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Coupling of different antioxidative systems in rice under the simultaneous influence of selenium and cadmium☆

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ABSTRACT

Selenium (Se) elevates the antioxidant ability of rice against cadmium (Cd) stress, but previous studies only focused on the variation in antioxidant enzymes or nonenzymatic substances induced by Se under Cd stress and ignored the relationships between different antioxidant parameters during the interaction. Here, hydroponic experiments with rice were performed by adding both Cd and Se at doses in the range of 0–50 μM to explore the physiological responses of rice and their relationships in the presence of different levels of Se and Cd. Exogenous Cd markedly promoted the activity of antioxidant enzymes with the exception of catalase (CAT) and the concentration of nonenzymatic substances in aerial parts. Se enhanced the antioxidant capacity by improving the activities of all the enzymes tested in this study and increasing the concentrations of nonenzymatic compounds. The couplings among different antioxidant substances within paddy rice were then determined based on cluster and linear fitting results and their metabolic process and physiological functions. The findings specifically highlight that couplings among the ascorbic acid (AsA)-glutathione (GSH) cycle, glutathione synthase (GS)-phytochelatin synthetase (PCS) coupling system and glutathione peroxidase (GPX)-superoxide dismutase (SOD) coupling system in aerial parts helps protect plants from Cd stress. These coupling systems form likely due to the fact that one enzyme generated a product that could be the substrate for another enzyme. Noticeably, such coupling systems do not emerge in roots because the stronger damage to roots than other organs activates the ascorbate peroxidase (APX)-GPX-CAT and PCS-GS-SOD systems with distinct functions and structures. This study provides new insights into the detoxification mechanisms of rice caused by the combined effect of Se and Cd.

1. Introduction

Cadmium (Cd), which is one of the most detrimental and widespread pollutants in agricultural soils, is readily absorbed by roots and then migrates to other plant tissues, and this element is thus toxic to living cells at very low concentrations(Gong et al., 2017). The exposure of plants to Cd results in water imbalance(Perfus-Barbeoch et al., 2002), damage to photosystems (PSs)(Perez-Romero et al., 2016), and alterations in mineral nutrition(Li et al., 2016). Cd can interfere with metabolic processes to impair cellular functions by reacting with the sulfhydryl groups (SH) of enzymes and tissue proteins(Benavides et al., 2005). Moreover, plants can produce reactive oxygen species (ROS), such as superoxide anions (O_2^-), hydroxyl radicals (OH^-) and hydrogen peroxides (H_2O_2)(Heyno et al., 2008), which lead to toxic effects at the biochemical, physiological and molecular levels(Asgher et al., 2015; Pedro et al., 2013; Tang et al., 2013). To eliminate the ROS caused by Cd stress, plants have evolved several different detoxification mechanisms by generating phytochelatins to bind to heavy metals with phytochelatin synthetase (PCS) as well as activating antioxidant systems, including catalyzing the decomposition of H_2O_2 by catalase (CAT) and ascorbate peroxidase (APX), removing superoxide anions via superoxide dismutase (SOD), and eliminating peroxides by glutathione peroxidase (GPX) and nonenzymatic scavengers such as ascorbic acid (AsA) and glutathione (GSH), which are produced and reduced by glutathione synthase (GS) and glutathione reductase (GR) respectively(Bari et al., 2019; Li et al., 2022; Vazquez et al., 2006; Wu et al., 2020).

Selenium (Se), as an essential element for animals and humans, exerts positive effects on plants at low concentrations by promoting

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growth, increasing the antioxidative capacity, and scavenging excessive ROS(Birringer et al., 2002; Gupta and Gupta, 2016; Paciolla et al., 2011; Zhang et al., 2014). Recent studies have shown that Se antagonizes the detrimental effects of plants under biotic and abiotic stresses, including those caused by heavy metal stress, salt stress, and senescence(Amit et al., 2014; Camara et al., 2018; Djanaguiraman et al., 2005; Hasanuzzaman et al., 2011). Noticeably, at higher dosages, Se exerts a dual effect on plant growth. On the one hand, Se may act as a pro-oxidant and damaging plant(Kolbert et al., 2019), and on the other hand, Se can defend plants against Cd stress by (1) reducing Cd migration from soils to plant grains(Chang et al., 2022; Lin et al., 2012), (2) improving the antioxidant capacity of plants(Saidi et al., 2014; Wang et al., 2014), (3) increasing the respiration, photosynthesis and electron transfer rates of PS I and PS II(Haghighi and da Silva, 2016), (4) protecting reproductive organs and their functions, and (5) maintaining normal pollen germination(Ismael et al., 2018).

Rice is a staple food crop worldwide and plays an important role in food security(Zhang et al., 2010). Rice can uptake and accumulate Cd readily due to the high mobility and hydrophilic properties of Cd and the physiological features of rice(Meharg et al., 2013; Rizwan et al., 2016). Consequently, many studies have focused on strategies for minimizing the adverse health effects caused by Cd accumulation in paddy rice. In the past, Se has frequently been adopted to antagonize Cd due to its interactive effects with Cd(Lin et al., 2012; Liu et al., 2020). Although the variation in physiological indicators under the interaction of Se and Cd has been studied, most studies have focused on the changes in the antioxidant system arising from the combined effect of Se and Cd, e.g., increases in the antioxidative capacity and decreases in the Cd concentration caused by the interaction of Se and Cd(Huang et al., 2019; Lin et al., 2012; Wan et al., 2016). Almost no studies have explored the relationship among different enzymes, antioxidant compounds, pigments and other biomolecules, which is critical for exploring physiological processes and mechanisms under these interactions(Gill et al., 2013; Liu et al., 2021). Furthermore, whether an antioxidant coupling system exists under this interaction remains unclear, whereas similar systems, such as AsA-GSH, have been observed in the response to oxidative stress due to heat shock, salt stress and drought(Gill et al., 2013; Liu et al., 2021). Because the interaction could also generate oxidative stress, a coupling system may exist.

Therefore, hydroponic experiments were performed in this study to highlight the interaction among enzymes and nonenzymatic substances within the antioxidant system. This study aimed to provide new insights into the physiological responses of paddy rice under the interaction of Se and Cd. The activities of seven enzymes, namely, SOD, CAT, GPX, GS, GR, PCS, and APX, and the concentrations of nonenzymatic antioxidant substances, such as GSH and ASA, in rice grown in the presence of different levels of Se and Cd were determined to reveal their responses to the interaction of Se and Cd due to their critical function in alleviating metal stress and their proximity in the metabolic network(Gill et al., 2013; Seregin and Kozhevnikova, 2023). Hierarchical cluster analysis and linear correlation analysis were then performed to explore their relationships and verify the existence of coupling systems. We hope that this study will clarify the existence of a coupling system in the presence of exogenous Se and Cd and deepen the understanding of the antioxidant system of rice.

2. Materials and methods

2.1. Plant material and experimental design

Rice seeds of *Oryza sativa* L. spp. *japonica* were first disinfected by immersion in 10% H_2O_2 solution for half an hour and then rinsed with deionized water. Sterilized seeds were soaked in deionized water in the dark for 3 day at 30 °C, and the water-soaked seeds were then grown on a plastic net floating in deionized water dissolved with 0.5 mM $CaCl_2$ (pH 5.5) for 7 days. Then seedlings of similar size were transplanted to quarter-strength Kimura-B nutrient solution (pH 5.5, adjusted with 1 M HCl and NaOH), and the strength was increased from quarter to ½ and finally to 100% after 7 days. The final solution (100% Kimura-B nutrient solution) was renewed every 3 days. The seedlings were grown in a greenhouse with a 16-h day/8-h night photoperiod with temperatures of 25 °C/30 °C.

Four weeks after seedling transplantation to full-strength Kimura-B nutrient solution, Na_2SeO_3 and $CdCl_2$ were added to the media to perform the following treatments: (1) Se + Cd treatments, which included various combinations of Se and Cd at different concentrations, and (2) preSe treatments, 24-h pretreatment with different Se concentrations before Cd exposure, followed by performing similar to the Se + Cd treatments. The concentrations of Se and Cd applied in the aforementioned treatments were 0, 0.5, 2.5, and 5.0 μM and 0, 2, 5, 10, and 50 μM, respectively. In general, the Se concentration was lower than the Cd concentration because Se is usually utilized to alleviate Cd stress while low Se and high Cd levels are widespread(Huang et al., 2021; Lin et al., 2012). The experiment was conducted using a totally randomized design with three replicates, and the culture solutions were changed every 3 days.

After 7 days of incubation, rice was collected and classified into roots, stems, leaves and leaf sheaths. The roots were soaked in 20 mM EDTA+2% HNO_3 for 20 min to remove adsorbed metals on their surfaces and rinsed with distilled water. The other parts, including the leaves, leaf sheaths, and stems, were thoroughly washed with deionized water to eliminate possible chemical contaminants. Thereafter, the samples were stored at −80 °C before further analyses.

2.2. Determination of the Se and Cd concentrations

Fresh plant samples were collected and divided into roots, stems, leaf sheaths and leaves. These samples were freeze-dried (−79 °C) to a constant weight, ground into a powder and passed through a 100-mesh screen. Thereafter, the samples were digested with 3 mL of HNO_3 and 1 mL of 30% (v/v) H_2O_2 (original digestion solution), according to a previously described method(Liang et al., 2000). The concentration of Cd was directly analysed by inductively coupled plasma–mass spectrometry (ICP–MS) (US PE, NexION 300X). The determination of Se concentration requires further processing. The 1 mL digestion solution was added to 3 mL of 6 mol/L HCl, heated in a water bath (95 °C) for 2 h, and then determined by hydride generation-atomic fluorescence spectrometry (HGAFS 9700, BJHG, China) after reduction to $Se4^+$(Zhu et al., 2008). The accumulation and translocation of Se and Cd have been discussed in our previous study(Guo et al., 2021), and will not be mentioned in this manuscript.

2.3. Determination of enzyme activities

Frozen tissue (0.5 g) was homogenized in 4 mL of ice-cold extraction buffer (50 mM phosphate buffer saline (PBS), pH 7.0, 0.2 mM ethylenediaminetetraacetic acid (EDTA), and 2% polyvinyl pyrrolidone (PVP) (w/v)) using a prechilled agate mortar and pestle. The homogenate was transferred to the centrifuge tube, and the mortar was then rinsed twice with the buffer and transported to the centrifuge tube. The tubes were centrifuged for 20 min at 12,000 g at 4 °C. The supernatant was retained for the determination of enzyme activities.

The activities of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), GPX (EC 1.11.1.9), APX (EC 1.11.1.11), GR (EC 1.6.4.2), PCS (EC 2.3.2.15) and GS (EC 6.3.1.2) were determined by enzyme-linked immunosorbent assays using kits (US Thermo, Multiskan FC) according to the provided manuals (Biddy).

2.4. Determination of antioxidative compound concentrations

The GSH concentration was determined by fluorescence staining as described by Hissin and Hilf(Hissin and Hilf, 1976). Root and leaf tissue

(0.5 g) was ground into homogenate using 4 mL of ice-cold extraction buffer composed of 3 mL of 100 mM PBS (pH 7.0, 5 mM EDTA) buffer and 1 mL of metaphosphoric acid. The homogenate was then centrifuged for 30 min at 10,000 g at 4 °C. The supernatant was then diluted 5 times with 100 mM PBS buffer (pH 8.0, containing 5 mM EDTA) to obtain the analytical solution with PBS-EDTA and phthalaldehyde. After 15 min of reaction, the fluorescence intensity was measured using a fluorescence spectrophotometer (F-4500) with an excitation wavelength of 350 nm and an emission wavelength of 430 nm.

AsA was determined according to the method described by Singh (Singh et al., 2006). Root and leaf tissue (0.5 g) was ground into homogenate with 3 mL of ice-cold 5% (w/v) trichloroacetic acid (TCA) solution utilizing a prechilled agate mortar and pestle. The homogenate was centrifuged at 18,000×*g* for 15 min at 4 °C. AsA was determined in a reaction mixture consisting of 0.2 mL of supernatant, 0.5 mL of 100 mM PBS buffer (pH 7.4) containing 5 mM EDTA and 0.2 mL of deionized water. Colour was developed in reaction mixtures with the addition of 0.4 mL of 10% (w/v) TCA, 0.4 mL of 44% (v/v) phosphoric acid, 0.4 mL of a,a'-dipyridyl in 70% (v/v) ethanol and 0.2 mL of 3% (w/v) $FeCl_3$. The reaction mixtures were incubated at 40 °C for 40 min and quantified spectrophotometrically (US Agilent, Cary series UV–Vis Spectrophotometer) at a wavelength of 525 nm.

The methods used for the determination of the proline, chlorophyll and carotenoid concentration, lipid peroxidation and H_2O_2 concentration are described in the Supporting Information and are thus not included here.

2.5. *Data analysis*

All results were analysed using SPSS 20.0 and Origin 9. Pearson

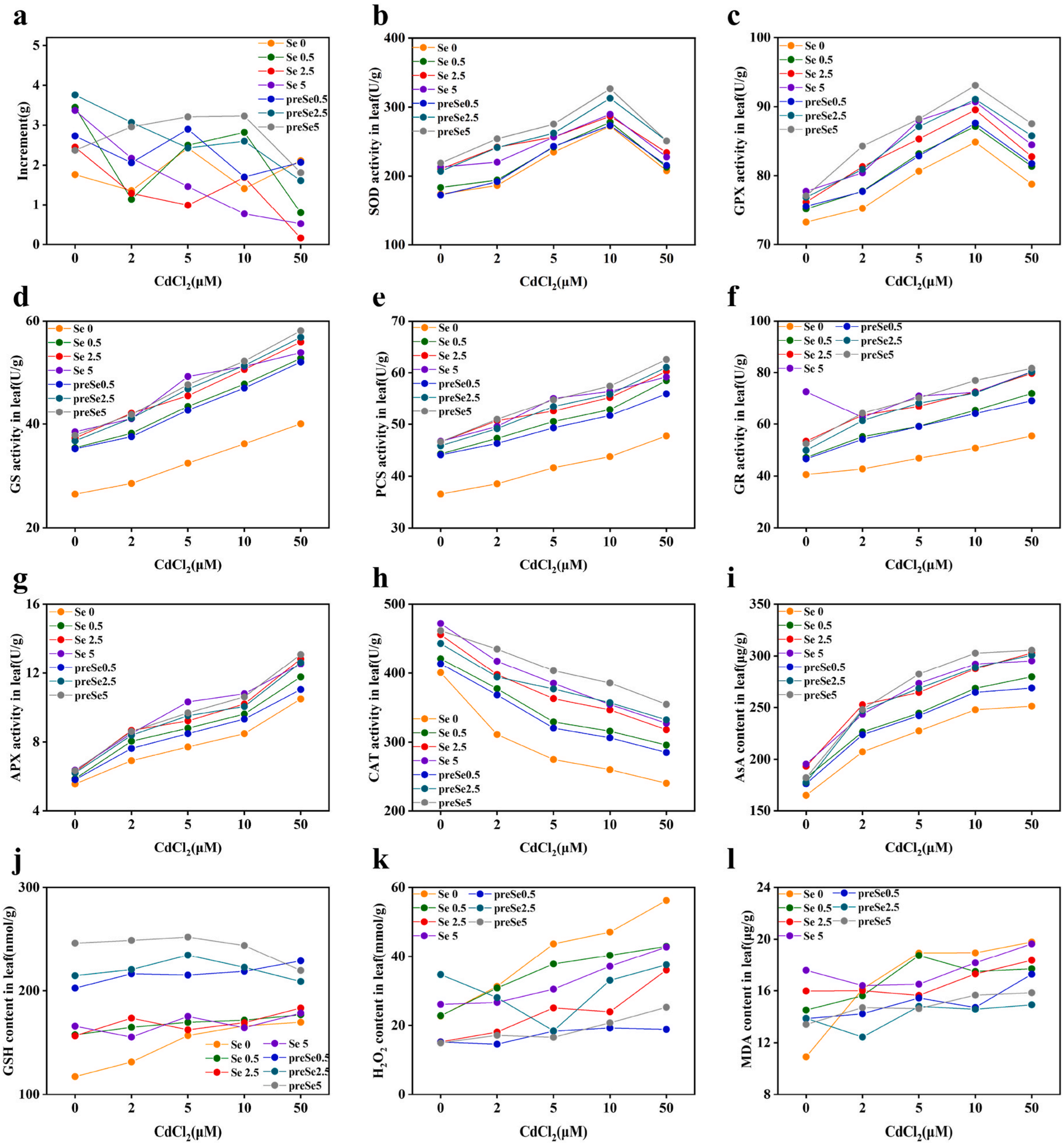

Fig. 1. Increment of rice, the activities of enzymes, the levels of nonenzymatic antioxidant substances and oxidation index in rice leaves under different Se and Cd treatments. a: increment of rice under different Se and Cd treatments. b–h: Activities of SOD, GPX, GS, PCS, GR, APX and CAT in rice leaves under different Se and Cd treatments. i–l: AsA, GSH, H_2O_2 and MDA concentrations in rice leaves under different Se and Cd treatments.

correlation analysis was used to analyse the correlations among data showing a normal distribution, whereas Spearman correlation was used for data that do not show a normal distribution. $p < 0.05$ was considered to indicate a significant difference. A hierarchical clustering method was adopted to conduct the clustering analysis. Thee repetitions of each experimental group were included in the experiment, and their relative standard deviations were all less than 5%. Blank samples were included to avoid contamination in the test. For the detection of Se and Cd, quince leaf (GSB-11) was adopted as a reference material for quality control, and the recovery rate was between 90 and 110%.

3. Results

3.1. Effect of Se and Cd on the increment and enzyme activities

The influence of Se and Cd on the growth of rice was first elevated by

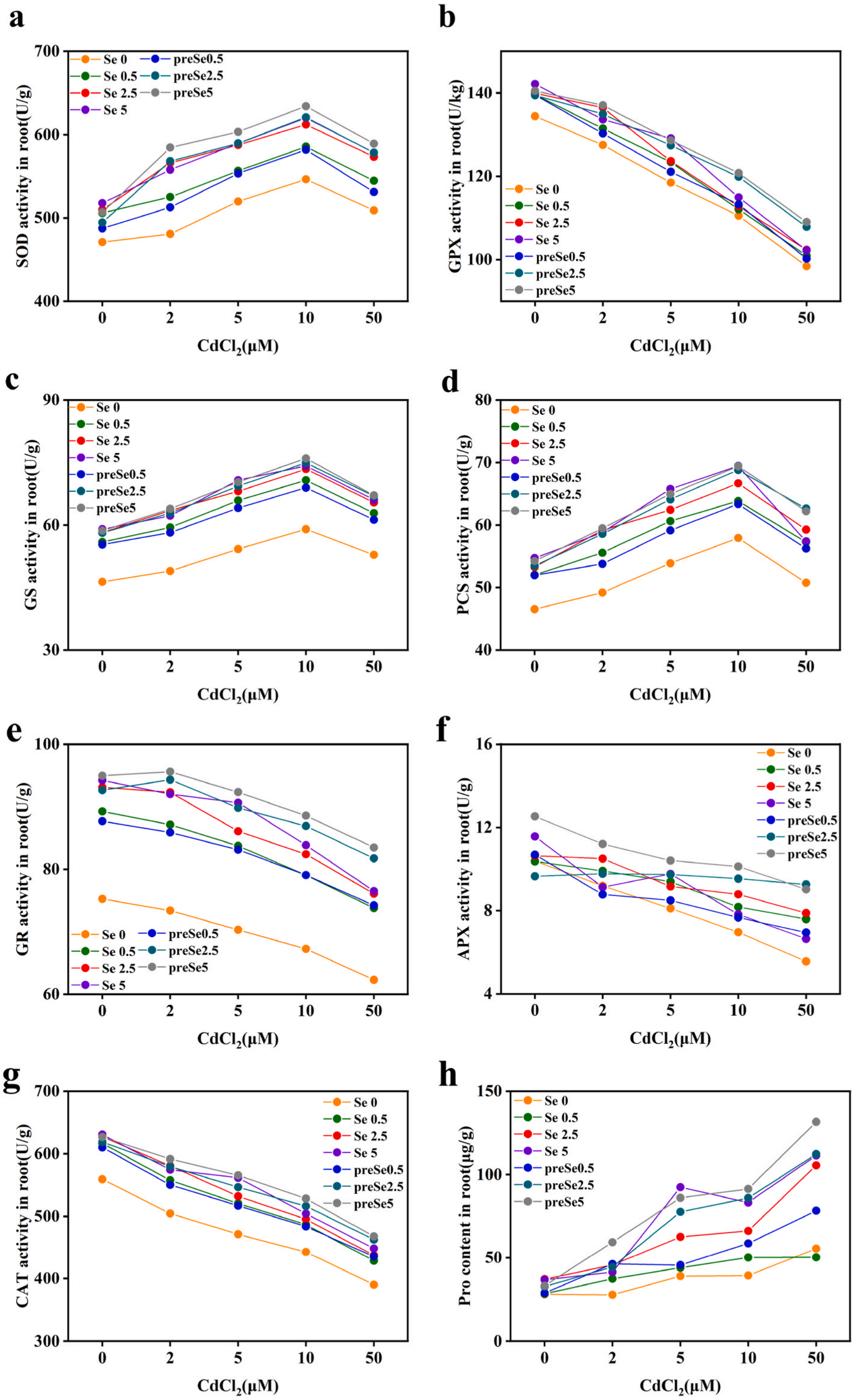

Fig. 2. Enzyme activities and proline concentrations in roots under different Se and Cd treatments. a–g: Activities of SOD, GPX, GS, PCS, GR, APX, CAT and proline concentrations in rice roots under different Se and Cd treatments.

their changes of weight. As shown in Fig. 1a, Se significantly promoted the growth of rice in the absence of Cd. However, the improvement effect appeared unrelated to the concentration of Se and method used for Se addition. Interestingly, the effects of Cd on rice growth were irregular rather than inhibitory. A similar phenomenon was also observed with the addition of both Se and Cd. Above all, the interaction of Se and Cd had an irregular effect on rice growth, which may have been caused by many factors in addition to the interaction that could influence the rice yield.

As shown in Figs. 1b and 2a and Figs. S1–2a, the activity of SOD increased markedly with increases in the Cd concentrations from 0 to 10 μM in solution, whereas SOD activity in roots and leaves was inhibited as the Cd concentration increased from 10 to 50 μM. In the leaf sheaths and stems, however, the activity of SOD increased with increases in the Cd concentrations over the experimental range. Compared with the addition of Cd alone, both the Se and preSe treatments, particularly with high Se concentrations, increased the activity of SOD in leaves and roots, and in general, the preSe treatments increased the activity of SOD more significantly than the Se treatments. SOD activity was highest in the roots, followed by the stems and leaf sheaths, and was lowest in the leaves. The activity of GPX exhibited a similar trend to that found for SOD activity, as revealed in Figs. 1c and 2b and Figs. S1–2b, except in roots, which showed decreases in GPX activity with increases in the Cd concentrations.

GS activity increased substantially with increases in the Cd concentration in solution, as demonstrated in Figs. 1d and 2c and S1-2c, except in the roots, where GS activity decreased with increases in the Cd concentration from 10 to 50 μM. The results found for PCS were similar to those obtained for GS, as can be easily concluded from the results shown in Fig. 1d and e, Fig. 2c and d andFig. S1-2c and d. The effect of Se on GS and PCS activity was similar to that found on SOD and GPX, but notable increases in GS and PCS were observed. The activity of the two enzymes in different organs showed similar characteristics: root $>$ leaf sheath $\approx$ stem $\approx$ leaf.

As shown in Fig. 1f and g, Fig. 2e and f and Fig. S1-2e and f, GR and APX activity showed obvious increases with increases in the Cd concentration, and this finding was observed in all tested parts except the roots, where a completely opposite trend was observed. Moreover, Se had similar effects on GR and APX activities as on GS activity.

CAT exhibited a distinctive pattern compared to the other enzymes, and the activity of this enzyme showed obvious decreases in all organs with increases in the Cd concentration over the experimental concentration range (Fig. 1h; Fig. 2g and S1-2 g). The effect of Se on CAT activity was similar to that on the other enzymes. The activity of CAT in different organs showed a decreasing tendency from roots to leaves.

3.2. *Effect of Se and Cd on AsA and GSH concentrations in leaves and roots*

The ascorbate concentration showed a Cd-dependent increase in the leaves, and the effect was markedly weaker in the roots (Fig. 1i and S4a). Se improved the concentration of AsA, and the effect was similar to that found on the antioxidant enzymes. Furthermore, the AsA concentration in the leaves was significantly higher than that in the roots.

Unlike AsA, GSH showed more complex responses under Cd stress (Fig. 1j and S4b). Cd generally induced a sharp increase in the GSH concentration in the roots but caused either an increase or a decrease in the GSH concentration in the leaves. Se also increased the concentration of GSH.

3.3. *Effect of Se and Cd on lipid peroxidation and the H_2O_2 concentration in leaves*

Cd induced a significant increase in the H_2O_2 concentration, and this increase was clearly inhibited by the application of exogenous Se (Fig. 1k). Compared with the results obtained from the simultaneous addition of Se and Cd, pretreatment with Se more strongly inhibited the increase in the H_2O_2 concentration.

The level of lipid peroxidation can be indicated by the MDA concentration. In contrast to the results found for the H_2O_2 concentration, the MDA concentration showed markedly smaller changes under Cd stress (Fig. 1l). Se also decreased the MDA levels in the leaves, especially after pretreatment with Se, but this alleviation was slight.

3.4. *Effect of Se and Cd on the proline concentrations in leaves and roots*

As shown in Fig. S3e and Fig. 2h, Cd induced a significant increase in the proline concentration, and the effect became increasingly obvious with increases in the Cd concentration. The addition of Se strongly enhanced the concentration of proline in the roots and leaves, especially in the pretreatment groups, and this finding was the same as those found for the enzymes. Moreover, the proline concentration in the roots was clearly lower than that in the leaves.

3.5. *Effect of Se and Cd on the chlorophyll and carotenoid concentrations in leaves*

As shown in Fig. S3a-d, a decrease in the chlorophyll concentration, including the chlorophyll *a* and *b* concentrations, was observed after the addition of Cd, and this decrease could be relieved by the addition of Se when the Cd concentration was higher than 2 μM. Both Se and Cd had a slight effect on the carotenoid concentration.

3.6. *Effect of Se and Cd on the total protein levels in leaves and roots*

As shown in Fig. S3f and Fig. S4c, the addition of Cd to the growth solution markedly increased the total protein concentration in both the leaves and roots. The effect of Se on the total protein concentration was similar to that of Cd, and the pretreatment resulted in a stronger increase than the other treatments. The total protein in the leaves was significantly higher than that in the roots.

4. Discussion

4.1. *Grouping of physiological indicators under the interaction of Se and Cd*

First, a hierarchical cluster analysis, which utilizes correlation as the basis for clustering, was conducted to classify the physiological indicators due to that it could better reveal the relationship between different antioxidant substances. In leaves, the indicators could be divided into several groups (Fig. 3a). Antioxidant systems can be categorized into three groups. The first group included APX, AsA, Pro, GR, GS, and PCS, and the second group included SOD and GPX, whereas the last group was only composed of CAT. These results imply that these systems are not regulated by the same mechanism. The plant pigments were divided into two groups, namely, chlorophylls and carotenoids, because these showed different behaviours under the interaction of Se and Cd. This finding can also be confirmed by the results shown in Fig. S3, which demonstrated that Cd caused more damage to chlorophylls than carotenoids. Among oxidant stress indicators, H_2O_2 and MDA were classified into the same group, which was reasonable due to their close relationship. Leaf Cd and solution Cd were classified into identical groups, suggesting that Cd was readily absorbed from the solution and translocated into leaves.

The grouping of physiological indicators in the leaf sheaths and stems was similar to that in the leaves, whereas the grouping in the roots was completely different (Fig. 3). Moreover, as shown in Figs. 1–2 and S1-S2, some enzyme activities, such as those of GPX, GR and APX, exhibited completely different trends in the roots with increases in the Cd concentrations compared with those in the aerial parts. Previous studies also found similar results. For example, Lin et al. and Ding et al.

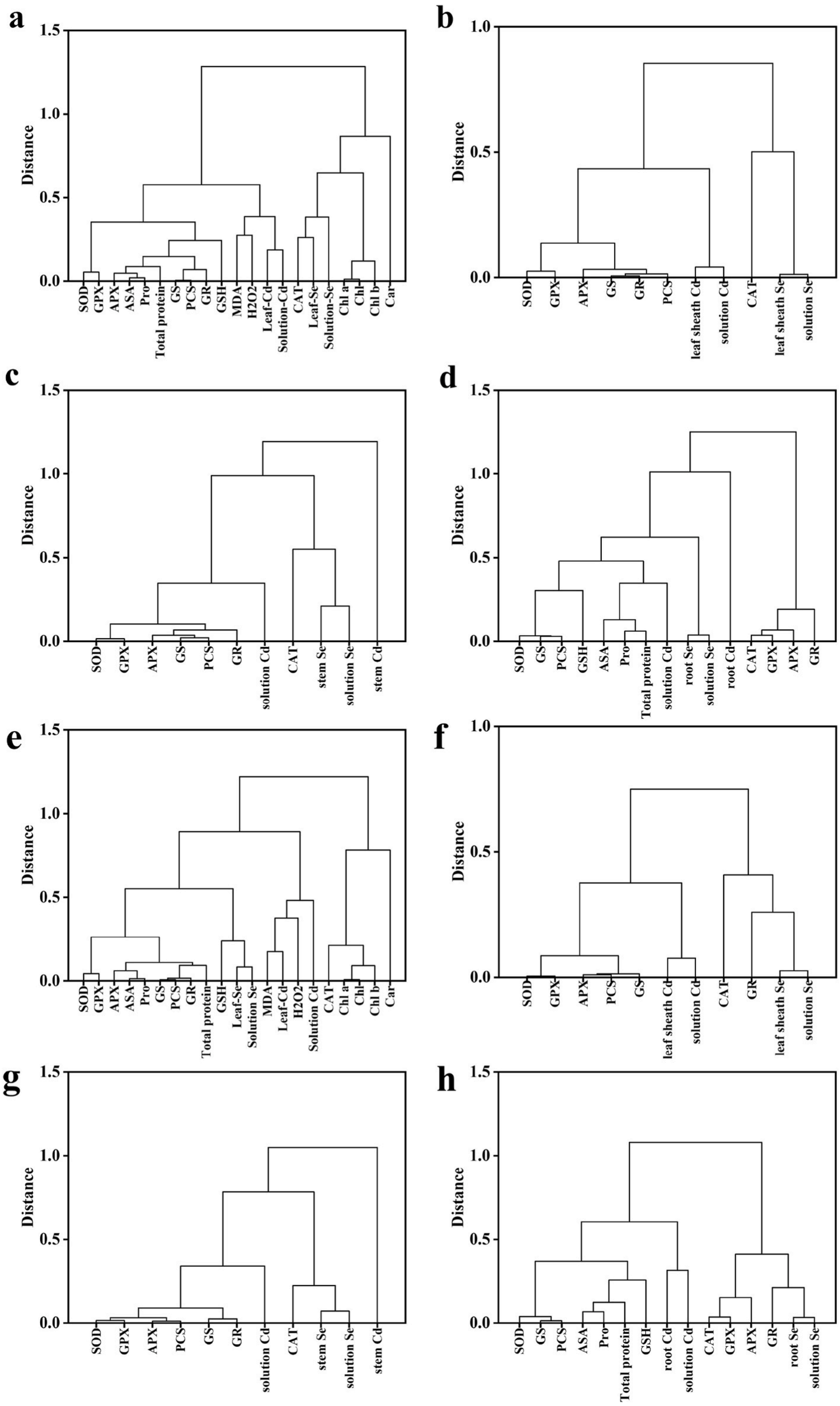

Fig. 3. Cluster analysis of physical indictors in different rice organs subjected to the Se + Cd and preSe treatments. Cluster analysis of physical indicators of the leaf (a), leaf sheath (b), stem (c) and root (d) of rice subjected to Se + Cd treatments. Cluster analysis of physical indicators of the leaf (e), leaf sheath (f), stem (g) and root (h) of rice subjected to preSe treatments.

demonstrated that the activities of APX exhibited different trends in roots and shoots, suggesting that the physiological response in the roots was distinct from that in the aerial parts(Ding et al., 2013; Lin et al., 2012). The reasons for these differences will be discussed below.

The grouping of physiological indicators in the preSe treatments was similar to that in the Se + Cd treatments, as shown in Fig. 3, indicating that the time of Se application does not change the relationship among physiological indicators.

Linear correlation analysis was used to explore the relationship between different compounds in the same group. We then found that the compounds in the same group exhibited a highly significant linear correlation.

4.2. AsA-GSH cycle-GS-PCS coupling system in aerial parts

The AsA-GSH cycle plays a crucial role in maintaining ROS homeostasis in cells by efficiently scavenging H_2O_2(Jia et al., 2023; Khan et al., 2019). Since the cycle was discovered in the mid-1970s, the enzyme-catalysed reactions of this pathway have been widely reported (Pandey et al., 2015). The AsA-GSH cycle is induced by various biotic and abiotic stresses, such as drought(Sofo et al., 2010) and salt stress (Mittova et al., 2004). However, the response of the cycle to heavy metals has not been studied intensively.

The cluster analysis showed that GR, APX, AsA, and GSH, which as enzymes or nonenzymatic compounds that comprise the AsA-GSH cycle, were usually divided into the same group in the aerial parts, which indicated that these compounds might protect cells from ROS as a whole. The linear correlation analysis further supported the highly significant linear relationship between the compounds comprising the cycle (Fig. 4, S5-S9).

Notably, the substances in the AsA-GSH cycle as well as GS, PCS, and proline were divided into identical groups, which suggests that the AsA-GSH cycle might be coupled with other systems to defend plants from Cd stress. Compared with the relationship between substances of the AsA-GSH cycle, GS, PCS and proline had an even stronger significant linear correlation with some of the substances in the cycle, as shown in Fig. 4 and Figs. S5–S9. For example, the r^2 found for the relationships of GR and GS, GR and PCS, and Pro and AsA in the leaves of the pretreatment group reached 0.95887, 0.97153, and 0.97116, respectively (Fig. 4, S7). Therefore, the PCS, GS, Pro and AsA-GSH cycle might form a coupling system to respond to the interaction. The coupling system may be formed by the sharing of GSH for phytochelatin synthesis and the AsA-GSH cycle, such as GS providing GSH for both the reduction of DHAR and the generation of PCS(Mittler et al., 2004; Nocito et al., 2011). Although proline appears not present in the metabolic network, it could chelate metal chelators, scavenge ROS and, more importantly, promote the accumulation of GSH(Höller et al., 2014; Mansour and Ali, 2017). Therefore, proline should also be included in the coupling system. Compounds such as DHA and DHAR were not further detected for two reasons. First, these compounds were only present in the cycle or involved in the synthesis of phytochelatin but did not participate in the coupling of the two processes(Mittler et al., 2004; Nocito et al., 2011). Second, the two processes have already been widely demonstrated. The exact mechanisms are shown in Fig. 6. Above all, the results demonstrated that under Cd stress and the alleviating effect of Se, these enzymes and nonenzyme substances may act as a coupling system to defend plants against adversity.

4.3. GPX-SOD coupling system and other compounds in aerial parts

As shown in the cluster analysis, GPX and SOD were always classified into one group in the aerial parts, and further analysis showed that the two enzymes had a significant linear relationship (Fig. 5). For instance, the r^2 found for GPX and SOD in the leaf sheaths of the preSe group even reached 0.9902 (Fig. 5), which also suggested that these enzymes may act as a system to defend plants from stress. The observed tendency was similar to that found by Srivastava et al. (2014). This system may be caused by the GPX-mediated detoxification of H_2O_2, which is formed from the reaction catalysed by SOD.

Chlorophylls and carotenoids have different biological functions; thus, it is easy to understand that these compounds may exhibit different behaviours under the interaction of Se and Cd.

The response of CAT was completely different from that of the other enzymes because its activity decreased with the addition of Cd (Figs. 1–2 and S1-S2). However, as shown in Fig. 1k, the H_2O_2 concentration increased with the addition of Cd, which means that the decrease in CAT activity was not due to a decrease in the substrate level. In contrast, the decrease in CAT activity may have led to an increase in the H_2O_2 level (Dat et al., 2003; Fujiki and Bassik, 2021). Moreover, previous studies have demonstrated that CAT is more sensitive to heavy metal stress than other antioxidant enzymes(Sun et al., 2015). Therefore, CAT was seriously damaged, which may be one important reason for its different trends compared with those of other enzymes.

4.4. Root antioxidant systems

The root antioxidant systems were completely different from those of the aerial parts. The AsA-GSH cycle-GS-PCS coupling system and GPX-SOD were not found in the roots. Moreover, a new coupling system including APX-GPX-CAT and PCS-GS-SOD emerged (Fig. 3, S10) for two reasons. The first is that the Cd concentration in the roots was markedly higher than that in the aerial parts, which means that the roots suffered much more serious injuries leading to critical damage to antioxidant

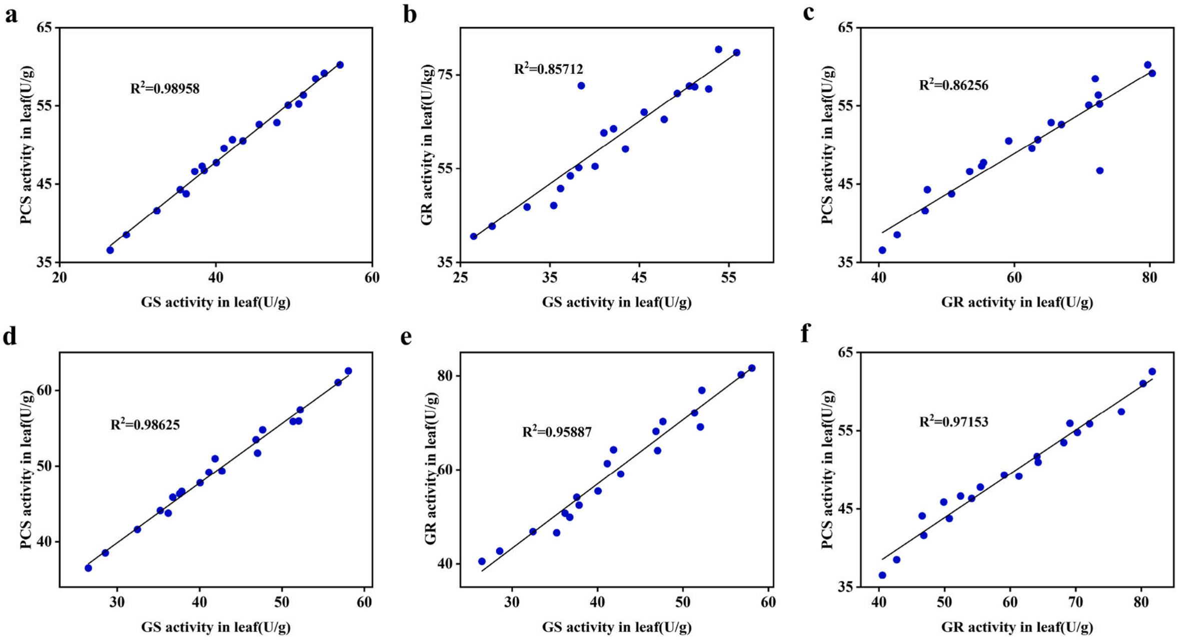

Fig. 4. Fitting curve between AsA-GSH cycle-GS-PCS coupling system enzymes in leaves of the Se + Cd group (a–c) and preSe group (d–f).

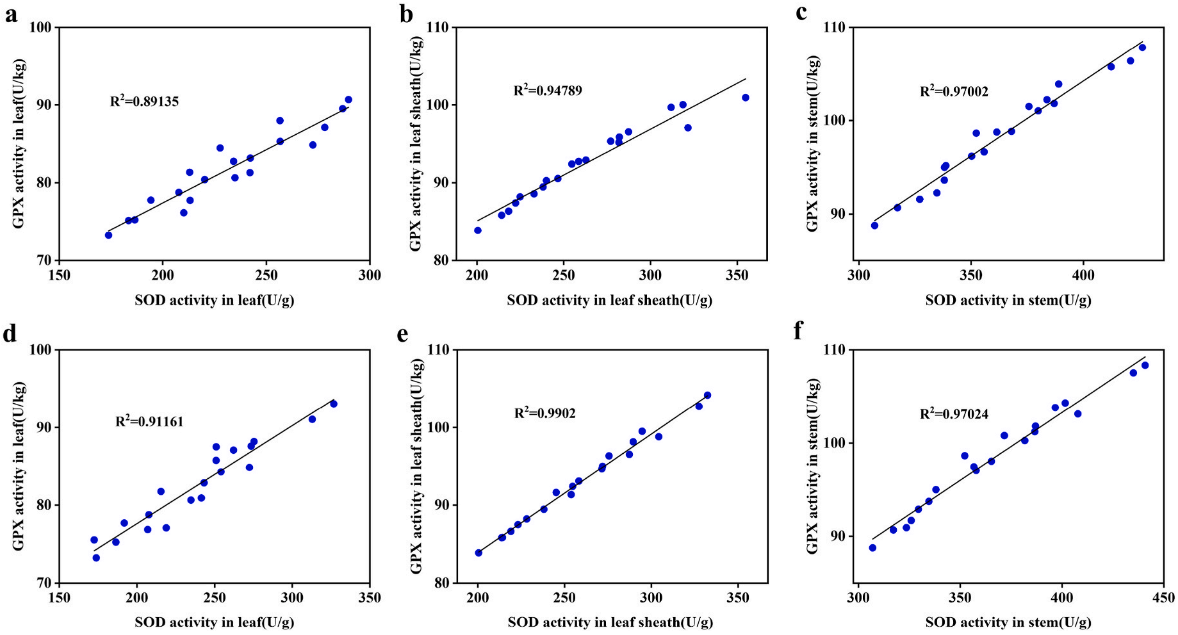

Fig. 5. Fitting curve between GPX and SOD in aerial rice parts in the Se + Cd group (a, b, c) and preSe group (d, e, f).

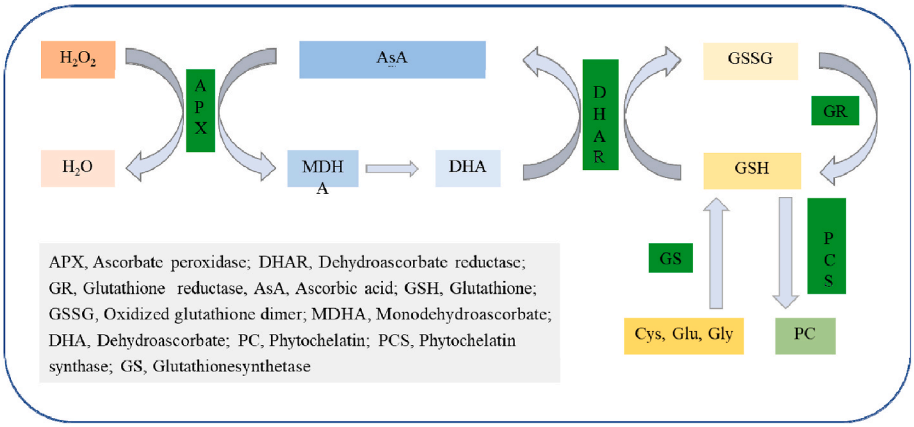

Fig. 6. AsA-GSH cycle-GS-PCS coupling system.

systems(Balestrasse et al., 2001). Decreases in the activities of enzymes and the concentrations of nonenzymatic compounds in the roots might prove this idea. In addition, the different functions and cell structures of roots may also contribute to this phenomenon. For instance, there are no chloroplasts in root cells that play a critical role in the production of ROS (Khorobrykh et al., 2020). Notably, the activities of enzymes in the APX-GPX-CAT coupling system decreased with increases in the Cd concentrations, which implies that these enzymes were sensitive to Cd (Fig. 2). Moreover, PCS and GS were still classified into one group, which implies that the synthesis of GSH was still important for the production of PCS. The activities of PCS and GS did not decrease until the Cd concentrations increased from 10 μM to 50 μM, which means that these enzymes were tolerant to Cd. This finding may have been caused by the function of roots. Roots are the most important organs for inhibiting Cd translocation, and phytochelatin generated by PCS is one of the most important substances for the chelation of Cd(Ricachenevsky et al., 2018).

5. Conclusion

In this study, a hydroponic experiment was performed to explore the variations in antioxidant materials, including enzymatic and nonenzymatic reducing substances, as well as their relationships in the presence of various levels of exogenous Se and Cd. Exogenous Cd stimulated the antioxidant system and improved the activities of enzymes, with the exception of CAT, in the aerial parts, whereas Se enhanced the antioxidant capacity by enhancing enzyme activities. The concentrations of nonenzymatic antioxidant substances were also increased by the addition of Se and Cd. However, the activities of antioxidant enzymes, including GPX, APX, GR and CAT, decreased with the addition of Cd to the roots. Through cluster analysis, the antioxidant systems were divided into several groups. The significant linear correlation between substances in the same group implied the presence of a coupling system consisting of these substances. Based on these findings, an AsA-GSH cycle-GS-PCS coupling system and a GPX-SOD coupling system in the aerial plant parts were proposed. Their activities or concentrations exhibited similar trends in the presence of Cd and Se, suggesting that these systems respond to stress together. However, the timing of Se application did not change these systems. These coupling systems form likely due to the fact that one enzyme generated a product that could be the substrate for another enzyme in this study. For instance, GSH generated by GS is a substrate used by PCS. However, the coupling systems of the aerial parts did not exist in the roots; instead, APX-GPX-CAT and PCS-GS-SOD appeared in the roots. The former system may be formed by the serious damage caused by a high concentration of Cd, whereas the latter was caused by the different biological functions in roots. Our study provides the first demonstration of the existence of a coupling system under the interaction of Se and Cd, suggesting that either antioxidant enzymes or nonenzymatic substances do not function alone, which provides new insights for further study. In the future, genomics and proteomics techniques could be adopted to further confirm the existence of coupling systems and explore their regulatory mechanisms.

Author contributions

Da Lei, Haorui Cao: Investigation, Methodology, Writing – original draft, Kuankuan Zhang, Kang Mao, and Yongkun Guo: Investigation; Jen-How Huang and Guili Yang: writing – review & editing; Hua Zhang and Xinbin Feng: Supervision.

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.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.122526.

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