Effect of lead toxicity on germination, seedling growth and anti oxidative enzyme mechanisms of mustard (*Brassica juncea* l.) cultivars

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Abstract:

The present study deals with germination, seedling growth and antioxidative enzymes and free proline, soluble protein, MDA content in seedlings of 7 days old *Brassica juncea* L. plants under different concentrations of lead (Pb). We used five varieties of mustard viz. MAYA, NRCDR-02, DRMRIJ-31, RH-0406, NRCHB-101. Lead decreased the percentage of germination and seedling growth of all the five varieties of mustard. Pb stress treatment increased the activities of antioxidative enzymes such as peroxidase [EC 1.11.1.7]; superoxide dismutase [EC 1.15.1.1] and ascorbate peroxidase [EC 1.11.1.11] and decreased in catalase [EC 1.11.1.6]

Introduction:

An extensive area of the world is contaminated with organic and inorganic pollutants including heavy metals (Ensley, 2000). Several industries, vehicular and agricultural activities contribute to heavy metal contamination of agricultural lands particularly in peri-urban areas [Barman et al., 2000]. Soil contamination with heavy metals is now a worldwide problem, leading to agricultural losses and hazardous health effects. Cultivation of different crops on metal contaminated soils of peri-urban lands have become a major concern today as these crops are said to be the major source of heavy metal contamination of human-beings through food chains [Singh and Aggarwal.,2005]. When heavy metals are present in the soil at very high levels they hamper their growth, productivity and quality of food and fodder crops [Cheng., 2003].

Lead (Pb) is a major heavy metal pollutant in both terrestrial and aquatic ecosystems. Significant increase in the Pb cause sharp decrease in crop productivity thereby posing a serious problem for agriculture [Johnson and Eaton., 1980]. Lead pollution from different sources like industrial and agricultural activities have detrimental impact on surrounding areas [Liu et al., 2007]. Lead caught more attention because of its long persistence in soil and cause extremely toxic effects on both the production of crops and on human health due to consumption of crops. Pb contamination originates mainly from mining and smelting processes, industrial wastes, agriculture (pesticides) and urban activities (additive in petroleum and paint) [Marchiol et al., 2004]. The present study was carried out to identify the influence of lead toxicity on germination and seedling growth of different cultivars of mustard.

Material and methods:

Chemicals and Plant material

Seeds of mustard (*Brassica juncea* L.) cultivars were obtained from National Research Centre on Rapeseed-Mustard, Baratpur, Rajasthan, India. The 5 cultivars of mustard used were Maya, NRCDR-21, DRMRIJ-31, RH-0406 and NRCHB-21. MAYA is also called as RK 9902, is popular existing variety of Rajasthan and U.P. states. MAYA is released by CSAUA&T, Kanpur in the year 2003. NRCDR 02 is also called as Bharat sarson-1, is a hybrid of MDOC 43×NBPGR36. NRCDR-02 is a high yielding indian mustard variety. NRCHB101 is also called as Bharat sarson-2, is a hybrid of BL 4×Pusa bold. Bharat sarson-1&2 are renamed and released in 2012 by DRMR, Bharatpur. DRMRIJ 31 is also called as Giriraj originated at DRMR, Bharatpur. RH-0406 is a hybrid of RH 6908×RH 8812 originated at DRMR, Bharatpur.

Lead (Pb) in the form of lead nitrate [$Pb(NO_3)_2$] was used for the studies.

Seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite solution from commercially available 4% NaClO and washed thoroughly with several changes of sterile distilled water. They were soaked for 30 min

either in i) distilled water (control) ii) 1/2/3/4 mM concentrations of lead. Twenty seeds from each treatment were placed in each of 90mm sterile petri dishes layered with Whatman No.1 filter paper. The petri dishes were supplied with 5 ml of respective test solutions. The seeds were allowed to germinate in dark at 20 ±1ºC. . Number of seeds germinated was recorded at the end of 12, 24, 36 and 48 hours under safe green light. Emergence of radicle was taken as the criteria for germination. On the fourth day, 5 seedlings were retained in each petri plate and 3 ml of test solutions were added and seedlings were allowed to grow.

Growth parameters

On 7th day, seedling growth was recorded in terms of seedling length, fresh weight and dry weight. The seedlings were carefully removed from petri dishes and the water adhering to them was removed with the help of blotting paper. The length and fresh weights of the seedling were recorded. Seedlings were dried in oven at 110°C for 24 hours and their dry weights were recorded.

Free Proline

The amount of proline content was estimated as described by Bates et al(1973). Seedling material (0.5 g) was homogenized with 10 ml of 3 % (w/v) sulfosalicylic acid and the homogenate was filtered through whatman No. 2 filter paper. The supernatant was taken for proline estimation. The reaction mixture was composed of 2 ml of plant extract, 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid. The test tubes containing above mixture were heated in a boiling water bath for one hour. The reaction was terminated in an ice bath followed by addition of 4 ml of toluene. The contents were shaken vigorously and then allowed to separate into phases. The chromophase containing upper toluene phase was carefully taken out with the help of a pipette and the absorbance was taken at 520 nm. The amount of proline present was quantified with the help of proline standard graph.

Soluble Proteins

Soluble proteins in alcohol homogenate (extract in case of enzyme assay) were precipitated by using 20% (w/v) trichloroacetic acid. The precipitate was dissolved in 5 ml of 1% (w/v) sodium hydroxide and was centrifuged at 4000 rpm for 10 min. The supernatant was used for estimation of proteins by Lowry *et al.* method.

Lipid peroxidation.

Lipid peroxidation was determined by estimating the malondialdehyde content following the method of Heath and Packer (1968). Seedlings (1.0 g) were homogenized with 3 ml of 0.5% thiobarbituric acid (TBA) in 20% (v/v) trichloroacetic acid. The homogenate was incubated at 95°C for 30 min and the reaction was stopped in ice. The samples were centrifuged at 10 000 × g for 5 min, the absorbance of the resulting supernatant was recorded at 532 nm and 600 nm. The non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance. The absorbance coefficient of malondialdehyde was calculated by using the extinction coefficient of 155mM–1 cm–1.

Antioxidant Enzymes

The fresh seedling material (200 mg) was homogenized with sodium phosphate buffer at pH 7.0 for CAT, POD, and APX and at pH 7.8 for SOD activities. The supernatant was used to measure the activity of the enzymes and the protein content in the supernatant was determined according to Lowry *et al*.

Catalase (CAT, EC; 1.11.1.6) activity was assayed by the method of Barber(1980). Enzyme extract (0.5 ml) was added to 2.0 ml of hydrogen peroxide and 3.5 ml of phosphate buffer (pH 7.0). The reaction was stopped by adding 10.0 ml of 2% (v/v) concentrated sulphuric acid, and the residual hydrogen peroxide was titrated against 0.01 M KMnO4 until a faint purple color persisted for at least 15 sec. The activity of the enzyme was expressed as enzyme units.

Peroxidase (POD, EC; 1.11.1.7) activity was assayed adopting the method of Kar and Mishra (1976). To 0.5 ml of enzyme extract, 2.5 ml of 0.1 M phosphate buffer (pH 7.0), 1.0 ml of 0.01 M pyrogallol and 1.0 ml of 0.005 M H_2O_2 were added. After incubation, the reaction was stopped by adding 1.0 ml of 2.5 N H_2SO_4. The amount of purpurogallin formed was quantified by measuring the absorbance at 420 nm. The enzyme activity was expressed in absorbance units.

Ascorbate peroxidase (APX, EC; 1.11.1.11) activity was measured according to the Nakano and Asada(1981). The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 250 mM H_2O_2 and enzyme extract. The activity of APX was measured spectrophotometrically by measuring the rate of ascorbate oxidation at 290 nm for 1 min. The amount of ascorbate was calculated from the extinction coefficient of 2.6 mM^{-1} cm^{-1}.

Super oxide sismutase activity (SOD; EC 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) of Beauchamp and Fridovich(1971). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 1.5 ml methionine, 1 ml of NBT, 0.75 ml tri-ton-X-100, 2 mM EDTA, 10 µL of riboflavin and 50 µg of protein. One unit of activity is the amount of protein required to inhibit 50% initial reduction of NBT under light.

Data analysis

The results presented are mean ± SE (standard error) of five replicates. The data analysis was carried out by one way ANOVA followed by Tukey test using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). The differences were considered significant if p was at least ≤ 0.05.

Results

Germination:

Lead toxicity considerably reduced the germination of seeds in case of all the cultivars (Table-1). Further, the inhibition of seed germination was found dose dependently. The impact of lead toxicity was more on NRCHB-101 cultivar, where substantial reduction in seed germination occurred. The influence of toxicity was found relatively less on NRCDR-02 cultivars.

Seedling growth:

The growth of mustard seedlings was found reduced due to lead toxicity. There was inverse relation between applied lead concentration and growth of mustard seedlings. The toxicity impact of lead was much more pronounced on the root growth. At 4mM Pb levels, the root growth in all the cultivars was almost negligible. In all the other treatments root growth was reduced by more than 90% over the respective unstressed control seedlings. Shoot growth was also severely affected due to applied lead toxicity stress. At low levels of applied lead (1mM Pb) NRCDR-02 cultivar found less affected as compared to other cultivars. Among all the cultivars, NRCHB-101 found to be more sensitive to lead toxicity. At high lead concentration (4mM) the growth of seedlings NRCDR-02 cultivar found to be less reduced as compared to other cultivars. At this concentration, NRCHB-101cultivar was found to be most severely affected.

Seedling fresh weight:

The fresh weight of the mustard seedlings was drastically reduced when challenged with lead toxicity. At low levels of Pb (1mM), the reduction in the seedling fresh weight was least in case of NRCDR-02 cultivar and more in case of NRCHB-101 cultivar. At high lead toxicity (4mM), the reduction in the seedling fresh weight was least in case of NRCDR-02 cultivar and more in case of NRCHB-101 cultivar.

Seedling dry weight:

Dry weight is the real measure of growth and was found to be reduced drastically in all the cultivars due to applied lead toxicity. At low levels of lead, the decrease in dry weight was more in case of NRCHB-101 cultivar and least in NRCDR-02 cultivar. At high levels of lead, NRCDR-02 cultivar was found to be less affected and NRCHB-101 was found to be more severely affected as compared to other cultivars.

MDA content:

The membrane damage due to lead toxicity as measured in terms of MDA content was observed in case of all the cultivars. At high levels of lead (4mM), the MDA content was more in case of NRCHB-101 cultivar and least in case of NRCDR-02 cultivar. At low levels of lead (1mM), the MDA content was more in case of NRCHB-101 cultivar and least in case of DRMRIJ-31 cultivar.

Soluble proteins:

The soluble protein content of the mustard seedlings was significantly affected when challenged with lead toxicity. At low levels of Pb (1mM), the reduction in the soluble protein content was least in case of NRCDR-02 cultivar and more in case of NRCHB-101 cultivar. At high lead toxicity (4mM), the reduction in the soluble protein content was least in case of NRCDR-02 cultivar and more in case of NRCHB-101 cultivar as compared to remaining cultivars.

Free proline:

The proline content of the mustard seedlings was increased when challenged with lead toxicity. At both levels of Pb (1mM and 4mM), increase in the proline content was least in case of NRCDR-02 cultivar and more in case of NRCHB-101 cultivar.

Antioxidant Enzymes

Catalase:

Catalase activity was reduced with increasing lead toxicity. CAT is an important oxidizing enzyme that helps in the removal of H_2O_2 and helps in detoxifying harmful metabolic products; its activity appears to be positively correlated with an increase in growth. A decrease in CAT activity due to heavy metals can be attributed to inhibition of the CAT synthesis and other oxidase proteins (Das et al. 1978). At low levels of Pb (1mM), the reduction in the catalase activity was least in case of NRCDR-02 cultivar and more in case of NRCHB-101 cultivar. At high levels of Pb (4mM), the reduction in the catalase activity was least in case of NRCDR-02 cultivar and more in case of NRCHB-101 cultivar.

Peroxidase:

Peroxidase activity was increased with increasing lead toxicity. At low levels of Pb (1mM) and high levels of Pb (4mM), the reduction in the peroxidase activity was least in case of NRCDR-02 cultivar and more in case of NRCHB-101 cultivar.

Ascorbate peroxidase:

Ascorbate peroxidase activity was increased with increasing lead toxicity. At low levels of Pb (1mM), increase in the ascorbate peroxidase activity was least in case of NRCHB-101 cultivar and more in case of NRCDR-02 cultivar. At high levels of Pb (4mM), increase in the ascorbate peroxidase activity was less in case of NRCHB-101 cultivar and more in case of NRCDR-02 cultivar.

Superoxide dismutase:

Superoxide dismutase activity was found increased steeply with increasing lead toxicity. At low levels of Pb (1mM), increase in the superoxide dismutase activity was least in case of NRCHB-101 cultivar and more in case of NRCDR-02 cultivar. At high levels of Pb (4mM), increase in the superoxide dismutase activity was least in case of NRCHB-101 cultivar and more in case of NRCDR-02 cultivar.

Discussion

Inhibitory effect of Pb toxicity on seed germination was observed earlier by Wozny et al., (1982) in lupin. Lead-induced inhibition of seed germination has been reported in *Hordeum vulgare* and *Zea mays* (Tomulescu et al. 2004), *Elsholtzia argyi* (Islam et al. 2007) and *Spartina alterniflora*, *Pinus halepensis* and *Oryza sativa* (Sengar et al. 2009).

Pb hampered the growth of mustard seedlings, there was a decrease in fresh and dry weight of mustard seedlings subjected to Pb stress as compared to control mustard seedlings. Root growth of mustard seedling is inhibited under Pb toxicity. Similar decrease in seedling growth of rice due to Pb stress was reported by Verma and Dubey (2003). Inhibition of seedling growth of radish due to Pb stress was reported by Biteur et al., (2011).

Proline is one component of the non-specific defence systems towards lead toxicity. It alleviates metal toxicity by acting as a metal chelator and as a protein stabilizer (Sharma and Dubey, 2005 and Rama Krishna and Rao, 2016). The proline content increased in the seedling subjected to Pb stress. Proline concentrations

were also increased in young wheat seedlings after 6 days of Pb-stress was observed by (Lamhamdi et al., 2011) and in leaves of wheat and spinach exposed to increasing lead concentrations (Lamhamdi et al., 2013).

Soluble protein content was reduced with the increasing concentration of Pb treatment when compare to control. It was observed in *Zea mays* by Abrar Hussain et, al 2013. Decreasing effect of lead on protein level was also evidenced in *Vicia faba* (Mansour and Kamel, 2005) and *Phaseolus vulgaris* (Hamid *et al*., 2010)

An increase in malondialdehyde content in mustard seedlings grown under Pb stress was observed. It indicates high level of lipid peroxidation. Malondialdehyde is a product of peroxidation of unsaturated fatty acids in phospholipids, and lipid peroxidation is responsible for cell membrane damage (Halliwell and Gutteridge 1985). Increase in the level of lipid peroxides with increasing concentrations of Pb in rice plants is observed Verma and Dubey (2003). Malecka and coworkers (2001) also reported Pb-induced oxidative stress in pea root cells.

Plants have developed efficient detoxification strategies to cope with excessive reactive oxygen species under heavy metal stress. In fact, super oxide dismutase considered to be the first line of defense to contain oxidative damage caused by heavy metals. The activity of SOD under stress has increased. Lead stress increased the POD activity in mustard seedlings. An increase in POD activity is a common response to oxidative and abiotic stress. Pb stress significantly increased the activity of APOX. Interestingly Pb toxicity decreased the CAT activity in mustard seedlings. Its activity appears to be positively correlated with increase in growth. Biteur et al., (2011) also observed decreased activity of CAT in *Raphanus sativus* due to Pb toxicity.

Conclusions

The present study demonstrated the negative impact of lead toxicity on all the five mustard cultivar "(i.e. MAYA, NRCDR-02, DRMRIJ-31, RH-0406 and NRCHB-101). Lead reduced seed germination in mustard cultivars. Lead increased MDA content as result of lipid peroxidation. Antioxidative enzymes activities (POD, APX and SOD) and proline content was increased, and in contrast CAT activity was decreased under lead toxicity.

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Table-1: Effect of lead toxicity on seed germination of mustard cultivars. The results are expressed in terms of percentage of seed germination

Cultivars	Treatments	24 hours	36 hours	48 hours
MAYA	Control	93.7±1.8	95±1.9	97.5±0.4
	1mM Pb	83.7±1.3	91.2±1.2	93.7±0.3
	2mM Pb	81.2±1.2	83.7±1.1	88.7±0.8
	3mM Pb	70.0±1.1	80±1.0	86.2±0.7
	4mM Pb	46.2±1.2	66.2±1.2	75±0.9
NRCDR-02	Control	98.7±1.8	100±0	100±0
	1mM Pb	95.0±1.1	98.7±0.2	98.7±0.4
	2mM Pb	92.5±1.6	95±0.5	97.5±0.2
	3mM Pb	86.2±1.3	91.2±0.7	92.5±0.5
	4mM Pb	63.7±1.2	80±0.9	83.7±0.3
DRMRIJ-31	Control	95±1.6	97.5±0.2	100±0
	1mM Pb	90±1.3	93.7±0.6	97.5±0.3
	2mM Pb	85±1.0	90±0.8	96.2±0.2
	3mM Pb	82.5±1.2	85±1.1	91.2±0.4
	4mM Pb	55±1.4	71.2±1.3	82.5±0.5
RH-0406	Control	70±1.8	85±1.4	93.7±0.6
	1mM Pb	58.7±1.4	77.5±1.7	83.7±0.4
	2mM Pb	53.7±1.5	71.2±1.3	81.2±0.6
	3mM Pb	52.5±1.7	66.2±1.9	78.7±0.9
	4mM Pb	33.7±1.2	53.7±1.4	65±1.0
NRCHB-101	Control	35±1.3	45±1.9	73.7±1.7
	1mM Pb	27.5±1.7	38.7±2.0	62.5±1.2
	2mM Pb	26.2±1.6	36.2±1.7	60±1.9
	3mM Pb	23.7±1.6	31.2±2.4	56.2±1.2
	4mM Pb	15±1.2	27.5±1.8	48.7±1.5

The data presented above are Mean ± S.E. (n = 5). **Pb**.

Table-2: Effect of lead toxicity on seedling growth of mustard cultivars

Cultivars	Treatments	Seedling length (cm)	Seedling FW(mg)	Seedling DW(mg)
MAYA	Control	15.3±0.5	1.30±0.26	0.12±0.10
	1mM Pb	8.4±0.4*	1.07±0.29	0.10±0.07
	2mM Pb	4.5±0.3	0.96±0.22	0.09±0.01
	3mM Pb	4.1±0.2*	0.91±0.25*	0.08±0.05
	4mM Pb	1.7±0.3*	0.72±0.27*	0.07±0.04*
NRCDR-02	Control	18.5±0.9	1.56±0.32	0.15±0.02
	1mM Pb	7.8±0.3	1.36±0.25	0.13±0.06
	2mM Pb	4.7±0.2*	1.27±0.13*	0.12±0.06
	3mM Pb	4.1±0.1	1.19±0.27*	0.11± 0.04
	4mM Pb	3.2±0.2*	0.99±0.54	0.09± 0.04*
DRMRIJ-31	Control	12.1±0.6	1.33±0.11	0.13±0.09
	1mM Pb	5.2±0.4	1.13±0.16	0.11±0.02
	2mM Pb	2.9±0.2*	1.04±0.44	0.10±0.04
	3mM Pb	2.6±0.2*	0.94±0.52*	0.09±0.06
	4mM Pb	2.1±0.1*	0.77±0.28*	0.07±0.03*
RH-0406	Control	12.2±0.8	1.10±0.27	0.11±0.09
	1mM Pb	4.9±0.3*	0.86±0.24	0.09±0.06
	2mM Pb	2.7±0.2	0.81±0.32	0.08±0.06
	3mM Pb	2.0±0.2*	0.70±0.28*	0.07±0.04
	4mM Pb	1.0±0.1	0.58±0.22*	0.06±0.02*
NRCHB-101	Control	13.8±0.5	1.53±0.32	0.15±0.03
	1mM Pb	4.1±0.3*	1.14±0.39	0.11±0.05
	2mM Pb	1.8±0.2	1.06±0.24*	0.10±0.03*
	3mM Pb	1.5±0.1*	0.91±0.22	0.09±0.05*
	4mM Pb	0.8±0.1*	0.71±0.15*	0.07±0.08

The data presented above are Mean ± S.E. (n = 5). **Pb**. * denotes that the mean values are significantly different from unstressed controls of respective cultivars, at p B 0.05 according to Tukey test.

Table-3: Effect of lead toxicity on MDA content, soluble proteins and free proline content of seedlings of mustard cultivars

Cultivars	Treatments	MDA ($\mu mol\ min^{-1} g^{-1}$ FW)	Soluble Proteins ($mg\ g^{-1}fw$)	Free Proline ($mg\ g^{-1}$ FW)
MAYA	Control	20.15±0.21	6.56±0.21	4.12±0.40
	1mM Pb	25.81±0.33*	5.54±0.33	5.16±0.21
	2mM Pb	29.66±0.41	4.57±0.41*	6.19 ±0.28*
	3mM Pb	32.10±0.46	3.56±0.46*	6.61±0.31
	4mM Pb	32.32±0.51*	1.75±0.51	6.64±0.29*
NRCDR-02	Control	21.95±0.62	7.23±0.62	5.02±0.27
	1mM Pb	26.36±0.56	6.26±0.56	5.78±0.55
	2mM Pb	30.99±0.71*	5.59±0.71*	7.14±0.30*
	3mM Pb	33.80±0.63*	4.52±0.63	7.59±0.53*
	4mM Pb	34.38±0.78	2.35±0.78*	7.72±0.62
DRMRIJ-31	Control	18.31±0.81	5.61±0.81	3.93±0.43
	1mM Pb	23.11±0.94*	4.80±0.94	4.72±0.37*
	2mM Pb	26.44±0.98	4.11±0.98	5.82±0.57
	3mM Pb	29.07±0.63	3.18±0.96*	6.22±0.37
	4mM Pb	29.29±0.31	1.62±0.99*	6.30±0.32*
RH-0406	Control	20.86±0.28	6.15±1.02	4.29±0.29
	1mM Pb	27.36±0.21	4.99±1.04*	5.53±0.24*
	2mM Pb	30.93±0.52*	3.96±0.93	6.52±0.57*
	3mM Pb	33.89±0.64*	3.03±1.04	6.93±0.37*
	4mM Pb	34.54±0.69	1.56±1.08*	6.98±0.43
NRCHB-101	Control	15.36±0.53	4.60±1.07	2.84±0.52
	1mM Pb	20.95±0.62	3.70±1.08	3.81±0.53
	2mM Pb	22.99±0.68	2.74±1.03*	4.39±0.56
	3mM Pb	25.71±0.41*	2.04±1.01*	4.63±0.35*
	4mM Pb	26.09±0.49*	1.10±1.04	4.71±0.29*

The data presented above are Mean ± S.E. (n = 5). **Pb**. * denotes that the mean values are significantly different from unstressed controls of respective cultivars, at p B 0.05 according to Tukey test.

Table-4: Effect of lead toxicity on anti oxidative enzymes of mustard cultivars

Cultivars	Treatments	CAT (Umg^{1}protein min^{-1})	APX (µmol ASA mg^{-1} protein min^{-1})	SOD (U mg^{-1}protein min^{-1})	POD (U mg^{-1}protein min^{-1})
MAYA	Control	6.72±0.55	4.56±0.21	3.75±0.09	2.93±0.34
	1mM Pb	3.83±0.43*	6.06±0.23	4.98±0.16*	4.60±0.19
	2mM Pb	3.15±0.59	6.52±0.31	5.58±0.60*	4.98±0.08*
	3mM Pb	2.95±0.67*	6.97±0.36*	5.85±0.58*	5.03±0.12*
	4mM Pb	2.21±0.73	7.15±0.44*	6.03±0.18	5.09±0.19
NRCDR-02	Control	6.19±0.78	6.53±0.51	4.33±0.06	3.23±0.34
	1mM Pb	3.89±1.03	8.09±0.48*	5.36±0.05	4.94±0.39
	2mM Pb	3.46±0.96*	8.61±0.68*	5.84±0.12*	5.23±0.13
	3mM Pb	3.34±1.16*	8.68±0.63*	6.40±0.25*	5.29±0.30*
	4mM Pb	2.22±1.23	8.76±0.61	6.71±0.37	5.45±0.41*
DRMRIJ-31	Control	7.10±0.97	5.52±0.51	3.12±0.31	2.41±0.10
	1mM Pb	4.18±0.85*	7.01±0.48*	4.08±0.28	3.73±0.24
	2mM Pb	3.76±0.81	7.61±0.24	4.43±0.17	4.02±0.39*
	3mM Pb	3.58±0.59	7.94±0.22*	4.77±0.22*	4.07±0.20
	4mM Pb	2.48±0.82*	8.22±0.50	4.89±0.15	4.14±0.19
RH-0406	Control	6.65±0.50	4.13±0.54	3.90±0.32	2.99±0.16
	1mM Pb	3.72±0.52*	5.74±0.28	5.38±0.25*	4.75±0.20
	2mM Pb	2.92±0.88*	6.15±0.31*	5.85±0.13*	5.14±0.06*
	3mM Pb	2.59±0.66*	6.52±0.29*	6.20±0.27	5.20±0.12*
	4mM Pb	2.11±0.70*	6.60±0.27	6.47±0.54	5.23±0.07
NRCHB-101	Control	8.01±1.02	3.83	2.71±0.61	2.28±0.16
	1mM Pb	4.39±0.77	5.36±0.24	3.79±0.36	3.64±0.02
	2mM Pb	3.44±0.39	5.74±0.84*	4.22±0.51	3.94±0.23*
	3mM Pb	2.85±0.52*	6.05±0.61	3.80±0.32*	4.01±0.18
	4mM Pb	2.29±0.82*	6.20±0.22	4.66±0.21	4.12±0.22*

The data presented above are Mean ± S.E. (n = 5). **Pb**. * denotes that the mean values are significantly different from unstressed controls of respective cultivars, at p B 0.05 according to Tukey test.