## Introduction

Accumulation of heavy metals in agricultural soils mainly results due to anthropogenic activities such as application of contaminated effluents, industrial waste, phosphate fertilizers, herbicides and pesticides, agrochemicals, and sewage sludge (Adrees et al. 2015; Rehman et al. 2015; Rizwan et al. 2016a). Coupled with these anthropogenic activities, volcanic eruption, forest fires, run off, and production of sea salt aerosols are among the various natural sources of heavy metal release into the environment (Nagajyoti et al. 2010). Among heavy metals, cadmium (Cd) is widely distributed in world soils both naturally as well as anthropogenically (Choppala et al. 2014; Du et al. 2014). Several studies have reported that Cd toxicity decreased the plant growth and biomass by generating reactive oxygen species at cellular and subcellular levels (Rizwan et al. 2016b, 2016c). The Cd toxicity also decreased the photosynthetic pigments and gas exchange characteristics in a variety of plant species (Li et al. 2015; Lysenko et al. 2015; Tauqeer et al. 2016). Cd mainly enters into humans via food crops grown in Cd-contaminated soil (Rizwan et al. 2016a, 2016b). The Cd toxicity in humans has caused a number of disorders such as bone fracture, kidney failure, mental abnormalities, hypertension, and various skin disorders (Song et al. 2015). Similarly, Cd is also toxic to animals, causing disturbances in their metabolic activities along with various lethal diseases, which occur by grazing or engulfing the Cd-contaminated fodder and other crops (Wang et al. 2016a).

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Plants have developed a range of natural defense systems against metal stress comprising of both enzymatic and non-enzymatic antioxidants, production of osmolyte, and synthesis of chelating agents (Artiushenko et al. 2014; Adrees et al. 2015; Rizwan et al. 2015). Tolerance against metal stress varies depending upon plant species, variety, and the type of metal stress (Artiushenko et al. 2014). Maize (*Zea mays* L.) is a hyper accumulator plant and has the ability to accumulate and tolerate a certain Cd concentration without exhibiting toxicity symptoms (Yang et al. 2016). Maize has the potential to enhance its biomass production that act as supporting pillar for Cd tolerance in this crop (Rizwan et al. 2016b). Privileged biomass production accumulates the Cd in legislating volume and transports Cd towards the aerial parts (Broadhurst et al. 2015; Wang et al. 2016b). The genetic variations exist in Cd uptake by crop plants (Naeem et al. 2016; Rizwan et al. 2016c). For instance, Cd uptake and accumulation was higher in maize cultivar (31P41-Pioneer) sensitive to Cd stress compared to cultivar (3062-Pioneer) tolerant to Cd stress (Tanwir et al. 2015). Similarly, Cd stress decreased the growth and biomass of the Agatti-2002 maize cultivar compared to the EV-1098 cultivar and 50  $\mu\text{M}$  Cd applied in the sand medium did not affect the growth parameters of the latter cultivar (Hussain et al. 2012). In another study, Cd was accumulated up to 45  $\text{mg kg}^{-1}$  of maize dry weight without appearing with visible toxicity symptoms with a growth medium pH of greater than 6 (Broadhurst et al. 2015). Thus, selection of Cd-tolerant maize cultivars might be an effective strategy for the cultivation of Cd-contaminated soils (Rizwan et al. 2016b). However, varieties developed from traditional lines are not known for Cd accumulation potential, which may have variations in Cd uptake and root to shoot translocation compared to traditional parents (Naeem et al. 2016). This suggests that existing varieties/cultivars should be characterized for Cd uptake and translocation ability. The present study was conducted to evaluate the Cd accumulating ability of different maize hybrids and the interaction of Cd with micronutrient uptake and translocation.

## Materials and methods

### Plant culture and treatments

A hydroponic experiment was conducted in the warehouse of the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad (UAF) Pakistan. Seeds of seven maize hybrids, approved for general cultivation in Punjab, Pakistan, were collected from Monsanto (DKC 65-25, DKC 61-25, and DKC 919), Pioneer (32-T-16, 32-B-33, and 31-P-41), and Syngenta (Syn-hybrid) seed distributors in Faisalabad. Seeds were surface sterilized with 10%  $\text{H}_2\text{O}_2$  and rinsed thoroughly with distilled water. The disinfected

seeds were sown in polyethylene-lined iron trays containing moist sand, prewashed with a hydrochloric acid solution (10%) followed by washing with distilled water. Ten-day-old seedlings were transplanted into foam-plugged holes (one seedling per hole) of polystyrene sheets floating on plastic tubs with 25 L capacity. Each tube was filled with a modified nutrient solution containing the following: 16.0 mM  $\text{KNO}_3$ , 2.0 mM  $\text{NH}_4\text{H}_2\text{PO}_4$ , 6.0 mM KCl, 4.0 mM  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 1.0 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.5 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 2.0  $\mu\text{M}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5  $\mu\text{M}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 2.0  $\mu\text{M}$   $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.5  $\mu\text{M}$   $\text{H}_2\text{MoO}_4$ , 25  $\mu\text{M}$   $\text{H}_3\text{BO}_4$ , and 50  $\mu\text{M}$  Fe-EDTA (Johnson et al. 1957). Additional N produced by  $\text{NH}_4\text{H}_2\text{PO}_4$ , and  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  supplementation was subtracted from  $\text{KNO}_3$ . After 2 weeks of growth in the nutrient solution, the plants were subjected to different Cd concentrations (0, 5, 10, and 15  $\mu\text{M}$  as  $\text{Cd}(\text{NO}_3)_2$ ) in each plastic tube. After every week of growth, the old nutrient solution was replaced with fresh one. To ensure oxygen supply to the roots, nutrient solution was kept aerated continuously using aeration pumps connected to each tub via plastic tubes. The pH of the nutrient solution was monitored daily and adjusted to  $6.5 \pm 0.05$  with 0.1 N HCl or NaOH. The experiment was laid out in completely randomized factorial design with four replicates.

### Measurements of gas exchange parameters and chlorophyll contents

Two weeks after Cd exposure, gas exchange parameters such as photosynthetic rate, transpiration rate, and stomatal conductance were measured from the third upper fully expanded leaf, using system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Readings were taken by clamping the central part of the leaf in the chamber of the instrument between 11:00 am to 12:00 am to ensure unchanged photon flux density and temperature. Chlorophyll measurement, in terms of SPAD value, was performed on the third upper leaf with the help of chlorophyll SPAD meter (SPAD-502).

### Plant harvesting and biomass

The plants were harvested after 3 weeks of Cd stress then washed with tap water followed by two washings with distilled water. Plants were blotted dry and separated into roots and shoots while their fresh biomasses were recorded. Root and shoot length was recorded using stainless steel scale prior to plant drying. Then, samples were stored in labeled paper bags and air-dried. Followed by air drying, samples were dried at  $65 \pm 5$  °C in a forced air-driven oven till a constant weight and dry biomass of root and shoot was recorded.

## Measurement of Cd and micronutrients

Plant samples (1.0 g each sample) were mixed in 5 ml of each concentrated HNO<sub>3</sub> and HClO<sub>4</sub> in a conical flask and kept for overnight. Then, 5 ml of concentrated HNO<sub>3</sub> was added in the next day and digestion was performed using hot plate until the material became clear. Followed by digestion, samples were cooled and diluted to 50 ml with distill water (AOAC 1990). This diluted material was filtered with Whatman filter paper number 42 and stored in airtight plastic bottles.

Cadmium, zinc (Zn), copper (Cu), and manganese (Mn) concentrations were measured by using calibrated atomic absorption spectrometer (Model Thermo Electron S-Series). The regression relation between concentration and absorbance of the standard solutions were used to calculate the concentration of unknown samples. The enrichment factor (ability of plants to accumulate metal) was calculated as the ratio of shoot Cd concentration to Cd concentration in nutrient solutions (Chen et al. 2004). The translocation factor was calculated as the ratio of shoot Cd concentration to root Cd concentration (Baker and Whiting 2002). Both roots and shoots fresh biomass Cd tolerance index (FBTI) of maize hybrids was calculated by the following formula;

$$\text{FBTI} = \left( \frac{\text{Treatment FBM} - \text{Control FBM}}{\text{Control FBM}} \right) \times 100$$

## Statistical analysis

The data were statistically analyzed following the ANOVA technique (Steel et al. 1996). The least significant difference (LSD) test was applied to differentiate the treatment means differences using the Statistix 8.1 computer software (version 8.1 Software package).

## Results

### Physiological traits

Effect of maize hybrids (V), Cd levels, and their interactions were significant ( $p \leq 0.05$ ) for studied plant physiological traits (photosynthesis rate, transpiration rate, chlorophyll content, and stomatal conductance) except for V × Cd interaction for leaf chlorophyll content (Table 1). By increasing the Cd levels in the growth medium, a decline in maize physiological traits was observed and the highest reduction in these parameters was attained at maximum Cd stress (15 μM Cd) compared to the control. Effects of Cd stress were more prominent on transpiration rate and stomatal conductance as compared to photosynthesis rate and chlorophyll content. Maize hybrids denoted variable susceptibility to Cd stress depending upon applied Cd concentrations and studied physiological traits.

The maize hybrid DKC 61-25 reported significantly higher photosynthesis rate and stomatal conductance while the hybrid DKC 919 and Syn resulted in a maximum transpiration rate and chlorophyll content, respectively. Among hybrids, Cd-associated photosynthesis reduction was minimum in 32-B-33 and maximum in DKC 65-25 as compared to other hybrids. Least tolerance to applied Cd stress was observed for DKC 65-25 for all studied traits at all Cd levels.

### Agronomic traits

Plant agronomic traits were significantly ( $p \leq 0.05$ ) affected under Cd stress except for root dry biomass at 10 and 15 μM Cd levels (Table 1). No significant interaction of V × Cd was documented for most of the studied traits except shoot dry biomass, which was significant (Table 1). By increasing Cd levels, a gradual decline in studied agronomic traits was observed for all hybrids.

### Micronutrient uptake

The potential of maize hybrids for micronutrients (Cu, Zn, and Mn) accumulation in plant shoot and root was evaluated both under Cd-stressed and non-stressed conditions (Table 2). Both Cd concentration and maize hybrid background significantly ( $p \leq 0.01$ ) affected the plant shoot and root potential to uptake these elements. No significant interaction of V × Cd was evaluated for both root and shoot for Cu and Mn while Zn accumulation was unaffected by this combination (Table 2). Accumulation of micronutrients was higher at a minimum Cd stress level and gradually decreased with increasing Cd concentrations in the nutrient solution. All hybrids responded differently depending upon Cd levels and plant part for studied elements accumulation. The maize hybrid 32-B-33 accumulated the highest Cu concentrations while the DKC 65-25 hybrid accumulated the highest Zn concentrations in both shoot and roots compared to the other hybrids. 31-P41 and DKC 919 exhibited greater accumulation of Mn in shoot and roots, respectively. The difference in shoot and root Mn concentration was the least compared to Cu and Zn accumulation. The Cd stress produced the highest negative effect on Zn accumulation in both shoot and roots in all hybrids.

### Cadmium tolerance and translocation

Plant Cd tolerance index (CTI) responded similarly as for micronutrient accumulation. Increasing Cd levels in the growth medium gradually decreased the CTI in all maize hybrids compared to the control (Fig. 1). Effects of Cd levels and hybrids were highly significant for all calculated CTI except for root length CTI that was non-significant. The hybrid 32-B-33 showed minimum CTI for shoot biomass accumulation (0.24) while DKC 919 exhibited least CTI (0.62) for shoot

**Table 1** Maize hybrids response to cadmium stress levels for selected agronomic and physiological traits

Maize hybrid	Nutrient solution Cd level ( $\mu\text{M}$ )					Photosynthesis rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )					Transpiration rate ( $\text{mmol O}_2 \text{ m}^{-2} \text{ s}^{-1}$ )					Chlorophyll contents (SPAD value)					Stomatal conductance ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )				
	0	5	10	15	0	5	10	15	0	5	10	15	0	5	10	15	0	5	10	15	0	5	10	15	
DKC 65-25	485 w	476 x	468 y	460 z	3.24 g	2.54 i	1.85 l	1.26 o	0.83 hi	0.79 k	0.75 m	0.70 o	13.38 o	11.27 s	9.17 v	7.06 x									
23-T-16	576 s	566 t	556 u	546 v	3.34 e	3.34 e	2.44 j	1.66 n	0.88 e	0.84 gh	0.79 k	0.74 m	16.70 i	14.07 l	11.44 r	8.82 w									
DKC 61-25	772 a	758 b	746 d	732 f	4.48 c	4.48 c	3.27 fg	2.22 k	0.92 c	0.88 e	0.83 hi	0.78 kl	22.51 a	18.96 e	15.42 j	11.88 p									
32-B-33	656 m	644 o	634 q	622 r	5.72 a	3.62 d	2.65 h	1.80 m	0.96 b	0.91 d	0.86 f	0.81 j	22.39 b	18.86 f	15.34 k	11.82 q									
31-P-41	672 k	660 l	649 n	637 p	4.63 b	3.62 d	2.65 h	1.80 m	0.85 fg	0.82 ij	0.76 l	0.72 n	20.18 d	17.0 h	13.83 n	10.66 u									
DKC 919	744 d	731 f	719 h	706 j	4.62 b	4.51 c	3.30 f	2.24 k	0.94 c	0.89 e	0.84 gh	0.79 k	20.48 c	17.25 g	14.03 m	10.81 t									
Syn hybrid	751 c	737 e	725 g	712 i	5.76 a	4.47 c	3.27 fg	2.22 k	0.99 a	0.93 c	0.88 e	0.83 hi	20.46 c	17.23 g	14.02 m	10.80 t									
LSD value	Cd 0.9476 <sup>**</sup> variety (V) 1.2536 <sup>**</sup>					Cd 0.0159 <sup>**</sup> variety (V) 0.0210 <sup>**</sup> Cd $\times$ V					Cd 0.005759 <sup>**</sup> variety (V) 0.007618 <sup>**</sup> Cd $\times$ V					Cd 0.0110 <sup>**</sup> variety (V) 0.0146 <sup>**</sup>									
	Cd $\times$ V 2.5071 <sup>**</sup>					0.0420 <sup>**</sup>					0.0152 <sup>NS</sup>					Cd $\times$ V 0.0292 <sup>**</sup>									
	Shoot dry biomass ( $\text{g pot}^{-1}$ )					Root dry biomass ( $\text{g pot}^{-1}$ )					Shoot length (cm)					Root length (cm)									
	0	5	10	15	0	5	10	15	0	5	10	15	0	5	10	15	0	5	10	15	0	5	10	15	
DKC 65-25	7.20 a	5.74 bc	4.32 e-h	3.06 j-m	1.49 ab	1.16 d	0.86 fgh	0.62 j-m	102.5b	94.25 de	84 ghi	75.25 klm	38.9 b	34.3 f	29.7 jk	25.2 op									
23-T-16	3.57 hij	2.87 j-n	2.15 nop	1.59 p	0.75 g-j	0.78 g-j	0.58 k-n	0.42 n	100.5 bc	92.25 ef	82.25 hi	73.75 lm	33.8 f	29.8 jk	25.8 no	21.85 r									
DKC 61-25	4.00 ghi	3.22 i-l	2.41 mno	1.75 op	1.36 bc	1.07 de	0.81 f-i	0.59 klm	99 c	90.75 f	81 ij	72.75 m	34.2 f	30.03 j	26 mn	22.05 r									
32-B-33	5.97 b	4.81def	3.60 hij	2.63 k-n	1.2 cd	0.88 fg	0.65 i-l	0.47 mn	93 ef	85.75 g	76.5 kl	68.5 n	38 c	33.5 fg	29.08 k	24.72 pq									
31-P-41	5.03 cde	4.01 f-i	3.02 j-m	2.15 nop	1.23 cd	0.94 ef	0.70 h-k	0.50 lmn	93 ef	85.25 gh	75.75 klm	68.25 n	41.2 a	36 c	31.4 i	26.6 m									
DKC 919	5.49 bcd	4.35 e-h	3.27 ijk	2.33 m-p	1.50 ab	1.10 d	0.83 fgh	0.59 klm	99.25	91 ef	81.5 i	72.75 m	32.8 gh	28.95 kl	25.05 op	21.25 r									
Syn hybrid	5.69 bc	4.60 efg	3.55 hij	2.47 l-o	1.62 a	1.22 cd	0.91 fg	0.66 ijk	106 a	97.5 cd	87 g	78 jk	36.93 d	32.6 h	28.2 l	23.92 q									
LSD value	Cd 0.3020 <sup>**</sup> variety (V) 0.3996 <sup>**</sup>					Cd 0.0601 <sup>**</sup> variety (V) 0.0795 <sup>**</sup> Cd $\times$ V					Cd 1.3028 <sup>**</sup> variety (V) 1.7234 <sup>**</sup> Cd $\times$ V 3.4468 <sup>NS</sup>					Cd 0.3155 <sup>**</sup> variety (V) 0.4174 <sup>**</sup>									
	Cd $\times$ V 0.7991 <sup>NS</sup>					0.1590 <sup>**</sup>										Cd $\times$ V 0.8347 <sup>**</sup>									

NS non-significant, LSD least significant difference, CV coefficient of variation

\*Significant @  $p \leq 0.05$ , \*\*Significant @  $p \leq 0.01$

**Table 2** Maize hybrids response to cadmium stress levels for micronutrient accumulation in shoot and root

Maize hybrid	Shoot Cu concentration (mg kg <sup>-1</sup> DW)				Shoot Zn concentration (mg kg <sup>-1</sup> DW)				Shoot Mn concentration (mg kg <sup>-1</sup> DW)			
	Nutrient solution Cd level (μM)											
	0	5	10	15	0	5	10	15	0	5	10	15
DKC 65-25	40.1 a	34.4 d	29.2 g	24.4 i	63.7 a	55.3 b	45.6 c	36.5 f	54 bc	48.6 ef	43.3 hi	39.5 L
23-T-16	38.7 b	33.2 e	28.2 h	23.5 ij	46.5 c	40.5 de	33.3 hi	26.7 L	53.9 bc	48.5 ef	43.2 hi	39.4 L
DKC 61-25	37.7 bc	32.3 ef	27.4 h	22.9 j	41.6 d	36.2 f	29.8 j	23.8 m	54.6 b	49.2 ef	43.8 ghi	40 kl
32-B-33	40.4 a	34.6 d	29.4 g	24.6 i	39.5 e	34.4 gh	28.3 k	22.7 m	54.3 bc	48.9 ef	43.5 hi	39.8 kl
31-P-41	37.6 bc	32.3 ef	27.5 h	22.9 j	41.8 d	36.4 f	29.9 j	24.0 m	56.5 a	51 d	45.3 g	41.4 jk
DKC 919	37.4 c	32.1 f	27.2 h	22.7 j	37.0 f	32.2 i	26.5 L	21.2 n	54.8 ab	49.4 de	43.9 gh	40.2 kl
Syn hybrid	38.2 bc	32.8 ef	27.8 h	23.2 j	40.8 de	35.6 fg	29.3 jk	23.4 m	52.8 c	47.5 f	42.3 ij	38.6 L
LSD value	Cd 0.4028 <sup>**</sup> variety (V) 0.5328 <sup>**</sup> Cd × V 1.0657 <sup>NS</sup>				Cd 0.5565 <sup>**</sup> variety (V) 0.7362 <sup>**</sup> Cd × V 1.4725 <sup>**</sup>				Cd 0.6552 <sup>**</sup> variety (V) 0.8668 <sup>**</sup> Cd × V 1.7336 <sup>NS</sup>			
Maize hybrid	Root Cu concentration (mg kg <sup>-1</sup> DW)				Root Zn concentration (mg kg <sup>-1</sup> DW)				Root Mn concentration (mg kg <sup>-1</sup> DW)			
	Nutrient solution Cd level (μM)											
	0	5	10	15	0	5	10	15	0	5	10	15
DKC 65-25	37.6 def	33 jk	28.3 pq	23.5 tu	40.6 a	32.9 b	24.6 d	18.9 gh	47.9 c	43.5 g	38.7 jk	35.9 m
23-T-16	39.4 bc	34.6 hi	29.7 no	24.7 st	33.2 b	26.9 c	20.2 f	15.5 jk	48.8 bc	44.3 fg	39.5 j	36.6 m
DKC 61-25	40.6 ab	35.7 gh	30.6 mn	25.4 rs	33.4 b	27.0 c	20.2 f	15.6 jk	49.3 b	44.7 ef	39.8 ij	37 lm
32-B-33	41.9 a	36.8	31.5 Lm	26.2 r	24.6 d	20.0 fg	15.0 k	11.5 n	50.5 a	45.8 de	40.8 hi	37.9 kl
31-P-41	38.9 cd	34.1 ij	29.3 nop	24.3 st	21.9 e	17.7 hi	13.3 lm	10.2 o	50.5 a	45.8 de	40.8 hi	37.9 kl
DKC 919	36.5 fg	32.1 kl	27.5 q	22.8 u	27.3 c	22.1 e	16.6 ij	12.7 m	50.7 a	46.1 d	41 h	38 kl
Syn hybrid	38 cd	33.4 ij	28.4 opq	23.6 tu	23.7 d	19.2 fg	14.4 kl	11.1 no	50.8 a	46.1 d	41 h	38.1 kl
LSD value	Cd 0.4939 <sup>**</sup> variety (V) 0.6534 <sup>**</sup> Cd × V 1.3069 <sup>NS</sup>				Cd 0.4513 <sup>**</sup> variety (V) 0.5970 <sup>**</sup> Cd × V 1.1941 <sup>**</sup>				Cd (Cd) 0.4429 <sup>**</sup> variety (V) 0.5859 <sup>**</sup> Cd × V 1.1719 <sup>NS</sup>			

NS non-significant, LSD least significant difference, CV coefficient of variation

\*Significant @  $p \leq 0.05$ , \*\*Significant @  $p \leq 0.05$

length. Root biomass accumulation documented minimum value for CTI for DKC-61-25 while CTI for root length was statistically non-significant among hybrids (Fig. 1). Except the 32-B-33 hybrid, all maize hybrids showed non-significant effect for root length CTI at all levels of Cd treatments.

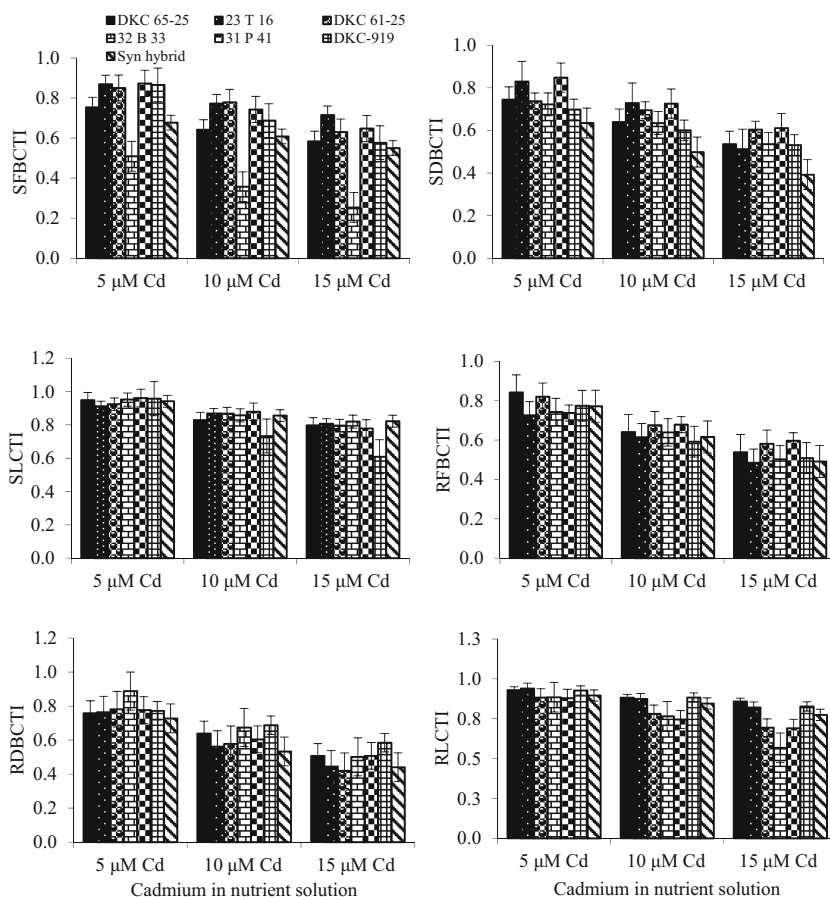
Cadmium translocation index and Cd concentration in shoot and root were significantly higher in those pots where the highest Cd level was applied (Fig. 2, Table 3). The genotypic differences in Cd translocation and accumulation in studied hybrids demonstrated the highest Cd translocation index for the 23-T-16 hybrid. The Cd concentration increased in both shoots and roots of all maize hybrids in a dose-additive manner (Table 3). At all Cd stress, the Syn hybrid accumulated the highest Cd concentration in shoots while the 31-P-41 hybrid accumulated the highest Cd concentration in the roots.

### Discussion

In the present study, six maize hybrids were tested in hydroponic medium to observe Cd-induced changes in growth and nutrient accumulation. Significant reduction in plant growth and gas exchange processes was recorded with increasing Cd stress in all maize hybrids with different extent. The highest negative effect was attained where the maximum level of Cd

was applied. Cadmium-induced growth reduction as recorded in this study is in line with previous studies such as shown in *Brassica juncea* (Iqbal et al. 2005) and *Pisum sativum* (Metwally et al. 2005). There are evidences of Cd-associated stunted growth and physiological traits under hydroponic conditions (Rizwan et al. 2016a). The Cd-based growth reduction in maize is possibly due to a reduction in photosynthetic activity and disturbance in leaf photosystems (Rizwan et al. 2016b). Cadmium denatures protein by disrupting the H-S (hydrogen-sulfur) bond which is responsible for stunted growth and development (Lin et al. 2007). In the present study, chlorophyll contents and gas exchange parameters as well as plant dry biomass decreased with increasing Cd levels in the growth medium (Table 1). Similar results related to Cd-mediated reduction in plant growth and photosynthesis have been observed in many plant species (Lysenko et al. 2015; Arshad et al. 2016; Rizwan et al. 2016a, 2016b). The maize hybrid DKC 61-25 reported significantly higher photosynthesis rate and stomatal conductance that may be due to its higher tolerance and adaptability to Cd treatments. The Cd-induced photosynthetic limitation is might be due to stomatal opening and closing and metabolic processes impairment (Wu et al. 2004, 2006). Another possible reason of the photosynthesis reduction under Cd stress is attributed to limited chlorophyll contents and decreased activity of rubisco and due to lowered

**Fig. 1** Shoot fresh biomass cadmium (Cd) tolerance index (SFBCTI), shoot dry biomass Cd tolerance index (SDBCTI), shoot length Cd tolerance index (SLCTI), root fresh biomass Cd tolerance index (RFBCTI), root dry biomass Cd tolerance index (RDBCTI), and root length Cd tolerance index (RLCTI) for different studied maize hybrids under exogenous applied Cd stress levels

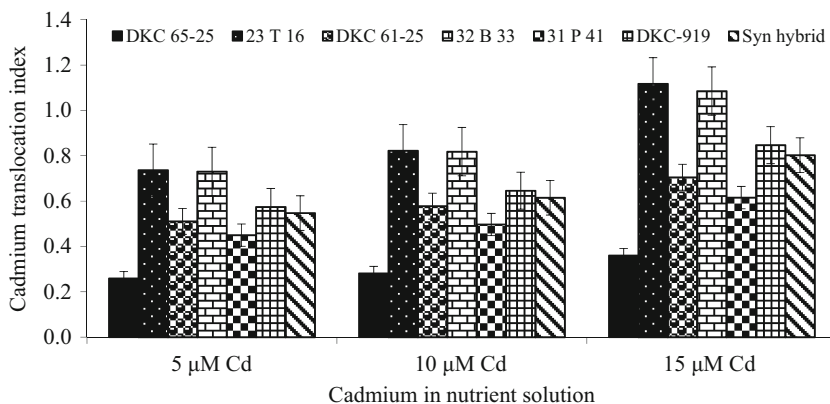


sub-stomatal CO<sub>2</sub> concentration (Wu et al. 2004; Cui and Wang 2006). Cd-mediated variation in gas exchange parameters in maize hybrids might be due to genetic variation in maize hybrids (Anjum et al. 2015). It is well documented that plants potentially develop a wide range of defense systems to minimize the toxic effects of metal stress (Artiushenko et al. 2014; Rizwan et al. 2016b). This defensive system may comprise of enzymatic and non-enzymatic antioxidants, production of osmolyte and chelate synthesis or enhanced cell wall lignification, and suberin lamella formation (Lux et al. 2011; Adrees et al. 2015). Higher level of Cd accumulation may

have affected the antioxidant enzymes (ascorbate peroxidase, superoxide dismutase, and glutathione reductase) that have resulted in inhibition of plant physiological activities. Plants could tolerate metal stress to a certain level by enhancing the activities of antioxidant enzymes, metallothioneins, and stress proteins; the response varies with plant species and metal applied (Xu et al. 2014; Anjum et al. 2015; Parrotta et al. 2015).

Our results reveal that on average, the concentration of Zn, Cu, and Mn in roots and shoots of the seven maize genotypes significantly decreased by the application of 15 μM Cd in the growth medium. The reduction in shoot and root Mn

**Fig. 2** Cadmium translocation index from roots to shoots of maize hybrids and exposed to different levels of Cd in the growth medium



**Table 3** Cadmium concentration in roots and shoot of maize hybrids

Maize hybrid	Root Cd concentration (mg kg <sup>-1</sup> DW)				Shoot Cd concentration (mg kg <sup>-1</sup> DW)			
	Nutrient solution Cd level (μM)							
	0	5	10	15	0	5	10	15
DKC 65–25	ND	19.2 i	32 e	43.1 b	ND	5 m	9 jk	15.5 h
23-T-16	ND	11.8 j	19 i	25.5 g	ND	8.7 k	15.8 h	28.4 e
DKC 61–25	ND	13.1 j	22 h	29.6 f	ND	6.7 L	12.6 i	20.9 f
32-B-33	ND	13.7 j	23 h	30.5 ef	ND	10 j	18.6 g	34.4 b
31-P-41	ND	22.6 h	38 cd	50.5 a	ND	10.2 j	18.6 g	31 d
DKC 919	ND	17.8 i	30 f	39.8 c	ND	10.2 j	19 g	33.2 c
Syn hybrid	ND	21.7 h	36 d	48.3 a	ND	11.9 i	22 f	38.7 a
LSD value	Cd 0.9049 <sup>**</sup> , variety (V) 1.3822 <sup>**</sup> Cd × V 2.3941 <sup>**</sup>				Cd 0.4575 <sup>**</sup> , variety (V) 0.6989 <sup>**</sup> Cd × V 1.2105 <sup>**</sup>			

Values are mean ± SD (*n* = 4). Different letters indicate that values are significantly different at *p* < 0.05

ND not detected, LSD least significant difference, CV coefficient of variation

<sup>\*\*</sup>Significant @ *p* ≤ 0.05

concentration due to Cd toxicity was minimum as compared to Cu and Zn (Table 2). Cd stress induced the highest negative effect on Zn accumulation in both shoot and root (Rizwan et al. 2016a). Cadmium reduces the uptake of these micronutrients by modifying the permeability of plasma membranes and contending for the same membrane transporters. This phenomenon alters the nutrient concentration and composition in plants (Sarwar et al. 2010). Resultantly, Cd causes nutrient deficiency and imbalance in plants (Rizwan et al. 2012). Furthermore, the alteration in micronutrient concentrations and uptake in plants could also be due to a Cd-induced reduction in enzyme activity (i.e., catalase, peroxidase, polyphenol oxidase, superoxide dismutase) and inhibition of root emergence and growth (Chen et al. 2003). In the present study, there existed a significant variation among maize genotypes for root and shoot micronutrient concentration, as affected by Cd levels. The maize hybrid DKC 65-25 showed higher potential for accumulation of Zn, Cu, and Mn, even with incremental Cd toxicity, as compared to other genotypes. It has been shown that there were genotypic differences in the five wheat genotypes for Fe, Zn, and Cu uptake and translocation by adding of 1 mg Cd L<sup>-1</sup> to the nutrient solution (Zhang et al. 2002). Their conclusion was that the effect Cd in solution on micronutrient content varies among elements, plant organ, and genotype. A study by Wang et al. (2007) revealed the variation in Cd, Fe, Cu, Mn, and Zn accumulation in two maize genotypes; one had a higher capacity to uptake Cd from solution and could be a potential hyperaccumulator for Cd toxicity.

Cadmium uptake by roots and shoots varied greatly among the hybrids and increased linearly with increasing concentration of applied Cd (Table 3). The Cd uptake and translocation varied among the wheat cultivars (Naeem et al. 2016) and maize cultivars (Hussain et al. 2012; Broadhurst et al. 2015;

Tanwir et al. 2015). The variation in Cd uptake among maize hybrids might be due to the difference in the genetic makeup of the plants. The roots accumulated the higher amount of Cd compared to shoots (Table 3). The first strategy of plants exposed to Cd stress is an accumulation or deposition of Cd in the cell wall (Fernandez et al. 2014; Rizwan et al. 2016a). The higher accumulation of Cd in roots compared to shoots is quite similar to the phenomenon that the maize plants increase their root biomass to cope with the heavy metal stress like Cd (Rizwan et al. 2016b). Root played a vital role in rhizosphere modification by transforming the architecture, solubility, mobility, and uptake of nutrients (Adrees et al. 2015; Keller et al. 2015). Uptake of Cd in roots mainly depends on root structure and root activities (Stritsis et al. 2014). The plant cell wall serves as a first barrier blocking heavy metal entry into cells and is considered as a pivotal site for storage and deposition of Cd that resulted as a crucial mechanism for plant heavy metal tolerance. Moreover, owing to being negatively charged, the plant cell wall of maize has significant potential for heavy metal binding and retention (Polle and Schützendübel 2004). Overall, the higher accumulation of Cd in roots may enhance the plant tolerance to Cd stress.

### Conclusion

Cadmium effects on growth, biomass, chlorophyll contents, and gas exchange parameters varied greatly among the maize hybrids and decreased with increasing Cd concentrations in the growth medium. The uptake of micronutrients varied greatly among maize hybrids and decreased with increasing the Cd levels. Among the studied hybrids, 31-P-41 and Syn hybrid accumulated the highest Cd concentrations in roots and

shoots, respectively. The higher Cd accumulating maize hybrids may be used for both phytoremediation of Cd-contaminated soils and in the selection and breeding for Cd-tolerant genotypes. However, the molecular basis of such differential accumulation needs to be further investigated.

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