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Full Length Article

Assessment of the Hazardous Effects of Cd on Physiological and Biochemical Characteristics of Soybean Genotypes

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Abstract

Heavy metal contamination in soil has become a serious problem, and cadmium (Cd) in particular is of increasing global concern due to its readily taken up by plants. The selection of plant genotypes with low Cd uptake in roots and its transport to edible parts is a realistic approach to alleviate adverse effects of Cd contamination. In view of these considerations, series of experiments were performed to identify the genotypic difference in physiological and biochemical characteristics of soybean genotypes by applying different Cd concentrations. Twenty three soybean genotypes were grown at 0 and 2.0 μmol L^{-1} Cd in nutrient solution to compare the difference in Cd tolerance. Biometric, physiological and biochemical parameters revealed better performance for S951-3 and high sensitivity of Q17-3 to Cd toxicity. S951-3 and Q17-3 were used in the succeeding experiment to investigate the mechanisms of Cd tolerance in soybean. The Cd (5.0 μmol L^{-1}) reduced growth, chlorophyll content and photosynthetic rate in both genotypes, but the extent of reduction was different. Cadmium, MDA contents, and activity of antioxidant enzymes were significantly increased with Cd treatment, being higher in Q17-3. These data suggest S951-3 as more resistant genotype than Q17-3 to Cd stress. © 2014 Friends Science Publishers

Keywords: Soybean; Cd accumulation; Vacuolar Cd retention; Symplastic Cd transport; Genotypic variation; Root/shoot ratio; Cd extraction capability; Antioxidant system

Introduction

Cadmium (Cd^{2+}) is categorized as a long biological half-life heavy metal. It is readily absorbed by roots and finds its way into the food chain, resulting in toxicity for living organisms (Sanita di Toppi and Gabbrieli, 1999). Moreover, high Cd concentration restrains seed germination, decreases photosynthesis and plant growth, and interferes with the supply of nutrients (Vecchia *et al*., 2005; Rodriguez *et al.,* 2006; Horemans *et al.,* 2007). Plant metabolism may be affected by Cd^{2+} in different ways, and the photosynthetic process in particular appears to be sensitive to this trace metal (Van Assche and Clijsters, 1990). Cadmium causes structural change in chloroplast (Vassiliev *et al*., 2004), inhibits chlorophyll biosynthesis, and its interaction with essential elements (Ciecko *et al*., 2004; Mazen, 2004).

The plant species and their cultivars showed different responses to Cd in the growth medium. The lowest observed effect concentration (LOEC) of Cd on plant biomass was 6.25 mg (lettuce), 12.5 mg (oat) and 50 mg (Chinese cabbage) Cd/kg dry soil (Da Rosa Correa *et al.,* 2006). Legume crops showed low tolerance ability to Cd toxicity as compared to cereals, and show great inhibition in even low Cd levels (Inouhe *et al.,* 1994). Bingham *et al*. (1980) gave the following order of decreasing sensitivity to Cd toxicity, based on the Cd concentration in the soil causing 25% decrease in yield: Spinach > Soybean > Turnip > Cabbage.

Cultivars within the same species can also differ widely in their response to trace elements. Genetic variation within the species for tolerance also exists in soybean (Zhang *et al*., 2002; Zhao *et al.,* 2002; Hassan *et al.,* 2005a). De Oliveira *et al.* (1994) reported genotypic difference in soybean for Cd accumulation, cv. 'Doko', in most cases, exhibited higher Cd content than cv. 'Bossier'. The difference in root Cd uptake and shoot accumulation is used as a marker to explain the genotypic variation in tolerance to Cd toxicity (Kochian, 1995; Wahid and Ghani, 2008).

Hence, it seems possible to find the cultivars with low Cd concentration in plant edible parts. This approach has been fruitfully applied so far in sunflower and durum wheat (Wang, 2002). Qadir *et al.* (2004) studied ten *Brassica juncea* cultivars to determine their Cd extraction capability and extent of resistance to Cd stress, and found that cv. Pusa Jai Kisan possessed a better Cd-binding and antioxidant system.

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Stolt *et al.* (2003) described the vacuolar Cd retention in the root cells is supposed to influence the symplastic Cd transport to the xylem and shoots, give rise to genotypic difference in grain Cd accretion. The mechanisms that affect the root uptake and Cd transportation in shoot can also influence the expression of Cd toxicity in plants (Dunbar *et al.*, 2003). As compared with the normal rice cultivars, the hybrid ones are intended to taken up more Cd from the same polluted soil by root and transport it to shoots and edible parts (Wang and Gong, 1996). The higher abilities of the hybrid rice cultivars for Cd accumulation is due to their higher root activity, increase root/shoot ratio and extra water consumption per gram of grain (Wu *et al.*, 1999).

In order to determine genotypic difference within different soybean genotypes on the basis of Cd stress, this study was conducted to screen and assess the response of genotypes to different Cd levels using growth, physiological, and biochemical characteristics as marker.

Materials and Methods

Plant Material and Growth Condition

Screening experiment: Twenty three soybean cultivars, differing in ecotypes and growth habits were used in this study for comparison of Cd tolerance. For the surface sterilization of seeds, H_2O_2 (2%) was used, washed five times with deionized water and germinated in sterilized sand. After second compound leaves, seedlings were resettled to plastic kettles, covered with plastic lid with equally distributed holes. In each hole, one seedling was planted with each container; 4-5 seedlings of each cultivar were planted. The composition of the basic nutritional solution is presented in Table 1.

At the seventh day after transplantation, two levels of Cd were used, 0 and 2 μmol L^{-1} with maintained pH to 6.0 every other day. The nutritional solution was changed once a week.

At 20 DAT, SPAD values, photosynthetic rate (Pn) were determined and plants were harvested for determination of Cd contents in root and shoot and for measuring root, shoot dry weight. Completely randomized design (CRD) with three replicates was used.

Evaluation experiments: Two soybean genotypes (*Glycine max* L.): S951-3(G2) and Q17-3(G1), with differential tolerance to Cd, based on previous experiment, were used in evaluation experiment. Genotypes were further compared at different Cd levels: T1=0, T2=0.2, T3=1.0 and T4=5.0 μmol L^{-1}, in a hydroponics study. The treatment procedure was kept same as for the screening experiment. At 20th DAT, data were collected for physiological parameters and plants were harvested for further processing.

Measurement of Chlorophyll and Photosynthetic Rate

At the 20th DAT, the 2nd fully emerged leaves were selected for the analysis of chlorophyll contents (Wang, 2002) (Minolta SPAD-502, Japan). Meanwhile, photosynthetic rate (Pn) was also measured with the help of an Infra red analyzer (LI-6400 System, USA) (Wang *et al.*, 2011).

Table 1: The composition of the basic nutrient solution

Nutrients	Amount (mg L^{-1})
$(NH_4)_2SO_4$	48.2
$MgSO_4$	65.9
K_2SO_4	15.9
KNO_3	18.5
$Ca(NO_3)_2$	59.9
KH_2PO_4	24.8
Fe-citrate	5.0
$MnCl_2$.4H2O	0.9
$ZnSO_4$.7H_2O	0.11
$CuSO_4$.5H_2O	0.04
HBO_3	2.9
H_2MoO_4	0.01

Determination of Cd Contents

The sampled shoots as well as roots were dry-ashed in a muffle furnace at 550^{o}C for 20 h, incubated with 1:1 acid mixture of nitric acid (HNO_3): hydrogen peroxide (H_2O_2) at 72^{o}C for 2 h, and dissolved in 0.1 N HCl (Hsu and Kao, 2003). The contents of Cd^{2+}, in these plant tissues were determined by atomic absorption spectrophotometer (Model AA-6800; Shimadzu, Kyoto, Japan).

Measurement of Plant Growth Traits

At 20th DAT, three plants per cultivar were harvested and separated into shoots (leaf blades and sheaths) and roots. Roots were soaked in 0.2% EDTA for 2 h and then rinsed with deionized water thoroughly. Both fresh shoot and roots were weighed, meanwhile shoot height and root length were also measured and then dried in an oven for 48 h at 85°C, and dry weight was recorded.

MDA Content and Antioxidant Enzyme Assay

At 20th DAT, samples were collected for the measurement of antioxidant enzymes. After washing with deionized water, the samples were ground under chilled condition in relevant buffer for each enzyme. The mixture was passed through muslin cloth for filtration and centrifuged (4^{o}C) for 20 min at 10,000, and the supernatant was used for enzyme assays. The MDA concentration and SOD/POD activities were determined as follow.

The MDA content was measured by the method described by (Heath and Packer, 1968). Plant material (0.2 g) were homogenized and extracted in 10 mL of 0.25% (w/v) TBA. Extract was heated at 95^{o}C for 30 min and ice cool immediately. MDA contents were measured at 532 nm after centrifugation at 10,000 *g* for 10 min.

Super oxide dismutase (SOD) activity was assayed with the method described by Giannopolitis and Ries (1977) with slight modifications. Briefly, 0.5 g of samples was

crushed in 5 mL extraction buffer. The photo-reduction of NBT was measured at 560 nm.

Peroxidase (POD) activity was measured with slight modifications (Zheng and Huystee, 1992). The reaction mixture consists of 50 mM potassium phosphate buffer (pH 6.1), 1% guaiacol, 0.4% H_2O_2 and enzyme extract. The absorbance was measured at 470 nm. Enzyme activity was calculated in terms of μ mol of guaiacol oxidized min^{-1} g^{-1} fresh weight at 25±2°C.

Statistical Analysis

All data were presented in mean values of three replicates. Statistical analyses of data were performed by two-way analysis of variance, and treatment means were compared with least significant difference test at P≤0.05 and P≤0.01 (Sokal and Rohlf, 1997).

Results

Biometric Traits

Screening experiment: The dry weight is a genetic variable and an inherent difference in growth and biomass production among genotypes was found, however it is distinctly influenced by Cd stress as well. Thus, the maximum dry weight of shoot without Cd treatment was of E931 and E932, being 7.98 and 7.39 g per plant, respectively; however, it was much reduced in E932 compared to other genotypes when subjected to Cd stress. But E931 performed better under Cd stress, with maximum dry weight, and it was followed by E935-3. The minimum shoot dry weight was of S932 and SB under control and of W7-3 and H18-4 under Cd stress (Fig. 1). The value of T/C ratio was found highest (0.94) for three genotypes (S951-3, E935-4 and JOPB), and lowest for W7-3 and H18-4 (Table 6). The root dry weight differed also among genotypes and between the treatments. Genotypes E935-4 and S951-28 had highest dry root weight in control, while S951-28, S951-23-1 and S951-29 had maximum root weight under Cd stress. The lowest dry root weight was observed for W7-3 and S932 in control and of Q17-3 and Q15-4 under Cd stress (Fig. 2). The maximum T/C ratio (0.95) was observed in S951-3 and S951-28, while the lowest value (0.67) was in Q17-3 and Q15-4 (Table 6). The T/C ratio indicates that S951-3 is most tolerant whereas Q17-3 and Q15-4 were found as most tolerant and sensitive genotypes to Cd stress, respectively.

Evaluation performance: S951-3 (tolerant) and Q17-3 (sensitive) were further subjected to different Cd levels (T1=0, T2=0.2, T3=1.0 and T4=5.0 μ mol L^{-1}) in order to evaluate their plant growth characteristics including plant height, root length and plant biomass (Table 2). The difference among various Cd concentrations was statistically significant for root length being lowest with Cd (5.0 μmol L^{-1}) level. Under the lowest Cd level (0.2 μmol L^{-1}), root growth and plant shoot height were enhanced compared with the plants exposed to highest (5.0 μmol L^{-1}) Cd treatment. The dry biomass of roots and shoots showed a similar response to variable doses of Cd. The lowest Cd level increased plant biomass. However, the toxic effect of cadmium was quite apparent under 5.0 μmol L^{-1}. The differences between two genotypes for all the studied growth parameters were not significant. However there was a significant interaction for root length, plant height and dry shoot weight, while non significant results were found for root dry biomass.

Table 2: Effect of different Cd levels on growth characters of two soybean genotypes

Treatment (T)/ Genotype (G)	Root length (cm)	Plant height (cm)	Dry biomass (g per plant)	
			Root	Shoot
T1	22.00 b	26.70 a	4.40 ab	5.28 ab
T2	25.40 a	28.90 a	4.65 a	5.63 a
T3	22.60 b	28.00 a	4.06 b	5.13 b
T4	14.00 c	16.10 b	2.49 c	2.94 c
LSD (P≤05)	1.90	2.70	0.37	0.43
G1	20.10	24.30	3.74	4.59
G2	21.90	25.50	4.06	4.89
LSD	ns	ns	ns	Ns
T x G (Interaction)	*	*	ns	*

Different letter after data within a column represents significant difference at *P 0.05, respectively

Table 3: Difference among genotypes in Cd contents (μg g^{-1}) of shoot and root as affected by Cd stress

Genotypes	Shoot		Root	
	Ck	Cd treated	Ck	Cd treated
S951-3	2.30 g	31.40 u	7.10 l	67.00 p
S951-23-1	2.10 h	34.70 p	6.80 n	65.00 q
S951-28	2.40 i	33.50 s	7.20 k	82.00 l
S951-29	2.00 a	33.90 r	8.00 j	85.00 k
E931	2.90 a	41.60 h	9.30 h	113.00 f
E932	2.90 a	42.30 f	10.80 e	132.00 c
E935-3	2.80 b	41.70 g	7.10 l	70.00 n
E935-4	2.90 a	39.20 j	6.50 o	60.00 r
E935-6	2.30 g	35.50 m	6.90 m	70.00 n
SB	2.60 d	34.20 q	6.50 o	67.00 p
S873-12	2.50 e	34.80 o	6.80 n	68.00 o
S873-13	2.60 d	37.40 k	6.00 p	60.00 r
HS4	2.90 a	43.10 b	11.30 d	132.00 c
JOPB	2.40 f	36.90 l	12.60 a	150.00 a
ZH1	2.90 a	42.60 e	12.20 b	123.00 d
ZH3	2.00 f	32.40 t	11.30 d	112.00 g
H18-4	2.80 b	45.70 a	6.80 n	70.00 n
H18-6	2.30 g	34.90 n	10.50 f	106.00 i
H18-25	2.70 c	43.00 c	6.90 m	80.00 m
S932	2.30 g	34.80 o	8.70 i	100.00 j
W7-3	2.60 d	39.30 i	10.50 f	120.00 e
Q15-4	2.30 g	36.90 l	9.60 g	110.00 h
Q17-3	2.60 d	42.70 d	11.70 c	138.00 b

[1]Different letter after data within a column represent significant difference at $P \leq 0.01$

Biochemical Components

Screening experiment: The soybean genotypes used varied in Cd content of both shoots and roots (Table 3). There was a small difference under control treatment;

however, under Cd stress genotypes H18-4, HS4, H18-25 and Q17-3 contained the highest Cd in their shoots, while S951-3 and ZH3 had low contents. The C/T ratio in shoots ranged from 0.6 to 0.8, with SB having highest value (0.8) and S951-23-1, S951-29, Q15-4 and Q17-3 the lowest (0.6) (Table 6).

The Cd content in roots was 3-4 times higher than shoot. The genotypes JOPB and ZH1 had the maximum contents in control, while under Cd stress JOPB and Q17-3 had highest Cd content. The Cd tolerance indicator, C/T ratio in roots was highest (0.11) for S951-3 and E935-4 and lowest (0.08) for E931, E932, Q17-3 and JOPB (Table 6).

Evaluation performance: With increased Cd level in the growth medium, Cd content in both roots and shoots increased markedly (Table 4). Moreover, Cd content in roots was 3-4 times higher than in shoots. The highest Cd content was observed in the plants exposed to 5.0 µmol L^{-1} Cd. Between the two genotypes; Q17-3 had significantly higher Cd content in both roots and shoots than S951-3. A significant interaction between treatment and genotype was also found (Table 4).

It is obvious that MDA content rose with increased Cd level (Table 5). The highest MDA content was recorded in the plants exposed to Cd level of 5.0 µmol L^{-1}. The difference between the two genotypes was also significant, with Q17-3 being higher than S951-3. In addition, the interaction between treatment and genotype was highly significant.

The enhanced activity of antioxidant enzymes, like SOD and POD has been observed in the plant species and genotypes that are more sensitive to heavy metal toxicity, so it might be used as biological indicator for detecting Cd tolerance in plants. The results revealed that activities of SOD and POD were significantly increased when the plants were exposed to higher Cd level in comparison to lower Cd levels. Genotypic difference was also significant for both enzymes with Q17-3 having higher activity than S951-3.

Physiological Parameters

Screening experiment: Cadmium stress affected the chlorophyll content adversely of all 23 genotypes in screening experiment, and was higher (25.5) in the control than in Cd treatments (21.4) (Fig. 3). The genotypes also much differed in chlorophyll contents because of genetic differences as well as due to Cd toxicity. Therefore, it was difficult to evaluate these genotypes on the basis of absolute SPAD value either under control or Cd treatments. For that reason, a ratio of chlorophyll content in treated/control was calculated. SPAD value was highest for the genotype ZH3 in control, and for S873-12 and JOPB under Cd treatment, respectively. The lowest chlorophyll content was observed in E931 in control and for Q17-3 under Cd treatment (Fig. 3). The ratio of T/C was highest in E935-3 and the lowest was for Q17-3 (Table 6). There was a pronounced difference among genotypes in T/C ratio, indicating the genotypic variation among soybean cultivars for Cd toxicity tolerance.

Table 4: Effect of different Cd levels on chlorophyll content, photosynthesis and Cd content in the two soybean genotypes

Treatment/ Genotype	Chl. content (SPAD value)	Pn (µmol CO_2 m^{-2} s^{-1})	Cd content (µg g^{-1}) Root	Shoot
T1	28.00 b[1]	9.60 c	8.50 d	2.60 d
T2	33.60 a	13.00 a	21.50 c	5.00 c
T3	29.90 b	10.00 b	50.60 b	13.10 b
T4	17.40 c	3.00 d	199.10 a	61.00 a
$LSD_{.05}$	*2.80*	*1.20*	*12.50*	*2.30*
G1	25.80 b	8.00 b	77.20 a	22.40 a
G2	28.60 a	9.80 a	62.60 b	18.40 b
LSD.05	*2.60*	*1.30*	*7.30*	*2.70*
Interaction (T × G)	*	*	**	**

[1]Different letter after data within a column represents significant difference at $*P \leq 0.01$ or $**P$ 0.05, respectively

Table 5: Effect of different Cd levels on MDA content and antioxidative enzyme activity in the two soybean genotypes

Treatment / Genotype	MDA content (µmol g^{-1} FW)	Enzyme activity (U g^{-1} FW) SOD	POD
T1	15.20 d[1]	123.10 d	28.80 c
T2	22.30 c	146.00 c	31.90 c
T3	37.30 b	175.60 b	37.00 b
T4	72.30 a	238.90 a	48.30 a
$LSD_{.05}$	*6.40*	*19.70*	*4.30*
Q17-3	41.20 A	184.80 A	39.80A
S951-3	32.30 B	156.90 B	33.20 B
$LSD_{.05}$	*4.20*	*17.80*	*3.70*
Interaction between T × G	**[2]	**	*

[1]Different letter after data within a column represents significant difference at $*P \leq 0.01$ or $**P$ 0.05, respectively

The change in chlorophyll content was directly correlated to photosynthetic rate (Pn). Cd stress reduced Pn in all soybean genotypes as compared to control. The highest Pn was recorded in ZH3 followed by SB under both control and Cd stress, while the lowest was for Q17-3 and E931 (Fig. 4). Large variation in Pn T/C ratio was also found among genotypes with the maximum value obtained in S951-3 (0.49), while the lowest value (0.44) for Q17-3, E931 and E932 (Table 6).

Evaluation experiment: On the basis of the screening experiment, the genotypes having with (Q17-3) and highest (S951-3) values of both chlorophyll contents and photosynthesis rate were further scrutinized at different Cd levels (T1=0, T2=0.2, T3=1.0 and T4=5.0 µmol L^{-1}) (Table 4). The chlorophyll content in both soybean genotypes slightly increased when the plants were exposed to low Cd (0.2 µmol L^{-1}). On the other hand, a significant decrease in chlorophyll content was found for the plants subjected to 1.0 µmol L^{-1} Cd compared to the control. The difference between the two soybean genotypes was also significant, with, S951-3 being higher than Q17-3. This difference was also observed in all Cd treatments. There was a significant interaction between Cd treatment and soybean genotype.

Table 7: Comparison of stress tolerance index of different traits among soybean genotypes

Genotypes	Shoot (Dry weight) T/C ratio	Root (Dry weight) T/C ratio	SPAD T/C ratio	Pn T/C ratio	shoot (Cd contents) T/C ratio	Root (Cd contents) T/C ratio
S951-3	0.94	0.95	0.93	0.49 a	0.07	0.11 a
S951-23-1	0.91	0.92	0.93	0.48 ab	0.06	0.10 ab
S951-28	0.88	0.95	0.87	0.48 ab	0.07	0.09 ab
S951-29	0.86	0.9	0.9	0.48 ab	0.06	0.09 ab
E931	0.85	0.94	0.88	0.44 d	0.07	0.08 b
E932	0.68	0.82	0.95	0.44 d	0.07	0.08 b
E935-3	0.91	0.9	0.97	0.46 bcd	0.07	0.10 ab
E935-4	0.94	0.68	0.76	0.48 ab	0.07	0.11 a
E935-6	0.83	0.79	0.95	0.45 cd	0.06	0.10 ab
SB	0.86	0.7	0.79	0.48 ab	0.08	0.10 ab
S873-12	0.91	0.79	0.81	0.48 ab	0.07	0.10 ab
S873-13	0.86	0.77	0.89	0.47 abc	0.07	0.10 ab
HS4	0.88	0.67	0.78	0.48 ab	0.07	0.09 ab
JOPB	0.94	0.69	0.83	0.48 ab	0.07	0.08 b
ZH1	0.85	0.76	0.9	0.48 ab	0.07	0.10 ab
ZH3	0.91	0.76	0.77	0.48 ab	0.06	0.10 ab
H18-4	0.77	0.76	0.77	0.46 bcd	0.06	0.10 ab
H18-6	0.86	0.93	0.81	0.47 abc	0.07	0.10 ab
H18-25	0.91	0.78	0.85	0.48 ab	0.06	0.09 ab
S932	0.93	0.87	0.77	0.48 ab	0.07	0.09 ab
W7-3	0.76	0.84	0.7	0.48 ab	0.07	0.09 ab
Q15-4	0.86	0.67	0.85	0.48 ab	0.06	0.09 ab
Q17-3	0.82	0.67	0.69	0.44 d	0.06	0.08 b

Different letter after data within a column represents significant difference at $P \leq 0.01$

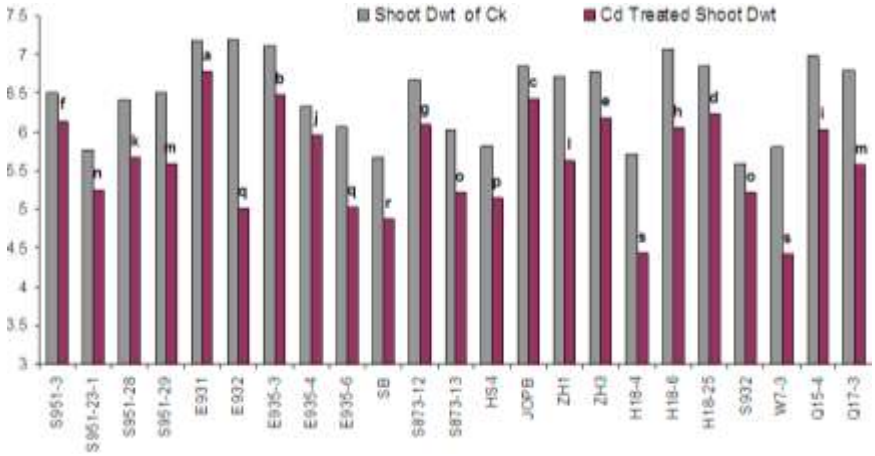

Fig. 1: Comparison of shoot dry weight of different genotypes under control and Cd stress conditions

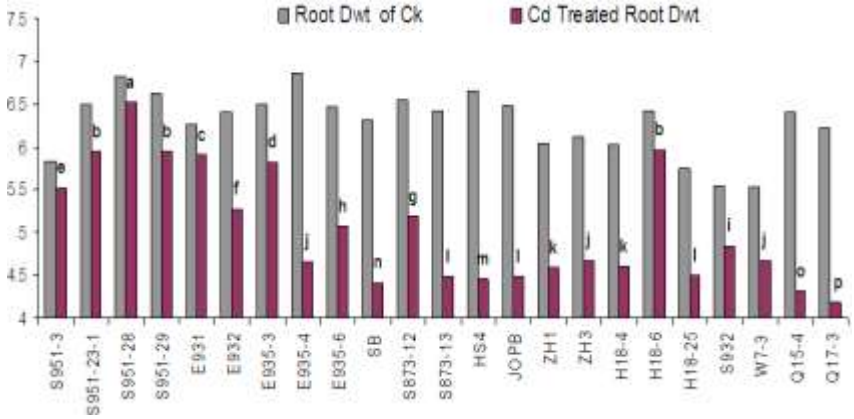

Fig. 2: Comparison of root dry weight of different genotypes under control and Cd stress conditions

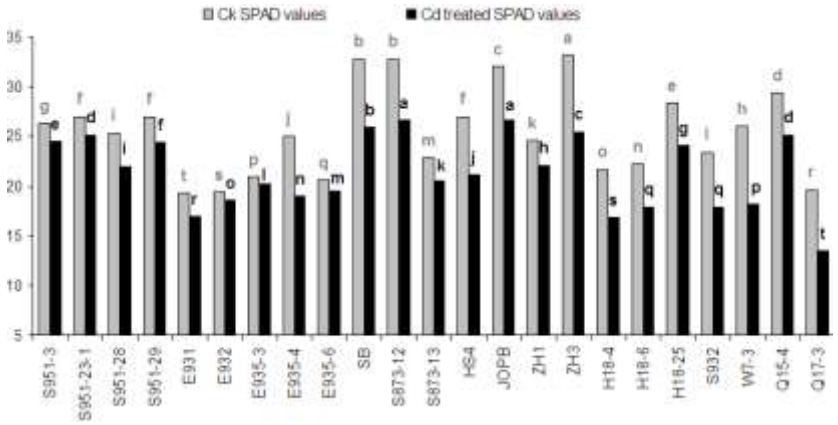

Fig. 3: The difference among soybean cultivars in chlorophyll content (SPAD values) as affected by Cd stress

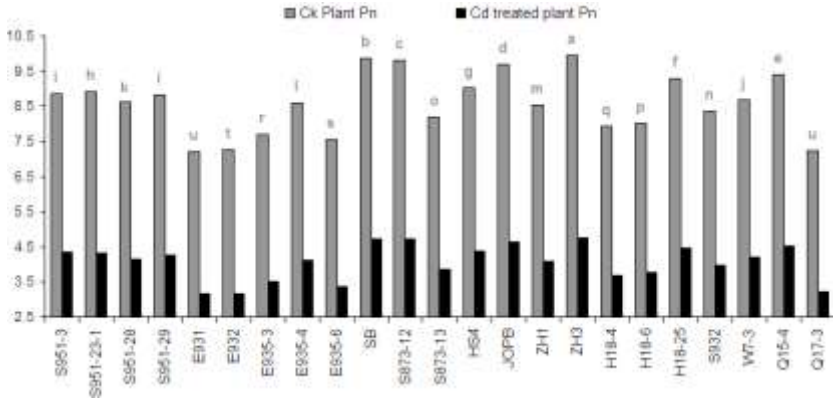

Fig. 4: The difference among soybean cultivars in photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) as affected by Cd stress

The photosynthetic rate in soybean plants was significantly affected by Cd toxicity (Table 4). It was found that lower Cd level (0.2 and 1.0 µmol L^{-1} Cd) enhanced Pn over control and high Cd level (5.0 µmol L^{-1}) reduced Pn significantly. The difference between two genotypes was also statistically significant. Genotype S951-3 had higher Pn than Q17-3 at all levels of cadmium, indicating Q17-3 a more sensitive to Cd toxicity.

Discussion

The study revealed that Cd stress significantly reduced the shoot (Fig. 1) and root (Fig. 2) biomass of all the soybean cultivars. The Cd tolerance index, was found highest (0.94) for S951-3, and lowest for W7-3. Dong *et al.* (2005) also reported the negative impact of different Cd levels on the growth of tomato plants grown under hydroponic conditions. The T/C ratio for root dry biomass in present study showed a large variation among genotypes (Table 6). The maximum value observed in S951-3 and S951-28, while the lowest T/C index of root dry biomass was observed in HS4, Q15-4 and Q17-3. Inouhe *et al.* (1994) reported that legume crops constantly showed strong inhibition under low amount of Cd while Metwally *et al.* (2005) reported that ten pea genotypes were significantly different from each others in growth response under Cd stress. Cadmium inhibited the roots biomass production greater as compared with the shoots in hydroponics, whereas contrary results were observed in sand culture of present study.

The evaluation of contrasting genotypes for plant growth at different Cd levels depicted that Cd concentration lower than 1.0 µmol L^{-1} had no deleterious effect and was beneficial for the growth of soybean. Peralta *et al.* (2000) also reported that a 5 ppm Cd promoted the root growth of alfalfa by 22% as compared to the root growth of the control plants. Oncel *et al.* (2000) found similar effect in wheat seedlings where low Cd concentration enhanced root growth. However in present study, 5.0 µmol L^{-1} Cd showed markedly deleterious effect on plant growth (Fig. 1 and 2).

Soybean showed intra-specific genetic variation for tolerance and Cd uptake (Bell *et al.*, 1997). All soybean genotypes varied significantly in response to Cd stress. Dry matter weight, SPAD value, Pn and Cd content in all genotypes decreased significantly. The chlorophyll content reduced under 2 µ M L^{-1} Cd stress. The T/C ratio for chlorophyll content was highest for E935-3 genotype and

lowest in Q17-3 (Table 6). Chlorophyll content, and net photosynthetic rate, was also reduced by Cd treatment in another experiment (Shamsi *et al.,* 2010). The decline of chlorophyll content in plants treated with Cd is mostly connected with its biosynthesis inhibition (Vassilev and Yordanov, 1997). Cd alone or in combination with Al inhibit Ca and Mg uptake, and reduced Fe, Cu, Mo, and B concentration in roots (Shamsi *et al.,* 2007). Based on low Fe and Mg concentrations in the foliar parts of Cd-treated sugar beet, Greger and Ogren (1991) recommended that reduction in chlorophyll concentration was linked with the deficiency of above mentioned elements. Chlorophyll concentration in plants under Cd stress could also be lowered by the activation of its enzyme degradation (Somashekaraiah *et al.,* 1992). Based on T/C value the genotype Q17-3 seemed to be most sensitive to Cd stress. Net photosynthesis declines in soybean plants at higher Cd concentration (Poschenrieder and Barcelo, 1990).

A highest Cd content was observed for the genotypes, with a marked reduction in growth and photosynthesis, while the reverse was true in Cd tolerant genotypes (Table 3 and 4). The susceptibility of some plants to heavy metal stress is actually a network including physiological and biochemical system i.e. uptake and accumulation of metals, and biochemical stress defense responses (Metwally *et al.,* 2005). The Cd tolerance index (T/C ratio) reflected the highest value in shoots of SB, while the lowest ratio was obtained for many genotypes, including Q17-3. For the root, the C/T ratio was highest for S951-3, E935-4, and the lowest for E931, E932, and Q17-3 and JOPB (Table 6).

Chlorophyll content and photosynthetic rate were also reduced at 5.0 μmol L^{-1} Cd, but not affected significantly at 1.0 μmol L^{-1} Cd (Table 4). Cadmium content in the roots and shoots was significantly enhanced with increased Cd level in the growth medium. The inhibition of Pn by Cd stress was either through the decline in chlorophyll content or/and stomatal conductance (Ouzoundidou *et al.,* 1997). The data also corresponded with those of Oncel *et al.* (2000), who found that Cd reduced chlorophyll *a* and *b* in wheat. Genotype Q17-3 had significantly lower chlorophyll content, Pn and higher Cd content in both roots and shoots as compared with S951-3. De Oliveira *et al*. (1994) reported genotypic difference in soybean for Cd accumulation, cv. 'Doko', in most cases, showed higher Cd concentration and content than cv. 'Bossier'. The decrease in photosynthetic rate and chlorophyll content due to Cd toxicity is genotypic dependent (Hassan *et al.,* 2005b). Intra-specific genetic variation in tolerance and Cd uptake in legume species including soybean had been reported by Bell *et al.* (1997).

Oxidative stress is the phenomenon, which has been considered as one of the key factor causing damage to plants under stress. The presence of toxic metals in cell results in the formation of free radical species, which are toxic to various cell organelles (Radotic *et al.,* 2000). The subsequence of the damage on cells by free radical species is characterized by lipid peroxidation, which may be implicated in the increased MDA content (Somashekaraiah *et al.,* 1992). The present study results proved a concomitant increase of MDA content with increasing Cd level in culture medium. Hegedus *et al.* (2001) reported increased in MDA level under Cd stress to the production of AOS.

Superoxide dismutase provides a defense mechanism against harmful ions, by converting O_2^- to H_2O_2 and further neutralized by POD. In our study, SOD and POD activities increased under Cd stress. Increase in POD activity was proportional to the ion concentration and was found increased by the progressive incubation time (Hegedus *et al.,* 2001). The findings of Da Rosa Correa (2006) in three crop species (lettuce, oats and Chinese cabbage) revealed that activity of all antioxidant enzymes increased significantly compared to those in control plants.

A significant difference between two soybean genotypes was observed for MDA content and activity of SOD and POD. The sensitive genotype Q17-3 had higher activity of antioxidant enzymes as compared to S951-3, which was relatively tolerant to Cd toxicity.

In conclusion, out of 23 genotypes, biometric, physiological and biochemical parameters revealed better performance for S951-3 and high sensitivity of Q17-3 to Cd toxicity. The Cd level 5.0 μmol L^{-1} was found to reduce growth, chlorophyll content and photosynthetic rate in both genotypes, but the extent of reduction was different. Cadmium, MDA contents, and activity of antioxidant enzymes were significantly increased with Cd levels, being higher in Q17-3. These data suggest S951-3 as more resistant genotype than Q17-3 sensitive to Cd stress. Intra-specific genetic variation in tolerance and uptake of Cd exists in almost every plant as depicted from the differential response of all the genotypes to Cd stress. Thus, Cd stress could be a valuable indicator to screen out the tolerant genotypes in crop species.

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