



Comparative analysis of cadmium-induced stress responses by the aromatic and non-aromatic rice genotypes of West Bengal

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Abstract

Constant exposure of the living ecosystems to heavy metals, like cadmium (Cd), induces a detectable change at the biochemical and genetic level. Repeated application of phosphate fertilizers in paddy fields, leads to increase in Cd content of soil. Cd being highly mobile is transported to shoot and grain, thereby entering into the food chain of animal system. In the present study, treatment of 7-day old rice seedlings with 10 μ M cadmium chloride resulted in Cd toxicity across the seven non-aromatic and six aromatic rice cultivars and landraces used for the study. Free proline and malondialdehyde content of treated samples were higher in comparison to the untreated samples, which indicated Cd induced tissue damage in plants. Photosynthetic pigment content of treated samples was also found to be much lower in comparison to the untreated samples, which is probably due to peroxidation of membrane, leading to compromised and lower photosynthetic efficiency of treated plants. At the genetic level, Randomly Amplified Polymorphic DNA assay was found to efficiently detect the genetic polymorphisms (caused by alterations in DNA bases) induced by Cd. Production of unique polymorphic bands in Cd-treated plants helps in assessment of the degree of damage Cd imparts on the plant system. Cluster analysis was performed and the rice genotypes were grouped into five distinct clusters, with IR64 and Tulsibhog in two distinct groups. Based on the variability in responses, the 13 rice genotypes were grouped into sensitive and tolerant ones.

Keywords Cadmium toxicity · Stress response · Aromatic rice · Non-aromatic rice

Introduction

One of the most toxic heavy metals being leached into the environment is Cd. Being highly soluble and mobile, its concentration gradually increases in the soil leading to bioaccumulation (Yu and Zhou 2009; Ci et al. 2010; Daud et al. 2009; Zhang et al. 2009). The sources of Cd release into the environment include both natural and anthropological origins. Volcanic eruptions and weathering of rocks act as the prime factors for release of Cd into the environment. About 25,000 t of Cd is being emitted into the atmosphere every year. In addition to the previously stated factors, forest fires and

human activities also play a role in Cd release. The most common causes of soil contamination with Cd is by industrial wastes released from metallurgical processes, electromining, paint industries, Cd battery industries (Moradi et al. 2005). Another natural source of Cd is phosphate rock, which is predominantly used in phosphate fertilizers; Cd content in arable soil depends on the application rate and frequency, and degree of Cd percentage present in phosphate fertilizers. Usage of phosphate fertilizers can increase flow of Cd into the food chain through direct phosphate fertilizer application to soil or through indirect changes in Cd bioavailability induced by fertilizers (Roberts 2014).

Various studies conducted by researchers have shown that 60–80% of toxic heavy metal accumulation in human bodies is mainly due to consumption of heavy metals contaminated food (Irfan et al. 2013). The risk reduction by Cd poisoning was regulated by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives at a Provisional tolerable monthly intake of Cd (PTMI) at 25 μ g/kg body weight (Food and Agriculture Organization 2010). The maximum limit of Cd

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content in rice is internationally set to be 0.4 mg Cd/kg of polished rice. Almost half of the world population consumes rice as the main staple food and the rate of consumption of rice tripled in a span of 3 years from 1960 to 2011 (International Rice Research Institute 2011). So indirectly, the Cd is getting mobilized from soil to both plant and animal system. The normal concentration of Cd in soil varies between 0 and 1 mg/kg, whereas low Cd contamination is considered where the Cd concentration in soil is between 1 and 3 mg/kg (Rodriguez and Rodriguez 1982).

Cadmium is absorbed by rice from soil, the ultimate accumulation to grain occurs after several intermediate processes. After absorption by the xylem, cadmium being highly mobile is transported from roots to shoots (Uraguchi et al. 2009). The four major processes for transport of cadmium and its subsequent accumulation involves uptake by root, translocation from root to shoot by xylem flow, nodal redirection, and recirculation from leaves (Ishikawa et al. 2005).

It was already reported that heavy metal (such as, arsenic and cadmium) induced genome instability can impair growth and development of plants and may result significant reduction in crop yield (Nagajyoti et al. 2010). Genotoxicity can take place in plants on exposure to heavy metals; even essential heavy metals can be genotoxic at higher concentrations. Heavy metal may cause genotoxicity through production of reactive oxygen species (ROS). ROS leads to base deletion, base modification, strand breaks, and other damages to DNA. The extent of damage depends on the concentration of genotoxic agent, duration of exposure on the one hand and innate tolerance potential of the plant on the other hand (Aslam et al. 2017). Genotoxic agent-induced DNA alterations can be assessed by DNA fingerprinting methods like Randomly Amplified Polymorphic DNA (RAPD) and amplified fragment length polymorphism (Labra et al. 2003; Liu et al. 2005; Cenkçi et al. 2009; Aksoy and Aras 2011). RAPD is one of the most simple and fastest methods that efficiently detect point mutations as well as temporary DNA alterations (Gupta and Sarin 2009; Cenkci et al. 2010). The genomic template stability percentage or GTS% can be utilized for efficiently assessing the genotoxic effects by evaluation of the RAPD banding profile (Atienzar et al. 1999).

One of the most widely cultivated and consumed cereal crops is rice (*Oryza sativa* L.) and in terms of cultivated area and consumption rates, it is the second most important cereal crop after wheat (Kosolsaksakul et al. 2014). Studies have reported that Cd stress affects rice physiologically and morphologically thereby resulting in several biochemical changes. Reduction in rice growth due to Cd pollution was reported by previous workers (Srivastava et al. 2014). Variations in Cd-induced stress responses among the cultivars may be corresponded to concentration of the pollutant, genotypic storage, and translocation potential (Cao et al. 2014). Studying the varietal responses will not only reveal the sensitivity or

tolerance potential of a particular genotype of commercial significance, but will also ensure the reliability of the biomarkers for assessing the stressed condition of widely consumed cultivars. In the highly polluted environment, where Cd is accumulating at several trophic levels, the biomarkers can act with high fidelity for genotoxicity assessment.

The objective of the present study is to compare the responses of non-aromatic and aromatic indica rice genotypes to moderate concentration of Cd, on the basis of physiological (root and shoot length), biochemical (Proline and MDA content) and molecular parameter assessment (changes in RAPD profiles), and according to Cd stress tolerance potential of these genotypes, categorize them as sensitive or tolerant cultivars irrespective of the presence or absence of characteristic fragrant trait. In our study, some known cultivars and landraces (mostly restricted to West Bengal) were taken. The results obtained may assure the use of these parameters as powerful biomarkers for genotoxicity assessment and applicability to ecotoxicology. The overall impact of Cd stress on aromatic and non-aromatic rice cultivars will also provide an insight on their differential responses.

Materials and methods

Plant material and treatment conditions Seeds of seven varieties of non-aromatic rice viz. IR64, Satabdi, Bandana, Palman, Swarna, Khanika, Kariagora, and six varieties of aromatic rice viz. Badshahbhog, Gobindobhog, Tulsibhog, Pusa Basmati, Tulaipanji, and Radhunipagol were procured from BCKV, Nadia, West Bengal, India. Seeds were surface sterilized with 5% sodium hypochlorite solution for 20 min, washed with distilled water to remove traces of the surface sterilizer, seeds were then soaked in water for 16 h in dark. The seeds were germinated in dark for 3 days on moist blotting paper bed followed by transference to beakers containing 50 ml $1\times$ Hoagland's solution (Hoagland and Snyder 1933) (1 mM KH_2PO_4 , 5 mM KNO_3 , $\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$, $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 11.8 μM $\text{MnSO}_4\cdot \text{H}_2\text{O}$, 0.7 μM $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$, 0.32 μM $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$, 0.16 μM $(\text{NH}_4)_6\text{MO}_7\text{O}_{24}\cdot \text{H}_2\text{O}$, 46.3 μM H_3BO_3 , 5 μM FeCl_3) and grown till seventh day, with 16 h photoperiod with a day/night temperature of $25\text{ }^\circ\text{C}\pm 2\text{ }^\circ\text{C}$, on seventh day plant samples were treated with 10 μM Cd and grown for another 7 days followed by analysis of different parameters.

Measurement of shoot and root length and estimation of photosynthetic pigment content Shoot and root length of control as well as treated plants were measured. The pigment content was estimated from treated and untreated rice leaves by acetone extraction method (Arnon 1949) with some modifications, 200 mg of fresh leaves were finely chopped and added to 10 ml of 80% acetone, left undisturbed for 16 h,

Table 1 DNA sequences of RAPD primers used for PCR amplification

Primers	Nucleotide sequences (5'-3')
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-05	AGGGGTCTTG
OPA-06	GGTCCCTGAC
OPA-07	GAAACGGGTG
OPA-08	GTGACGTAGG
OPA-09	GGGTAACGCC
OPA-10	GTGATCGCAG

absorbance of chlorophyll content was measured at 663 and 645 nm, whereas carotenoid content was measured at 470 nm using UV-VIS spectrophotometer (CompTech).

Stress-related parameters Free proline was estimated according to reported standard protocol (Bates et al. 1973) with a little modification, 500 mg leaves were homogenized in 5 ml 0.1 M sulphosalicylic acid and centrifuged at 5000 rpm for 15 min. To 2 ml supernatant equal volumes of glacial acetic acid and 2.5% ninhydrin solution was added, heated at boiling water bath for 1 h followed by addition of 2 ml toluene and brief vortexing. The toluene content was separated from the aqueous phase and absorbance was measured at 520 nm using Colorimeter (Abzyme). The proline content was calculated from the standard curve using proline as standard and expressed in $\mu\text{g/g}$ fresh weight. The extent of lipid peroxidation was estimated by determining the malondialdehyde content in accordance with the reported standard protocol (Heath

and Packer 1968) with some minor modifications, 250 mg of plant sample was homogenized in 2.5 ml of 0.1% Trichloroacetic acid(TCA) followed by centrifugation at 10,000 g for 5 min, for every 1 ml of aliquot, addition of 2 ml of 20% TCA containing 0.5% Thiobarbituric acid was done, this mixture was heated at 95 °C for 30 min, then quickly transferred to ice bath, the resulting mixture was centrifuged at 10,000 g for 15 min and the absorbance of the supernatant was taken at 532 and 600 nm using UV-VIS spectrophotometer. The concentration of MDA was calculated using the molar extinction coefficient of $155 \text{ mmol}^{-1} \text{ L}^{-1} \text{ cm}^{-1}$ and MDA content was expressed in moles/g fresh weight.

DNA extraction and RAPD profiling Genomic DNA isolation: 0.2 g of plant tissue of both treated and untreated samples were taken for isolation of DNA. The samples were crushed in liquid nitrogen, into fine powder, which were then transferred into sterile microfuge tubes, 2 ml of extraction buffer was added to it, extraction of total genomic DNA was done by modified CTAB method (Saghai et al. 1984) the solution was incubated at 65 °C for 30 min followed by phenol-chloroform-iso-amyl alcohol(25:24:1), after centrifugation the supernatant was again extracted with chloroform-iso-amyl alcohol, the DNA in the upper aqueous layer was transferred to new tube and precipitated with chilled isopropanol, after centrifugation the pellet was washed twice with 70% ethanol, air dried, and dissolved in $1 \times$ TE buffer.

Polymerase chain reaction Nine operon decamer random primers (sequences shown in Table 1) were used for PCR amplification. Total reaction volume for each PCR was set

Fig. 1 Graphical representation of shoot and root growth impairment under cadmium stress in **a** aromatic rice genotypes, **b** non-aromatic rice genotypes

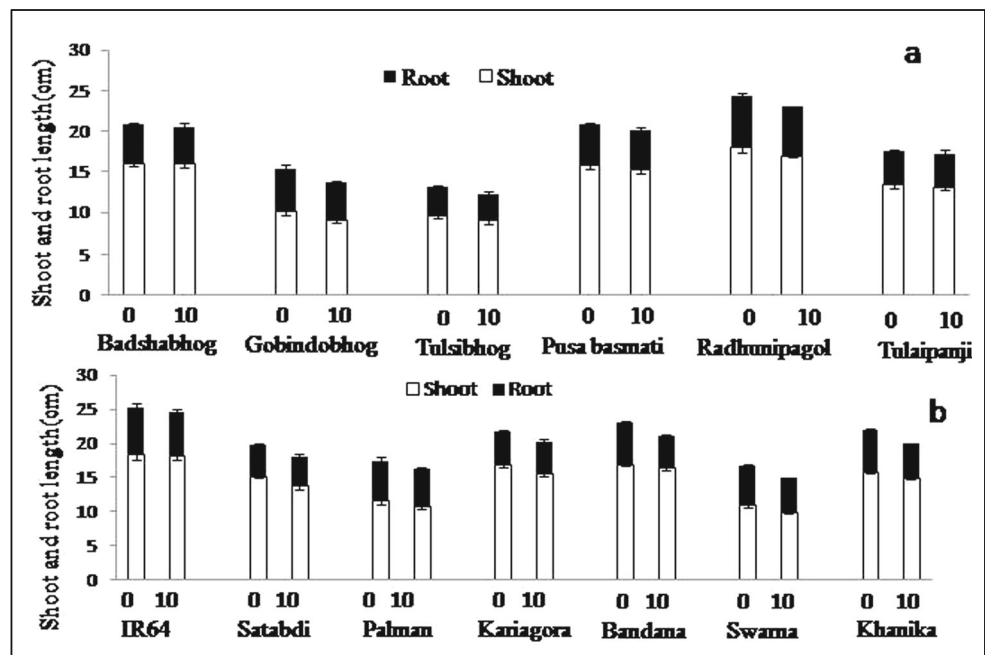
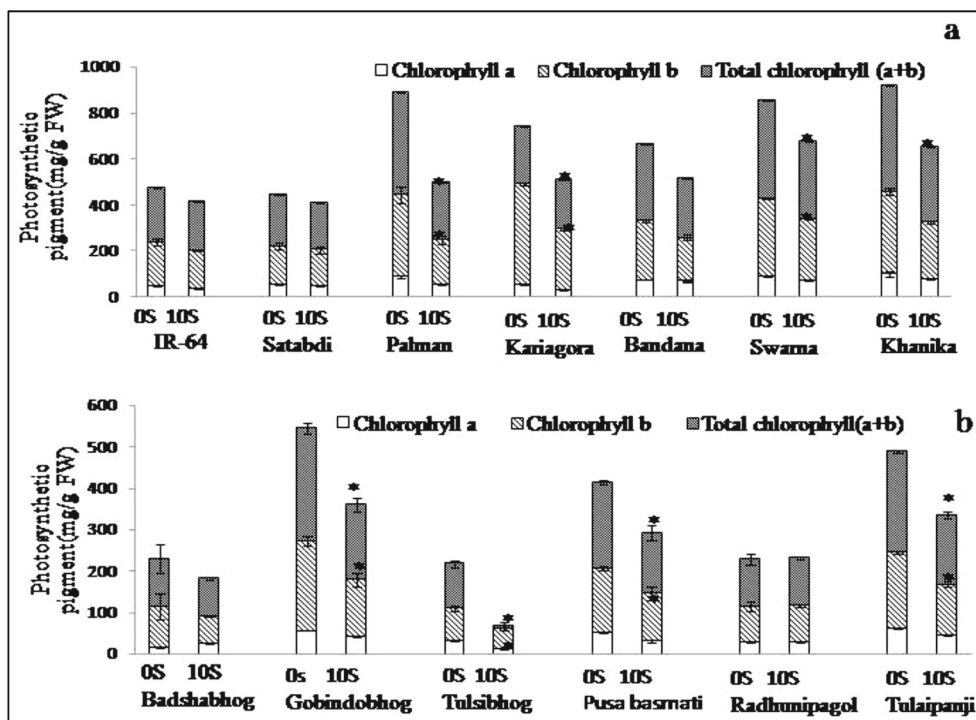


Fig. 2 Graphical representation of photosynthetic pigment contents in rice genotype., **a** Photosynthetic pigment content in non-aromatic rice genotypes. **b** Photosynthetic pigment content in aromatic rice genotypes, statistically significant group obtained from unpaired *t* test, having *P* value ≤ 0.05 is denoted by ‘*’



to be 20 μ l containing 45–50 ng of genomic DNA. The RAPD protocol comprised of initial denaturation of 3 min at 94 $^{\circ}$ C followed by a second denaturation at 94 $^{\circ}$ C for 45 s, annealing at 37 $^{\circ}$ C for 1 min, extension at 72 $^{\circ}$ C for 1 min, and final extension at 72 $^{\circ}$ C for 7 min. The PCR amplicons along with 5 μ l of loading dye were electrophoretically resolved in 1.8% agarose gel, using 1 \times Tris-acetate-EDTA(TAE) buffer at

pH 8.3. The gel was observed under ultraviolet (UV) transilluminator, and gel documentation system was used for photographing(UVP).

Calculation of genomic template stability percentage The RAPD profiles were exploited for calculating GTS values; GTS percentage quantitatively measures the changes in RAPD profiles generated in treated samples in comparison

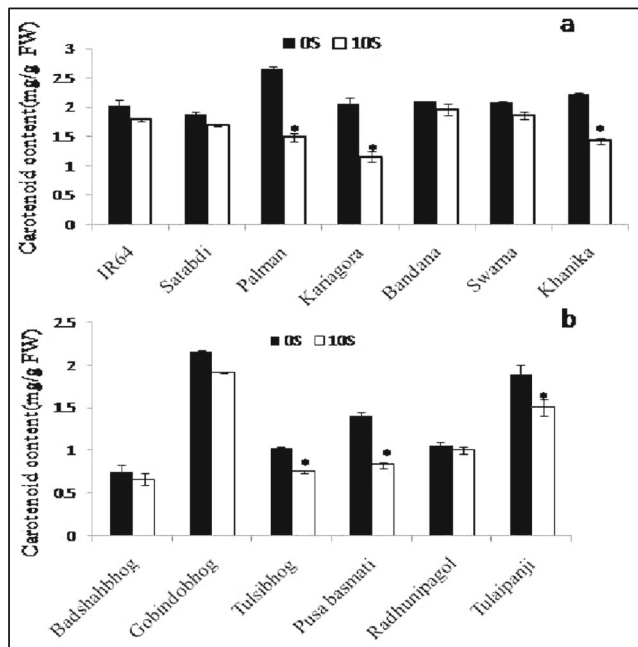


Fig. 3 Total carotenoid content of **a** non-aromatic and **b** aromatic cultivars, statistically significant group obtained from unpaired *t* test, having *P* value ≤ 0.05 is denoted by ‘*’

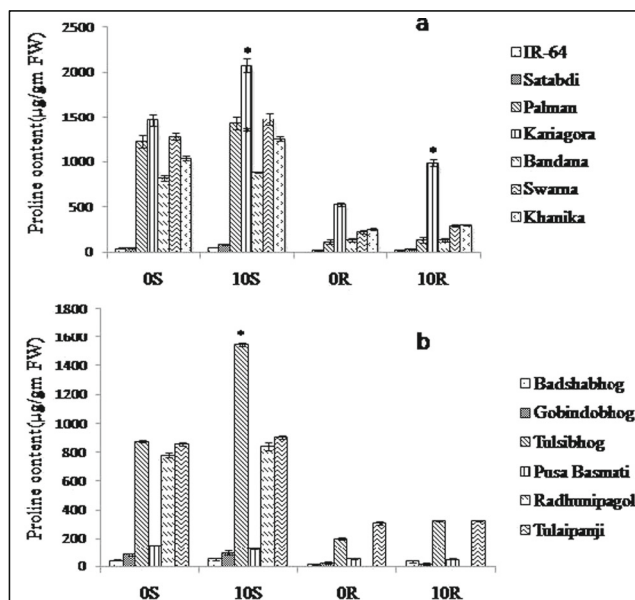
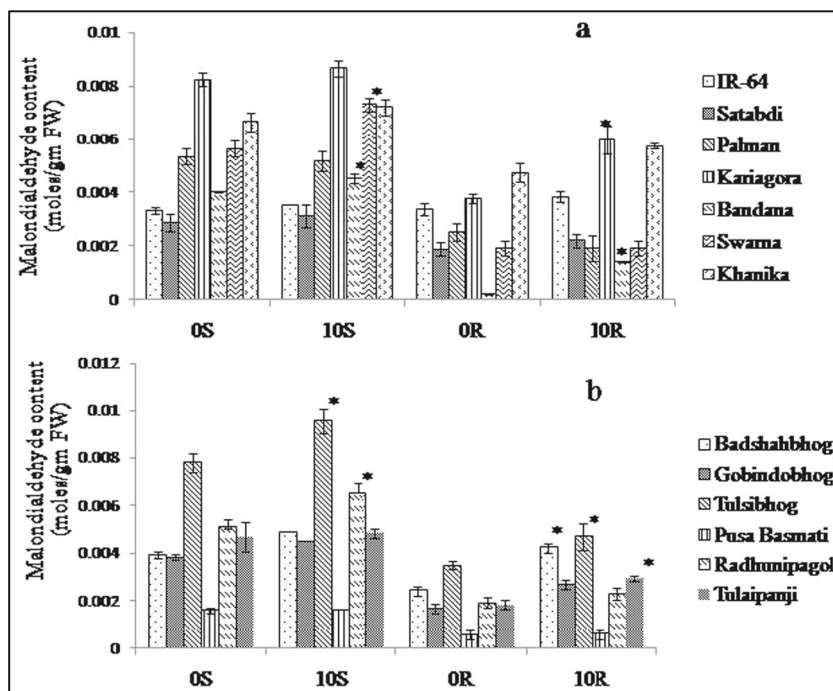


Fig. 4 Graphical representation of proline content in **a** non-aromatic rice genotypes and **b** aromatic rice genotypes, statistically significant group obtained from unpaired *t* test, having *P* value ≤ 0.05 is denoted by ‘*’

Fig. 5 Graphical representation of malondialdehyde content in **a** non-aromatic rice genotypes and **b** aromatic rice genotypes, statistically significant group obtained from unpaired *t* test, having *P* value ≤ 0.05 is denoted by ‘*’



to the control samples. GTS was calculated as $GTS = (1-a/n)$, where *a* = polymorphic profile (total number of bands appeared and disappeared, *n* = total number of bands found in control sample).

Statistical analysis All the data has been statistically tested for significance using R software, *t* test was done, the level of significance has been evaluated at $p \leq 0.05$. To explore the inherent pattern of effect of the Cd stress in rice varieties, we have performed k-mean cluster analysis on the data matrix of relative change using R software. Cluster analysis and grouping of genotypes for the respective parameters was studied. Principal component analysis has been carried out to understand the effect of the use of Cd on different physiological and biochemical parameters of rice plants from an experimental data set of 13 different aromatic and non-aromatic rice cultivars/landraces under Cd stress. ANOVA was done within species for the different parameters studied and between species for a particular parameter.

Results

Inhibition of root-shoot length and altered photosynthetic pigment content under Cd stress The Cd stress lead to decrease in shoot and root length in treated samples in comparison to control samples. Highest percentage of reduction in root length was observed in Tulsibhog (54%), whereas, the lowest percentage of reduction in root length was observed in Bandana, the percentage reduction in shoot length for these cultivars were 12 and 0.62%, respectively (Fig. 1). The

percentage reduction in root length was more in comparison to percentage reduction in shoot length. Cd stress has resulted in differential photosynthetic activity and responses across the genotypes. The pigment contents were found to be greatly reduced in Kariagora, while merely reduced in the cultivar IR-64 among non-aromatic cultivars (Fig. 2a). In aromatic cultivars, highest reduction in pigment content was observed in Tulsibhog and lowest reduction was observed in Tulaipanji genotype (Fig. 2b). Under stress conditions, reduced photosynthetic activity is correlated with reduced photosynthetic pigment content and reduced total carotenoid content. In

Table 2 GTS% of non-aromatic and aromatic rice genotypes

Cultivars	GTS%
Non-aromatic genotypes	
IR-64	92
Palman	74
Satabdi	68
Swarna	64
Khanika	59
Kariagora	49
Bandana	5
Aromatic genotypes	
Tulaipanji	80
Pusa Basmati	79
Gobindobhog	77
Badshahbhog	70
Radhunipagol	65
Tulsibhog	61

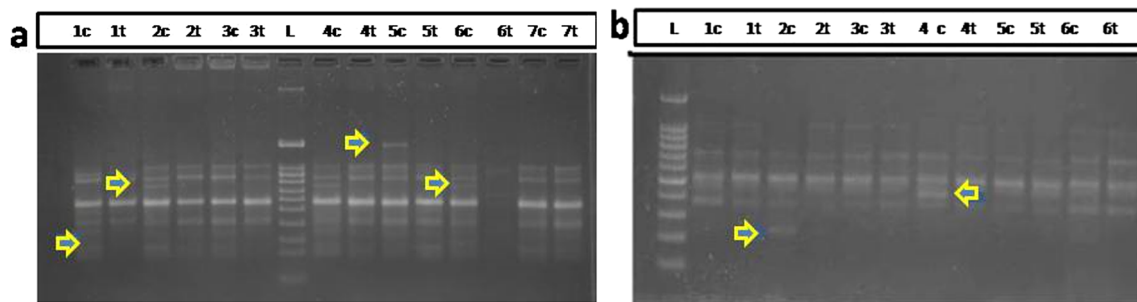


Fig. 6 1.8% agarose gel electrophoresis using RAPD Primer 2 in **a** non-aromatic rice genotypes: lanes 1c and 1t: control and 10 μ M treated IR-64 genotype, 2c and 2t: control and 10 μ M treated satabdi genotype, 3c and 3t: control and 10 μ M treated swarna genotype, L: 100 bp Ladder, 4c and 4t: control and 10 μ M treated Kariagora genotype, 5c and 5t: control and 10 μ M treated khanika genotype, 6c and 6t: control and 10 μ M treated Bandana genotype, 7c and 7t: control and 10 μ M treated

Palman genotype. **b** Aromatic rice genotypes: lanes 1c and 1t: control and treated Pusa Basmati genotype, 2c and 2t: control and treated Radhuniapagol genotype, 3c and 3t: control and treated Gobindobhog genotype, L: 100 bp Ladder, 4c and 4t: control and treated Tulsibhog genotype, 5c and 5t: control and treated Tulaipanji genotype, 6c and 6t: control and treated Badshahbhog genotype. *The arrows indicate polymorphic bands obtained after Cd treatment

non-aromatic rice genotypes highest reduction of carotenoid content was observed in Palman (Fig. 3a), whereas the reduction was least in the genotype IR-64, in aromatic rice cultivars, highest reduction was observed in Tulsibhog while the lowest reduction was observed in Badshahbhog (Fig. 3b).

Cd induced osmoregulant accumulation and membrane damage Accumulation of proline in higher plants is an indication of disturbed physiological condition, triggered by biotic or abiotic stress. Due to Cd stress, aromatic and non-aromatic cultivars and landraces produced various amounts of proline for osmoprotection. Highest accumulation of Proline was found to be in Kariagora (Fig. 4a), indicating its high sensitivity to Cd stress whereas the lowest was recorded for IR-64 among all the non-aromatic rice cultivars. Among the aromatic rice cultivars, the highest amount of proline was found in Tulsibhog and the lowest in Badshahbhog genotype (Fig. 4b). Cd induces membrane damage, a highly damaged membrane, will cause a greater lipid peroxidation and consequently a greater malondialdehyde accumulation which is the final product of lipid peroxidation. In non-aromatic rice cultivars, Kariagora, Swarna, and Khanika were found to accumulate higher concentration of malondialdehyde (Fig. 5a), among the aromatic rice cultivars, Tulsibhog and Radhuniapagol were found to contain higher amounts of malondialdehyde (Fig. 5b), indicating more tissue damage.

Altered RAPD profile RAPD analysis showed polymorphic bands in the treated samples. Appearance and disappearance of bands indicate addition/deletion (mutation) in the DNA template. Based on the GTS percentage observed from RAPD data analysis, overall GTS percentage of aromatic rice cultivars were more than the non-aromatic rice cultivars. In non-aromatic rice cultivars, IR-64 showed the highest GTS% of 92, whereas lowest GTS% was observed for bandana (5%), among aromatic rice genotypes, Badshahbhog was observed to have the highest GTS% of 80%, whereas the lowest GTS%

was observed in Tulsibhog. The GTS% of non-aromatic and aromatic cultivars are shown in Table 2. The RAPD profile of non-aromatic and aromatic rice genotypes are shown in Fig. 6a and Fig. 6b, respectively.

Grouping of genotypes according to their responses under Cd stress by cluster analysis and principal component (PCA) analysis Cluster analysis of all the 13 genotypes under Cd stress lead to their distribution into different groups. The best cluster is five cluster systems (Table 3). Cluster analysis on the basis of shoot and root growth under Cd stress distributed Gobindobhog, IR64, and Bandana into isolated groups (Fig. 7a). Clustering based on total carotenoid and total chlorophyll content grouped Badshahbhog, Tulsibhog, and Bandana into three isolated ranks (Fig. 7b). Ranking of genotypes on the basis of proline accumulation categorized IR64

Table 3 Clustering of genotypes on the basis of physiological and biochemical parameters

Species	Five cluster
<i>Tulsibhog</i>	1
Badshahbhog	2
Kariagora	2
Satabdi	2
<i>IR64</i>	3
Bandana	4
Tulaipanji	4
Gobindobhog	5
Khanika	5
Palman	5
Pusa Basmati	5
Radhuipagol	5
Swarna	5
*89.8% variation explained	

Tulsibhog and *IR64* were allotted to two separate groups

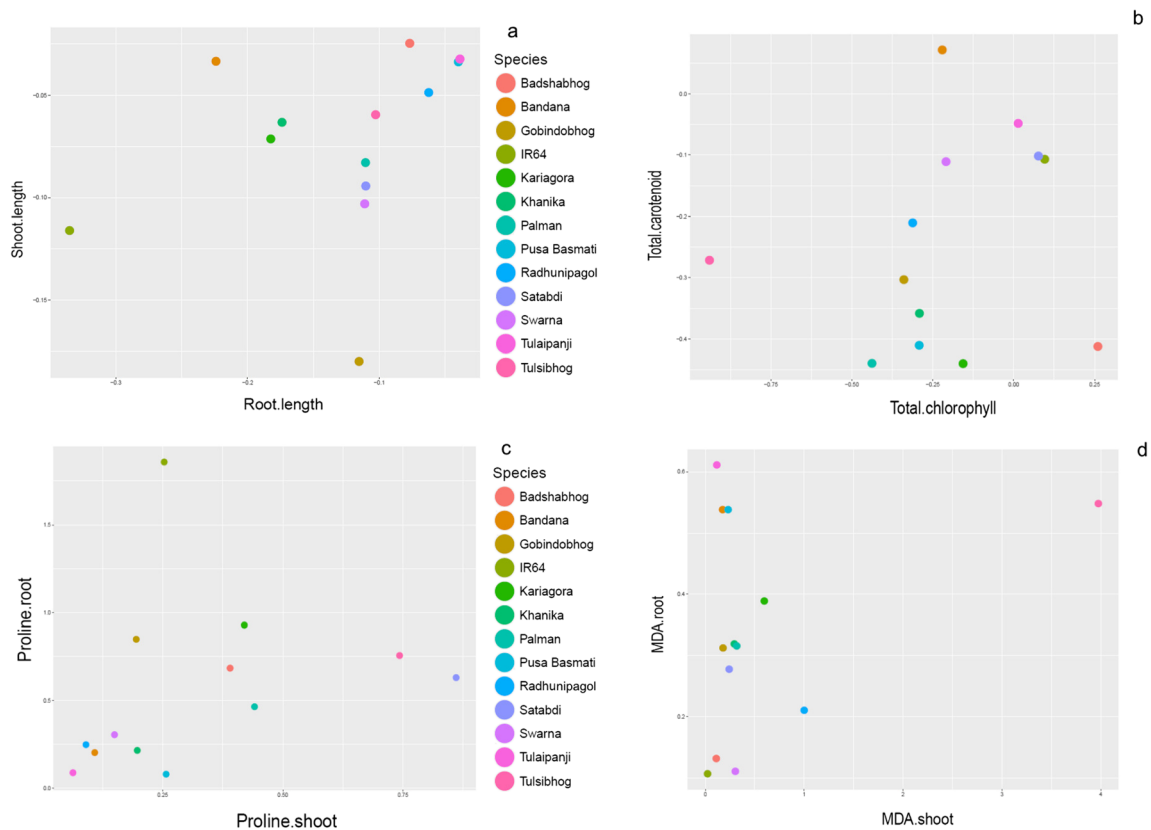


Fig. 7 Cluster analysis of all the 13 genotypes studied under cadmium stress. **a** Cluster analysis for the parameter of shoot and root growth. **b** Cluster analysis for the parameter of photosynthetic pigment and carotenoid content. **c** Cluster analysis for the proline content. **d** Cluster

analysis for Malondialdehyde (MDA) content, color coding for aromatic(A), and non-aromatic(NA) cultivars is given

A NA A NA NA NA NA A A NA NA A A

into an isolated group; Bandana was also found to be in isolation in comparison to the others (Fig. 7c). Distribution of genotypes on the basis of malondialdehyde production grouped IR64, Badshabhog, and Swarna in close ranks whereas Tulsibhog was grouped into a separate rank (Fig. 7d). For principal component analysis, the data matrix of the relative change of treated data over the control data was considered for different parameter values recorded for each of 13 rice cultivars under the stress. PCA results obtained from the data set are furnished in Table 4 and Table 5. It is observed that principle component 1 (PC1) explains 80.9% of the original variability and thus, serves as an important reflector of Cd effect on different physiological and biochemical parameters in some rice varieties. The biplots (Fig. 8a) provide a graphical idea regarding the amount of effect of Cd on the parameters with respect to the new transformed variables PC1 and PC2.

We have presented two zoomed versions of the biplot (Fig. 8b and Fig. 8c) for better understanding. Here, again it is clearly visible that the effect of the Cd stress on MDA-shoot is considerably high.

Discussion

Cd is one of the main environmental heavy metal contaminants; extreme abundance of which is seen near industries. Leaching of industrial effluents is one of the main Cd contaminants in agricultural fields; repeated use of phosphate fertilizers adds to the Cd content of the arable land. In our study, we have investigated the genotoxic effect of 10 μM (moderate level of Cd contamination in soil) Cd on 13 different local rice cultivars and landraces, which included both aromatic and non-aromatic

Table 4 Standard deviation, proportion of variance and cumulative proportion values of principal components

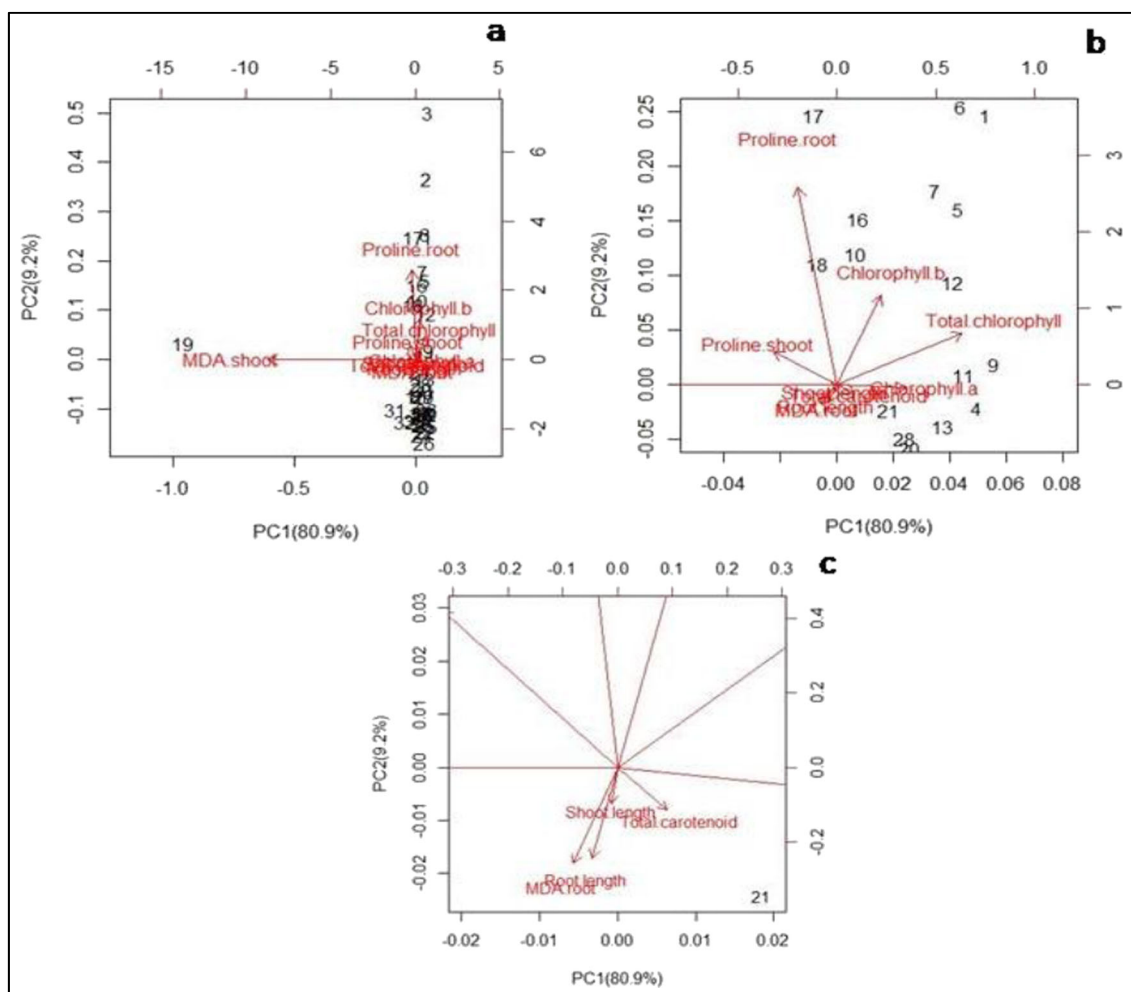
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Standard deviation	1.7539	0.59342	0.38588	0.28416	0.2659	0.21094	0.1186	0.09005	0.06665	0.03519
Proportion of variance	0.8093	0.09264	0.03917	0.02124	0.0186	0.01171	0.0037	0.00213	0.00117	0.00033
Cumulative proportion	0.8093	0.90194	0.94112	0.96236	0.9810	0.99267	0.9964	0.99851	0.99967	1.0000

Table 5 Contribution of the first 10 principal component axes to variation in rice varieties

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Proline shoot	-0.0364	0.1424	-0.0804	-0.4305	-0.5902	0.6563	-0.0380	0.0090	-0.0615	-0.0440
MDA shoot	-0.9951	0.0016	0.0775	0.0393	0.0390	0.0136	-0.0149	-0.0077	-0.0015	-0.0030
Shoot length	-0.0011	-0.0331	0.0616	-0.0332	-0.0363	-0.0058	0.1259	-0.1212	-0.3797	0.9043
Chlorophyll a	0.0404	-0.0180	0.1873	0.4315	0.0524	0.4298	-0.2148	0.0513	0.6609	0.3217
Chlorophyll b	0.0255	0.3926	0.6492	-0.1908	-0.1936	-0.2504	0.4579	-0.0232	0.2770	0.0031
Total chlorophyll	0.0723	0.2245	0.5853	0.1756	0.2093	0.2471	-0.4215	-0.0157	-0.5083	-0.1719
Total carotenoid	0.0102	-0.0387	-0.0640	0.5594	-0.0412	0.3338	0.6834	0.0569	-0.2606	-0.1729
Proline root	-0.0224	0.8705	-0.4156	0.1780	0.0576	-0.0738	-0.0859	0.1123	-0.0108	0.0911
MDA root	-0.0093	-0.0872	0.0499	0.4485	-0.7463	-0.3789	-0.2719	0.0760	-0.0913	-0.0128
Root length	-0.0053	-0.0823	0.0714	-0.1118	0.0509	-0.0127	0.0224	0.9798	-0.0592	0.0933

rice genotypes. Aromatic rice is favored for their characteristic aroma and has higher commercial significance. The aromatic rice variety Gobindobhog has earned geographical indication status recently for its indigenous cultivation to Burdwan, West Bengal, and Radhunipagol, and is a highly preferred aromatic genotype for its medicinal properties. Tulaipanji is a non-

Basmati aromatic rice indigenous to Uttar Dinajpur, West Bengal. Badshabhog and Tulsibhog are high yielding rice genotypes and has high marketability and indigenous to Bankura, West Bengal. Pusa Basmati is a cultivar released by PUSA, IARI, but is now commercially cultivated in West Bengal for its rich fragrance and resistance to biotic and abiotic stress.

**Fig. 8** a Biplot of the genotypes involving all the physiological and biochemical traits. b, c Zoomed in version of 'a'

Most of the aromatic rice genotypes studied in the present work are cultivated only in West Bengal and are maintained by the breeders and farmers. Our objective was to study, the tolerance response of the cultivars and landraces to Cd stress; simultaneously, we also wanted to investigate whether differential responses are present between aromatic and non-aromatic cultivars. Results indicated that Cd stress has impaired both shoot and root growth; a higher impairment rate of meristematic tissue was observed in case of root growth and it suggested the phytotoxicity of Cd for plant growth (Suzuki 2005). The highest reduction in root growth was observed for Tulsibhog, while the lowest was for Bandana. On subjecting the different plant genotypes to Cd stress, a clear decrease in photosynthetic pigment was observed; this decrease can be correlated to ROS mediated peroxidative breakdown of chloroplast membrane lipids and pigments. Heavy metal stress is known to impair the uptake of nutrients; this is also accompanied by chlorophyll degradation by enzymatic activity of chlorophyllase (Singh et al. 2006; Srivastava et al. 2007; Sharma and Dubey 2005). Studies indicated that Kariagora and Tulsibhog showed lower photosynthetic pigment contents in comparison to the other studied rice genotypes.

Maintenance of cell osmotic pressure is an important action as a primary defense mechanism in response to stress conditions; this is mediated by proline accumulation in high quantities. Proline is known to protect cell structure and function by maintaining the osmotic pressure (Koca et al. 2007). Proline is known as a proteogenic amino acid, an osmolyte and is also responsible for radical scavenging activity (Matysik et al. 2002). In our study, highest amount of proline accumulation was found in Kariagora and Tulsibhog, showing their sensitivity towards Cd stress, whereas the genotypes Badshahbhog and IR64 were found to contain lower amount of proline in comparison to the other genotypes.

Lipid peroxidation of the membrane leads to production of malondialdehyde as a result of membrane damage under stressed condition. Lipid peroxidation is mediated by free radical reaction that causes degeneration of cell membranes. Heavy metals is negatively affecting the plant membrane stability leading to damage and cell death (Sizova et al. 2004). Our results indicated that Cd stress has induced noticeable production of malondialdehyde in the treated plant samples compared to the control ones. The results suggest that the deficiency in antioxidants as a result of increased production of superoxide radical has led to the increased accumulation of malondialdehyde in the cells. From our studies, it was observed that Kariagora and Tulsibhog have highest concentrations of malondialdehyde content in comparison to untreated genotypes, suggesting their higher susceptibility towards Cd stress.

Any change in the RAPD banding profile consisted of disappearance or appearance of new bands in treated samples compared to that of untreated ones. Heavy metals have been

shown to induce various types of damages and alterations to DNA that might comprise of point and deletion mutations, single and double strand nicks, and base modification. In comparison to control set, when treated set produced lesser number of RAPD bands, then this might be correlated with several events of DNA modifications including DNA damage, single base mutations, or chromosomal alterations induced by genotoxic agents. Studies have revealed that appearance of new RAPD products indicate minute changes in primer annealing sites due to mutations, drastic deletions, or homologous recombination (Atienzar et al. 1999, 2000).

Our results indicate that treatment of different rice cultivars with 10 μ M Cd stress has produced noticeable change in treated samples in comparison to untreated ones. Among the aromatic rice cultivars, higher number of polymorphic bands were observed in the genotypes Tulsibhog and Radhunipagol (with a total of 13 and 12 polymorphic bands, respectively), while the least number of polymorphic bands were obtained for Badshahbhog and Pusa Basmati (which were 8 and 7, respectively). Tulsibhog having a GTS percentage of 61% has been identified as one of the most Cd-susceptible rice genotypes, high accumulation of MDA also correlates with this. Highest GTS value of 80% was obtained for Badshahbhog, indicating Badshahbhog as one of the Cd tolerant rice genotypes. Accumulation of lower amounts of malondialdehyde and proline compared to others and lesser reduction in chlorophyll content in Badshahbhog also supports this. The overall number of amplicons was more in case of non-aromatic rice cultivars compared to aromatic ones. Among the non-aromatic cultivars, the highest GTS% of 92 was observed for IR64 owing to polymorphic bands, whereas lowest GTS% of 5 was obtained for Bandana owing to 35 polymorphic bands followed by Kariagora having a GTS% of 49 and comprising of 15 polymorphic bands. The higher the genomic template stability percentage of a particular genotype, the higher is its tolerance ability to genotoxic agents, IR64 having the highest stability among all the non-aromatic rice cultivars have also been found to be more Cd tolerant corresponding to lower degree of proline and malondialdehyde accumulation as well as mere reduction in photosynthetic pigments under stress conditions. Kariagora has been found to be one of the most Cd susceptible rice genotypes, revealing its lower GTS% as well as having higher accumulation of stress indicators.

Cluster analysis was done using R software for all the physiological and biochemical parameters studied, the variations in Cd-induced stress responses among the 13 genotypes were statistically evaluated and differential responses among the genotypes can be best represented by ranking of genotypes into different groups. Five cluster grouping was found to represent and allot the genotypes in the best way possible, this led to the assignment of IR64 (the most tolerant of all) and Tulsibhog (the most sensitive of all) into completely two different groups, group 1 and group, 3 respectively. Badshahbhog,

Kariagora, and Satabdi are placed in group 2, whereas Bandana and Tulaipanji in group 4, rest of the genotypes are placed in group 5. Among the non-aromatic cultivars, IR64 was found to have the recurrent non-significant *P* values whereas the genotype Kariagora has significant *P* values for all the parameters studied. This shows the tolerance potential of the genotype IR64 towards Cd stress, whereas Kariagora was found to have the least tolerance potential among the non-aromatic cultivars studied. Among the aromatic rice genotypes studied, Tulsibhog was found to be the most sensitive to Cd stress, whereas the genotypes Badshabhog and Tulaipanji showed lesser responses in the treated seedlings in comparison to the untreated ones; this is also correlated with their non-significant *P* values obtained for malondialdehyde and total chlorophyll content studies.

The loading vectors of PC1 exhibits that the effect of the stress on MDA (shoot) is quite high (−0.995198358). We have also observed from the loading vector of PC1 that proline (shoot and root), MDA (shoot and root), root and shoot length form a group, all getting negative loads, whereas Chlorophyll a, Chlorophyll b, total Chlorophyll, and total carotenoid make a different set with positive loading. Variability explained by principal component 2 (PC2) is 9.26%, and like that, there are other principal components which are less relevant as the variability explained by them is much smaller. The biplots clearly indicate that MDA-root, root-length, and shoot-length get affected in a similar pattern while we can put the other parameters in a different group on the basis of how the application of Cd affects them.

Among the seven non-aromatic rice genotypes, non-significant *P* values were obtained for five non-aromatic cultivars (IR64, Satabdi, Palman, Bandana, and Swama), when statistically assessed by *t* test for four different parameters (proline content, malondialdehyde content, total chlorophyll content, root growth inhibition percentage). This clearly indicates that the changes in treated plants are not significant to their control counterparts. IR64 had the highest number of non-significant *P* values of all the genotypes. Among the six aromatic cultivars studied, only two cultivars showed non-significant *P* values for two parameters studied (malondialdehyde content and total chlorophyll content) which includes Badshabhog and Tulaipanji.

Conclusions

The present study showed the effects of 10 μM Cd stress on different rice cultivars, variations in responses were observed across the 13 studied genotypes, and Cd-sensitive and Cd-tolerant genotypes were grouped accordingly irrespective of presence or absence of the fragrant trait. The aromatic genotypes did not show any Cd-induced stress responses that could be solely attributed to aromatic genotypes only, rather the aromatic and non-aromatic genotypes behaved in a similar

manner under Cd stress where some of the genotypes were found to be sensitive (Tulsibhog, Kariagora) and some were found to be tolerant (IR64, Badshabhog). Effect of Cd stress in the commercially important aromatic and non-aromatic rice cultivars have been studied with a high degree of emphasis, and the use of molecular markers for stress-induced genotoxic studies jointly with different biochemical stress markers can be a reliable tool for ecotoxicological interventions.

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References

- Aksoy KD, Aras S (2011) Evaluation of copper-induced stress on eggplant (*Solanum melongena* L.) seedlings at the molecular and population levels by use of various biomarkers. *Mutat Res* 719:29–34
- Amon DI (1949) Copper enzymes in isolated chloroplasts polyphenol oxidases in *Beta vulgaris*. *Plant Physiol* 24:1–15
- Aslam R, Bhat TM, Choudhury S, Ansari MYK (2017) An overview on genotoxicity of heavy metals in a spice crop (*Capsicum annum* L.) in respect to cyto-morphological behavior. *Caryologia* 70:42–47
- Atienzar FA, Conradi M, Evenden AJ, Jha AN, Depledge MH (1999) Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* to benzo [a] pyrene. *Environ Toxicol Chem* 18:2275–2282
- Atienzar FA, Cordi B, Donkin ME, Evenden AJ, Jha AN, Depledge MH (2000) Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae. *Palmariapalmata*. *Aquat Toxicol* 50:1–12
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39:205–207
- Cao D, Zhang HZ, Wang YD, Zheng LN (2014) Accumulation and distribution characteristics of zinc and cd in the hyperaccumulator plant *Sedum plumbizincicola*. *Bull Environ Contam Toxicol* 93(2): 171–176
- Cenkçi S, Yıldız M, Cigerci İ H, Konuk M (2009) Toxic chemicals-induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris* L.) seedlings. *Chemosphere* 76:900–906
- Cenkci S, Cigerci IH, Yıldiz M, Ozay C, Bozdog A, Terzi H (2010) Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. *Environ Exp Bot* 67:467–473
- Ci D, Jiang D, Wollenweber B (2010) Genetic variance in Cd tolerance and accumulation in wheat materials differing in ploidy and genome at seedling stage. *J Agron Crop Sci* 196:302–310
- Daud MK, Sun Y, Dawood M (2009) Cd-induced functional and ultra-structural alterations in roots of two transgenic cotton cultivars. *J Hazard Mater* 161:463–473
- Food and Agriculture Organization/World Health Organization (2010) Joint FAO/WHO Expert Committee on Food Additives, Seventy-Third Meeting, Geneva, 8–17 June 2010. Summary and Conclusions. Available at: www.who.int/foodsafety/publications/chem/summary73.pdf

- Gupta M, Sarin NB (2009) Heavy metal induced DNA changes in DNA analysis and identification of sequence characterized amplified region marker. *J Environ Sci* 21:686–690
- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125:189–198
- Hoagland DR, Snyder WC (1933) Nutrition of strawberry plants under controlled conditions: (a) effects of deficiencies of boron and certain other elements: (b) susceptibility to injury from sodium salts. *Proceedings of the. Am Soc Horticul Sci* 30:288–294
- International Rice Research Institute (2011) World production and consumption of domestic milled rice. Available at: http://ricestat.irri.org/vis/wrs_quickCharts.php
- Irfan M, Hayat S, Ahmad A, Alyemen MN (2013) Soil Cd enrichment: allocation and plant physiological manifestations. *Saudi J Biol Sci* 20:1–10
- Ishikawa S, Ae N, Sugiyama M, Murakami M, Arao T (2005) Genotypic variation in shoot cadmium concentration in rice and soybean in soils with different levels of cadmium contamination. *Soil Sci Plant Nutr* 51:101–108
- Koca H, Bor M, Ozdemir F, Turkan I (2007) The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ Exp Bot* 60:344–351
- Kosolsaksakul P, Farmer JG, Oliver IW, Graham MC (2014) Geochemical associations and availability of Cd (Cd) in a paddy field system, northwestern Thailand. *Environ Pollut* 187:153–161
- Labra M, Di Fabio T, Grassi F, Regondi SM (2003) AFLP analysis as biomarker of exposure to organic and inorganic genotoxic substances in plants. *Chemosphere* 52:1183–1188
- Liu W, Li P, Qi XM, Zhou Q (2005) DNA changes in bar-ley (*Hordeum vulgare*) seedlings induced by Cd pollution using RAPD analysis. *Chemosphere* 61:158–167
- Matysik J, Alia BB, Mohanty P (2002) Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr Sci* 82:525–532
- Moradi A, Abbaspour KC, Afyuni M (2005) Modelling field-scale Cd transport below the root zone of a sewage sludge-amended soil in an arid region in Central Iran. *J Contam Hydrol* 79:187–206
- Nagajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett* 8(3): 199–216
- Roberts TL (2014) Cd and phosphorous fertilizers: the issues and the science. *Procedia Eng* 83:52–59
- Rodriguez FM, Rodriguez CE (1982) Lead and Cd levels in soil and plants near highways and their correlation with traffic density. *Environ Pollut Ser B* 4:281–290
- Saghai MA, Soliman KM, Jorgenson RA, Allard RW (1984) Ribosomal DNA spacer length polymorphism in barley, Mendelian inheritance, chromosomal location and population dynamics. *Proc Natl Acad Sci U S A* 81:8014–8018
- Sharma P, Dubey RS (2005) Lead toxicity in plants. *Braz J Plant Physiol* 17:35–52
- Singh S, Eapen S, D'Souza SF (2006) Cd accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant, *Bacopa monnieri* L. *Chemosphere* 62:233–246
- Sizova OI, Kochetkov VV, Validov SZ, Boronin AM, Kosterin PV, Lyubun YV (2004) Arsenic contaminated soils: genetically modified *Pseudomonas* spp. and their arsenic phytoremediation potential. *J. Soil Sediment* 2:19–23
- Srivastava S, Mishra S, Tripathi RD, Dwivedi S, Trivedi PK (2007) Phytochelatin and antioxidant systems respond differentially during arsenite and arsenate stress in *Hydrilla verticillata* (L.f.) Royle. *Environ Sci Technol* 41:2930–2936
- Srivastava RK, Pandey P, Rajpoot R, Rani A, Dubey RS (2014) Cd and lead interactive effects on oxidative stress and antioxidant responses in rice seedlings. *Protoplasma* 251:1047–1065
- Suzuki N (2005) Alleviation by calcium of Cd-induced root growth inhibition in *Arabidopsis* seedlings. *Plant Biotechnol* 22:19–25
- Uraguchi S, Mori S, Kuramata M, Kawasaki A, Arao T, Ishikawa S (2009) Root-to shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. *J Exp Bot* 60:2677–2688
- Yu Z, Zhou Q (2009) Growth responses and Cd accumulation of *Mirabilis jalapa* L. under interaction between Cd and phosphorus. *J Hazard Mater* 167:38–43
- Zhang F, Zhang H, Wang G (2009) Cd-induced accumulation of hydrogen peroxide in the leaf apoplast of *Phaseolus aureus* and *Vicia sativa* and the roles of different antioxidant enzymes. *J Hazard Mater* 168:76–84