Photosynthetic and antioxidant variability in soybean genotypes under Cadmium stress

Faheema Khan*, and Asma Al-Huqail
Department of Botany and Microbiology, College of Science King Saud University, Riyadh 11495, Kingdom of Saudi Arabia.
Received: 17-02-2016 Accepted: 25-12-2016 DOI: 10.18805/lr.v0i0.7853

ABSTRACT
Soybean, the worldwide main source of oil and high protein feeds for the livestock sector has a high cadmium (Cd) accumulation capacity. With this background, the hydroponic culture experiments were conducted to investigate the effects of different concentrations of Cd (0-100 µM) on growth, water relations, photosynthetic variables, oxidative stress, and antioxidant response in two soybean genotypes P-218 and P-898. Ten days old seedlings were subjected to (0-100 µM CdCl$_2$) for 15 days. The results indicated that the growth of genotype P-218 was not affected significantly up to 75 µM CdCl$_2$ treatment growth of P-828 was reduced significantly beyond 25 µM CdCl$_2$ treatments. Cd toxicity caused severe impairments in photosynthetic variables like photosynthetic rate, chlorophyll fluorescence and chlorophyll content, in P-828 than in P-218. The activities of antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase) were higher in P-218 than in P-828 at various levels of Cd treatments. Chlorophyll fluorescence measurements revealed that non-photochemical quenching increased in P-218 and decreased in P-828 whereas the electron transport rate increased under Cd stress in P-828 and decreased in P-218. It is concluded that tolerance capacity of P-218 against Cd can be associated with the capability of this genotype in keeping an active photosynthetic system and strong antioxidant defense system.

Key words: CdCl$_2$, Chlorophyll fluorescence, Lipid peroxidation, Oxidative stress, Soybean.

INTRODUCTION
Heavy metals are natural elements of the Earth’s crust. However, due to anthropological activities heavy metal contamination of soil and water resources has become a concern of scientific interest (Singh and Prasad, 2011; Gill et al., 2013). Cadmium is generally considered as highly toxic element and its negative effects on plant development and growth have been frequently observed in previous studies (Maksymiec and Krupa, 2006). Physiological effects of Cd toxicity in plants include inhibition of seed germination, major reductions in growth rates (Larbi et al., 2002), Cd also inhibits the photosynthetic pigment biosynthesis, affects the respiratory electron transport flow and also interacts with enzymes of Calvin cycle (Groppa et al., 2012). At the cellular level, Cd toxicity is known to cause alterations such as membrane damage, disruption of electron transport, inhibition/activation of enzymes and interaction with nucleic acids (Chen et al., 2003). In plant cell mitochondrial and photosynthetic electron transfer systems (ETS) are the major targets of Cd toxicity and that subsequently results into rapid production of reactive oxygen species (ROS) (Heyno et al., 2008). Cd at toxic level leads to excessive production of ROS causing cell death due to oxidative stress such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and damage to nucleic acid (Gill and Tuteja, 2010).

*Corresponding author’s e-mail: drfaheemakhan@gmail.com
growth, photosynthetic parameters, lipid peroxidation, and antioxidant response on two soybean genotypes. The promising genotype can be exploited either for direct use in moderately effected Cd soils, or for use in selection and breeding programs to make further advancement of soybean in response to Cd stress.

**MATERIALS AND METHODS**

Seeds of soybean genotypes (P-218 and P-898) were obtained from Indian Agricultural Research Institute, New Delhi, India. These genotypes were surface sterilized, and sown in soilrite mix (Keltech Energies Limited, Bangalore). After 3 days of germination, the seedlings were transferred to Hoagland’s solution (Hoagland and Arnon, 1950) in hydroponic culture system. The growth chamber was maintained at a photosynthetic photon flux density of 430 μmol m⁻² s⁻¹, 14 h of light, 10 h of dark and a relative humidity of 60%. 15-day-old seedlings were treated with 0, 25, 50, 75 and 100 M CdCl₂. Plants were used for growth measurements 15 day after imposing Cd treatments. All the experiments were conducted one time in replicates.

Fresh weights (FW) of shoots and roots were weighed on an electronic top pan balance (Model BL-210-S, Sartorius, Germany). For dry weight (DW) determination samples were oven dried at 65 °C ± 2°C for 72 h and then weighed independently. FW and DW were expressed in g per plant. Length of the plant was measured by using a metric scale and expressed in centimeter.

Photosynthetic rate (PN), stomatal conductance and transpiration rate was measured by infrared gas analyzer (e.g. Portable Photosynthesis System LI-6400, LI-COR Inc., Lincoln, NE, USA). Stomatal conductance and transpiration rate were also measured directly by IRGA using the same instrument. All these measurements were taken at 10:00 to 11:00 in the morning in triplicates when relative humidity, temperature, photosynthetic photon flux density, and CO₂ concentration ranged between 50–60%, 30–35°C, 1200 μmol (photon) m⁻² s⁻¹, and 350–360 ppm, respectively.

Chlorophyll (chl) content was estimated in the fresh leaf samples by the method of Hiscox and Israelstam (1979). The absorbance of DMSO, containing the pigments, was recorded at 663 and 645 nm, using a UV-Vis spectrophotometer (BIO 20, Perkin Elmer, Germany). Values of optical densities (ODs) were used to compute the chlorophyll a, chlorophyll b and total chlorophyll contents by using the following formulae given by Arnon (1949).

\[
\text{FM: FV} = \text{measured with a chlorophyll fluorescence meter (PAM-102, H. Walz, Effeltrich, Germany) after a 30-min dark period at room temperature. The procedure of Schreiber et al. 1986 was used, whereby the minimum (F0) and the maximum fluorescence following a saturating light pulse (Fm) were obtained, and then used to derive the variable fluorescence (Fv = Fm-F0).}
\]

The level of lipid peroxidation in the leaves was determined as malondialdehyde (MDA) content by the method of Heath and Packer (1968). Fresh tissue was grinded in 0.1% TCA with a pestle and mortar and centrifuged at 10,000 rpm for 5 min. 1.0 mL of supernatant was taken in a separate test tube, to which 4.0 ml of 0.5% TBA was added. The mixture was incubated at 95°C for 30 min. The absorbance of the supernatant was read at 532 nm and 600 nm, corrected for unspecified turbidity by subtracting the value at 600 nm. TBA reagent was used as blank. The concentration of MDA was calculated using an extinction (ε) of 155 mM⁻¹ cm⁻¹.

The amount of AsA in leaf samples was measured according to the method of Shigeoka et al. (1979) using sub-samples from the same leaf tissue that were used for the determination of enzyme activities.

**In vitro assay of SOD activity was determined by monitoring the ability of enzyme from the leaf tissue to inhibit photochemical reduction of NBT at 560 nm (Beauchamp and Fridovich, 1971). The reduction in NBT was measured by reading absorbance at 560 nm. One unit of SOD was defined as amount of enzyme which produced a 50% reduction of NBT under the assay conditions (Giannopolitis and Ries, 1977). The activity was expressed in enzyme unit (EU) mg⁻¹ protein h⁻¹.

**In vitro assay of APX activity was estimated by the method used by Nakano and Asada (1981). APX activity was determined spectrophotometrically by monitoring the decrease in ascorbate at 290 nm (ε=2.8 μM⁻¹ cm⁻¹) as described by Nakano and Asada (1981). One enzyme unit determines the amount of enzyme necessary to decompose 1 mmol ascorbate per mg of protein per min at 25°C, and expressed as enzyme unit (EU) mg⁻¹ protein.

**In vitro activity of CAT was determined by the method of Aebi (1984). The activity was determined by monitoring the disappearance of H₂O₂ measuring a decrease in the absorbance at 240 nm. The enzyme activity was calculated using the extinction coefficient 0.036 μM⁻¹ cm⁻¹. One enzyme unit (EU) determines the amount of enzyme necessary to decompose 1 mmol of H₂O₂ per mg protein per min at 25°C, and expressed as EU mg⁻¹ protein.

**In vitro assay of GR activity was determined by the method of Foyer and Halliwell (1976). The GR activity was determined by stoichiometric conversion of NADPH at the baseline level of NADPH absorbance at 340 nm. Protein concentration was determined according to Bradford (1976), using BSA as a standard.

Dried leaf samples (0.1g) were digested with 8 mL nitric acid and 2mL hydrogen peroxide. The digested material was then diluted to 25mL with MilliQ-water and mineral elemental analysis was carried out by optical inductively coupled plasma (ICP) spectrometry (ICP-OES spectrometer,
RESULTS AND DISCUSSION

Growth of soybean genotypes was measured in terms of plant fresh weight (FW) and dry weight (DW) of plants after 15 days of CdCl₂ treatment. Enormous variability in the growth of soybean genotypes was observed under various levels of Cd treatments. The growth of P-898 was significantly reduced at all the Cd treatments. The reduction in the FW and DW of this genotype was 7-64% and 33-73%, respectively. Growth of P-218 declined significantly at Cd concentrations greater than 75 µM. There was 2-23% and 2-60% reduction the FW and DW, when compared to control (Table 1).

Table 1: The effect of different Cd concentration on plant fresh weight (g) plant dry weight (g) and relative water content (RWC%)

<table>
<thead>
<tr>
<th>CdCl₂ (µM)</th>
<th>Plant fresh weight (g)</th>
<th>Plant dry weight (g)</th>
<th>RWC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-218</td>
<td>P-898</td>
<td>P-218</td>
</tr>
<tr>
<td>0</td>
<td>0.410^a</td>
<td>0.560^a</td>
<td>0.062^a</td>
</tr>
<tr>
<td>25</td>
<td>0.401^a</td>
<td>0.520^a</td>
<td>0.061^a</td>
</tr>
<tr>
<td>50</td>
<td>0.395^a</td>
<td>0.420^a</td>
<td>0.056^a</td>
</tr>
<tr>
<td>75</td>
<td>0.367^a</td>
<td>0.231^a</td>
<td>0.045^a</td>
</tr>
<tr>
<td>100</td>
<td>0.314^a</td>
<td>0.202^a</td>
<td>0.025^a</td>
</tr>
</tbody>
</table>

Data are means of three replications (n =3). Data (within each column) followed by same superscript letters are not significantly different according to the LSD test at P = 0.05.

The water relation data suggest that the Cd tolerant genotype P-218 achieved osmotic adjustment by lowering the osmotic potential in response to the externally imposed stress at 100 µM Cd. Photosynthetic rate (PN) decreased by 29-54% in P-898 when plants were subjected to increasing level CdCl₂ treatments (29-54) (2-100 µM) over control; however, reductions in stomatal conductance (gs) was, amounting to 9-23% when compare to control, respectively. Conversely, a reduction in internal CO₂ concentration (Ci) was, amounting to only 7-23% in this genotype. Significant reduction in (PN) and gs in P-218 was reported only at 100 µM Cd treatment only over control (Table 2). Moreover, cadmium chloride application reduced leaf chlorophyll content significantly in both the genotypes in dose dependent manner, however the reduction was much more pronounced in P-898 (31-91%) than P-218 (20-51%) when compare to control. The ratio of variable fluorescence in dark adapted leaves is shown in fig. 2. Leaves of P-898 showed decrease in Fv/Fm with increased level of CdCl₂ (25-100µM) stress amounting (12-84%) while Fv/Fm was reduced only to (3.4-24%) in P-218 with respect to control. Control level ranges from 0.70 to 0.82 during the whole experimental period (Fig. 2). Under Cd stress P-898 progressively reduces psII from control level amounting (11-64%) reduction. Slight reduction in psII was observed in P-218 (7-16%) over control. Control plants of the genotypes showed levels of psII ranging from 0.67-0.76 during the whole experimental period (Fig. 3). In order to evaluate the level of lipid peroxidation caused by oxidative stress, MDA level was determined in soybean genotypes subjected to different level of CdCl₂ for 15days. Maximum amount of the MDA (113%) was reported in P-
Figure 2: Chlorophyll fluorescence (Fv/Fm) of P-218 and P-898 genotypes of soybean exposed to various levels of CdCl₂ treatment (25µM-100µM) at 15 DAT. Data represent mean of three replicates. Vertical bars indicate ±S.E.

Figure 3: PSII quantum efficiency of P-218 and P-898 genotypes of soybean exposed to various levels of CdCl₂ treatment (25µM-100µM) at 15 DAT. Data represent mean of three replicates. Vertical bars indicate ±S.E.

Figure 4: Non photochemical quenching (NPQ) of P-218 and P-898 genotypes of soybean exposed to various levels of CdCl₂ treatment (25µM-100µM) at 15 DAT. Data represent mean of three replicates. Vertical bars indicate ±S.E.

Figure 5: SOD activity of P-218 and P-898 genotypes of soybean exposed to various levels of CdCl₂ treatment (25µM-100µM) at 15 DAT. Data represent mean of three replicates. Vertical bars indicate ±S.E.

Table 3: The effect of different Cd concentration on contents of MDA (nmol g⁻¹ FW) and ascorbate (nmol g⁻¹ FW) of soybean genotype

<table>
<thead>
<tr>
<th>CdCl₂ (µM)</th>
<th>MDA (nmol g⁻¹ FW)</th>
<th>AsA (nmol g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-218</td>
<td>P-897</td>
</tr>
<tr>
<td>0</td>
<td>0.152a</td>
<td>1.92a</td>
</tr>
<tr>
<td>25</td>
<td>0.155a</td>
<td>2.91b</td>
</tr>
<tr>
<td>50</td>
<td>0.158a</td>
<td>2.80b</td>
</tr>
<tr>
<td>75</td>
<td>0.159a</td>
<td>3.40c</td>
</tr>
<tr>
<td>100</td>
<td>0.161a</td>
<td>3.99d</td>
</tr>
</tbody>
</table>

Data are means of three replications (n = 3). Data (within each column) followed by same superscript letters are not significantly different according to the LSD test at P = 0.05.
Ascorbate content was measured at 10 day of CdCl₂ stress in soybean genotypes. There was significant increase in ascorbate content in dose dependent manner. Genotype P-898 showed relatively reduced ascorbate content with an increased level of CdCl₂ stress (28-117%) while genotype P-218 showed 3 fold change of (252%) at 100µM CdCl₂ treatment when compare to control (Table 3).

At the end of the experimental period, CdCl₂ treatment had led to the significant increase of Cd ions in the roots of the treated plant. Cd tolerant genotype P-218 showed (18-89%) Cd accumulation at 100µM CdCl₂ stress whereas genotype P-898 showed (21-70%) Cd accumulation at 100µM when compares to control. (Fig. 9)

According to Patra et al. (2004), Cd is a non-essential element that inhibits some vital plant processes such as photosynthesis, mitosis and water absorption with adverse effects on leaves, wilting of older leaves, stunted foliage, and brown short roots. The effects of Cd on plant growth were evaluated by examining the biomasses of soybean seedlings after 15 days of Cadmium treatment. Plant growth was reduced in both genotypes in dose dependent manner 25-100 µM CdCl₂. Dong et al. (2005) also reported the negative impact of different Cd levels on the growth of tomato plants grown under hydroponic conditions. The Plant biomass in present study showed a large variation among genotypes (Table 1). 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 other in growth response under Cd stress. Soybean showed intra-specific genetic variation for tolerance and Cd uptake (Bell et al., 1997). Soybean genotypes varied significantly in response to Cd stress. In this experiment, the loss of turgor, indicated by a considerable decrease in relative water content (RWC). Moreover the water relations data suggest that the Cd tolerant genotype achieved osmotic adjustment by lowering the osmotic potential in response to the externally imposed stress at 100 µM Cd. The contents of light harvesting pigments in Cd-treated soybean genotypes decreased progressively with rising Cd concentrations Cadmium-
induced declining effect on Chl content which could be explained on the basis of inhibitory effect of Cd co-enzymes involved in pigment biosynthesis (Qian et al., 2009). Total chlorophyll contents showed significant decline in both genotypes in dose dependent manner (Fig.1). Chlorophyll content, and net photosynthetic rate, was also reduced by Cd treatment in another experiment (Shamsi et al., 2010). The data also corresponded with those of Oncel et al. (2000), who found that Cd reduced chlorophyll a and b in wheat. To understand the effect of Cd stress on PS-II machinery, we measured the chlorophyll fluorescence parameters such as Fv/Fm (Fig.2). Photosynthetic efficiency of PS II was not much affected in P-218 under 100 µM Cd while it significantly decreased in P-898. (Fig.3). The Fv/Fm and qP are frequently used to measure the maximum photochemical efficiency of PS II and proportion of oxidized reaction centres PS II, respectively, while Fv/F0 (activity of PS II) and Fm/F0 (electron transport rate through PS II) are also being used to explore the photosynthetic efficiency of plants in fluctuating environments (Xing et al., 2010). Under Cd stress, the decline in qP and decrease in Fv/Fm and Fm/F0 ratios indicate about structural and functional alterations in photosynthetic process as evidenced by decreased biomass accumulation in P-218 and P-898 soybean seedlings. The enhanced NPQ value in genotype P-218 over P-898 pointed towards the mechanism involved in dissipation of excess excitation energy in order to protect the photosynthetic apparatus under Cd stress (Fig.4). Furthermore, rise in NPQ value under Cd stress may be explained on the basis of down-regulation of PS II activity to avoid over-reduction of QA in order to match the decreased demand for electrons through NADPH consumption (Xu et al., 2010). The higher qN in P-218 associated with a higher concentration of reduced AsA and higher activities of the scavenging enzymes probably suggested that the excess energy generated in this tolerant genotype under Cd stress is effectively dissipated through up-regulation of the reactive oxygen scavenging system. AsA can directly scavenge superoxide, hydroxyl radicals and can serve as an enzyme cofactor. The activities of SOD, CAT, APX and GR in P-218 increased significantly, while those of P-898 either declined or stayed unchanged. The superior performance of the Cd-tolerant genotype may therefore be partially attributed to their ability to detoxify effectively ROS generated during stress as a consequence of reduced photochemical carbon fixation and excess energy. These processes involve the maintenance of higher activity of SOD (8 fold increase at 100µM Cd) for the detoxification of O2 and production of H2O2 (Fig.1) CAT and APX regulate H2O2 level in plants. Our data showed that CAT and APX activities in leaves of P-218 increased with increased level of Cd treatment, in contrast, CAT activity in leaves of P-898 decreased with increasing Cd levels. We propose that CAT and APX (3 times increase in P-218 but a substantial reduction in P-898) which are both responsible for detoxification of H2O2, are probably equally important in the detoxification H2O2 in PK-327. Confirming our results findings Shamsi et al. (2014) reported a significant difference between two soybean genotypes. The sensitive genotype Q17-3 had higher activity of antioxidant enzymes as compared to S951-3, which was relatively tolerant to Cd toxicity. GR is important in protecting many plants from oxidative stress (Foyer et al., 1991). Although 100µM Cd remarkably enhanced GR activity of P-218, its activity in leaves of P-898 was decreased under all Cd levels. Because decreased GR activity enhances stress sensitivity (Bor et al., 2003). In addition to that, GR activity in P-218 leaves was significantly higher (5 fold at 100µM) under Cd stress. (Fig.8) Dixit et al. (2001) noted that Cd (50 and 250 µM) caused a progressive increase in GR activity in pea seedlings by 76% and 172%, respectively. However, Singh et al. (2008) observed that Cd (50 and 250 µM) caused a decrease in GR activity in wheat seedlings. The up-regulation of this antioxidant system in the tolerant P-218, together with the maintenance of a high level of reduced AsA and lower MDA compared with those observed in P-898, suggested that this pathway probably plays a functional role in mitigating the effects of Cd stress in soybean. 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 in response to Cd stress.

Variability for root Cd concentration observed here from hydroponics cultures is potentially valuable information for screening soybean genotypes in response to Cd stress. Moreover in our experiment the Cd tolerant genotype P-218 showed more pronounced Cd accumulation in root than in Cd sensitive genotype P-898. The results are in accordance with those of other studies that have shown genotypic variability in Cd accumulation in other crop plants. (Stolt et al., 2006; Perilli et al., 2010).

In conclusion in the present study, opposite patterns of response to Cd stress in P-218 and P-898 were obtained as judged by chlorophyll fluorescence photosynthetic and stomatal parameters with antioxidative enzyme activities. Better Cd stress tolerance of P-218 was associated with increased non photochemical quenching and its ability to maintain higher induced activities of SOD, CAT, APX and GR, resulting in lower level of lipid peroxidation. P-898 is Cd sensitive and P-218 is the Cd-tolerant genotype of soybean. The fact that genetic variability exists with a species in the tendency to accumulate Cd provides an opportunity to plant breeders and genetic engineers to select the Cd tolerant trait which might helpful in development of Cd-tolerant crop plants

ACKNOWLEDGEMENT

The authors would like to extend their sincere appreciation to the deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project no RGP-231.
REFERENCES


