Ecotoxicology and Environmental Safety 205 (2020) 111167

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Salicylic acid mediated reduction in grain cadmium accumulation and amelioration of toxicity in *Oryza sativa* L. cv Bandana

Snehalata Majumdar [a], Sonal Sachdev [b], Rita Kundu [a,*]

[a] Department of Botany, Centre of Advanced Study, University of Calcutta, 35 Ballygunge Circular Road, Kolkata, 700019, West Bengal, India
[b] Division of Plant Biology, Bose Institute, P-1/12, CIT Road, Scheme VIIM, Kankurgachi, Kolkata, 700054, West Bengal, India

ARTICLE INFO

Keywords:
Rice
Salicylic acid
Cadmium
Yield
Grain
Cancer risk

ABSTRACT

Contamination of agricultural fields with Cadmium (Cd) due to several agricultural practices is increasing worldwide. The rice plants can easily take up Cd and accumulate it into different parts, including the grains, posing a threat to human health even at low concentration exposure. Several phytohormones, including Salicylic acid (SA) have been investigated since long for its alleviating properties under various biotic and abiotic stress conditions. In the present study, 100 μM SA application to ameliorate 25 μM Cd stress was studied for 72 h in hydroponics in *Oryza sativa* cv. Bandana seedlings. Pot experiments were done with same treatment condition and plants were grown till maturity. SA application to Cd exposed rice seedlings alleviated the stress condition, which was established by several physiological, biochemical, histochemical and gene expression analysis. SA treatment to Cd stressed seedlings showed elevated photosynthetic pigment content, on-protein thiol content and relieved the Cd induced growth inhibition considerably. It lowered the accumulation of ROS like, $O2^-$ and H_2O_2 with a regulated antioxidative enzymatic activity. SA application in Cd exposed rice seedlings had upregulated expression of *OsHMA3* and *OsPCS1* whereas*OsNRAMP2* gene was downregulated. Co-application of SA and Cd led to higher yield and improved agronomic traits in comparison to only Cd exposed plants under pot experimentation. Daily intake of Cd and Carcinogenic risk were also reduced by 99.75% and 99.99% respectively in the SA treated Cd stressed plants. SA positively affected the growth and tolerance of rice seedlings to Cd stress. Hence, SA addition to Cd contaminated soil can ensure rice cultivation without posing health risk to consumers.

1. Introduction

Cd pollution is one of the major environmental concerns of recent times. It is ranked 7th among the toxic substance priority list (Cotton et al., 1999; ATSDR, 2019). In most cases, increased amount of Cd in soil is anthropogenic and its concentration beyond threshold level is a serious threat. Application of excess phosphate fertilizers and contaminated irrigation water (Du et al., 2013; Kosolsaksakul et al., 2014), are instrumental in increasing Cd burden of the arable land. Cd is highly mobile and has a very high soil to plant transfer rate, which facilitates its accumulation in the grains and secure its niche in the food web (Irshad et al., 2020). Even at minute concentration it causes considerable impairment in plant growth and development, ultimately compromising crop yield and quality significantly (Zhang et al., 2015; Wang et al., 2018). Rice is one of the primary sources of calorie for Asian population (Gao et al., 2018) and intake of Cd contaminated grain has led to serious health hazards like skeletal damage, lung and prostate cancer and cardiovascular diseases (Jan et al., 2019). Our preliminary data showed a varied range of Cd status (BDL to 0.49 mg kg^{-1}) in grains and (1.76 mg $Kg^{-1}$to 13.8 mg kg^{-1}) in soil samples collected from agricultural fields located at different regions of West Bengal, much higher than the allowable limits (Unpublished data). According to CODEX, the maximum allowable limit of Cd in polished rice is 0.4 mg kg^{-1} whereas the same in other food crops is 0.10 mg kg^{-1} (Fu et al., 2008; Xue et al., 2014).

Heavy metals interfere with various essential cellular biomolecules through oxidative stress causing physiological, morphological and metabolic anomalies in plants, resulting in leaf chlorosis, membrane damage and protein degradation. Cd is also found to reduce the final plant height, number of panicles and tillers per plant significantly, thereby affecting the total yield of the crop plants (Huang et al., 2017). To combat the heavy metal induced toxicity, plants have evolved several mechanisms to defend (Singh and Shah, 2015; Khan et al., 2013) which include antioxidative enzymes, like, superoxide dismutase (SOD),

* Corresponding author.
E-mail address: kundu_rita@yahoo.co.in (R. Kundu).

https://doi.org/10.1016/j.ecoenv.2020.111167
Received 12 April 2020; Received in revised form 19 July 2020; Accepted 10 August 2020
Available online 19 August 2020

catalase (CAT), guaiacol peroxidase (POD) and non-enzymatic components (carotenoid, NP-SH, proline, etc.). Toxic metals and metalloids induce Phytochelatin synthase (*PCS*) to produce ligands called phytochelatins, that detoxify metalloids and non-essential toxic metals like Cd by sequestering it into vacuoles (Uraguchi et al., 2017). *Os*PCS played a crucial role in mitigation of Cd stress and down regulation of the gene decreased Cd uptake in rice seeds (Shri et al., 2014; Das et al., 2017). The family of natural resistance-associated macrophage proteins (*NRAMP*), another important group of transmembrane proteins involved in metal transport and homeostasis in both root and shoot, are considered 'general metal ion transporters' due to their ability to transport Mn^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Cd^{2+},Ni^{2+} and Co^{2+} across plasma membrane into cytosol, or across tonoplast (Ullah et al., 2018; Mani et al., 2018). Xylem loading of Cd from symplasm takes place through the heavy metal transporting ATPases (*HMA*s) which belong to P1B-ATPases. *OsHMA3*, another ATPase coupled transporter, localized in the tonoplast play a significant role in vacuolar sequestration of heavy metals. Overexpression of *OsHMA3* is reported to decrease the Cd concentration in rice grains by 94–98% (Lu et al., 2019; Miyadate et al., 2011).

To reduce the Cd induced toxicity, amelioration of Cd stress is of paramount importance. Many plant molecules like Abscisic acid, Jasmonates and Salicylic Acid (SA) (Joseph et al., 2010) play a significant role in mitigating the heavy metal stress by modulating the gene expression of various transporters and altering the biochemical reactions (Emamverdian et al., 2020). Exogenous application of SA was reported to promote growth, increase photosynthetic efficiency, modify levels of osmoregulant, reduce ROS generation and up-regulate defense related genes (An and Mou, 2011; Zhang et al., 2013; Zengin et al., 2014; Anjum et al., 2018). Its anti-stress effects were studied in potato (Khan et al., 2015), wheat (Zengin et al., 2015) and tobacco (Abdul Halim &Phang, 2017) under aluminum, arsenic and lead stress respectively. Plants grown in soil treated with SA provided protection against heavy metal induced stress responses (Wani et al., 2017; Kohli et al., 2019), improved mineral status, reduced shoot and root Cd content in rice (Fatima et al., 2014). Other SA application methods (pre-treatment, foliar spray) were reported (Saruhan et al., 2012; Khan et al., 2014), where, pretreatment with SA was found to significantly reduce shoot Cd content (Cao et al., 2013). SA application under heavy metal stress and subsequent metal accumulation in grain has been studied across many crop species, but Cd accumulation pattern in different plant parts, especially in grains is nearly unexplored in rice. A recent study of SA application through foliar spray on leaves showed lowered Cd uptake in grains of brown rice (Wang et al., 2019). In Asian countries like India, pre-treatment or foliar spray application is laborious, time consuming and expensive, but application of SA can be done with irrigation water, saving cost and time.

We hypothesize that SA application in soil will be effective in minimizing grain Cd accumulation in *O. sativa* cv. Bandana. SA is believed to alleviate Cd toxicity, so, yield of the plants should remain unhampered upon SA application under Cd stress. Our initial field experiments have shown that Bandana, a high yielding variety, widely cultivated in West Bengal and different parts of India, is a moderate Cd accumulator. In the present study, various biochemical and physiological changes under Cd stress including plant growth, photosynthetic pigment content, lipid peroxidation, proline accumulation, non-protein thiol production, and ROS localization at the seedling stage were evaluated. To have an insight into the molecular mechanism behind it, expression of some selected Cd transport and sequestration related genes were also investigated. Pot experiment was conducted to assess the Cd status of different plant parts in the mature plants. Several agronomic traits including yield per plant, panicle length, panicle weight, tiller number, hollow seed percentage were studied to assess the ameliorating effect of SA. Health risk of Cd and SA treated rice was also assessed.

2. Materials and methods

2.1. Hydroponic experiment

2.1.1. Growth and treatment condition

Seeds of *Oryza sativa* cv. Bandana (procured from Bidhan Chandra Krishi Viswavidyalaya, West Bengal) were surface sterilized with 0.2% Dithane solution for 5–7 min, rinsed thoroughly with distilled water and soaked overnight (about 16 h), the soaked seeds were plated on 100mmX10mm borosilicate glass petri plates supplied with clean, moist filter paper, seeds were allowed to germinate in dark conditions at room temperature for 72 h. Upon germination (appearance of coleoptile), the plates were supplemented with 20 ml of half strength Hoagland's nutrient solution (Hoagland and Snyder, 1933) (pH 6.0) [1 mM KH_2PO_4, 5 mM KNO_3, 5 mM $Ca(NO_3)_2.4H_20$, 2 mM $MgSO_4.7H_2O$, micronutrients-11.8 μM $MnSO_4.7H_2O$, 0.7 μM $ZnSO_4.7H_2O$, 0.32 μM $CuSO_4.5H_2O$, 0.16 μM $(NH_4)_6Mo_7O_{24}.H_2O$, 6.3 μM H_3BO_3, 5 μM Fe-EDTA] and grown at 14/10 h photoperiod at 30±2 °C temperature and 70% relative humidity. Six days old seedlings with uniform size were subjected to treatment. Initial screening was done with different concentrations of Cd and SA, the following concentrations were chosen, based on half maximal inhibitory concentration (IC50) of Cd and the best growth enhancing concentration of SA. Four treatment sets were designed as follows, i) C (0 Cd + 0 SA), ii) T1 (25 μM or 4.52mgL^{-1} $CdCl_2$ + 100 μM or 13.81 mgL^{-1} SA), iii) T2 (25 μM $CdCl_2$ or 4.52mgL^{-1}$CdCl_2$+ 0 μM SA), iv) T3 (0 $CdCl_2$+100 μM or13.81 mgL^{-1} SA), with triplicates of each. Seedlings were treated for 72 h, shoots and roots from each set were harvested on the 9th day after plating.

2.1.2. Estimation of RWC, root and shoot length

Plantlets from each set were harvested and their fresh weight was taken. The shoot and root length of plantlets from each set were also recorded. RWC was calculated as [{(FW-DW)/(turgid weight-DW)} × 100] (Barr and Weatherley, 1962).

2.1.3. Analysis of oxidative damage related parameters

2.1.3.1. Estimation of chlorophyll. 100 mg of leaf samples from each set was chopped and extracted with 80% acetone. Absorbance was measured at 645 nm and 663 nm. The concentration of chlorophyll a, chlorophyll b were measured according to Arnon, 1949 and expressed as mg g^{-1} fresh weight.

2.1.3.2. Estimation of lipid peroxidation. Lipid peroxidation in shoot and root tissue was determined by the estimation of malondialdehyde (MDA) (Heath and Packer, 1968). 200 mg of fresh plant samples from each set was homogenized in 2 ml of 0.1% trichloroacetic acid (TCA) solution, the homogenate was centrifuged at 10,000×*g* for 5 min. For every 1 ml aliquot, 2 ml of 20% TCA containing 0.5% TBA was added, heated at 95 °C for 30 min and centrifuged at 10,000×*g* for 15 min. Absorbance of supernatant was taken at 532 nm and 600 nm. The concentration of MDA was calculated by using the molar extinction coefficient of 155.1 mM^{-1}cm^{-1} and expressed as nmol g^{-1} fresh weight.

2.1.3.3. Estimation of hydrogen peroxide (H_2O_2). The concentration of endogenous H_2O_2 was determined (Sergiev et al., 1997). Briefly, 100 mg of tissue was homogenized in 1 ml of chilled 0.1% Trichloroacetic acid (TCA) and centrifuged at 12,000×*g* for 15 min. 0.5 ml of the supernatant was mixed with 0.5 ml of 10 mM Potassium Phosphate buffer (pH 7.0) and 1 ml 1 M Potassium Iodide, the final reaction mixture was read at 390 nm. The H_2O_2 content was calculated from standard curve of H_2O_2.

2.1.3.4. Localization of ROS accumulation in leaves. Localization of O_2^- and H_2O_2 radicals within the plant tissue was detected. Plant tissue was immersed in 50 mM phosphate buffer containing 0.2% Nitro blue tetrazolium (NBT) (Jabs et al., 1996). H_2O_2 was detected by immersing the tissue in 3,3′-diaminobenzidine (DAB) (1 mg/ml) (Thordal-Christensen et al., 1997).

2.1.4. Analysis of tolerance related parameters

2.1.4.1. Estimation of non-protein thiol (NP-SH). The concentration of non-protein thiol (NPSH) was determined according to Cakmak and Marschner (1992). 100 mg of plant tissue was extracted with 1 ml of 5% orthophosphoric acid and centrifuged at 15,000×*g* for 15 mins at room temperature. To 0.5 ml of the aliquot, 2.4 ml of 150 mM phosphate buffer (pH 7.4) containing 5 mM EDTA and 0.5 ml of 6 mM 5,5-dithio-2, 2-dinitrobenzoic acid (Ellman's reagent) were added. The mixture was incubated for 15 min at room temperature and the end product was read at 412 nm.

2.1.4.2. Analysis of enzymatic antioxidant activity. 100 mg fresh tissue was homogenized in 600 μl of 50 mM Potassium Phosphate buffer (pH6.8) containing 0.1 mM EDTA and centrifuged at 13000 rpm for 15 min at 4 °C. The supernatant was used for the following assays:: The SOD activity was determined according to Beyer and Fridovich (1997). To 100 μl of the supernatant, 3 ml of solution containing 100 μM EDTA, 13 mM L-methionine, 75 μM NBT, 2 μM Riboflavin and 50 mM Potassium Phosphate buffer (pH7.8) were added. The reaction was illuminated under fluorescent lamp (Philips, 40 W) for 1.5 min, the light was turned off to stop the reaction. The absorbance was recorded at 630 nm. SOD activity was calculated by determining the amount of extract that led to 50% inhibition of NBT-reduction. The CAT activity as determined according to Havir and McHale (1987). To 100 μl of the extract, 3 ml solution containing 50 mM Potassium Phosphate buffer (pH7.0) and 20 mM H_2O_2were added. The decrease in absorbance was recorded at 240 nm.

2.1.4.3. Estimation of carotenoid content. 100 mg of leaf tissue from each set was chopped and extracted with 80% acetone. Absorbance was measured at 480 nm for presence of carotenoid according to (Arnon, 1949).

2.1.5. Analysis of molecular parameters

Total RNA was isolated from the samples using HiPurA Plant and Fungal RNA Miniprep Purification Kit according to manufacturer's instructions**.** The cDNA was synthesized from the RNA by First A synthesis kit (Thermo Scientific). This was followed by amplification using the designed primers. The primer sequences are *18SrRNA* (internal standard) (5′-TTC TAT GGG TGG TGG TGC AT-3′, 5′-GTG CGC CCA GAA CAT CTA AG-3′), *PCS1*(5′-AGG TCC TAC AGC AAA TCC GT-3′, 5′-ATT CCC ACT TAG CAA TGC GG-3′), *HMA3* (5′-GTT CAG CAT CGA CTC GTT CC-3′, 5′-CCA CAT TTT CCG GGT TTG GT-3′) and *NRAMP2*(5′-GCC CTC GTT GTT TCG TTC TT-3′, 5′-AAC AGC CCA ATA GCC CAG AT -3′).

2.2. Pot experiment

All the pot experiments were conducted at the Agricultural Experimental Farm, University of Calcutta, Baruipur, 24 paragana (South), West Bengal (22.35°N, 88.44°E) located in the Gangetic alluvial region, during June–September 2018–19 (with 14/10 h photoperiod, 30±2 °C temperature and 70% relative humidity). 100 germinated seedlings were placed in each pot with 2 kg of soil (16cm × 6cm × 10cm), then the seedlings were allowed to grow for 21days. 3–4 seedlings of uniform sizes were then transferred to separate pots (8cm × 10cm × 19cm) containing 3 kg of soil and grown till maturity. Plants were treated in the following concentrations and combinations, i) CP (0 $CdCl_2$+0 SA), ii) P1 (25 μM or 4.52 mg kg^{-1} $CdCl_2$ + 100 μM or 13.81 mg Kg^{-1}SA), iii) P2 (25 μM or 4.52 mg $Kg^{-1}CdCl_2$ + 0 μM SA),iv) P3 (0 $CdCl_2$+100 μM or13.81 mg kg^{-1} SA), the treatment was given by adding aqueous solution of $CdCl_2$ and SA in required amounts for 3 Kg of soil per pot, once during the growth period. Plant height was recorded during vegetative phase, at 45 days. Several agronomic traits were analyzed after complete maturation of the plants, including final height, total tiller number, effective tiller number, panicle length, panicle weight, total number of seed and hollow seed per panicle. The experiment was performed in triplicates. Data are represented as agronomic trait values per plant. Yield of the plants was calculated and expressed as g $plant^{-1}$.

2.2.1. Estimation of Cd content

The grain, shoot, root and soil samples were oven dried at 80 °C for 72 h 100 mg of each of the sub-sampled dried tissue was crushed individually and digested in HNO_3:HCl: $HClO_4$ (4:2:1), after complete digestion, the solution was made up to 25 ml volume with deionized distilled water (Barman et al., 2020), Cd content in the samples were determined by Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP- AES) (SPECTRO Analytical Instruments GmbH, Germany).

2.2.2. Analysis of Cd content dependent quantitative factors

The extent of Cd transference from soil to crop and associated intake and risk factors were calculated as follows:

1. Bioconcentration factor (BCF) = $C_{root/soil}$ (Kabata-Pendias and Mukherjee, 2007)
2. Translocation factor (TF) = C_{grain}/C_{shoot}orC_{grain}/C_{root} or C_{shoot}/C_{root} (Kalavrouziotis et al., 2012).
3. Daily intake of metal (DIM) in mg kg^{-1} day^{-1}= (C X IR)/BW (US EPA, 2004).
4. Cd exposure related cancer risk (CR) = DIM X SF (Rais et al., 2017).

Where C_{soil}, C_{root}, C_{shoot}, C_{grain} are Cd content in soil, root, shoot and grain respectively, C is metal content in grains in mg kg^{-1}, IR is ingestion rate in mg day^{-1} (an adult person of age range 20–40 years old consumes on an average 300 g rice per day, IR was calculated on this basis), SF is slope factor.

2.3. Statistical analysis: Levene's test was performed for homogeneity testing of variances, Shapiro wilk test was done for normality testing. One way analysis of variance (ANOVA) was performed with Tukey's honestly significant difference (hsd) test, using SPSS 17.0. Significant differences among the treatments were determined at 5% level of significance ($P < 0.05$). The experimental data was represented as mean ± standard deviation (SD), with triplicates for each set. Graphs were illustrated using Graphpad Prism 6 tool.

3. Results

3.1. SA escalated growth and RWC

The shoot and root growth of T2 set were reduced by 29.6% and 31.1% respectively, with regards to the untreated rice seedlings, while, the growth of T1 set increased by 0.4% and 2.3% with respect to shoot and root length of untreated rice seedlings (Fig. 1a). In comparison to control set, 6% and 21.4% reduction in shoot and root length of T3 set were observed. RWC was reduced by 35.5% in T2 set with respect to control, whereas a mere reduction of 1.6% was recorded in T1 set (Fig. 1b).

3.2. SA induced drop in oxidative damage related parameters

The total chlorophyll pigment content depleted in T2 set by 34.3% with respect to C set whereas, the simultaneous SA + Cd exposure elevated the amount of total chlorophyll content by 0.6% in T1 set (Fig. 2a). Accumulation of MDA in T2 shoot was 68.3% higher than C shoot, whereas MDA accumulation in T1 root increased by only 4.4% with respect to control, T3 shoot had lower accumulation of MDA,

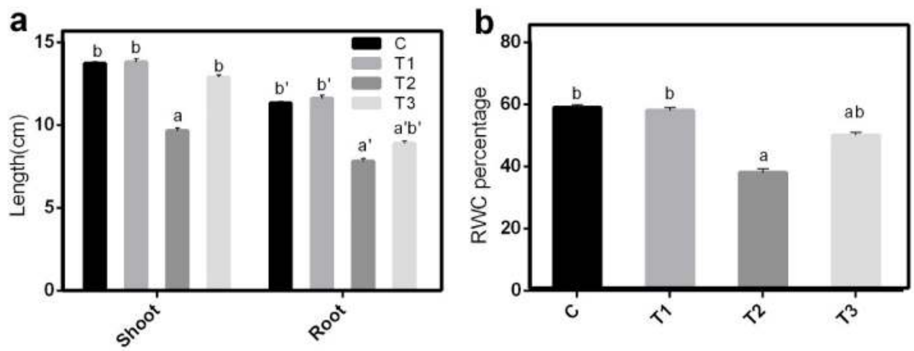

Fig. 1. Effect of Cd on physiological parameters a) shoot and root length b) Relative water content percentage. Values represent the mean of three replicates. Mean values represented by same letters are not significantly different, different letters represent statistical significance at $p < 0.05$, C (0 Cd + 0 SA), T1 (25 μM Cd + 100 μM SA), T2 (25 μM Cd + 0 μM SA), T3 (0 Cd + 100 μM SA).

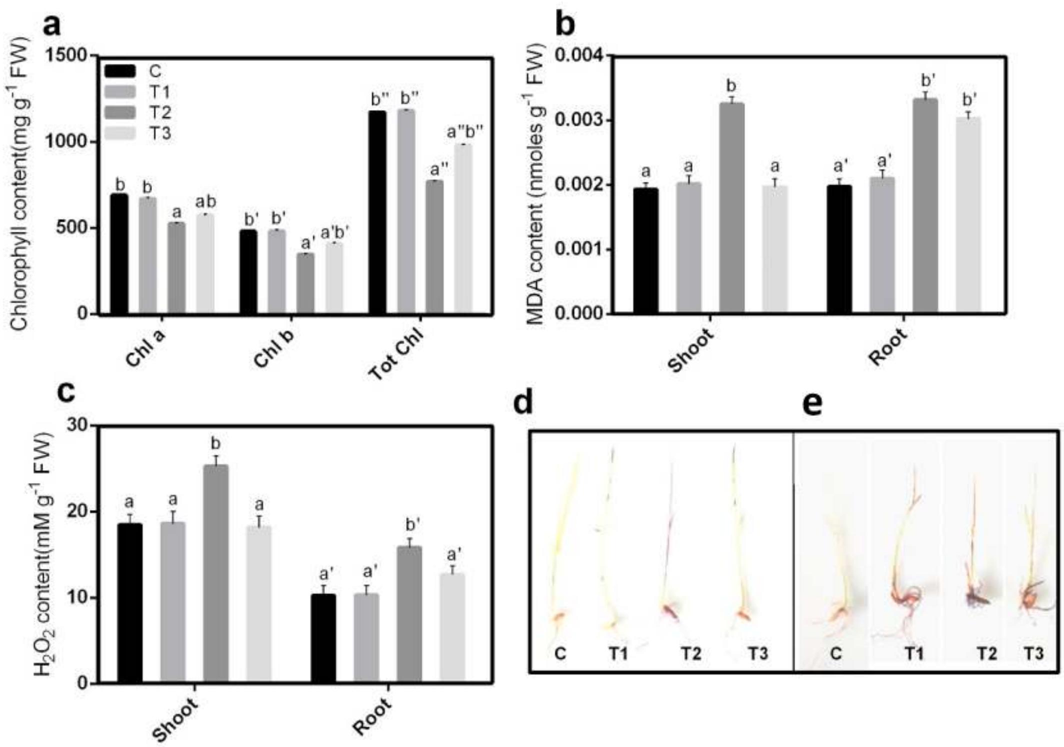

Fig. 2. Effect of Cd induced oxidative damage related parameters a) Chlorophyll content b)Malondialdehyde content c) Endogenous H_2O_2 content d) Localization of Superoxide anion e) Localization of H_2O_2. Values represent the mean of three replicates. Mean values represented by same letters are not significantly different, different letters represent statistical significance at $p < 0.05$, C (0 Cd + 0 SA), T1 (25 μM Cd + 100 μM SA), T2 (25 μM Cd + 0 μM SA), T3 (0 Cd + 100 μM SA).

accounting to 1.8% higher than c set (Fig. 2b). A similar trend was also observed in the accumulation of endogenous hydrogen peroxide. 0.38% higher accumulation of hydrogen peroxide with respect to control, was observed in T1 root, compared to a considerable increase of 53.7% in T2 root (Fig. 2c). Superoxide and hydrogen peroxide was detected by histochemical staining and it was observed that more Reactive Oxygen Species accumulated in T2 set in comparison to T1 set (Fig. 2d,2e).

3.3. SA altered stress scavengers

SA elevated the SOD activity in roots significantly by 62.3% in comparison to C set. It was found to be higher than theT2 seedlings which had 54.8% increase in root, T3 root exhibited a mere increase of 9% in the SOD activity (Fig. 3a). Root catalase activity increased by 85.3% and 157.8% in T1 and T2 sets respectively, in comparison to C set, however, a miniscule increase of 14.8% was observed in T3 set (Fig. 3b). SA application was able to modulate the antioxidant enzyme activities. T1 set showed 36.6% increase amount of non-protein thiol content in roots, with respect to C plants, whereas a slight increase of 14.4% was observed in T2 roots (Fig. 3c). No significant change was observed in carotenoid content (Fig. 3d).

3.4. Semi quantitative expression of OsHMA3, OsPCS1 and OsNRAMP2 genes

According to densitometric analysis the *OsHMA3* and *OsPCS1* gene expression were found to be significantly upregulated in T1 set by 1.22 and 1.05 folds respectively, whereas the *OsNRAMP2* gene was found to be down regulated in T1 set by 0.85 folds. *OsHMA3* gene was down regulated by 0.85 folds, whereas *OsPCS1* and *OsNRAMP2* genes were up regulated by 1.015 and 1.028 folds in T2 set (Fig. 3e).

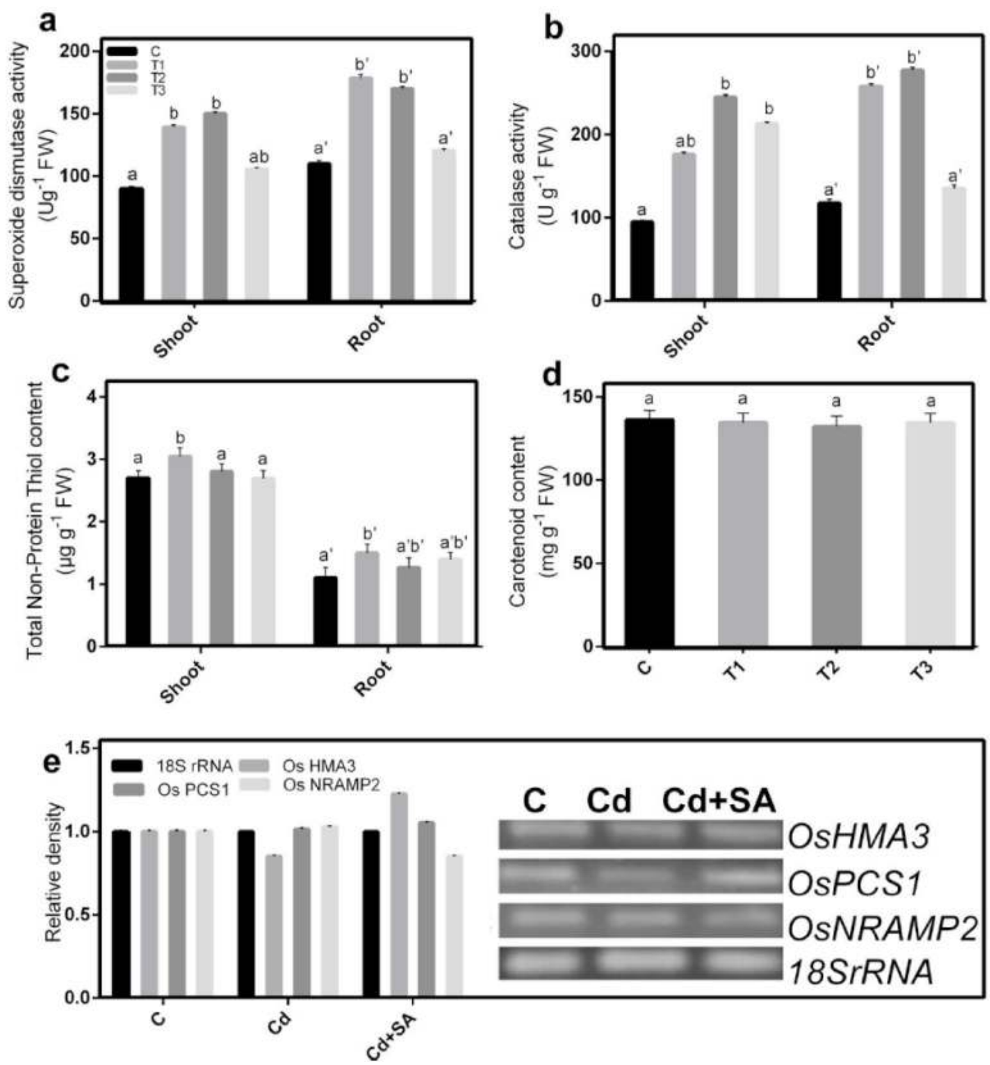

Fig. 3. SA induced alteration in stress scavengers and gene expression a)Superoxide dismutase activity b)Catalase activity c) Total non protein thiol content d)Carotenoid content in leaves e), i) Densitometric analysis using NIH ImageJ and Semi quantitative reverse transcriptase PCR amplification analysis documented by 1.8% Agarose gel analysis of amplicons. Values represent the mean of three replicates. Mean values represented by same letters are not significantly different, different letters represent statistical significance at $p < 0.05$, C (0 Cd + 0 SA), T1 (25 μM Cd + 100 μM SA), T2 (25 μM Cd + 0 μM SA), T3 (0 Cd + 100 μM SA).

3.5. Reduced Cd uptake and health risk potential

Cd concentration in grains of P2 set was 0.8 mg kg^{-1} rice, which was significantly reduced by 95% in grains of P1 set accounting to 0.004 mg kg^{-1} rice, the Cd concentration in shoot and root of P1 set was reduced by 82.6% and 54.1% respectively. Cd accumulation in P3 plants was not detected. The BCF of Cd was reduced by 54.16% in P1 plants in comparison to the P2 plants, in a similar way the TF (C_{grain}/C_{root}, C_{grain}/C_{shoot}, C_{shoot}/C_{root}) values were also reduced by 89.1%, 87.2% and 62.1% respectively in P1 plants with respect to the severely affected P2 plants. The DIM for P1 rice grains was reduced by 99.69% with respect to P2 rice. The lowered DIM for P1 rice also reduced the CR for P1 rice by 99.99% with respect to P2 rice grains (Table 1).

Table 1
Effect of SA application on Cd content and associated factors.

	CP	P1	P2	P3
Cd content (mg kg^{-1})				
Seed	ND	0.04 ± 0.0021	0.8 ± 0.0024	ND
Soil[a]	ND	2.68 ± 0.0022	2.68 ± 0.0021	ND
Shoot	ND	0.16 ± 0.0017	0.92 ± 0.0013	ND
Root	ND	0.44 ± 0.0020	0.96 ± 0.0019	ND
Associated factors				
BCF(g/s)	ND	0.164 ± 0.012	0.358 ± 0.0111	ND
TF (g/r)	ND	0.090 ± 0.021	0.833 ± 0.013	ND
TF (g/s)	ND	0.111 ± 0.031	0.869 ± 0.043	ND
TF (s/r)	ND	0.363 ± 0.01	0.958 ± 0.055	ND
DIM	ND	0.0008 ± 0.0009	0.32 ± 0.00046	ND
CR	ND	3.2×10^{-5}±0.00027	5.12 ± 0.00041	ND

[a] Residual Cd content.

3.6. Differential changes in agronomic traits and yield related components

Upon Cd exposure, all the studied agronomic parameters including plant height (both at 45 days and final), tiller number, grain attributes were hampered. Simultaneous application of SA and Cd were able to aid the recovery of the plants from the Cd induced toxicity. In P1 plants, yield attributes were significantly close to C and P3 plants. As the effective tillers in P1 plants increased by 81.48% with respect to P2 plants, total yield also considerably increased by 81.91% upon co-application of SA with Cd. The number of hollow seeds was decreased by 32.69% in P1 plants with respect to P2 plants. Traits like initial plant height, final plant height, total tiller number, total seed, and panicle weight changed trivially in P3 plants with respect to C plants (decrease percentage ranging from 0% to 7%). However, the yield was reduced by 23.6% in P3 plant with respect to C plants (Fig. 4a-i).

4. Discussion

The genotype Bandana used for the study, is one of the stable donor varieties, with high yield and productivity, widely used in breeding

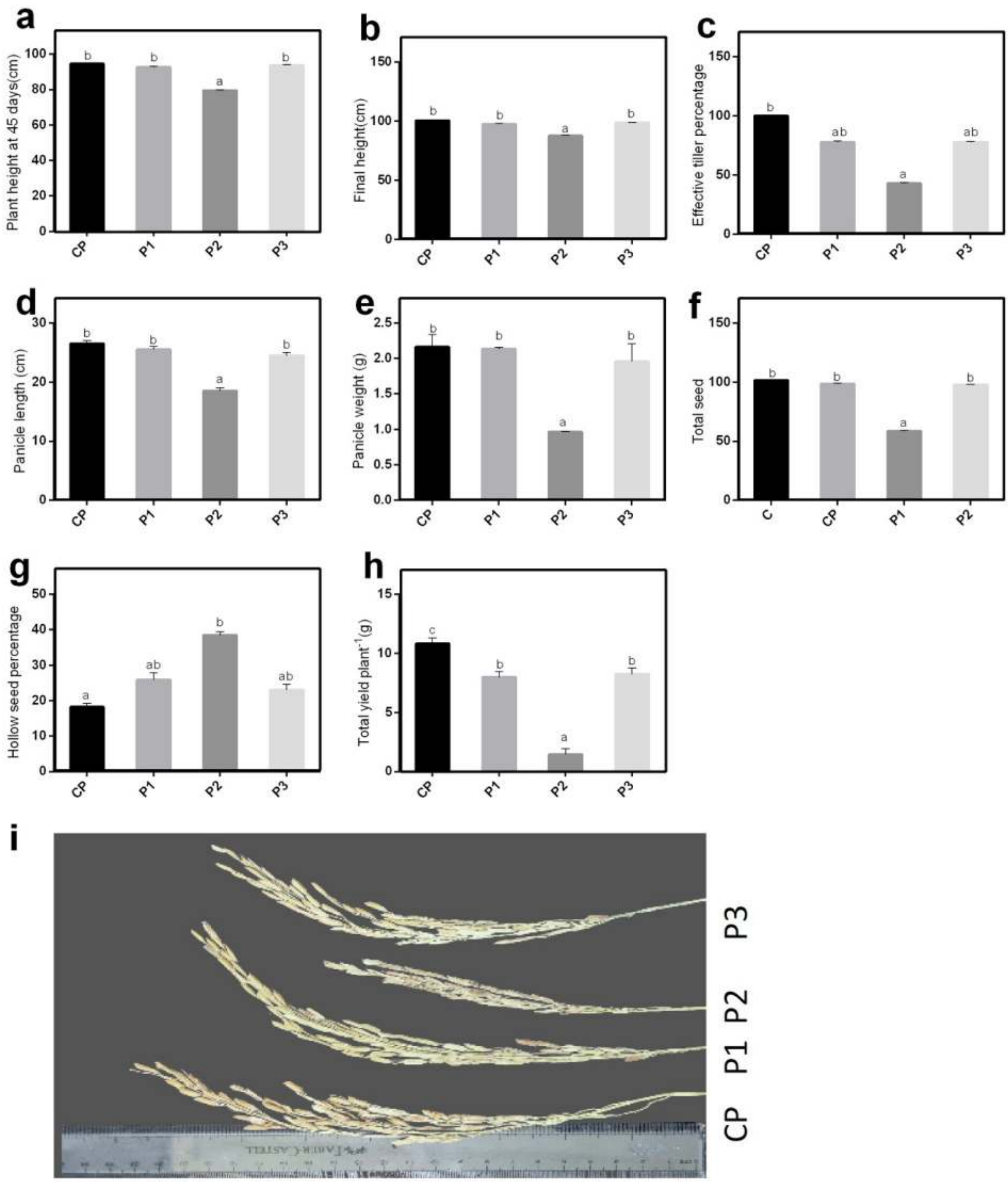

Fig. 4. Effect of SA application on various agronomic traits a) Plant height at 45 days b)Final plant height c) Effective tiller d) Panicle length e)Total seed f)Hollow seed percentage g)Panicle weight h)Total yield i) Pictographic representation of Panicles after maturity. Mean values represented by same letters are not significantly different, different letters represent statistical significance at $p < 0.05$, CP (0 Cd + 0 SA), P1 (25 μM Cd + 100 μM SA), P2 (25 μM Cd + 0 μM SA), P3 (0 Cd + 100 μM SA).

programs for its beneficial attributes (Jadhao et al., 2014; Poudel, 2018), but use of a Cd accumulating variety for breeding might lead to generation of Cd accumulating lines, posing threat to human health. Therefore, use of high yielding Cd accumulator varieties, as donors, can be accepted, provided Cd translocation to the grain is checked. In West Bengal, Cd contamination in agricultural soil may range from 1.76 mg kg^{-1} to 13.8 mg kg^{-1} (unpublished data), presenting high probability of Cd translocation and accumulation in grains. The Cd concentration selected for this study lies in the above mentioned limit and was found to cause moderate toxicity by significantly constraining normal growth and development of the plants. Simultaneous SA application in Cd stressed plants was found to ameliorate the stressed condition by enabling several defensive responses. It was observed that, application of only SA (at the present concentration) to plants was non-toxic.

SA application has been reported to alleviate several heavy metal toxicities including Cd (Hayat et al., 2010). Cd toxicity cause decreased uptake of water which leads to retarded growth of the root and shoot (Mondal et al., 2013), as well as biomass and may cause insufficient crop yield (Ahmad et al., 2016). Hampered development is also reported in mungbean, and ground nut plants (Siddhu and Khan, 2012). Significant decrease in plant growth was seen in the present study under Cd stress, reduction in shoot and root length was observed in T2 set, in comparison to C set. In T1 set no reduction in shoot length was seen rather 2.3% increase in root length was observed. SA has been reported to alleviate Cd toxicity by several ways (Krantev et al., 2008; Guo et al., 2007; Belkhadi et al., 2010). Previously it was reported that, application of SA provided protection to arsenite exposed rice seedlings against stress and restored the plant growth parameters to a certain extent (Singh et al., 2017).

Chlorophyll metabolism and chloroplast ultrastructure are negatively affected due to the mineral stress induced by Cd (Djebali et al., 2005; Hakmaoui et al., 2007; Parmar et al., 2013; Arivazhagan and Sharavanan, 2015). We found that Cd negatively affected the chlorophyll synthesis in rice plants, plant growth and development were also compromised, only SA exposed plants had growth indices higher than Cd treated plants and close to T1 plants. Cd also hinders the uptake of nutrients like Fe, Mg which play significant role in chlorophyll synthesis, as reported in soybean (Xu et al., 2015), *Lemna minor* (Lu et al., 2018). Cd binds to essential Ca^{2+}sites and hinders the PSII photoactivation, by alteration of light harvesting complexes responsible for transfer of light to PSII reaction centre, the chlorophyll antenna complexes are responsible for conversion of light energy to electron transport from PSII, decreased oxygen evolution from PSII leads to modulated photochemical reactions, the energy storage process by synthesis of ATP and NADPH is impeded leading to impaired biochemical functions of the cell (Sytar et al., 2016; Rastogi et al., 2017; Dewez et al., 2018). We observed that SA application to Cd stressed plants improved the pigment content and growth, reports suggest that SA reduced Cd uptake and enhanced the uptake of Fe, Mg, Ca, thereby promoting chlorophyll synthesis (Lu et al., 2018). Hence SA application lowered Cd availability for Ca binding sites and leads to increased oxygen evolution by restoring PSII photoactivation. SA application improved the pigment content and growth as SA is reported to influence the RuBisCo activity, redox homeostasis and stomatal switch (Rivas-San Vicente and Plasencia, 2011). Similar effect was also seen in maize plants (Krantev et al., 2008).

Cd is known to induce production of hydrogen peroxide in many plants (Maksymiec and Krupa, 2006; Rodrı'guez-Serrano et al., 2009; Vestena et al., 2011; Zhao et al., 2012). Cd has the ability to compete and replace several important nutrients, like, Fe is replaced in several proteins leading to escalated free cellular Fe levels, which can directly induce ROS generation through Fenton and Haber-Weiss reactions (Cuypers et al., 2010). Cd induced increase in H_2O_2 is mainly synthesized by NADPH oxidase of plasma membrane or mitochondria or peroxisomes, which is subsequently transferred to other plant parts, this also induces accumulation of superoxide radicals and fatty acid hydroperoxides, ultimately leading to membrane damage (Garnier et al., 2006). T2 seedlings exhibited higher accumulation of ROS in the tissue, which was lowered significantly by SA application, this was congruent with previous reports. Our findings of histochemical detection of hydrogen peroxide and superoxide, indicated that application of SA to Cd treated seedlings could reduce the amount of endogenous ROS accumulation in the seedlings to considerable levels in comparison to T2 seedlings. Similar results were also reported in duckweed and perennial rye grass (Wang et al., 2013; Li et al., 2017). SA is reported to directly scavenge ROS by acting as an antioxidant (Popova et al., 2009), it jointly acts in a feed forward loop with glutathione and detoxifies ROS (Herrera-Vásquez et al., 2015). SA confers protection against heavy metal toxicity by detoxification of ROS thus reduces the degree of lipid peroxidation (Guo et al., 2007; Moussa and El-Gamal, 2010; Wang et al., 2013; Tamás et al., 2015; Khan et al., 2015). Cell membrane is prone to damage by redox active metals (Yılmaz and Parlak, 2011), higher the accumulation of MDA, higher is the level of oxidative stress (Hou et al., 2007). Cd induces oxidative stress, leading to enhanced MDA content, H_2O_2 content and electrolyte leakage (Schützendübel et al., 2002; Liu et al., 2003; Singh et al., 2006; Guo et al., 2007; Hsu and Kao, 2007; Xu et al., 2010; Srivastava et al., 2014). Our findings indicate lowered membrane damage with lowered amount of endogenous hydrogen peroxide accumulation in rice roots in T1 seedlings. Similar results were also reported in duckweed and perennial rye grass (Wang et al., 2013; Li et al., 2017, 2018). Damage indices were lower in T3 plant in comparison to T2 plants and were close to C plants, ensuring that SA alone had no toxicity in T3 plants.

NPT are one of the main components to detoxify ROS during heavy metal toxicity, they chelate with heavy metals facilitating their vacuolar sequestration and limiting their translocation to different plant tissues, they also act as signaling complexes and antioxidants (Li et al., 2011). We observed escalated levels of NPT in T1 as well as T2 set, however higher accumulation of NPT was observed in T1 set. The co-application of SA and Cd was reported to act synergistically resulting in increased synthesis of NPT, phytochelatin and glutathione (Gu et al., 2018).

The presence of antioxidant enzymes like SOD and CAT in different cellular organelles, protect the plants from oxidative damages. In the present study, SA application not only helped in alleviation of Cd induced growth impairment but also regulated the enzymatic activities. We observed that Cd stress significantly increased SOD and CAT activity in both shoot and root of T2 seedlings. Increased ROS generation due to Cd toxicity is counteracted by escalated activity of enzymatic antioxidants, higher SOD and CAT activity was observed under Cd stress in rice (Bari et al., 2019). However, SA application to Cd exposed plants enhanced the SOD activity whereas the CAT activity was reduced. SA applied Cd exposed kentucky blue grass was also found to have higher SOD activity than only Cd treated seedlings, (Guo et al., 2013), similar trend in SOD activity was observed in *Nymphaea tetragona* Georgi (Gu et al., 2018), *Lemna minor* (Lu et al., 2018), Chinese cabbage (Mba et al., 2007) and mustard plants (Ahmad et al., 2011). SA application has been reported to lower the CAT activity, a major H_2O_2 detoxifying enzyme in wheat and tomato (Sahu and Sabat, 2018; Yüzbaşıoğlu et al., 2019). SA is reported to donate electron for peroxidative cycle of catalase and lower its enzymatic activity by competitive inhibition (Ma et al., 2017). Hence, absence of such inhibitory role of SA on SOD activity might delineate the differential responses induced by SA on SOD and CAT activity. However, the enzymatic activity in T3 plants was similar to C plants, revealing that SA in absence of Cd exposure was unable to elicit anti-oxidative responses. The overall fold changes of biochemical, physiological and agronomic parameter analysis of SA and Cd exposed rice seedlings with respect to untreated ones is represented by color coded heat map (Fig. 5).

The chelation and sequestration of heavy metals are mainly carried out by metallothioneins and phytochelatins. The phytochelatins are synthesized as defense response, involving binding of sulphahydryl and carboxyl groups to a wide range of elements like Cd,Pb,Cu, Ni etc. (Cobbett, 2000; Emamverdian et al., 2015). *PCS* plays a significant role in the sequestration of Cd in *A. thaliana* (Chen et al., 2006; Liu et al., 2010; Kühnlenz et al., 2016). Function of *PCS* in Cd accumulation was established by *OsPCS* mutant studies, which showed lower accumulation. Constitutive expression of *PCS* occurs in plants, but they are upregulated in presence of metal/metalloid due to post transcriptional activation of the *PCS* gene (Cobbett, 2000; Vatamaniuk et al., 2000). Phytochelatins synthesized by *PCS* bind to Cd and form PC-Cd complexes which are sequestered in the vacuoles. Forming PC-Cd complexes and their subsequent sequestration into the vacuoles is crucial for Cd tolerance (Clemens et al., 1999; Ha et al., 1999; Vatamaniuk et al., 1999). Expression of *PCS1* slightly increased in T1 seedlings in comparison to the untreated ones, indicating higher PC synthesis, resulting in higher sequestration of Cd into the vacuoles. Cd sequestration is also mediated by Os*HMA3* (Morel et al., 2009). As observed in our study, the upregulated expression of *OsHMA3* in T1 set is statistically significant to the C and T2 set. There are no dedicated transporters for Cd transportation, so other ion transporters are used to transport Cd. *OsNRAMP2* is highly expressed in seedlings with high Cd accumulation properties.

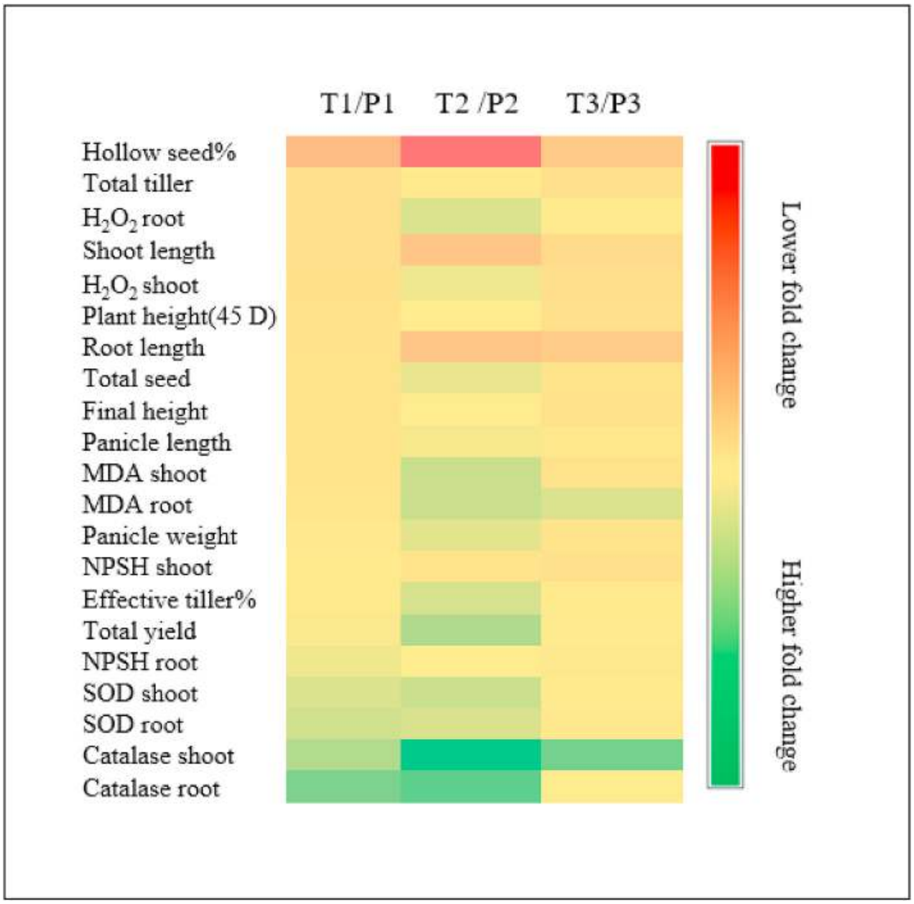

Fig. 5. Heat map analysis on the basis of fold change of treated rice seedlings with respect to untreated set, the assessed physiological, biochemical and agronomic are represented.. T1/P1 (25 μM Cd + 100 μM SA), T2/P2 (25 μM Cd + 0 μM SA), T3/P3 (0 Cd + 100 μM SA).

NRAMP2 allele from low Cd accumulating plants increased Cd sensitivity, SA application reduced *NRAMP2* expression suggesting lowered sensitivity of SA exposed plants (Zhao et al., 2018). The *OsNRAMP2* gene was downregulated in T1 seedlings. Increase of *OsNRAMP2* expression under Cd stress was observed which decreased on application of SA. The upregulation of the gene under Cd stress is similar to reports in ryegrass (Li et al., 2017). Application of SA to Cd exposed seedlings reduced the *NRAMP* expression owing to alleviating property of SA. The whole reaction cascade which follows after application of SA is represented schematically (Fig. 6).

The hydroponics is a well-balanced and controlled nutrient delivery system for lab-based experimentations, full term growth of rice plants in hydroponics require several infrastructural facilities and is expensive (Bado et al., 2016). In dearth of such prerequisites, the plants were grown in pot soil at the experimental farm, the soil used for pot experiments were Gangetic alluvial in nature with loamy clay consistency, the pH and organic carbon contents were reported to be 6.7 and 7.4 g kg^{-1} respectively (Mukherjee et al., 2019; Ghosh et al., 2020), providing favorable growth conditions for the plants. Additionally, owing to the near neutral pH and soil characters like high organic carbon content, loamy texture, the basal Cd uptake in Cd unexposed plants might be too low to be detected, as the Cd mobility is highly impaired in soil with clay loam texture (Hattori et al., 2006) with high organic carbon (Christensen et al., 1996) and near neutral pH (Xiaofang et al., 2019). Co-application of SA and Cd in rice plants have shown reduced accumulation of Cd in the root, shoot and grains of P1 plants. The influx of Cd is reported to reduce under Cd stress by SA application in peanuts and perennial ryegrass, the rearrangement of cell wall components might be a contributing factor here (Wang et al., 2013; Xu et al., 2015; Bai et al., 2015). Although the application of several organic acids (for eg. Citric, acetic, malic, succinic, oxalic) to soil (Sidhu et al., 2019) have exhibited increase in heavy metal bioavailability, yet, exogenous SA application has reduced elemental uptake by increasing citrate efflux from roots of treated seedlings, decreasing the Cd content in the root tips (Yang et al., 2003). Rhizospheric exudation of several secondary metabolites is reported to be a dynamic strategy to impede Cd uptake by roots (Bali et al., 2020). Unlike other low molecular weight organic acids, SA up to concentrations of 0.5 mM had no significant impact on soil anionic charges (Zhang et al., 2008). The TF(C_{shoot}/C_{root}) was greatly reduced in the P1 plants under influence of SA. The application of SA is reported to considerably reduce Cd uptake and toxicity in radish roots (Raza and Shafiq, 2013), Cd content reduced considerably in different parts of flax plant upon SA application (Belkhadi et al., 2010), wheat (Shakirova et al., 2016), oilseed rape (Ali et al., 2015), ryegrass (Wang et al., 2013; Bai et al., 2015), Kentucky bluegrass (Guo et al., 2013). Apart from cell wall rearrangement, SA is also reported to modify the functionality of various metal translocators resulting in reduced accumulation of the toxic metal in the aerial parts, due to its sequestration in the root vacuoles (Shi et al., 2009; Drazic et al., 2005), interaction between SA and Cd increased the synthesis of sulfhydryl groups (Metwally et al., 2003), promoting chelation of Cd ions, these cumulatively lowered the Cd translocation in the shoot, subsequently relieving the grains from Cd accumulation. The lowered *OsNRAMP*2, elevated *OsPCS*1 and *OsHMA*3 transcripts of the T1 seedlings contributed to hindered xylem loading, limiting the Cd deposition in shoot and grains. The reduced toxicity of Cd as a result of impaired Cd uptake by SA application, positively affected the yield related components in P1 plants in comparison to the P2 plants. SA application for amelioration of various stress have been

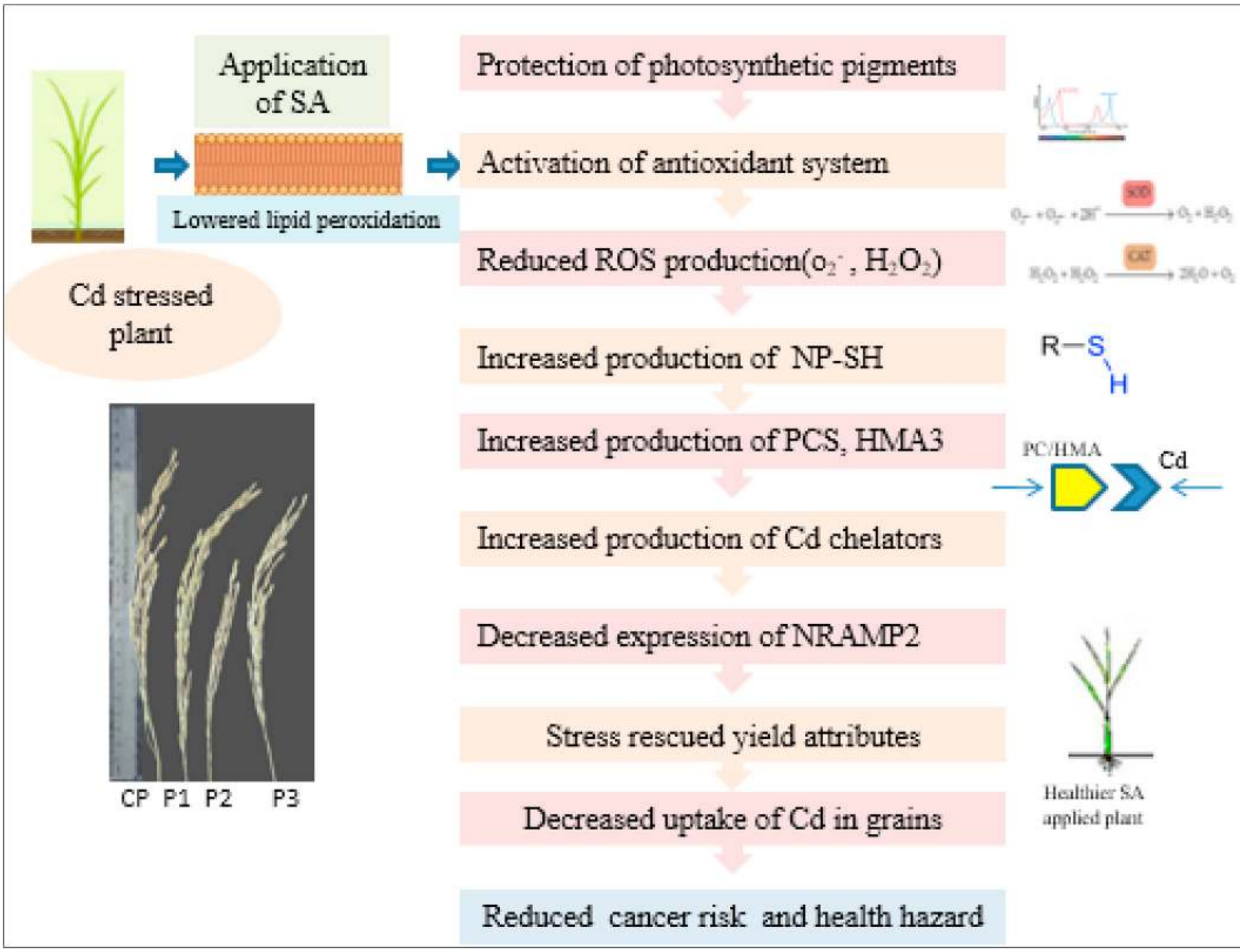

Fig. 6. Simplified schematic representing mechanism of SA providing Cd stress tolerance to rice plants, application of SA to Cd exposed rice seedling results in lowered membrane damage, enhanced photosynthetic pigment production, SA causes antioxidative burst, producing higher levels of ROS scavengers, minimizing free radicals from the plant cells, SA application increases production of NP-SH, required for production of thiol containing Cd chelators like phytochelatins. This causes chelation and sequestration of free divalent Cd ions, NRAMP2 expression is also reduced on SA application increasing tolerance of the seedlings towards Cd stress. Plants on reaching maturity exhibits better yield, lowered Cd uptake in P1 set, ultimately making the seeds of P1 set safe for consumption.

reported to enhance the yield related traits (like total panicle number, tiller number, total seed per panicle and total yield per plant). P1 plants had higher plant height in comparison to P2 plants, as, SA not only plays a major role in restricting Cd toxicity and uptake, but it also supports plant growth and development, by regulating photosynthetic efficiency, osmotic balance, ion homeostasis and anti-oxidative defense system (Khan et al., 2010, 2012a,b,c, 2013b, 2014; Nazar et al., 2011; Miura and Tada, 2014). These protective roles imparted by SA are highly congruent with our findings of hydroponic and pot experiments.

The bioavailable Cd is almost 40–50% lower than the total Cd content of soil, due to association of Cd with carbonates, oxides and other organic forms (Barman et al., 2020). The Cd contents of P2 seed (0.8 mg kg^{-1}) were much higher than the recommended limit (0.4 mg kg^{-1}) by CODEX, 2011, application of SA reduced 95% Cd accumulation in P1 with respect to P2. This is highly impactful, for further application in highly Cd contaminated areas or high Cd accumulating plants. 40.3% of total dietary intake of Cd is contributed by rice, which is reported to range between 0.021 and 0.022 mg kg^{-1} (Kim et al., 2019). The DIM for this cultivar is several folds higher than recent reports by Barman et al. (2020). The application of SA successfully reduced the Cd content in the grains of P1 plants, which resulted in 99.69% lower DIM. The higher the DIM higher will be the occurrence rates of Cd induced disease manifestations ranging from acute bone problems to fatal conditions like cancer. In non-smokers, dietary Cd is strongly associated with gastric cancer (Kim et al., 2019), breast cancer (Grioni et al., 2019). The CR was also reduced on application of SA in P1 plants in comparison to P2 plants. People who are more exposed to heavy metals through grains, vegetables and water are at a higher risk for the development of cancer in future (Zhang et al., 2018; Rezapour et al., 2018).

5. Conclusion

SA application to soil was found to be highly promising in reducing grain Cd content, increasing crop yield in comparison to Cd stressed SA unapplied plants, the cancer risk was also significantly reduced making it safe for consumption. In the present scenario, the rising exposure of the mankind to hazardous materials, is allowing elevated occurrence of diseases and fatalities. The unrestricted use of phosphate fertilizers in agricultural field is subjecting the cereals and crops to uptake Cd substantially, ultimately entering the food chain. So, finding mitigation strategies to reduce Cd deposition in grains, is of utmost importance. SA was found to ensure restricted Cd entry into the rice grains, making it a cost effective and easy way to combat the serious Cd threat.

Author contributions

Snehalata Majumdar: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft, Writing-review & editing, Sonal Sachdev: Methodology, Validation, Rita Kundu: Conceptualization, Investigation, Formal analysis, Visualization, Supervision, Writing - review & editing.

Funding

This study was funded by Department of Biotechnology (West Bengal) [76(Sanc.)-BT/P/Budget/RD-14/2017; March 27, 2018].

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of competing interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

S M acknowledges Department of Biotechnology, Government of India, for her fellowship. S S acknowledges UGC for her fellowship. Authors would like to acknowledge CAS, Dept. of Botany, University of Calcutta, DST-FIST for infrastructural and instrumentation facilities. Authors are grateful to Dr. S Tripathy for providing the experimental field facility in University's Agricultural farm, Baruipur. Authors also acknowledge I.I.T Bombay for ICP/AES analysis. Financial assistance from WBDBT is also gratefully acknowledged. The authors are also thankful to Prof. S. Bhattacharya, Bidhan Chandra Krishi VishwaVidyalaya for providing the rice seed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2020.111167.

References

Agency for Toxic Substance and Disease Registry, 2019. https://www.atsdr.cdc.gov/spl/index.html.

Ahmad, P., Allah, E.A., Hashem, A., Sarwat, M., Gucel, S., 2016. Exogenous application of selenium mitigates cadmium toxicity in Brassica juncea L.(Czern& Cross) by up-regulating antioxidative system and secondary metabolites. J. Plant. Growth. Regul. 35 (4), 936–950. https://doi.org/10.1007/s00344-016-9592-3.

Ahmad, P., Nabi, G., Ashraf, M., 2011. Cadmium-induced oxidative damage in mustard [Brassica juncea (L.) Czern. &Coss.] plants can be alleviated by salicylic acid. South Afr. J. Bot. 77 (1), 36–44. https://doi.org/10.1016/j.sajb.2010.05.003.

Ali, E., Maodzeka, A., Hussain, N., Shamsi, I.H., Jiang, L., 2015. The alleviation of cadmium toxicity in oilseed rape (Brassica napus) by the application of salicylic acid. Plant Growth Regul. 75 (3), 641–655. https://doi.org/10.1007/s10725-014-9966-0.

An, C., Mou, Z., 2011. Salicylic acid and its function in plant immunity F. J. Integr. Plant Biol. 53 (6), 412–428. https://doi.org/10.1111/j.1744-7909.2011.01043.x.

Arivazhagan, V., Sharavanan, P.S., 2015. Effect of cadmium on photosynthetic responses and biochemical contents of maize plants. Am. J. Environ. Eng. Sci. 2 (3), 32.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 24 (1), 1. https://doi.org/10.1104/pp.24.1.

Bado, S., Forster, B.P., Ghanim, A.M., Jankowicz-Cieslak, J., Berthold, G., Luxiang, L., 2016. Protocol for screening for salt tolerance in rice. In: Protocols for Pre-field Screening of Mutants for Salt Tolerance in Rice, Wheat and Barley. Springer, Cham, pp. 21–31. https://doi.org/10.1007/978-3-319-26590-2_1.

Bai, X., Dong, Y., Kong, J., Xu, L., Liu, S., 2015. Effects of application of salicylic acid alleviates cadmium toxicity in perennial ryegrass. Plant Growth Regul. 75 (3), 695–706. https://doi.org/10.1007/s10725-014-9971-3.

Bali, A.S., Sidhu, G.P.S., Kumar, V., 2020. Root exudates ameliorate cadmium tolerance in plants: a review. Environ. Chem. Lett. https://doi.org/10.1007/s10311-020-01012-x.

Bari, M.A., Akther, M.S., Reza, M.A., Kabir, A.H., 2019. Cadmium tolerance is associated with the root-driven coordination of cadmium sequestration, iron regulation, and ROS scavenging in rice. Plant Physiol. Biochem. 136, 22–33. https://doi.org/10.1016/j.plaphy.2019.01.007.

Barman, F., Majumdar, S., Arzoo, S.H., Kundu, R., 2020. Genotypic variation among 20 rice cultivars/landraces in response to cadmium stress grown locally in West Bengal, India. Plant Physiol. Biochem. https://doi.org/10.1016/j.plaphy.2020.01.019.

Barr, H.D., Weatherley, P.E., 1962. A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust. J. Biol. Sci. 15 (3), 413–428. https://doi.org/10.1071/bi9620413.

Belkhadi, A., Hediji, H., Abbes, Z., Nouairi, I., Barhoumi, Z., Zarrouk, M., Chaïbi, W., Djebali, W., 2010. Effects of exogenous salicylic acid pre-treatment on cadmium toxicity and leaf lipid content in Linumusitatissimum L. Ecotoxicol. Environ. Saf. 73 (5), 1004–1011. https://doi.org/10.1016/j.ecoenv.2010.03.009.

Cakmak, I., Marschner, H., 1992. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol. 98 (4), 1222–1227. https://doi.org/10.1104/pp.98.4.1222.

Cao, F., Liu, L., Ibrahim, W., Cai, Y., Wu, F., 2013. Alleviating effects of exogenous glutathione, glycinebetaine, brassinosteroids and salicylic acid on cadmium toxicity in rice seedlings (Oryza sativa). Agrotechnology 2 (1), 107–112.

Chen, A., Komives, E.A., Schroeder, J.I., 2006. An improved grafting technique for mature Arabidopsis plants demonstrates long-distance shoot-to-root transport of phytochelatins in Arabidopsis. Plant Physiol. 141 (1), 108–120. https://doi.org/10.1104/pp.105.072637.

Christensen, J.B., Jensen, D.L., Christensen, T.H., 1996. Effect of dissolved organic carbon on the mobility of cadmium, nickel and zinc in leachate polluted groundwater. Water Res. 30 (12), 3037–3049. https://doi.org/10.1016/s0043-1354(96)00091-7.

Clemens, S., Kim, E.J., Neumann, D., Schroeder, J.I., 1999. Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. EMBO J. 18 (12), 3325–3333. https://doi.org/10.1093/emboj/18.12.3325.

Cobbett, C.S., 2000. Phytochelatins and their roles in heavy metal detoxification. Plant Physiol. 123 (3), 825–832. https://doi.org/10.1104/pp.123.3.825.

Codex Alimentarius Commission, 2011. Joint FAO/WHO food standards programme codex committee on contaminants in foods. fifth session. In: Working Document for Information and Use in Discussions Related to Contaminants and Toxins in the GSCTFF, CF/5 INF/1. FAO/WHO, The Hague.

Cotton, F.A., 1999. Survey of transition-metal chemistry. In: Advanced Inorganic Chemistry, sixth ed. John Wiley and Sons, ISBN 0-471-19957-5, p. 633. https://doi.org/10.1016/s0039-9140(99)00269-6.

Cuypers, A., Plusquin, M., Remans, T., Jozefczak, M., Keunen, E., Gielen, H., Opdenakker, K., Nair, A.R., Munters, E., Artois, T.J., Nawrot, T., 2010. Cadmium stress: an oxidative challenge. Biometals 23 (5), 927–940. https://doi.org/10.1007/s10534-010-9329-x.

Das, N., Bhattacharya, S., Bhattacharyya, S., Maiti, M.K., 2017. Identification of alternatively spliced transcripts of rice phytochelatin synthase 2 gene OsPCS2 involved in mitigation of cadmium and arsenic stresses. Plant Mol. Biol. 94 (1–2), 167–183. https://doi.org/10.1007/s11103-017-0600-1.

Dewez, D., Goltsev, V., Kalaji, H.M., Oukarroum, A., 2018. Inhibitory effects of silver nanoparticles on photosystem II performance in Lemna gibba probed by chlorophyll fluorescence. Curr. Plant Biol. 16, 15–21. https://doi.org/10.1016/j.cpb.2018.11.006.

Djebali, W., Zarrouk, M., Brouquisse, R., El Kahoui, S., Limam, F., Ghorbel, M.H., Chaïbi, W., 2005. Ultrastructure and lipid alterations induced by cadmium in tomato (Lycopersiconesculentum) chloroplast membranes. Plant Biol. 7 (4), 358–368. https://doi.org/10.1055/s-2005-837696.

Drazic, G., Mihailovic, N., 2005. Modification of cadmium toxicity in soybean seedlings by salicylic acid. Plant Sci. 168 (2), 511–517. https://doi.org/10.1016/j.plantsci.2004.09.019.

Du, Y., Hu, X.F., Wu, X.H., Shu, Y., Jiang, Y., Yan, X.J., 2013. Affects of mining activities on Cd pollution to the paddy soils and rice grain in Hunan province, Central South China. Environ. Monit. Assess. 185 (12), 9843–9856. https://doi.org/10.1007/s10661-013-3296-y.

Emamverdian, A., Ding, Y., Xie, Y., 2020. The role of new members of phytohormones in plant amelioration under abiotic stress with an emphasis on heavy metals. Pol. J. Environ. Stud. 29 (2) https://doi.org/10.15244/pjoes/108687.

Emamverdian, A., Ding, Y., Mokhberdoran, F., Xie, Y., 2015. Heavy metal stress and some mechanisms of plant defense response. Sci. World J. 2015, 1–18. https://doi.org/10.1155/2015/756120.

EPA, A., 2004. Risk assessment guidance for superfund. In: Volume I: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment), vol. 5. EPA/540/R/99.

Fatima, R.N., Javed, F., Wahid, A., 2014. Salicylic acid modifies growth performance and nutrient status of rice (Oryza sativa) under cadmium stress. Int. J. Agric. Biol. 16 (6) https://doi.org/10.12692/ijb/6.4.177-183.

Fu, J., Zhou, Q., Liu, J., Liu, W., Wang, T., Zhang, Q., Jiang, G., 2008. High levels of heavy metals in rice (Oryzasativa L.) from a typical E-waste recycling area in southeast China and its potential risk to human health. Chemosphere 71 (7), 1269–1275. https://doi.org/10.1016/j.chemosphere.2007.11.065.

Gao, M., Zhou, J., Liu, H., Zhang, W., Hu, Y., Liang, J., Zhou, J., 2018. Foliar spraying with silicon and selenium reduces cadmium uptake and mitigates cadmium toxicity in rice. Sci. Total Environ. 631, 1100–1108. https://doi.org/10.1016/j.scitotenv.2018.03.047.

Ghosh, S., Chakrabarti, K., Mukherjee, A.K., Tripathi, S., 2020. Different rice based cropping systems can influence various soil organic carbon pools in a clay loam soil

of West Bengal. Int. J. Curr. Microbiol. App. Sci 9 (3), 1884–1897. https://doi.org/10.20546/ijcmas.2020.903.219.
Grioni, S., Agnoli, C., Krogh, V., Pala, V., Rinaldi, S., Vinceti, M., Contiero, P., Vescovi, L., Malavolti, M., Sieri, S., 2019. Dietary cadmium and risk of breast cancer subtypes defined by hormone receptor status: a prospective cohort study. Int. J. Canc. 144 (9), 2153–2160. https://doi.org/10.1002/ijc.32039.
Gu, C.S., Yang, Y.H., Shao, Y.F., Wu, K.W., Liu, Z.L., 2018. The effects of exogenous salicylic acid on alleviating cadmium toxicity in Nymphaea tetragona. Georgi. S. Afr. J. Bot. 114, 267–271. https://doi.org/10.1016/j.sajb.2017.11.012.
Guo, B., Liang, Y.C., Zhu, Y.G., Zhao, F.J., 2007. Role of salicylic acid in alleviating oxidative damage in rice roots (Oryza sativa) subjected to cadmium stress. Environ. Pollut. 147 (3), 743–749. https://doi.org/10.1016/j.envpol.2006.09.007.
Guo, Q., Meng, L., Mao, P.C., Jia, Y.Q., Shi, Y.J., 2013. Role of exogenous salicylic acid in alleviating cadmium-induced toxicity in Kentucky bluegrass. Biochem. Systemat. Ecol. 50, 269–276. https://doi.org/10.1016/j.bse.2013.05.002.
Ha, S.B., Smith, A.P., Howden, R., Dietrich, W.M., Bugg, S., O'Connell, M.J., Goldsbrough, P.B., Cobbett, C.S., 1999. Phytochelatin synthase genes from Arabidopsis and the yeast Schizosaccharomyces pombe. Plant Cell 11 (6), 1153–1163. https://doi.org/10.1105/tpc.11.6.1153.
Hakmaoui, A., Ater, M., Boka, K., Baron, M., 2007. Copper and cadmium tolerance, uptake and effect on chloroplast ultrastructure. Studies on Salix purpurea and Phragmitesaustralis. ZeitschriftfürNaturforschung C 62 (5–6), 417–426. https://doi.org/10.1515/znc-2007-5-616.
Hattori, H., Kuniyasu, K., Chiba, K., Chino, M., 2006. Effect of chloride application and low soil pH on cadmium uptake from soil by plants. Soil Sci. Plant Nutr. 52 (1), 89–94. https://doi.org/10.1111/j.1747-0765.2006.00007.x.
Havir, E.A., McHale, N.A., 1987. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol. 84 (2), 450–455. https://doi.org/10.1104/pp.84.2.450.
Hayat, Q., Hayat, S., Irfan, M., Ahmad, A., 2010. Effect of exogenous salicylic acid under changing environment: a review. Environ. Exp. Bot. 68 (1), 14–25. https://doi.org/10.1016/j.envexpbot.2009.08.005.
Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125 (1), 189–198. https://doi.org/10.1016/0003-9861(68)90654-1.
Herrera-Vásquez, A., Salinas, P., Holuigue, L., 2015. Salicylic acid and reactive oxygen species interplay in the transcriptional control of defense genes expression. Front. Plant Sci. 6, 171. https://doi.org/10.3389/fpls.2015.00171.
Hoagland, D.R., Snyder, W.C., 1933. Effects of deficiencies of boron and certain 16 other elements: (b) Susceptibility to injury from sodium salts. In: Proc Am SocHort 17 Sci, vol. 30, pp. 288–294.
Hou, W., Chen, X., Song, G., Wang, Q., Chang, C.C., 2007. Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (Lemna minor). Plant Physiol. Biochem. 45 (1), 62–69. https://doi.org/10.1016/j.plaphy.2006.12.005.
Hsu, Y.T., Kao, C.H., 2007. Toxicity in leaves of rice exposed to cadmium is due to hydrogen peroxide accumulation. Plant Soil 298 (1–2), 231–241. https://doi.org/10.1007/s11104-007-9357-7.
Huang, Y., Liao, M., Ye, Z., Li, T., 2017. Cd concentrations in two low Cd accumulating varieties of rice and their relationships with soil Cd content and their relation under field conditions. J. Ecol. Rural Environ. 33 (8), 748–754.
Irshad, M.K., Chen, C., Noman, A., Ibrahim, M., Adeel, M., Shang, J., 2020. Goethite-modified biochar restricts the mobility and transfer of cadmium in soil-rice system. Chemosphere 242, 125152. https://doi.org/10.1016/j.chemosphere.2019.125152.
Jabs, T., Dietrich, R.A., Dangl, J.L., 1996. Initiation of runaway cell death in an Arabidopsis mutant by extracellular superoxide. Science 273 (5283), 1853–1856. https://doi.org/10.1126/science.273.5283.1853.
Jadhao, K.R., Samal, K.C., Pradhan, S.K., Rout, G.R., 2014. Studies on molecular characterization of DREB gene in Indica rice (Oryza sativa L.). Hered. Genet. 3 (3) https://doi.org/10.4172/2161-1041.1000133, 2161-1041.
Jan, M., Shah, G., Masood, S., Iqbal Shinwari, K., Hameed, R., Rha, E.S., Jamil, M., 2019. Bacillus cereus enhanced phytoremediation ability of rice seedlings under cadmium toxicity. BioMed Res. Int. https://doi.org/10.1155/2019/8134651, 2019.
Joseph, B., Jini, D., Sujatha, S., 2010. Insight into the role of exogenous salicylic acid on plants grown under salt environment. Asian J. Crop Sci. 2 (4), 226–235. https://doi.org/10.3923/ajcs.2010.226.235.
Kabata-Pendias, A., Mukherjee, A.B., 2007. Trace Elements from Soil to Human. Springer Science & Business Media. https://doi.org/10.1007/978-3-540-32714-1.
Kalavrouziotis, I.K., Koukoulakis, P., Kostakioti, E., 2012. Assessment of metal transfer factor under irrigation with treated municipal wastewater. Agric. Water Manag. 103, 114–119. https://doi.org/10.1016/j.agwat.2011.11.002.
Khan, A.L., Waqas, M., Hussain, J., Al-Harrasi, A., Hamayun, M., Lee, I.J., 2015. Phytohormones enabled endophytic fungal symbiosis improve aluminum phytoextraction in tolerant Solanum lycopersicum: an examples of Penicillium janthinellum LK5 and comparison with exogenous GA3. J. Hazard Mater. 295, 70–78. https://doi.org/10.1016/j.jhazmat.2015.04.008.
Khan, M.I.R., Asgher, M., Khan, N.A., 2014. Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean (Vigna radiata L.). Plant Physiol. Biochem. 80, 67–74. https://doi.org/10.1016/j.plaphy.2014.03.026.
Khan, N., Syeed, S., Masood, A., Nazar, R., Iqbal, N., 2010. Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mungbean and alleviates adverse effects of salinity stress. Int. J. Plant Biol. 1 (1) https://doi.org/10.4081/pb.2010.e1 e1-e1.
Kim, H., Lee, J., Woo, H.D., Kim, D.W., Choi, I.J., Kim, Y.I., Kim, J., 2019. Association between dietary cadmium intake and early gastric cancer risk in a Korean population: a case–control study. Eur. J. Nutr. 58 (8), 3255–3266. https://doi.org/10.1007/s00394-018-1868-x.
Kohli, S.K., Bali, S., Tejpal, R., Bhalla, V., Verma, V., Bhardwaj, R., Alqarawi, A.A., Abd_Allah, E.F., Ahmad, P., 2019. In-situ localization and biochemical analysis of bio-molecules reveals Pb-stress amelioration in Brassica juncea L. by co-application of 24-Epibrassinolide and Salicylic Acid. Sci. Rep. 9 (1), 1–15. https://doi.org/10.1038/s41598-019-39712-2.
Kosolsaksakul, P., Farmer, J.G., Oliver, I.W., Graham, M.C., 2014. Geochemical associations and availability of cadmium (Cd) in a paddy field system, northwestern Thailand. Environ. Pollut. 187, 153–161. https://doi.org/10.1016/j.envpol.2014.01.006.
Krantev, A., Yordanova, R., Janda, T., Szalai, G., Popova, L., 2008. Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. J. Plant Physiol. 165 (9), 920–931. https://doi.org/10.1016/j.jplph.2006.11.014.
Kühnlenz, T., Hofmann, C., Uraguchi, S., Schmidt, H., Schempp, S., Weber, M., Lahner, B., Salt, D.E., Clemens, S., 2016. Phytochelatin synthesis promotes leaf Zn accumulation of Arabidopsis thaliana plants grown in soil with adequate Zn supply and is essential for survival on Zn-contaminated soil. Plant Cell Physiol. 57 (11), 2342–2352. https://doi.org/10.1093/pcp/pcw148.
Li, T., Di, Z., Islam, E., Jiang, H., Yang, X., 2011. Rhizosphere characteristics of zinc hyperaccumulator Sedum alfredii involved in zinc accumulation. J. Hazard Mater. 185 (2–3), 818–823. https://doi.org/10.1016/j.jhazmat.2010.09.093.
Li, T., Xu, W.H., Chai, Y.R., Wang, Z.Y., Xie, D.T., 2017. Differences of Cd uptake and expression of Cd-tolerance related genes in two varieties of ryegrasses. Bulg. Chem. Commun. 49 (3), 697–705.
Liu, J.G., Liang, J.S., Li, K.Q., Zhang, Z.J., Yu, B.Y., Lu, X.L., Yang, J.C., Zhu, Q.S., 2003. Correlations between cadmium and mineral nutrients in absorption and accumulation in various genotypes of rice under cadmium stress. Chemosphere 52 (9), 1467–1473. https://doi.org/10.1016/s0045-6535(03)00484-3.
Liu, W.J., Wood, B.A., Raab, A., McGrath, S.P., Zhao, F.J., Feldmann, J., 2010. Complexation of arsenite with phytochelatins reduces arsenite efflux and translocation from roots to shoots in Arabidopsis. Plant Physiol. 152 (4), 2211–2221. https://doi.org/10.1104/pp.109.150862.
Lu, C., Zhang, L., Tang, Z., Huang, X.Y., Ma, J.F., Zhao, F.J., 2019. Producing cadmium-free Indica rice by overexpressing OsHMA3. Environ. Int. 126, 619–626. https://doi.org/10.1016/j.envint.2019.03.004.
Lu, Q., Zhang, T., Zhang, W., Su, C., Yang, Y., Hu, D., Xu, Q., 2018. Alleviation of cadmium toxicity in Lemna minor by exogenous salicylic acid. Ecotoxicol. Environ. Saf. 147, 500–508. https://doi.org/10.1016/j.ecoenv.2017.09.015.
Ma, X., Deng, D., Chen, W., 2017. Inhibitors and activators of SOD, GSH-px, and CAT. Enzyme inhibitors and activators 29, 207. https://doi.org/10.5772/65936.
Maksymiec, W., Krupa, Z., 2006. The effects of short-term exposition to Cd, excess Cu ions and jasmonate on oxidative stress appearing in Arabidopsis thaliana. Environ. Exp. Bot. 57 (1–2), 187–194. https://doi.org/10.1016/j.envexpbot.2005.05.006.
Mani, A., Sankaranarayanan, K., 2018. In silico analysis of natural resistance-associated macrophage protein (NRAMP) family of transporters in rice. Protein J. 37 (3), 237–247. https://doi.org/10.1007/s10930-018-9773-y.
Mba, F.O., Zhi-Ting, X., Hai-Jie, Q., 2007. Salicylic acid alleviates the cadmium toxicity in Chinese cabbages (Brassica chinensis). Pakistan J. Biol. Sci. 10, 3065–3071. https://doi.org/10.3923/pjbs.2007.3065.3071.
Metwally, A., Finkemeier, I., Georgi, M., Dietz, K.J., 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiol. 132 (1), 272–281. https://doi.org/10.1104/pp.102.018457.
Miura, K., Tada, Y., 2014. Regulation of water, salinity, and cold stress responses by salicylic acid. Front. Plant Sci. 5, 4. https://doi.org/10.3389/fpls.2014.00004.
Miyadate, H., Adachi, S., Hiraizumi, A., Tezuka, K., Nakazawa, N., Kawamoto, T., Katou, K., Kodama, I., Sakurai, K., Takahashi, H., Satoh-Nagasawa, N., 2011. OsHMA3, a P1B-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. New Phytol. 189 (1), 190–199. https://doi.org/10.1111/j.1469-8137.2010.03459.x.
Mondal, N.K., Das, C., Roy, S., Banerjee, A., 2013. Effect of varying cadmium stress on chickpea (Cicer arietinum L) seedlings: an ultrastructural study. Ann. Environ. Sci. 7 (1), 5.
Morel, M., Crouzet, J., Gravot, A., Auroy, P., Leonhardt, N., Vavasseur, A., Richaud, P., 2009. AtHMA3, a P1B-ATPase allowing cd/Zn/co/Pb vacuolar storage in Arabidopsis. Plant Physiol. 149 (2), 894–904. https://doi.org/10.1104/pp.108.130294.
Mukherjee, A.K., Tripathi, S., Mukherjee, S., Mallick, R.B., Banerjee, A., 2019. Effect of integrated nutrient management in sunflower (Helianthus annuus L.) on alluvial soil. Curr. Sci. 117 (8), 1364. https://doi.org/10.18520/cs/v117/i8/1364-1368.
Nazar, R., Iqbal, N., Syeed, S., Khan, N.A., 2011. Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. J. Plant Physiol. 168 (8), 807–815. https://doi.org/10.1016/j.jplph.2010.11.001.
Parmar, P., Kumari, N., Sharma, V., 2013. Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. Bot. Stud. 54 (1), 45. https://doi.org/10.1186/1999-3110-54-45.
Popova, L.P., Maslenkova, L.T., Yordanova, R.Y., Ivanova, A.P., Krantev, A.P., Szalai, G., Janda, T., 2009. Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings. Plant Physiol. Biochem. 47 (3), 224–231. https://doi.org/10.1016/j.plaphy.2008.11.007.
Poudel, A.P., 2018. Analysis of growth and yield attributing characteristic of direct seeded upland rice genotypes in western hills of Nepal. Acta sci. agric.(ISSN: 2581-365X) Acta sci. agric 2 (7), 117–122.

Rastogi, A., Zivcak, M., Sytar, O., Kalaji, H.M., He, X., Mbarki, S., Brestic, M., 2017. Impact of metal and metal oxide nanoparticles on plant: a critical review. Front. Chem. https://doi.org/10.3389/fchem.2017.00078, 2017.

Raza, S.H., Shafiq, F., 2013. Exploring the role of salicylic acid to attenuate cadmium accumulation in radish (Raphanussativus). Int. J. Agric. Biol. 15 (3).

Rezapour, S., Kouhinezhad, P., Samadi, A., 2018. The potential ecological risk of soil trace metals following over five decades of agronomical practices in a semi-arid environment. Chem. Ecol. 34 (1), 70–85. https://doi.org/10.1080/02757540.2017.1404585.

Rivas-San Vicente, M., Plasencia, J., 2011. Salicylic acid beyond defence: its role in plant growth and development. J. Exp. Bot. 62 (10), 3321–3338. https://doi.org/10.1093/jxb/err031.

Rodríguez-Serrano, M., Romero-Puertas, M.C., Sparkes, I., Hawes, C., Luis, A., Sandalio, L.M., 2009. Peroxisome dynamics in Arabidopsis plants under oxidative stress induced by cadmium. Free FreeRadic. Biol. Med. 47 (11), 1632–1639. https://doi.org/10.1016/j.freeradbiomed.2009.09.012.

S Schützendübel, A., Nikolova, P., Rudolf, C., Polle, A., 2002. Cadmium and H2O2-induced oxidative stress in Populus× canescens roots. Plant Physiol. Biochem. 40 (6–8), 577–584. https://doi.org/10.1016/s0981-9428(02)01411-0.

Sahu, G.K., Sabat, S.C., 2018. Salicylic acid modulates isoenzyme pattern of enzymatic antioxidants in wheat. Indian J. Plant Physiol. 23 (3), 486–493. https://doi.org/10.1007/s40502-018-0384-6.

Saruhan, N., Saglam, A., Kadioglu, A., 2012. Salicylic acid pretreatment induces drought tolerance and delays leaf rolling by inducing antioxidant systems in maize genotypes. Acta Physiol. Plant. 34 (1), 97–106. https://doi.org/10.1007/s11738-011-0808-7.

Sergiev, I., Alexieva, V., Karanov, E.V., SERGIEV, I., Karanov, E., Sergiev, L., Karanova, E., ALEXIEVA, V., Alexieva, V.V., 1997. Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. Comp. Ren. Del. Academie Bul. des Sci. 51, 121–124.

Shakirova, F.M., Allagulova, C.R., Maslennikova, D.R., Klyuchnikova, E.O., Avalbaev, A. M., Bezrukova, M.V., 2016. Salicylic acid-induced protection against cadmium toxicity in wheat plants. Environ. Exp. Bot. 122, 19–28. https://doi.org/10.1016/j.envexpbot.2015.08.002.

Shri, M., Dave, R., Diwedi, S., Shukla, D., Kesari, R., Tripathi, R.D., Trivedi, P.K., Chakrabarty, D., 2014. Heterologous expression of Ceratophyllumdemersumphytochelatin synthase, CdPCS1, in rice leads to lower arsenic accumulation in grain. Sci. Rep. 4, 5784. https://doi.org/10.1038/srep05784.

Siddhu, G., Khan, M.A., 2012. Effects of cadmium on growth and metabolism of Phaseolus mungo. J. Environ. Biol. 33 (2), 173.

Sidhu, G.P.S., Bali, A.S., Bhardwaj, R., 2019. Role of organic acids in mitigating cadmium toxicity in plants. In: Cadmium Tolerance in Plants. Academic Press, pp. 255–279. https://doi.org/10.1016/b978-0-12-815794-7.00010-2.

Singh, A.P., Dixit, G., Kumar, A., Mishra, S., Kumar, N., Dixit, S., Singh, P.K., Dwivedi, S., Trivedi, P.K., Pandey, V., Dhankher, O.P., 2017. A protective role for nitric oxide and salicylic acid for arsenite phytotoxicity in rice (Oryza sativa L.). Plant Physiol. Biochem. 115, 163–173. https://doi.org/10.1016/j.plaphy.2017.02.019.

Singh, I., Shah, K., 2015. Evidences for suppression of cadmium induced oxidative stress in presence of sulphosalicylic acid in rice seedlings. Plant Growth Regul. 76 (1), 99–110. https://doi.org/10.1007/s10725-015-0023-4.

Sytar, O., Brestic, M., Taran, N., Zivcak, M., 2016. Plants used for biomonitoring and phytoremediation of trace elements in soil and water. In: Ahmad, Parvaiz (Ed.), Plant Metal Interaction: Emerging Remediation Techniques. Elsevier, ISBN 978-0-12-803158-2, pp. 361–384. https://doi.org/10.1016/b978-0-12-803158-2.00014-x, 2016.

Tamás, L., Mistrík, I., Alemayehu, A., Zelinová, V., Bočová, B., Huttová, J., 2015. Salicylic acid alleviates cadmium-induced stress responses through the inhibition of Cd-induced auxin-mediated reactive oxygen species production in barley root tips. J. Plant Physiol. 173, 1–8. https://doi.org/10.1016/j.jplph.2014.08.018.

Thordal-Christensen, H., Zhang, Z., Wei, Y., Collinge, D.B., 1997. Subcellular localization of H2O2 accumulation in papillae and hypersensitive response during the barley—powdery mildew interaction. Plant J. 11 (6), 1187–1194. https://doi.org/10.1046/j.1365-313x.1997.11061187.x.

Ullah, I., Wang, Y., Eide, D.J., Dunwell, J.M., 2018. Evolution, and functional analysis of Natural Resistance-Associated Macrophage Proteins (NRAMPs) from Theobroma cacao and their role in cadmium accumulation. Sci. Rep. 8 (1), 1–15. https://doi.org/10.1038/s41598-018-32819-y.

Uraguchi, S., Tanaka, N., Hofmann, C., Abiko, K., Ohkama-Ohtsu, N., Weber, M., Kamiya, T., Sone, Y., Nakamura, R., Takanezawa, Y., Kiyono, M., 2017. Phytochelatin synthase has contrasting effects on cadmium and arsenic accumulation in rice grains. Plant Cell Physiol. 58 (10), 1730–1742. https://doi.org/10.1093/pcp/pcx114.

Vatamaniuk, O.K., Mari, S., Lu, Y.P., Rea, P.A., 1999. AtPCS1, a phytochelatin synthase from Arabidopsis: isolation and in vitro reconstitution. Proc. Natl. Acad. Sci. Unit. States Am. 96 (12), 7110–7115. https://doi.org/10.1073/pnas.96.12.7110.

Vatamaniuk, O.K., Mari, S., Lu, Y.P., Rea, P.A., 2000. Mechanism of heavy metal ion activation of phytochelatin (PC) synthase blocked thiols are sufficient for PC synthase-catalyzed transpeptidation of glutathione and related thiol peptides. J. Biol. Chem. 275 (40), 31451–31459. https://doi.org/10.1074/jbc.m002997200.

Vestena, S., Cambraia, J., Ribeiro, C., Oliveira, J.A., Oliva, M.A., 2011. Cadmium-induced oxidative stress and antioxidative enzyme response in water hyacinth and salvinia. Braz. J. Plant Physiol. 23 (2), 131–139. https://doi.org/10.1590/s1677-04202011000200005.

Wang, F., Tan, H., Zhang, Y., Huang, L., Ding, Y., Chen, Z., Zhu, C., 2019. Salicylic Acid Application Alleviates Cadmium Accumulation in Brown Rice by Modulating its Shoot to Grain Translocation in Rice, 31 December 2019. PREPRINT (Version 1) available at Research Square. https://doi.org/10.21203/rs.2.19653/v1.

Wang, J., Fang, Y., Tian, B., Zhang, X., Zeng, D., Guo, L., Hu, J., Xue, D., 2018. New QTLs identified for leaf correlative traits in rice seedlings under cadmium stress. Plant Growth Regul. 85 (2), 329–335. https://doi.org/10.1007/s10725-018-0393-5.

Wang, Q., Liang, X., Dong, Y., Xu, L., Zhang, X., Kong, J., Liu, S., 2013. Effects of exogenous salicylic acid and nitric oxide on physiological characteristics of perennial ryegrass under cadmium stress. J. Plant Growth Regul. 32 (4), 721–731. https://doi.org/10.1007/s00344-013-9339-3.

Wani, A.B., Chadar, H., Wani, A.H., Singh, S., Upadhyay, N., 2017. Salicylic acid to decrease plant stress. Environ. Chem. Lett. 15 (1), 101–123. https://doi.org/10.1007/s10311-016-0584-0.

Xiaofang, L.I., Dongmei, Z.H.O.U., 2019. A meta-analysis on phenotypic variation in cadmium accumulation of rice and wheat: implications for food cadmium risk control. Pedosphere 29 (5), 545–553. https://doi.org/10.1016/s1002-0160(19)60828-3.

Xu, J., Wang, W., Yin, H., Liu, X., Sun, H., Mi, Q., 2010. Exogenous nitric oxide improves antioxidative capacity and reduces auxin degradation in roots of Medicagotruncatula seedlings under cadmium stress. Plant Soil 326 (1–2), 321. https://doi.org/10.1007/s11104-009-0011-4.

Xu, L.L., Fan, Z.Y., Dong, Y.J., Kong, J., Bai, X.Y., 2015. Effects of exogenous salicylic acid and nitric oxide on physiological characteristics of two peanut cultivars under cadmium stress. Biol. Plant. (Prague) 59 (1), 171–182. https://doi.org/10.1007/s10535-014-0475-9.

Xue, D., Jiang, H., Deng, X., Zhang, X., Wang, H., Xu, X., Hu, J., Zeng, D., Guo, L., Qian, Q., 2014. Comparative proteomic analysis provides new insights into cadmium accumulation in rice grain under cadmium stress. J. Hazard Mater. 280, 269–278. https://doi.org/10.1016/j.jhazmat.2014.08.010.

Yang, Z.M., Wang, J., Wang, S.H., Xu, L.L., 2003. Salicylic acid-induced aluminum tolerance by modulation of citrate efflux from roots of Cassia tora L. Planta 217 (1), 168–174. https://doi.org/10.1007/s00425-003-0980-0.

Yılmaz, D.D., Parlak, K.U., 2011. Changes in proline accumulation and antioxidative enzyme activities in Groenlandiadensa under cadmium stress. Ecol. Indicat. 11 (2), 417–423. https://doi.org/10.1016/j.ecolind.2010.06.012.

Yüzbaşıoğlu, Elif, Dalyan, Eda, 2019. Salicylic acid alleviates thiram toxicity by modulating antioxidant enzyme capacity and pesticide detoxification systems in the tomato (Solanum lycopersicum Mill.). Plant Physiol. Biochem. 135, 322–330. https://doi.org/10.1016/j.plaphy.2018.12.023.

Zengin, F., 2014. Exogenous treatment with salicylic acid alleviating copper toxicity in bean seedlings. Proc. Natl. Acad. Sci. India B Biol. Sci. 84 (3), 749–755. https://doi.org/10.1007/s40011-013-0285-4.

Zengin, F., 2015. Effects of exogenous salicylic acid on growth characteristics and biochemical content of wheat seeds under arsenic stress. J. Environ. Biol. 36 (1), 249.

Zhang, J.Q., Dong, Y.H., 2008. Effect of low-molecular-weight organic acids on the adsorption of norfloxacin in typical variable charge soils of China. J. Hazard Mater. 151 (2–3), 833–839. https://doi.org/10.1016/j.jhazmat.2007.11.046.

Zhang, P.-J., et al., 2013. Feeding by whiteflies suppresses downstream jasmonic acid signaling by eliciting salicylic acid signaling. J. Chem. Ecol. 39, 612–619. https://doi.org/10.1007/s10886-013-0283-2.

Zhang, X., Chen, H., Jiang, H., Lu, W., Pan, J., Qian, Q., Xue, D., 2015. Measuring the damage of heavy metal cadmium in rice seedlings by SRAP analysis combined with physiological and biochemical parameters. J. Sci. Food Agric. 95, 2292–2298. https://doi.org/10.1002/jsfa.6949.

Zhang, Y., Yin, C., Cao, S., Cheng, L., Wu, G., Guo, J., 2018. Heavy metal accumulation and health risk assessment in soil-wheat system under different nitrogen levels. Sci. Total Environ. 622, 1499–1508. https://doi.org/10.1016/j.scitotenv.2017.09.317.

Zhao, F.Y., Han, M.M., Zhang, S.Y., Wang, K., Zhang, C.R., Liu, T., Liu, W., 2012. Hydrogen peroxide-mediated growth of the root system occurs via auxin signaling modification and variations in the expression of cell-cycle genes in rice seedlings exposed to cadmium stress. J. Integr. Plant Biol. 54 (12), 991–1006. https://doi.org/10.1111/j.1744-7909.2012.01170.x.

Zhao, J., Yang, W., Zhang, S., Yang, T., Liu, Q., Dong, J., Fu, H., Mao, X., Liu, B., 2018. Genome-wide association study and candidate gene analysis of rice cadmium accumulation in grain in a diverse rice collection. Rice 11 (1), 61. https://doi.org/10.1186/s12284-018-0254-x.