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Mitigation effects of selenium on accumulation of cadmium and morpho-physiological properties in rice varieties

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ABSTRACT

Selenium (Se) is a beneficial element, but only when present within its permissible range. Its hyper-accumulation in edible plant parts can cause Se toxicity. This study aimed to develop an agronomic plan for biofortification of rice with Se and reclamation of cadmium (Cd)-contaminated soil, utilizing sodium selenite (Na_2SeO_3) and cadmium chloride ($CdCl_2$) as soil treatments. Biofortification was performed on two target rice varieties: genotypes 5097A/R2035 and GangYou725, in field trials by applying Cd at a concentration of 0–8 mg kg soil^{-1} and Se at 0–1 mg kg soil^{-1}. Since these rice varieties have different metabolic specificity, the degree of elemental accumulation, deviations in chlorophyll concentration, activity of photosynthetic apparatus and grain yield were assessed. It was found that application of 1 mg kg^{-1} Se_2O_3 decrease Cd content and increased chlorophyll content and photosynthetic activity while grain yield was unaffected by application of the metallic trace-elements. Comparing effects at different stages, we found that the 50% heading stage was most sensitive to metal application. In sum, Se mitigates Cd toxicity, but hyperaccumulation of Se (4 mg kg^{-1}) in polished rice was observed with Cd at 4 and 8 mg kg^{-1}. The elevated level of Cd stress in pot experiments resulted in over-accumulation of Se in the germ and endosperm that poses serious health concerns.

1. Introduction

Selenium (Se) is an essential element for human health, but the requirement is very low. Thus, biofortifying certain crops with this trace element could provide consumers with the necessary dietary amount. As one of the world's major staple crops, rice could serve as a good source of essential metals such as Se. However, cadmium (Cd) can accumulate up to hazardous levels in polished rice and poses serious health concerns. Ingestion of Cd is associated with the onset of many serious diseases (Peijnenburg et al., 2000) such as kidney (Rana et al., 2018) and lung cancer (Nescu et al., 1977). Cd can also displace other metals from metalloenzymes and inactivate them (Kjellström, 1979). Plants are considered to have little risk from metal contamination, as no obvious symptoms of toxicity have been observed in their morphology and growth. Zhang and colleagues found that 5 mg kg^{-1} Cd concentration in the soil was nontoxic (Zhang et al., 2010) with no obvious symptoms of necrosis (Ismael et al., 2018), chlorosis (Wei et al., 2005) or reduced shoot biomass (Zhang et al., 2013). However, consumption of plants

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from soils with low levels of Cd contamination could lead to metal toxicity if the plants hyperaccumulate the metal. Elevated metal concentrations can affect plant functions viz., decreased photosynthesis and sugar content (Shanying et al., 2017) and activation of anti-stress genes for superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) and other downstream enzyme activity (Rizwan et al., 2016). Some reports indicated that increased Cd stress in soil resulted in increased levels of phenolics and flavonoids, and higher total antioxidant capacity, and reduced chlorophyll content in exposed crops (Hassan et al., 2005). Studies describing trace metal uptake and accumulation are of value in assessing changes in morphology and physicochemical properties as well as subsequent yield reduction. Thus, the safe range for metal application relative to its accumulation in brown rice and polished rice need to be rigorously studied to establish the critical levels for various metal treatments.

Deficiency of an essential nutrient in the soil imposes stress on a plant, causing a subsequent reduction in antioxidant enzyme activities. Some plants, grown under conditions of low soil fertility, may show increased production of phytochemicals and antioxidant compounds (Ibrahim et al., 2013). One of the strategies for farming on metal-contaminated soils is to use cultivars that exclude Cd or are Cd-tolerant, or to treat the crop with elements such as Se that are able to mitigate the effects of Cd toxicity by replacing it. It was found that certain enzyme activities were specifically altered when Se was used as catalyst (Fairweather-Tait et al., 2011). Many recent studies showed that Se increased the sequestration of Cd in the cell walls and vacuoles, decreased the active chemical form of Cd in rice seedlings (Xu et al., 2020), altered the subcellular Cd distribution, and decreased the ROS induced by Cd stress (Wan et al., 2019). In cereals such as wheat, supplemental Se influenced the uptake of Fe (91%) and Na (16%) by enhancing the activity of the antioxidant system and maintaining normal characteristics of gas exchange and expansion (Fahim et al., 2015). It can also increase chlorophyll and carotenoid levels in plant leaves (Jing et al., 2013). The biofortification of crops by means of breeding is a long-lasting and comparatively risk-free process. Likewise, the simple addition of supplemental micronutrients to the soil in fertilizers can stimulate the activities of certain enzymes in plants. Se supplementation is a double-edged sword, because both a lack and an excess can be harmful to human health, and the boundary between the two levels is quite narrow (Brozmanova et al., 2010). A daily Se intake of 40 μg or less is considered insufficient and the human body lapses into a state of selenium deficiency; but, if the daily Se intake is > 200 μg it can result in symptoms of selenosis (Se poisoning) (Fordyce, 2005). In plants, both Se and Cd can accumulate above the permissible limits (Cd, 0.2 mg kg^{-1}; Se, 0.04–0.30 mg kg^{-1}) set by the National Food Safety Standards determination for Se and Cd in foods (Ei et al., 2020; Farooq et al., 2019b), which could cause serious health concerns as part of the food-chain. Sources of Se and Cd contamination in paddy water soil could be either industrial waste, overuse of Se fertilizers or artificial applications (Jafari et al., 2018).

The alleviation of Cd toxicity by Se application results in part from a reduction in Cd uptake, a decrease in ROS accumulation and a balancing of nutrients as indicated by various hydroponic studies (Lin et al., 2012a, b). Considering the known reactions of these elements, the performance under actual field conditions could be entirely different due to multiple environmental and epigenetic effects. Hence a pilot study is needed to truly reflect the outcomes of application of these elements. The study will be useful in providing basic information on the responses of different plant varieties to Se biofortification, the threshold levels for this essential element, the window of toxicity, and the crosstalk of Se and Cd at different levels in altering the nutritional architecture of rice. This investigation was designed to determine: (1) the relationship between application of trace metals and grain yield, (2) the effect of metal treatment on morphology and physiological traits such as chlorophyll content (SPAD), net photosynthetic rate (Photo; P_N), stomatal conductance (Cond; g_s), intercellular CO_2 (C_i), transpiration rate (Trmmol; E), water use efficiency (WUE) and stomatal limitation to CO_2 (SlCi) at four different rice developmental stages (tillering, 50% heading, flowering and 50% maturity stages), (3) health risk assessment in rice grain fractions and tissues (stems, leaves, bran, embryo, endosperm, brown rice and polished rice) to determine responses to supplementation with Se and Cd.

2. Materials and methods

2.1. Study design

The experiments were conducted in the experimental field of Sichuan Agricultural University, Wenjiang, Chengdu, China (longitude 103.8253°east, latitude 30.70254°north, and altitude 497 m). The rice genotypes, *5097A/R2035* (high-Se) (Liang et al., 2018) and *GangYouR725* (low-Se) (Farooq et al., 2019b; Farooq and Zhu, 2019), were used to assess the effects of Se and Cd application on uptake into roots, stem, leaves, grain, rice bran, embryo and endosperm. The permissible limits of these elements in food grains as set by the China National Food Safety Standards (Ei et al., 2020) are Cd, 0.2 mg kg $soil^{-1}$ and Se, 0.04–0.30 mg kg $soil^{-1}$. The low-Se rice (GangYou 725) was tested and its low-accumulation genotype was confirmed: 0.007 mg kg^{-1}Se in polished rice. Its Se content was below the standards for a Se-rich paddy, and hence was termed low Se-rich rice. It was used as positive control and as the standard reference material (SRM) for comparison because its Se content was below the permissible range under natural environment conditions (Farooq et al., 2019a).

For testing, samples of dry experimental soil were taken at depths of 0–20 cm from five different locations in the same field (by a five-spot-sampling method), and total Se and Cd, and plant-bioavailable Se (GB-5009.93-2017) and Cd (GB-5009.15-2014) were determined (Table 1) (Ei et al., 2020). One week before transplanting, plastic pots (28 cm × 35 cm × 27.3 cm) were filled with 10 kg soil, leaving 10 cm at the top for watering. Then, solutions of sodium selenite (Na_2SeO_3) and cadmium chloride ($CdCl_2.2½H_2O$) were added to each pot and the pots were watered every two days. The treatment groups used in these experiments to measure morpho-physiological features (SPAD, IRGA) and determine the nutritional content of rice are shown in Tables 2 and 3, respectively. Each treatment included three replicates.

2.2. Measurement of morpho-physiological parameters (SPAD units and IRGA values) of rice

This experiment compared the effect of treatment of rice plants with Cd alone to treatment with Cd plus Se. Plants in group 1 received Cd treatment only at 3, 4, 5, 6, 7, and 8 mg per kg of soil while group 2 plants were treated with 3, 4, 5, 6, 7, and 8 mg of Cd per kg of soil along

Table 1
Chemical properties of experimental dry soil.

	Index	Content	Method
1	pH	5.8	Potentiometry (NYT 1377–2007)
2	Organic carbon (g/kg of soil)	38.4	Potassium dichromate volumetric method (NYT 1121–6-2006)
3	Extractable nitrogen (mg/kg of soil)	141	Universal extraction colometric method (NYT, 1849–2010)
4	Extractable phosphorus (mg/kg of soil)	28.1	Universal extraction colometric method (NYT, 1849–2010)
5	Extractable potassium (mg/kg of soil)	113	Universal extraction colometric method (NYT, 1849–2010)
6	Total selenium (mg/kg of soil)	0.24	Fluorescence spectrophotometry (GB-5009.93-2017)
7	Bioavailable selenium (mg/kg of soil)	0.026	Extraction with 0.016 M potassium dihydrogen phosphate
8	Cadmium (mg/kg of soil)	0.13	Graphite furnace atomic absorption spectrophotometry (GB-5009.15-2014)

Table 2
Treatment levels used for morphophysiological features (SPAD, IRGA) determination.

Index	Cadmium treatment Only group		Cadmium along with Selenium treatment group	
	Na_2SeO_3 (mg kg^{-1} dry soil)	$CdCl_2.2½H_2O$ (mg kg^{-1} dry soil)	Na_2SeO_3 (mg kg^{-1} dry soil)	$CdCl_2.2½H_2O$ (mg kg^{-1} dry soil)
Treatment 0	0	0	0	0
Treatment I	0	3	1	3
Treatment II	0	4	1	4
Treatment III	0	5	1	5
Treatment IV	0	6	1	6
Treatment V	0	7	1	7
Treatment VI	0	8	1	8
Remarks	High Cd and Deficient Se		High Cd and High Se	

with 1 mg of Se per kg of soil. Cd was administered as a solution of $CdCl_2.2½H_2O$ and Se as Na_2SeO_3 in the paddy water/soil environment (Table 2).

2.3. Determination of grain yield (g per plant)

For the measurement of grain yield per plant all panicles of one plant were harvested, threshed and grains were weighed on an electronic balance (Compax, RS 232C) (Farooq et al., 2018). Data from three plants were averaged and used for further analysis.

2.4. Determination of chlorophyll concentration (SPAD units)

Three plants were chosen from each experimental unit at the stages of tillering, 50% heading, flowering and 50% maturity. Two leaves were selected from each plant and light absorbance was recorded using a digital chlorophyll meter (SPAD-502, Konica Minolta) and measured as chlorophyll SPAD units (Ling et al., 2011). Readings were taken from three different areas of each leaf–tip, middle and base–and averaged to represent the chlorophyll content of the leaf.

2.5. Determination of gas exchange parameters

The gaseous exchange parameters, net photosynthesis rate (Photo; P_N $_{(\mu mol \cdot m}$$^{-2}$ S^{-1})), stomatal conductance (Cond; g_S $_{(mmol\ CO2 \cdot m}$$^{-2}$ S^{-1})), intercellular CO_2 (C_i $_{(\mu mol\ CO2 \cdot m}$$^{-2}$ S^{-1})), transpiration rate (Trmmol; E $_{(mmol\ H2O \cdot m}$$^{-2}$ S^{-1})), water use efficiency (WUE) and stomatal limitation to CO_2 (SlCi) were measured with a portable photosynthesis analyser (LI-6400, LI-COR, Inc., Lincoln, NE) at the 50% heading and 50% maturity stages. The latest, fully expanded leaves were selected to measure photosynthetic parameters on plants between 08:00 and 11:00 h using the following settings: PARi = 1,200, stomatal ratio = 0.5, flow = 500 μmol mol^{-1} and reference CO_2 concentration = 400 μmol mol^{-1} (Hussain et al., 2019). Physiological measurements were taken when P_N, g_s and fluorescence rates were stable (P_N: slope< 1 and g_s: slope< 0.05 for 45 s).

2.6. Measurement of nutrient content in rice following Se and Cd treatment

To assess the effects of Cd and Se treatment on the nutrient content of rice grain fractions, plants were treated as follows: T0 (control); T1 (Se, 0 mg kg^{-1} + Cd, 4 mg kg^{-1}); T2 (Se, 0 mg kg^{-1} + Cd, 8 mg kg^{-1}), T3 (Se, 1 mg kg^{-1} + Cd 4 mg kg^{-1}), and T4 (Se, 1 mg kg^{-1}+ Cd 8 mg kg^{-1}) (Table 3). Rice plant samples were collected after complete maturity and the contents of selected nutritional factors were determined using analytical methods adapted from (Farooq and Zhu, 2019).

2.7. Statistical analysis

For analysis of morpho-physiological traits (chlorophyll concentration as SPAD value), photosynthetic apparatus function (IRGA) and yield, one-way and two-way ANOVA was performed using SAS 9.0 to compare the differences between treatments (T_0 to T_6), at the stages of tillering, 50% heading, flowering and 50% maturity in the two rice genotypes, 5097A/R2035 and GangYou 725. The data for photosynthetic apparatus function, chlorophyll concentration and grain yield were presented as the mean ± standard error (SEM) of three replicates. Duncan's multiple-range test was used to verify significance at the 5% level of probability. SigmaPlot 12.5, Origin 8.0 and Microsoft Excel 2016 were used for the graphical representation of data. For assessment of Se and Cd accumulation in rice, samples were collected from two plants and two biological repeats were made for each tissue (root, stem, leaves, rice bran, embryo, endosperm, brown rice and polished rice). Statistix 8.1 was used for correlation analysis and all pairwise interaction effects (2-factor factorial analysis design), and differences between treatments (T_0 to T_4), Se and Cd treatment, and the rice genotypes, 5097A/R2035 and GangYou 725 (Table 4). The least significance difference (LSD) test was performed to compare means for significance at the 5% probability level. The data are expressed as mg per kg of soil.

3. Results

3.1. Growth and biomass yield

The effect of Cd and Se application on grain yield per plant is shown in Fig. 1. The overall grain yield averaged 20–40 g per plant' and exposure to heavy metal stress had no significant effect on grain yield compared to control plants (Table 5). However, the yield of GangYou 725 was relatively higher than the yield of 5097A/R2035.

3.2. Chlorophyll content

The effect of Cd and Se on the chlorophyll index is shown in Fig. 2. The application of Se improved the chlorophyll content over that of plants treated with Cd alone. The SPAD values indicated that the 50% heading stage experienced the greatest pigment production, while lower levels were recorded at flowering and the 50% maturity stage (Fig. 2). The effect was most obvious at the T3 to T6 treatment levels. The chlorophyll content in GangYou 725 was higher relative to 5097A/R2035. A reduction in index was observed at the flowering stage with Cd application alone compared to control. Cd treatment significantly influenced chlorophyll content and gaseous exchange (IRGA) parameters (Table 5).

Table 3
Treatment levels used for rice nutritional content determination.

Index	Na_2SeO_3 (mg kg^{-1} dry soil)	$CdCl_2.2½H_2O$ (mg kg^{-1} dry soil)	Remarks
Treatment 0 (T_0)	0	0	No outside Cd and Se
Treatment 1 (T_1)	0	4	High Cd and Deficient Se
Treatment 2 (T_2)	0	8	High Cd and Deficient Se
Treatment 3 (T_3)	1	4	High Cd and High Se
Treatment 4 (T_4)	1	8	High Cd and High Se

Table 4
Two-factor factorial analysis design (varieties × stress, treatment × stress interaction effects all pairwise possible combinations).

Interaction Effects	*Roots*	*Stem*	*Leaves*	*panicle*	*Rice Bran*	*Embryo*	*Endosperm*
Varieties Vs Stress							
V1 × Se	4.995^{a}	3.219^{a}	1.969	4.011	1.663	1.514	0.833
V2 × Se	5.404^{a}	1.368 ^{ab}	0.730	4.156	1.655	1.674	0.827
V1 × Cd	3.099 ^{ab}	0.502 ^{b}	0.446	3.684	0.990	1.554	1.139
V2 × Cd	1.420 ^{b}	0.469 ^{b}	0.310	5.534	1.669	2.117	1.748
Treatments Vs Stress							
T_0 × Se	0.399 ^{cd}	0.078^{d}	0.084	0.487^{d}	0.154^{d}	0.095^{c}	0.033 ^{b}
T_0 × Cd	0.171^{d}	0.081^{d}	0.089	0.480^{d}	0.101^{d}	0.055^{c}	0.048 ^{b}
T_1 × Se	0.730^{c}	0.282^{c}	0.122	0.695^{c}	0.293^{c}	0.135 ^{b}	0.058 ^{b}
T_1 × Cd	1.151^{c}	0.386^{c}	0.236	4.473 ^{ab}	1.172 ^{bc}	1.851 ^{ab}	1.450 ^{ab}
T_2 × Se	0.982^{c}	0.101^{c}	0.167	0.963 ^{bc}	0.409^{c}	0.217 ^{b}	0.336 ^{ab}
T_2 × Cd	2.590 ^{bc}	0.802 ^{bc}	0.728	5.255^{a}	1.626 ^{abc}	1.889 ^{ab}	1.741^{a}
T_3 × Se	6.411 ^{b}	4.185 ^{ab}	2.930	7.002^{a}	2.724 ^{ab}	2.792^{a}	1.486^{a}
T_3 × Cd	1.726^{c}	0.115^{c}	0.110	4.127 ^{abc}	1.495 ^{abc}	1.627 ^{ab}	1.004 ^{ab}
T_4 × Se	12.675^{a}	4.632^{a}	2.262	7.882^{a}	3.210^{a}	3.232^{a}	1.440 ^{ab}
T_4 × Cd	3.574 ^{bc}	0.638^{c}	0.460	4.581 ^{ab}	1.026 ^{bc}	1.975 ^{ab}	1.580^{a}
S. E	0.999	0.767	0.674	0.873	0.389	0.503	0.316
LSD (0.05)	3.180	2.440	2.145	2.778	1.239	1.603	1.007
Interaction Effects (Variety × Stress)	*	*	ns	ns	ns	ns	ns
(Treatment × Stress)	*	*	ns	*	*	*	*

Note: V1: 5097A/R2035, V2: GangYou 725, Treatment groups: T_0 (Control), T_1 (Se; 0 mg kg^{-1} + Cd; 4 mg kg^{-1}), T_2 (Se; 0 mg kg^{-1} + Cd; 8 mg kg^{-1}), T_3 (Se; 1 mg kg^{-1} + Cd; 4 mg kg^{-1}), T_4 (Se; 1 mg kg^{-1} + Cd; 8 mg kg^{-1}). Means do not share the same letters in the column differ significantly at $p \leq 0.05$; * = Significant, ns = non-significant, S.E = Standard Error, LSD(0.05) = Least Significant Differences at 5% probability.

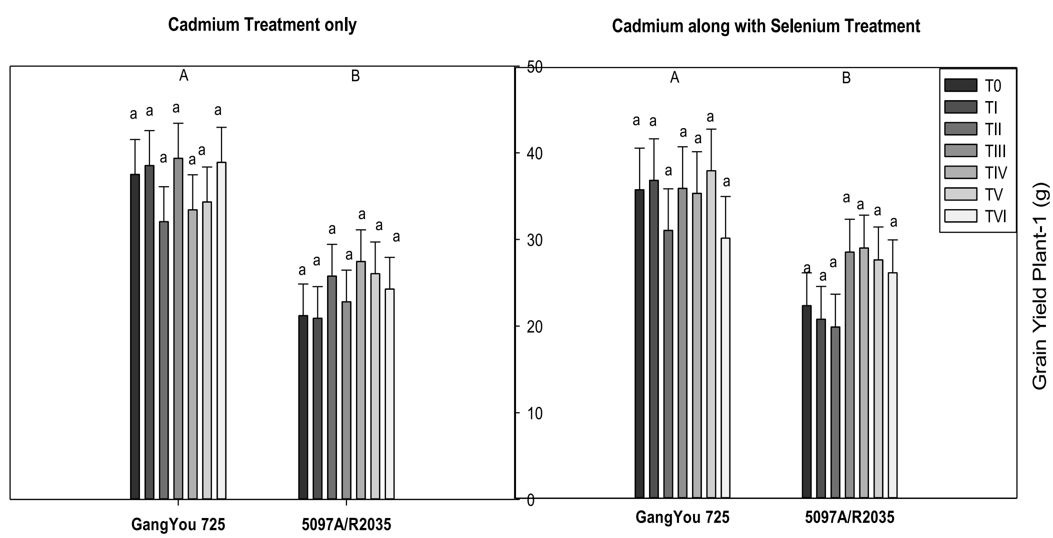

Fig. 1. Effect of metallic trace element application on grain yield per plant. Treatment groups: T0=control; Group 1: cadmium only; T1 = 3, T2 = 4, T3 = 5, T4 = 6, T5 = 7, T6 = 8 mg Cd/kg of soil; Group 2: cadmium + selenium (1 mg Se/kg soil); T1 = 3, T2 = 4, T3 = 5, T4 = 6, T5 = 7, T6 = 8 mg Cd/kg soil.
Note: Histograms denote mean ± standard error (SEM). Means that do not share the same letters differ significantly at $p \leq 0.05$. Capital letters indicate significant differences between varieties; lower case letters indicate differences between treatment levels within a variety.

3.3. Gaseous exchange parameters

Cd treatment decreased the gaseous exchange parameters relative to the control at the 50% heading stage. However, the CO_2 assimilation rate was increased by the application of Cd in 5097A/R2035 (Fig. 3). In the GangYou725 rice cultivar, the application of Cd significantly increased the WUE and net photosynthetic rate while reducing the CO_2 assimilation rate (Fig. 4). The Cd accumulation trend was higher in GangYou725 than 5097A/R2035 (Suppl. Table A, B). The supplementation of Se to Cd-stressed plants enhanced the net photosynthetic rate, CO_2 assimilation rate, stomatal conductance, and transpiration rate, relative to plants treated with Cd alone (Fig. 5). Control plants supplemented with Se exhibited significantly enhanced gas exchange parameters (Fig. 6). At 50% maturity stage the gaseous exchange parameters were significantly decreased comparative to the 50% heading stage. The plants experience stress when applied with high levels of Cd treatments indicating high chlorophyll and gaseous exchange parameters (Table 5). However, the application of exogenous Se at 1 mg kg^{-1} significantly reduced the stress level.

3.4. Uptake of Cd and Se and recovery of applied metal compounds

Seedlings grown under Cd stress exhibited decreased Cd and Se uptake relative to the control (Table 6); 75%–85% of the total applied was lost in the soil. Rice plants treated with Se (1 mg kg^{-1}; T_3, T_4) exhibited a 50% improvement in total Cd + Se recovery in the roots, stems, leaves and grain fractions (Table 4). The total nutrient uptake was >25 mg kg^{-1} in both rice cultivars (Table 6). Significant accumulation of Se was also observed in plants treated with Cd + Se relative to plants treated with Cd alone. The GangYou725 strain showed a 16%¬24% elemental recovery from the test soil, while for 5097A/R2035, the proportion was 12%¬14%.

Table 5
Correlation matrix (Pearson (n)) analysis among the measured traits for different treatment groups.

	Grain Yield	Se Contents	Cd Contents	Chlorophyll Contents	Photo ($_{PN}$)	Cond (gs)	Ci	E	WUE	SLCi	
Grain Yield	1	−0.01 ^{ns}	0.51^{ns}	0.87**	0.87**	0.86**	0.66*	0.84**	−0.64*	0.81**	Cd treatment Only
Se Contents	0.04^{ns}	1	0.36 *	0.05 ^{ns}	0.00 ^{ns}	−0.18 ^{ns}	−0.27 ns	−0.33^{ns}	0.47*	0.10^{ns}	
Cd Contents	−0.03 ^{ns}	0.93**	1	0.50*	0.61*	0.47*	0.47*	0.37^{ns}	−0.38*	0.61*	
Chlorophyll Contents	0.63*	0.20 *	0.23 ^{ns}	1	0.93**	0.82**	0.53*	0.77**	−0.58*	0.92**	
Photo ($_{PN}$)	0.69**	0.15 *	−0.33 ^{ns}	0.35 *	1	0.91**	0.59*	0.87**	−0.62*	0.86**	
Cond (gs)	0.57*	0.13 ^{ns}	−0.02 ^{ns}	0.28 ^{ns}	0.85**	1	0.54*	0.88**	−0.54*	0.69**	
Ci	0.23 ^{ns}	0.05 ^{ns}	0.04 ^{ns}	−0.20 ^{ns}	0.09 ^{ns}	0.45 ^{ns}	1	0.61*	−0.90**	0.66*	
E	0.69**	0.02 ^{ns}	−0.11 ^{ns}	0.38 ^{ns}	0.92**	0.94**	0.32 ^{ns}	1	−0.66**	0.68**	
WUE	0.22 ^{ns}	0.32 ^{ns}	0.28 ^{ns}	0.43 ^{ns}	0.22 ^{ns}	0.01 ^{ns}	−0.61*	0.12 ^{ns}	1	−0.71**	
SLCi	0.69**	0.00 ^{ns}	−0.15 ^{ns}	0.44 ^{ns}	0.94**	0.78**	−0.03 ns	0.89**	0.49*	1	
	Cd + Se Treatment Group										

Note: The significance was computed by comparing P-value (0.00). *Abbreviations*: Grain yield plant^{-1}(g), Se: Selenium (mg kg $^{-1}$), Cd: Cadmium (mg kg $^{-1}$), Chlorophyll contents (SPAD value), IRGA values: (Photo; $_{PN}$): Net photosynthesis rate, (Cond; gs): stomatal conductance, (Ci): intercellular CO_2, (Trmmol; E): transpiration rate, (WUE): water use efficiency and (SlCi): stomatal limitation to CO_2.

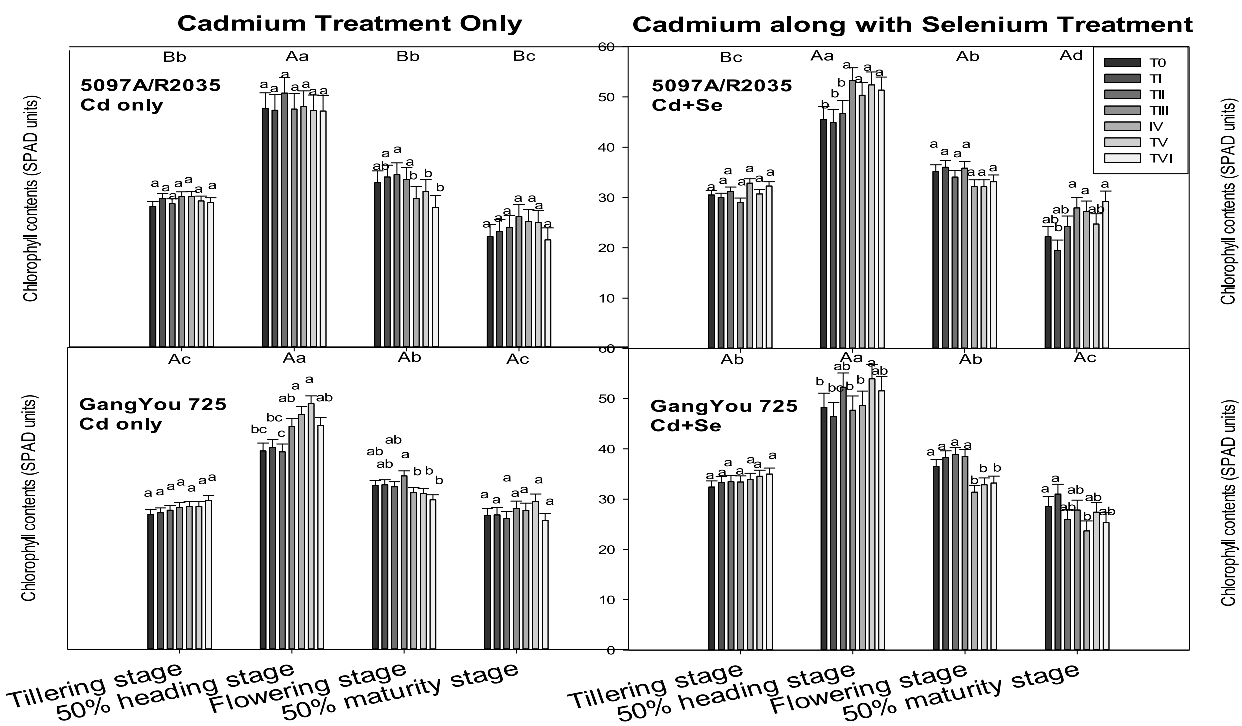

Fig. 2. Effect of metallic trace element application on chlorophyll content (SPAD units). Treatment groups: T0 = control; Group 1: cadmium only; T1 = 3, T2 = 4, T3 = 5, T4 = 6, T5 = 7, T6 = 8 mg Cd/kg of soil; Group 2: cadmium + selenium (1 mg Se/kg soil); T1 = 3, T2 = 4, T3 = 5, T4 = 6, T5 = 7, T6 = 8 mg Cd/kg soil. Note: Histograms denote mean ± standard error (SEM). Means that do not share the same letters differ significantly at p ≤ 0.05. Three types of lettering exist in each block (1) lower case letters on histograms (error bars) indicate significant differences within treatments, (2) capital letters indicate significant differences between varieties, (3) a capital letter followed by a lower case letter (Ac) indicates significant differences between stages.

3.5. Accumulation of Cd and Se

A decreasing Se accumulation pattern was observed as roots > panicle > stem > leaves in both cultivars, while for Cd the intake pattern was panicle > roots > stem > leaves (Table 4). In the GangYou725 cultivar, the panicle parts had Cd contents that were comparatively higher than in 5097A/R2035. The accumulation of Cd in panicle fractions decreased in the order of embryo > endosperm > rice bran. However, the contents of both elements were still too toxic for consumption. The rice cultivars were unable to keep the Cd content at or below the permissible limit. When T3 was compared with T4, it was found that the application of 1 mg kg^{-1} Se significantly mitigated Cd toxicity (Suppl. Fig. 1, Suppl. Fig. 2). The association analysis revealed strong correlation between Se and Cd content specifically in the Se + Cd treatment group (Table 5). The rice grains of both cultivars were unfit for consumption due to elemental hyperaccumulation issues at the higher treatment levels (Suppl. Table A, B). The rice Cd tolerance index was not so evident at this stage. Hence, we conducted association studies in detail to determine the actual content and percentage of Cd and Se that was accumulated in different rice tissues.

The elemental uptake (content and percentage).

Our study revealed that 5097A/R2035 accumulated Se to a higher

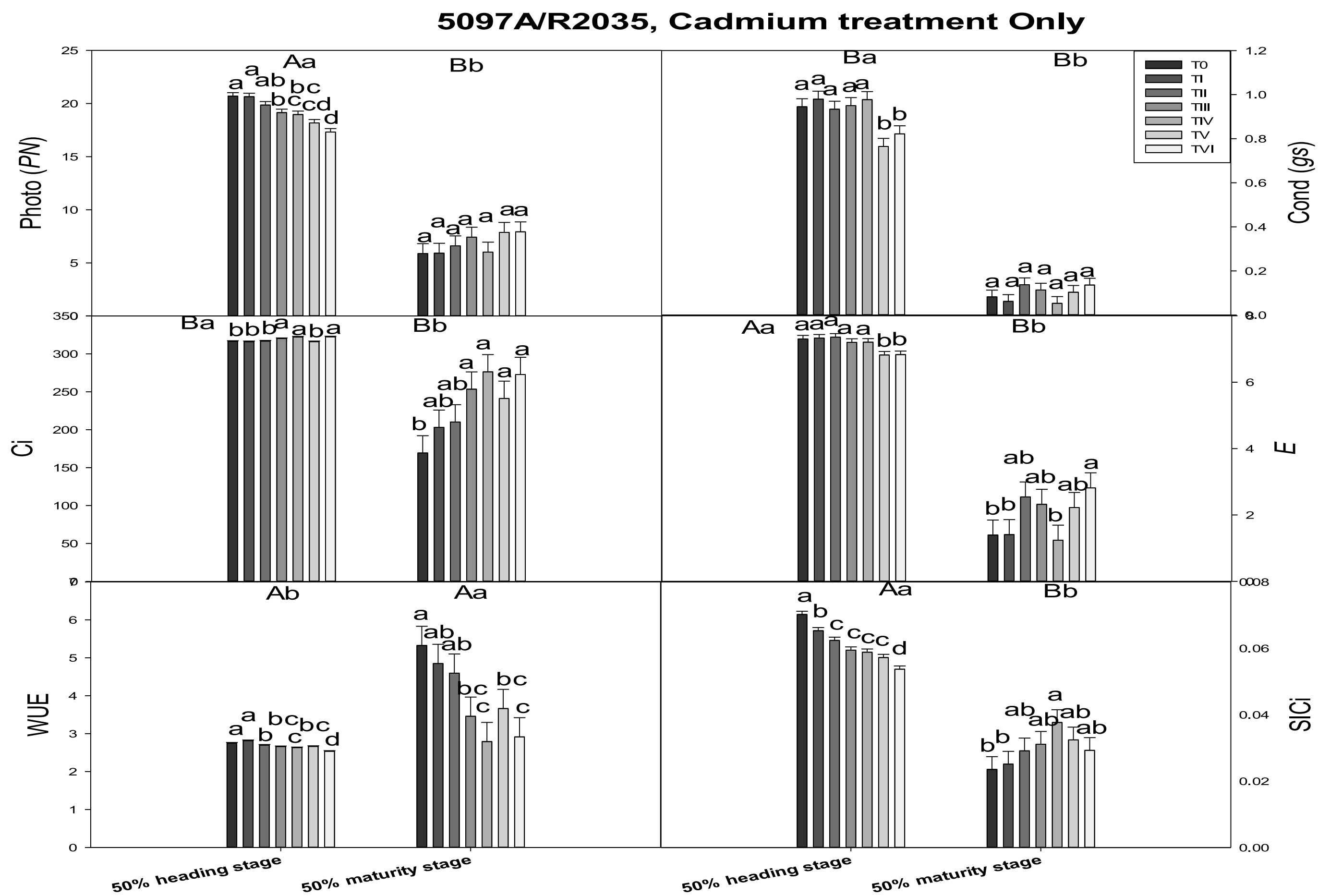

Fig. 3. Effect of Cd application on photosynthetic apparatus (IRGA values) in Se-rich rice (5097A/R2035). Treatment group: T0 = control; cadmium only, T1 = 3, T2 = 4, T3 = 5, T4 = 6, T5 = 7, T6 = 8 mg Cd per kg of soil.
Note: Histograms denote mean ± standard error (SEM). Means that do not share the same letters differ significantly at p ≤ 0.05. Three types of lettering exist in each block. (1) the small letters on histogram (error bars) indicate significant differences within treatments, (2) capital letters indicate significant differences between varieties (group = cadmium treatment only; 5097A/R2035 with GangYou 725; Figs. 7 and 8), (3) capital letters followed by lower case letters (Ac) indicate significant differences between stages. Net photosynthesis rate (Photo; P_N), stomatal conductance (Cond; g_s), intercellular CO_2 (C_i), transpiration rate (Trmmol; E), water use efficiency (WUE) and stomatal limitation to CO_2 (SlCi).

degree than GangYou725 because of the latter's higher Cd content. The percentage of Cd was found to be maximal at T1 and T2, where there was no exogenous Se (Fig. 7, Fig. 9). The application of Se (T3, T4) significantly reduced Cd uptake by the different plant parts, as shown by the histograms (Fig. 8, Fig. 10). The Se levels (Fig. 7) were in the safe range within polished rice at T1 and T2 but Cd toxicity was still prevalent (Fig. 8) in 5097A/R2035. The application of 1 mg Se per kg of soil along with 4 mg Cd per kg (T3) and 8 mg Cd per kg (T4) resulted in an increase in Cd + Se content up to a hypertoxic level of 4–7 mg kg^{-1}) in polished rice (Fig. 10). Although the Cd levels were reduced by exogenous Se (1 mg kg^{-1}), hypertoxicity was still observed in brown rice and milled rice (Suppl. Table C). The metal contents in rice grain fractions (bran, polished rice and germ) continued to be above the permitted consumable levels: Cd, >0.2 mg kg^{-1}; (GB/T 2762–2017), Se, >0.04–0.3 mg kg^{-1}; (GB/T 22499–2008), making the rice unsafe to eat.

4. Discussion

Plants take up nutrients from the soil and utilize them for their development, ultimately producing edible products in the form of fruits, vegetables and forage. The protective role of Se in human health has been widely discussed in the academic community (Lin et al., 2012a,b). In the present study, we demonstrated an ameliorative role of Se against Cd stress and identified the associated hyperaccumulation risks. The absorption and assimilation of Se by a plant depends on the soil's holding capacity and physicochemical properties (Clemens et al., 2013; Stadlober et al., 2001).

The threshold concentration for Cd toxicity varies according to the cultivar, species (hyper-vs hypo-accumulators) (Saengwilai et al., 2017), the ecotype (He et al., 2015), and the presence of other ions and nutrients (Volpe et al., 2015). Regarding alterations in the nutritional status of the plant, different trends have been observed by various sources. Cd can interfere with the uptake of mineral nutrients Fe, Ca, Zn, Mn, Cu, Mg, Si, K (Khan et al., 2015) and N, S, and P (Khan et al., 2016), leading to imbalances in their accumulation and translocation in plants. Cd treatment tends to increase water use efficiency (Ismael et al., 2019) and the transfer of plant reserves via plasmodesmata. Uraguchi et al. (2009) proposed that high transpiration rate and high xylem loading ability could be the reason for high Cd content in grain of some rice (Habataki) genotypes (Uraguchi et al., 2009). Stomatal closure caused by increased ABA levels in leaves approaching maturity has been observed (De Smet et al., 2006). Warming could also be a contributing factor for this phenomenon. The mutual contribution of root and xylem mobilization results in Cd accretion in upper plant parts (grain and fruits) (Wu et al., 2015), which then enters the food chain. Heavy metal ions mediate specific types of physiological, biochemical and nutritional changes in

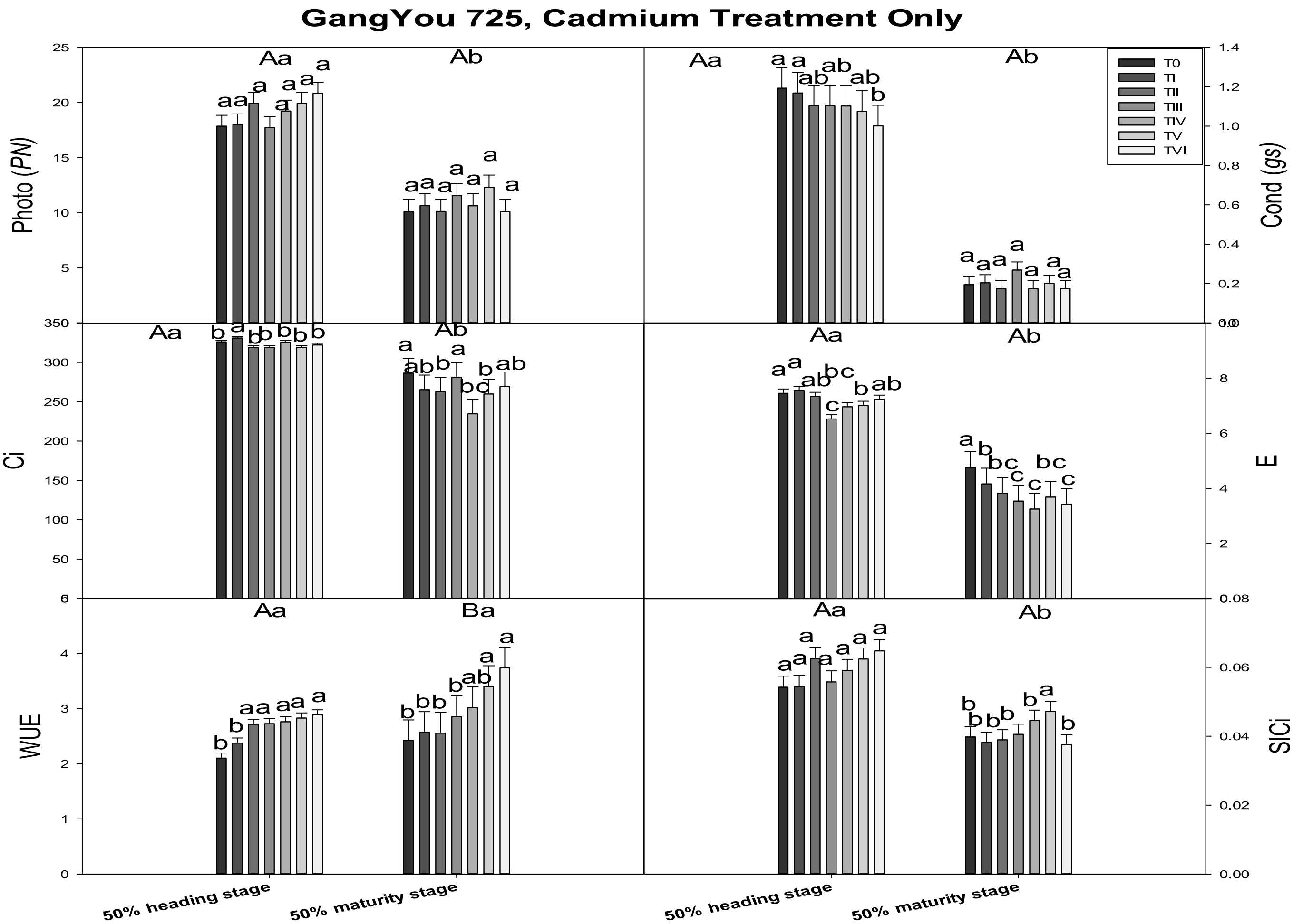

Fig. 4. Effect of Cd application on photosynthetic apparatus (IRGA values) in low-Se rice (GangYou 725). Treatment groups: T0 = control; cadmium only, T1 = 3, T2 = 4, T3 = 5, T4 = 6, T5 = 7, T6 = 8 mg Cd per kg of soil.
Note: Histograms denote mean ± standard error (SEM). Means that do not share the same letters differ significantly at $p \leq 0.05$. Three types of lettering exist in each block. (1) the small letters on histogram (error bars) indicate significant differences within treatments, (2) capital letters indicate significant differences between varieties (group = cadmium treatment only; 5097A/R2035 with GangYou 725; Figs. 7 and 8), (3) capital letters followed by lower case letters (Ac) indicate significant differences between stages. Net photosynthesis rate (Photo; P_N), stomatal conductance (Cond; g_s), intercellular CO_2 (C_i), transpiration rate (Trmmol; E), water use efficiency (WUE) and stomatal limitation to CO_2 (SlCi).

plants, leading to alterations in photosynthesis and respiration, disturbances in metabolism, lipid peroxidation and stunted growth (Hossain et al., 2012).

Rice plants exposed to heavy metals such as Cd undergo subsequent changes during growth to maturity. The 50% heading stage is the most productive in terms of chlorophyll content and photosynthetic functional characteristics. Se mitigates Cd-induced inhibition of photosynthesis, oxidative stress and loss of chlorophyll. Similar increases in photosynthesis rate were observed after application of silicon (Hussain et al., 2021). A Se-mediated reduction in oxidative stress induced by Cd was observed in tomatoes and radishes (cv. 'Cherry Belle') by Alyemeni (Alyemeni et al., 2018) and Amirabad (Auobi Amirabad et al., 2020) in greenhouse pot experiments. However, the basic differences and complex phenomena associated with uptake of the trace-metal elements Se and Cd, and the mechanism of Se mitigation of Cd toxicity in an actual field environment is still not well-understood. Cadmium treatment (150 mg L^{-1}) caused a significant reduction in growth, measured as height and biomass accumulation, and altered the level of chlorophyll pigments, gas exchange parameters, and chlorophyll fluorescence. Selenium application (10 μM) mitigated the adverse effects of cadmium on growth, chlorophyll and carotenoid contents, leaf relative water content, and other physiological attributes. Supplementation of Cd-treated plants with selenium (Cd + Se) enhanced the activity of antioxidant enzymes and co-application of Se significantly reduced Cd uptake. It was observed that grain yield was unaffected by Cd or Se uptake up to 8 mg kg^{-1} (Fig. 1) The results were in accordance with De Maria and colleagues who reported that high soil Cd concentrations (2.5–15 mg kg^{-1}) had no obvious effect on plant growth (De Maria et al., 2013).

Because of environmental variations and other confounding factors, it is difficult to make general statements about what constitutes a toxic Cd level in experiments under greenhouse, field or hydroponic conditions. One key factor to consider is the time of plant exposure to the toxic metal, which varies among different studies from several hours to months. There is some evidence regarding the physiology, responses and molecular competition between these elements from uptake to final sequestration. This interaction and competition between Cd and Se can lead to an induced deficiency of certain metals (Clemens, 2001), but some recent studies indicated that Se was unable to mitigate Cd toxicity at higher levels (Yang et al., 2019). Se is a required mineral, albeit at a low concentration, and is necessary for the normal functioning of living organisms (Abd_Allah et al., 2016). The high concentration of Se application can affect normal anti-oxidant activities of plants leading to hyper-toxic accretion in grain and altered biochemical functions (Abd_Allah et al., 2016). Selenite ions (SeO_3^{2-}) have a tendency to

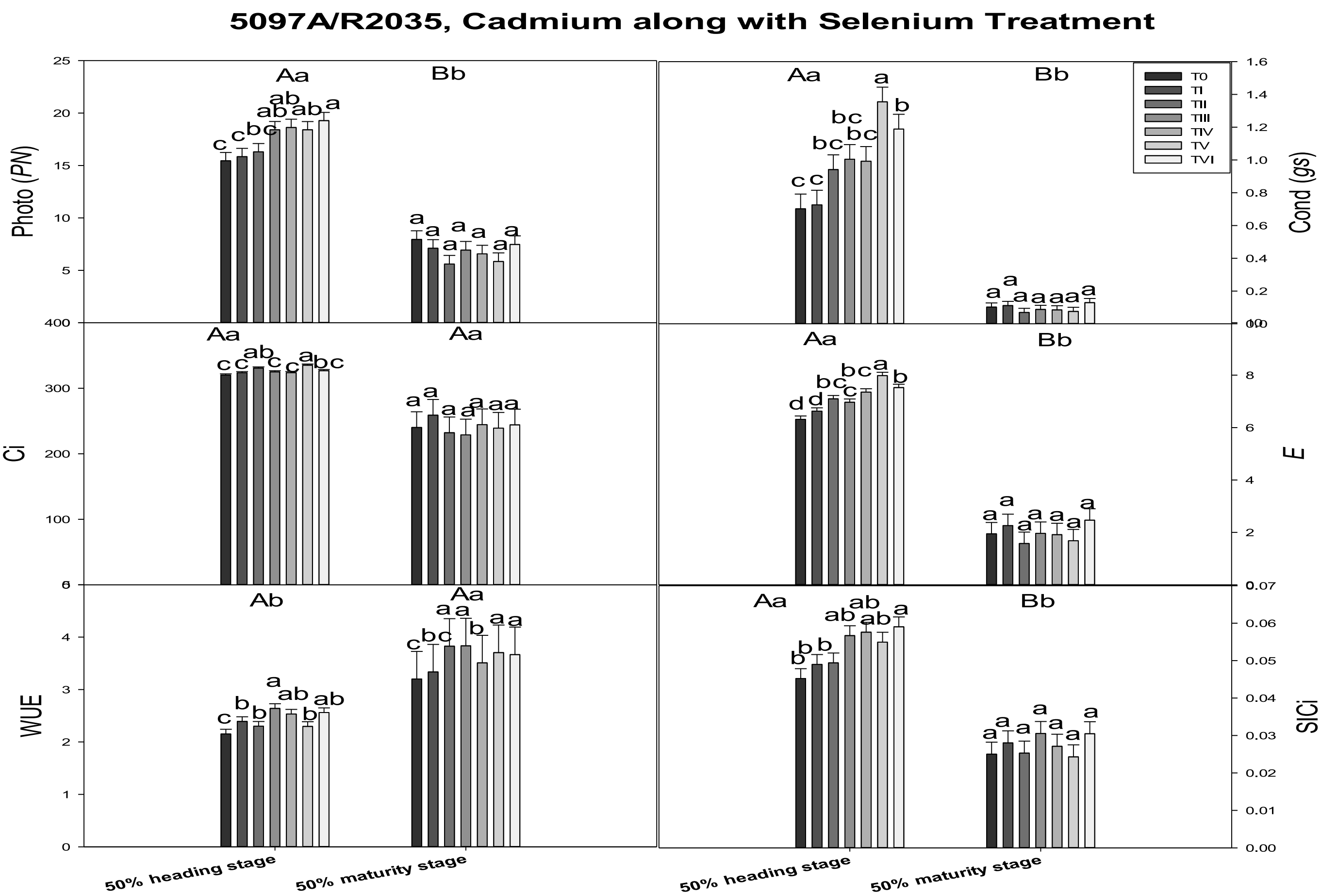

Fig. 5. Effect of Cd plus Se application on photosynthetic apparatus (IRGA values) in Se-rich rice (5097A/R2035). Treatment groups: T0 = control; cadmium + selenium (1 mg Se/kg soil); T1 = 3, T2 = 4, T3 = 5, T4 = 6, T5 = 7, T6 = 8 mg Cd/kg soil.
Note: Histograms denote mean ± standard error (SEM). Means that do not share the same letters differ significantly at p ≤ 0.05. Three types of lettering exist in each block. (1) the small letters on histogram (error bars) indicate significant differences within treatments, (2) capital letters indicate significant differences between varieties (group = cadmium + selenium treatment; 5097A/R2035 with GangYou 725; Figs. 9 and 10), (3) capital letters followed by lower case letters (Ac) indicate significant differences between stages. Net photosynthesis rate (Photo; P_N), stomatal conductance (Cond; g_s), intercellular CO_2 (C_i), transpiration rate (Trmmol; E), water use efficiency (WUE) and stomatal limitation to CO_2 (SlCi).

accumulate mostly in roots with lesser translocation into the aerial parts, whereas selenate (SeO_4^{2-}) has greater affinity for the aerial parts. Concentrations of either ion above 1.0 mg/L are toxic to rice plants. However, it was found that biofortification with sodium selenite resulted in a higher accumulation of selenium in the grain (Farooq et al., 2019a). A high level of the metallic elements Cd + Se in soil can change their electron affinity and physicochemical properties. In some studies, the redox potential was found to be reduced to −200 mV, resulting in a reduction of Se^{4+} to elemental Se^0 which help in reactions with Cd^{2+} to form the stable compound, cadmium selenide, CdSe (Affholder et al., 2019). Anaerobic soil conditions and acidic pH due to elemental treatment, result in the formation of iron plaque on the root surface enabling uptake of CdSe into the root vacuoles (Huang et al., 2017). The application of Se to Cd-stressed plants appreciably enhanced the uptake of elements (Alyemeni et al., 2018); however, too high of a Se concentration can decrease Cd uptake and accumulation. The inverted accumulation behaviour of both elements at the higher application levels in the present study (Se at 1 mg/kg + Cd at 8 mg/kg) could be due to this concentration effect. The mitigation of Cd toxicity by Se treatment was also observed in hydroponic experiments, however Cd mitigation depends on Se dose for crops growing under aerobic conditions (Affholder et al., 2019). Under the same treatment conditions, the responses of different cultivars were different. The crosstalk between Se and Cd at elevated stress levels determines the characteristics of metal uptake in plants.

Human risk from exposure to heavy metals through rice consumption has been assessed (Jafari et al., 2018). The accumulation of Cd and Se was found to be far from the levels given in the 2010 national standards for Cd (<0.2 mg/kg; GB 2762–2017) and Se (0.04–0.3 mg/kg; GB/T 22499–2008; GB 5009.93) (Liang et al., 2018). Se reduces Cd toxicity at both low and high levels of Cd stress, but the total metal accumulation of these elements was still high. The high Cd levels (4 and 8 mg/kg) also prevented over-accumulation of Se (4–7 mg/kg) in the brown rice and polished rice cultivars studied here. It is apparent that Se uptake affects the Cd content in rice tissues. If the level of Cd in the soil is low, Se application is able to mitigate Cd toxicity. But at high concentrations of Cd (4 and 8 mg per kg of dry soil), the plant's Se uptake and Cd mitigation responses differed, leading to Se hyperaccumulation in polished rice (4 mg/kg). The milling and polishing of rice grown in high-Cd soil seems to be of no use as the metals accumulated in brown rice and polished rice were still above the standard permissible range, raising the potential for serious health threats. The diffusion of ions through the plasmodesmata results in the transfer of hazardous metallic trace elements into the edible plant parts. Rice strains that over-accumulate Se, also activate a defense mechanism to reduce WUE and evapotranspiration losses, hence having less Cd localization as a result of the application of Se may reverse this response in the low-Se responsive cultivars. The mechanism of uptake and transport of Se to

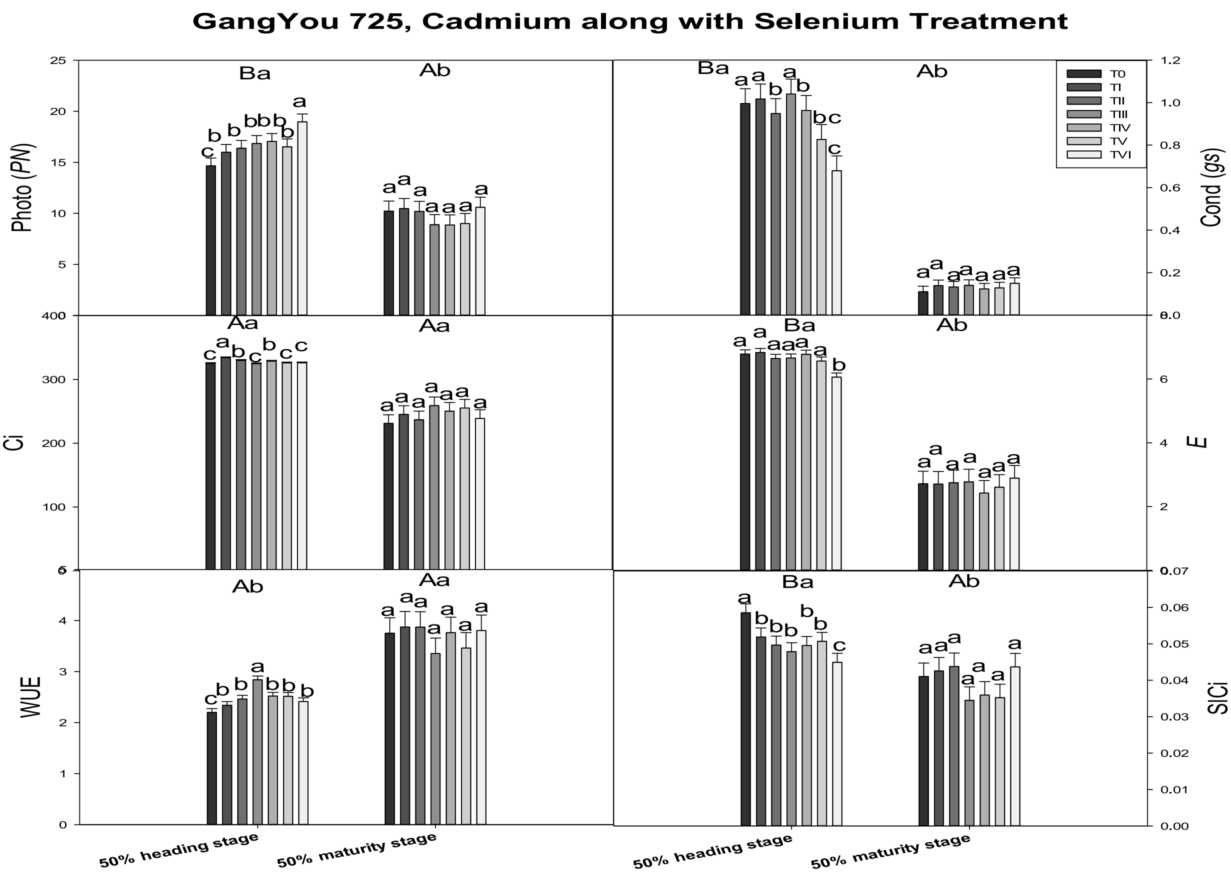

Fig. 6. Effect of Cd plus Se application on photosynthetic apparatus (IRGA values) in low-Se rice (GangYou 725). Treatment groups: T_0 = control; cadmium + selenium (1 mg Se/kg soil); T1 = 3, T2 = 4, T3 = 5, T4 = 6, T5 = 7, T6 = 8 mg Cd/kg soil.
Note: Histograms denote mean ± standard error (SEM). Means that do not share the same letters differ significantly at $p \leq 0.05$. Three types of lettering exist in each block. (1) the small letters on histogram (error bars) indicate significant differences within treatments, (2) capital letters indicate significant differences between varieties (group = cadmium + selenium treatment; 5097A/R2035 with GangYou 725; Figs. 9 and 10), (3) capital letters followed by lower case letters (Ac) indicate significant differences between stages. Net photosynthesis rate (Photo; P_N), stomatal conductance (Cond; g_s), intercellular CO_2 (C_i), transpiration rate (Trmmol; E), water use efficiency (WUE) and stomatal limitation to CO_2 (SlCi).

Table 6
The total elemental (Cd + Se) accumulation recovery and lost (percentage) in rice genotypes.

Treatments	Cd + Se Application (Na_2SeO_3) and cadmium chloride ($CdCl_2.2½H_2O$)	Total elemental (Cd + Se) accumulation by rice tissues (mg kg $^{-1}$)		Recovered (%)		Lost (%)	
	Soil (10 kg)	5097A/R2035	GangYou 725	5097A/R2035	GangYou 725	5097A/R2035	GangYou 725
T_0	5	3.88	2.45	61.40	57.60	38.60	42.40
T_1	40	5.88	9.86	14.70	24.65	85.30	75.35
T_2	80	9.77	13.19	12.21	16.49	87.79	83.51
T_3	50	26.43	26.74	52.86	53.48	47.14	46.52
T_4	90	45.62	27.79	50.69	30.88	49.31	69.12

Note: T_0 (Control), T_1 (Se; 0 mg kg^{-1} + Cd; 4 mg kg $^{-1}$), T_2 (Se; 0 mg kg^{-1} + Cd; 8 mg kg $^{-1}$), T_3 (Se; 1 mg kg $^{-1}$ + Cd; 4 mg kg $^{-1}$), T_4 (Se; 1 mg kg $^{-1}$ + Cd; 8 mg kg $^{-1}$).

hypertoxic levels in rice needs to be explored in future molecular studies to determine how to overcome this problem.

5. Conclusions

In this study, we assessed the effect of Se and Cd exposure on grain yield, chlorophyll content, gaseous exchange and nutritional aspects in two rice genotypes, utilizing Se to mitigate Cd toxicity. Cadmium application reduced the chlorophyll content and gaseous exchange properties, while Se treatment prevented the toxic heavy metal effects. Among the different rice fractions, the roots were most responsive to Cd, followed by panicles. Selenium application reduced Cd toxicity, but pro-oxidant effects of Se at 0.143–4.71 mg kg^{-1} were observed in polished rice at elevated Cd stress levels in soil. Our data demonstrate that Se can reduce Cd accumulation at low levels, but that high concentrations of these elements in the soil and plant tissues may be dangerous to persons consuming polished rice with such a high Se content. This potential entry of the metals into the food chain poses a serious threat to human health because rice is a major worldwide food crop. For biofortification purposes, 1 mg kg^{-1} of Se is a very high dose, resulting in rice grains with hypertoxic amounts of metallic trace elements, raising serious health concerns; and milling and polishing seem to have little effect on

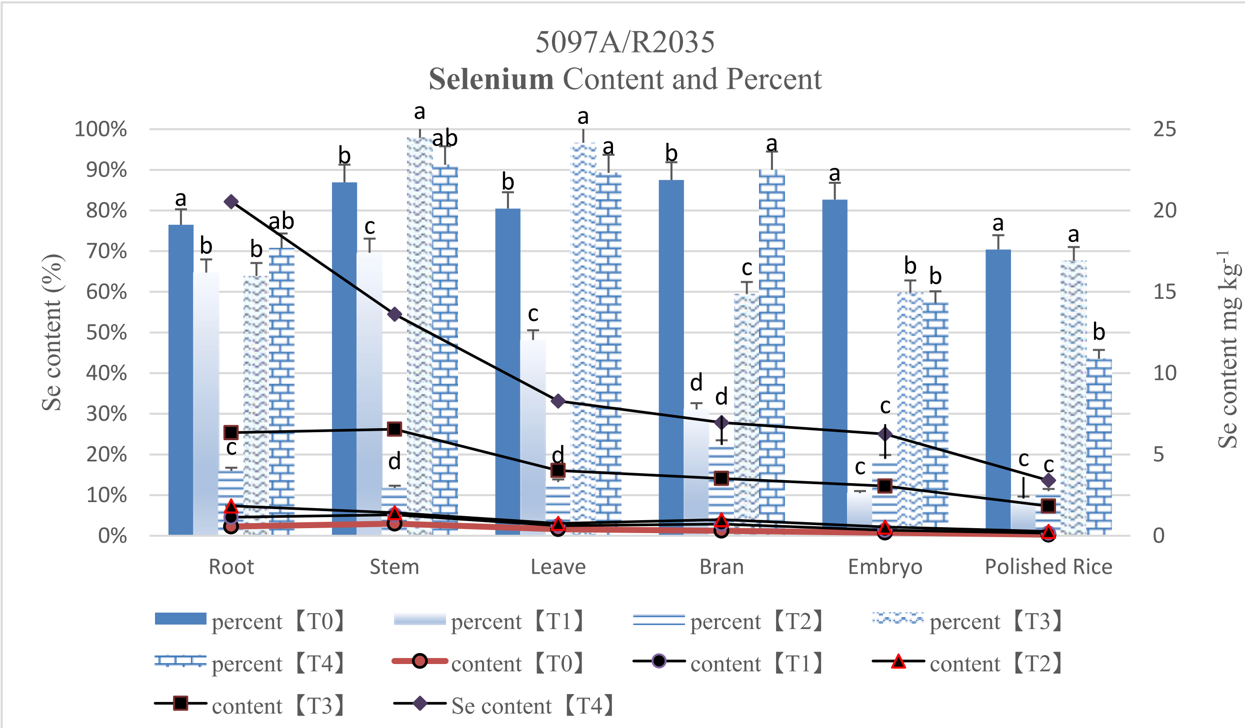

Fig. 7. Selenium content and percentages in Se-rich rice (5097A/R2035) fractions. Se, selenium. X-axis: rice fractions; Y-axis (left): The percentage of Se after T0, T1, T2, T3 and T4 treatments (indicated with histogram); Y-axis (right): Se content ($mg \cdot kg^{-1}$) in rice fractions after T0, T1, T2, T3 and T4 treatments (indicated with trend lines). Note: T0 = control; cadmium alone: T1 = 4 mg Cd/kg, T_2 = 8 mg Cd/kg; cadmium + selenium: T3 = 1 mg Se/kg + 4 mg Cd/kg, T4 = 1 mg Se/kg + 8 mg Cd/kg.

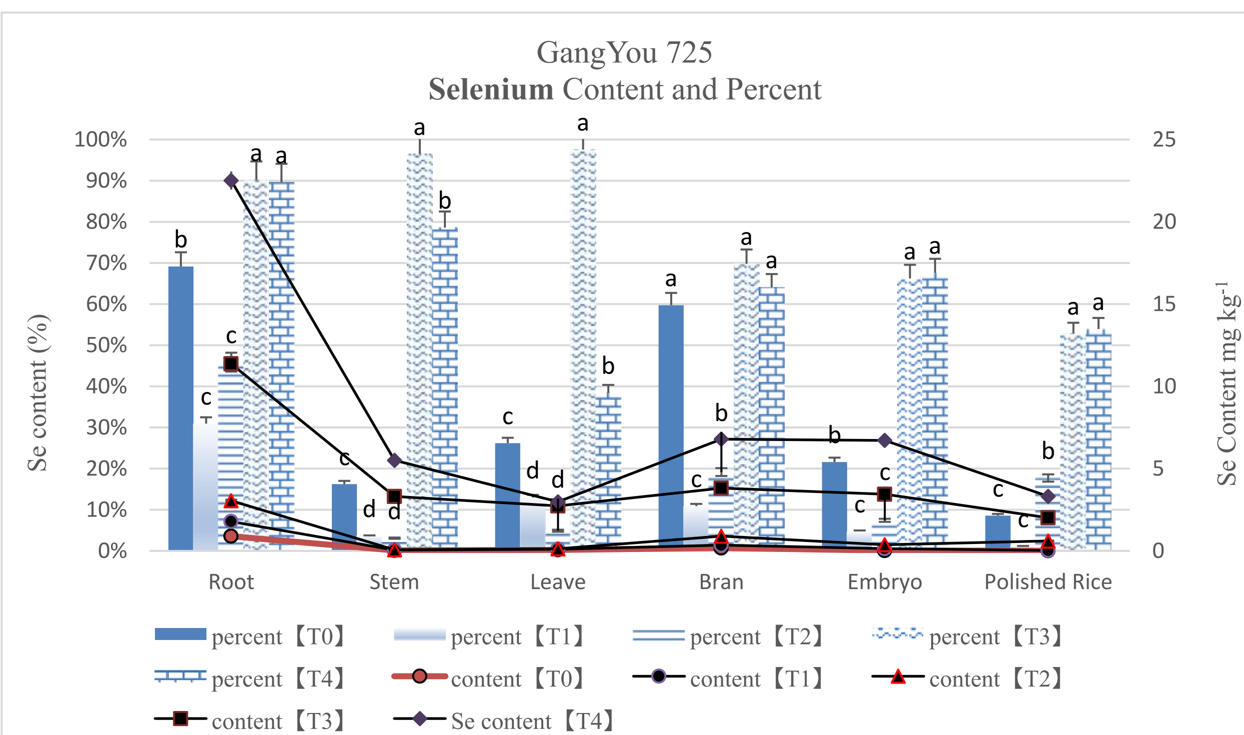

Fig. 8. Cadmium content and percentages in Se-rich rice (5097A/R2035) fractions. Cd; cadmium. X-axis: rice fractions, Y-axis (left): percentage of Cd after T0, T1, T2, T3 and T4 treatments (indicated with histogram), Y-axis (right): Cd content ($mg \cdot kg^{-1}$) in rice fractions after T0, T1, T2, T3 and T4 treatments (indicated with trend lines). Note: T0 = control; cadmium alone: T1 = 4 mg Cd/kg, T_2 = 8 mg Cd/kg; cadmium + selenium: T3 = 1 mg Se/kg + 4 mg Cd/kg, T4 = 1 mg Se/kg + 8 mg Cd/kg.

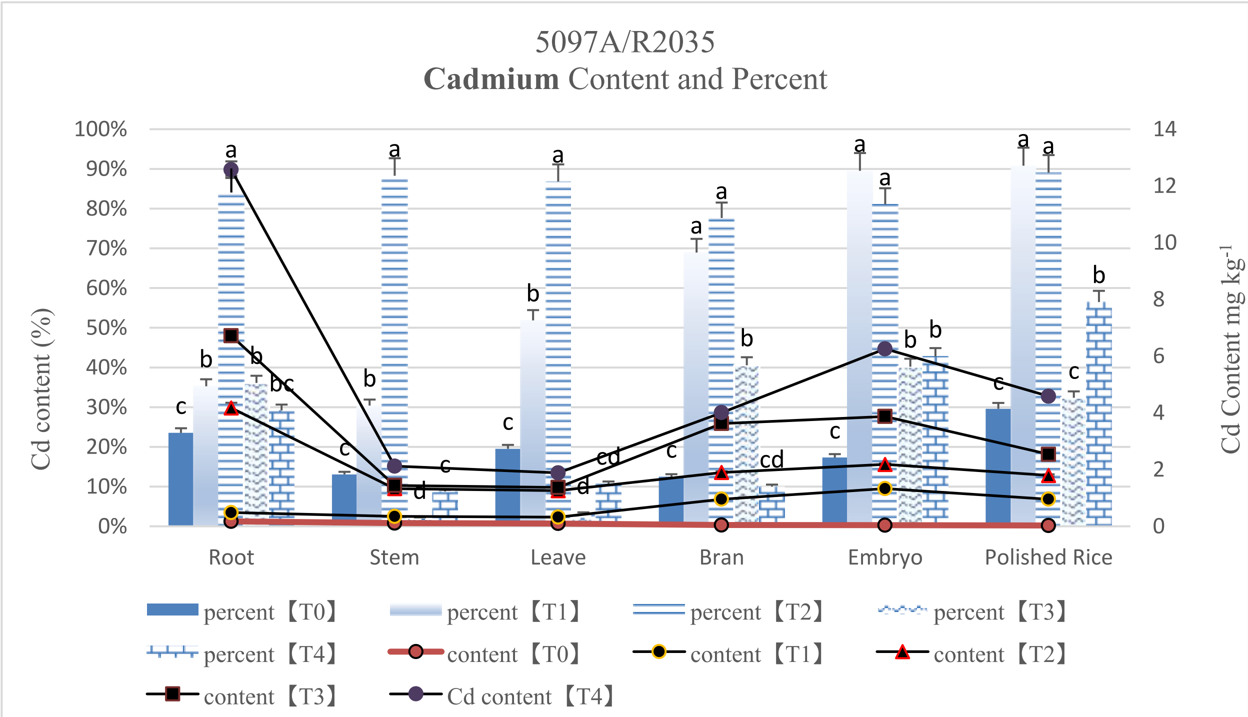

Fig. 9. Selenium content and percentages in low-Se rice (GangYou 725) fractions. Se; Selenium. X-axis: rice fractions; Y-axis (left); percentage of Se after T0, T1, T2, T3 and T4 treatments (indicated with histogram), Y-axis (right): Se content ($mg \cdot kg^{-1}$) in rice fractions after T0, T1, T2, T3 and T4 treatments (indicated with trend lines). Note: T0 = control; cadmium alone: T1 = 4 mg Cd/kg, T_2 = 8 mg Cd/kg; cadmium + selenium: T3 = 1 mg Se/kg + 4 mg Cd/kg, T4 = 1 mg Se/kg + 8 mg Cd/kg.

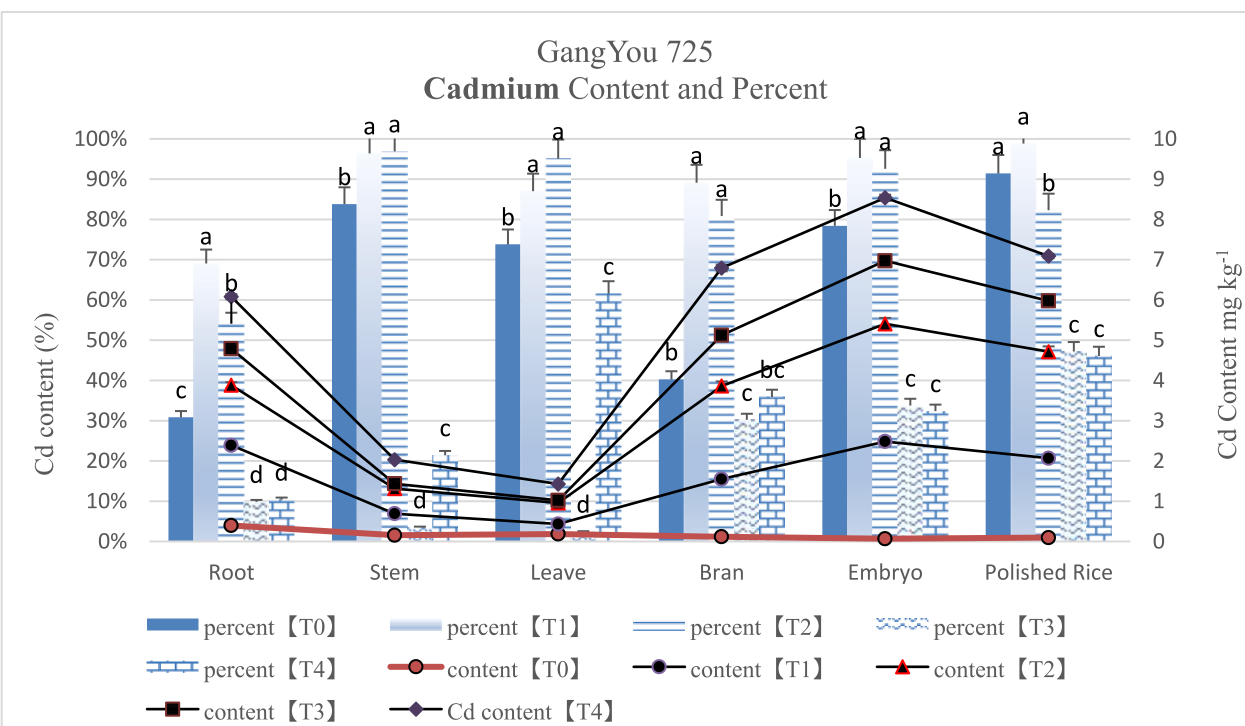

Fig. 10. Cadmium content and percentages in low-Se rice (GangYou 725) fractions. Cd; cadmium. X-axis: rice fractions; Y-axis (left): percentage of Cd after T0, T1, T2, T3 and T4 treatments (indicated with histogram), Y-axis (right): Cd content ($mg \cdot kg^{-1}$) in rice fractions after T0, T1, T2, T3 and T4 treatments (indicated with trend lines). Note: T0 = control; cadmium alone: T1 = 4 mg Cd/kg, T_2 = 8 mg Cd/kg; cadmium + selenium: T3 = 1 mg Se/kg + 4 mg Cd/kg, T4 = 1 mg Se/kg + 8 mg Cd/kg.

metal content in the grain. A comprehensive molecular, physicochemical and nutritional response study is needed to dissect out the mechanism governing the relative uptake of Se and Cd from soil, and the cellular responses of the plant that promote over-accumulation of Se in plant tissues.

Author contributions

MUF and JZ conceived the project and designed the experiments. MUF conducted the experiment. MUF, II, AR, SH and SIA wrote the manuscript. RM, CB and MS help in proofreading the manuscript. All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate (Not applicable).

Availability of data and material

The supplementary data is provided in extra file.

The authors have no relevant financial or non-financial interests to disclose.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2021.11.035.

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