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1 **Efficient regulation of cadmium accumulation by carboxymethylammonium**
2 **chloride in rice: correlation analysis and expression of transporter gene *OsGLR3***

3 Lin Fu ^{†a}, Jiawei Deng ^{†a}, Shuangyue Liu ^{†a}, Changbo Zhang ^{*a}, Weijie Xue ^{*a} Gilles
4 Mailhot ^b, Davide Vione ^c, Yun Deng ^d, Changrong Wang ^a, Lei Wang ^e

5 ^a *Key Laboratory of Original Agro-Environmental Pollution Prevention and Control,*
6 *Agro-Environmental Protection Institute of Ministry of Agriculture and Rural Affairs,*
7 *Tianjin, 300191, China*

8 ^b *Université Clermont Auvergne, CNRS, Institut de Chimie de Clermont-Ferrand, F-*
9 *63000 Clermont-Ferrand, France*

10 ^c *Università degli Studi di Torino, Dipartimento di Chimica, Via P. Giuria 5, 10125*
11 *Torino, Italy*

12 ^d *School of Environment and Ecology, Jiangnan University, Wuxi 214122, China*

13 ^e *Key Laboratory of Environmental Biotechnology, Research Center for Eco-*
14 *Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*

15 * Corresponding author. E-mail address: zhangchangbo@caas.cn (C.-B. Zhang),
16 tzfelicity@163.com (W.-J. Xue)

17 † These authors contributed equally to this work

18 **Abstract**

19 The mechanism of carboxymethylammonium chloride (CC) regulating cadmium
20 (Cd) accumulation in rice was studied in field and hydroponic experiments. Field
21 experiments showed that 0.2-1.2 mmol L⁻¹ CC spraying effectively reduced Cd
22 accumulation by 44%-77% in early rice grains and 39%-78% in late rice grains,
23 significantly increased calcium (Ca) content and amino acids content in grains, as

24 well as alleviated Cd-induced oxidative damage in leaves. Hydroponic experiments
25 further verified the inhibition effect of CC on Cd accumulation. 1.2 mmol L⁻¹ CC
26 made the highest decrease of Cd content in shoots and roots of hydroponic seedlings
27 by 45% and 53%, respectively. Exogenous CC significantly increased glutamate (Glu),
28 glycine (Gly) and glutathione (GSH) content, and improved the activities of catalase
29 (CAT) and superoxide dismutase (SOD) by 41-131% and 11-121% in shoots of
30 hydroponic seedlings, respectively. Exogenous CC also increased the relative
31 expression of *OsGLR3.1-3.5* in the shoots and roots of hydroponic seedlings. The
32 quantum computational chemistry was used to clarify that the Gly radical provided by
33 CC could form various complexes with Cd through carboxyl oxygen atoms. These
34 results showed that exogenous application of CC improved the tolerance to Cd by
35 enhancing the antioxidant capacity; inhibited the absorption, transport and
36 accumulation of Cd in rice by (1) promoting chelation, (2) increasing the GLRs
37 activity through upregulating the content of Glu, Gly, as well as the expression of
38 *OsGLR3.1-3.5*.

39 **Keywords:** Rice; Cadmium; Carboxymethylammonium chloride; Foliar resistance
40 control

41 **1. Introduction**

42 As a non-essential toxic element, cadmium (Cd) is easily absorbed and enriched
43 in rice grains, posing a potential threat to human health as it accumulates in the food
44 chain (Wang et al., 2019; Zou et al., 2021). The absorption and transport of Cd in rice
45 is the key process affecting the accumulation of Cd in grains, which is regulated by

46 chelating substances, transporters and non-selective cation channels (NSCCs) (Jing et
47 al., 2023; Kulsum et al., 2023; Wang et al., 2021; Han et al., 2019).

48 There are many chelating substances in rice cells that can chelate with Cd
49 (Zhang et al., 2009). For example, the cell wall is rich in various chelating Cd
50 functional groups such as carboxyl, carbonyl, and hydroxyl groups, so most Cd could
51 be trapped in the cell wall to prevent them from being transported across cell
52 membranes (Yu et al., 2020a; Yu et a., 2020b). But there is still a portion of Cd with
53 high activity that transmembrane transport into the cytoplasm. During the perennial
54 evolution of rice, various substances like small molecule acids such as citrate, amino
55 acids such as glutamate (Glu) and glycine (Gly), glutathione (GSH), and Cd-induced
56 phytochelatins (PCs) are formed in the cytoplasm to chelate with Cd, trapping Cd in
57 vacuoles and reducing Cd activity to inhibit Cd transport (Li et al., 2014; Xue et al.,
58 2022; Yuan et al., 2020; Xue et al., 2023). Most of the Cd in rice grains comes from
59 the re-transformation during the flowering to grain filling period of Cd stored in rice
60 leaves and stems before flowering (Xue et al., 2019; Zhou et al., 2018). Research has
61 shown that the ability of vegetative organs in low Cd accumulation (LCA) rice to
62 inhibit Cd transport to grains is higher than that of high Cd accumulation (HCA) rice
63 because LCA rice has a higher ability to synthesize chelating substances (Yuan et al.,
64 2020). These indicated that increasing chelating substances content in vegetative
65 organs is one of the important measures of reducing Cd content in rice. Glu and Gly
66 are precursors for the synthesis of GSH and PCs (Cao et al., 2018; Pál et al., 2018).
67 Gly could generate Glu by transferring amino groups to α -Ketoglutaric acid. However,

68 further exploration is needed to determine whether exogenous supplementation of Gly
69 regulates chelating substances content in rice.

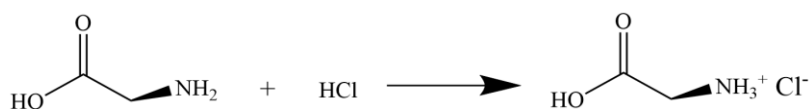
70 As is well known, Cd does not have specialized transporters in plants, and the
71 transport of Cd in rice mainly relies on the transporters of essential divalent cations
72 (Li et al., 2017). The functions of most well-known genes mediating Cd uptake,
73 transport, and accumulation in rice have been widely identified (Kulsum et al., 2023;
74 Feng et al., 2017; Tan et al., 2020). For example, *OsNramp1*, 5 and *OsIRT1*, 2
75 mediates the absorption of Cd^{2+} , Mn^{2+} and Fe^{2+} by rice roots (Sasaki et al., 2012;
76 Chen et al., 2019; Chen et al., 2020). *OsHMA3*, located on the vacuolar membrane of
77 rice roots, mediates the compartmentalization effect of vacuoles on Cd (Yan et al.,
78 2016); *OsHMA2*, located on the cell membrane of vascular bundle in rice roots,
79 mediates the transport of Cd^{2+} and Zn^{2+} from the roots to the aboveground tissues
80 (Ismael et al., 2019). *OsLCT1* and *OsLCD* mediate the transport of Cd^{2+} from the
81 phloem to the grains (Uraguchi et al., 2011; Shimo et al., 2011). *OsCdl* is a plasma
82 membrane protein involved in the absorption and accumulation of Cd in rice (Yan et
83 al., 2019). Studies have shown that any measure to increase endogenous Glu content
84 have the potential to regulate the expression levels of genes related to Cd uptake and
85 transport (Jiang et al., 2020). In addition to transporters, NSCCs are also an important
86 pathway for Cd uptake and transport in rice (Han et al., 2019). However, it is not clear
87 whether Gly could increase endogenous Glu content and thus affect the NSCCs for
88 Cd transmembrane transport.

89 NSCCs can simultaneously transport multiple cations with different valence
90 states, including Cd^{2+} (Demidchik et al., 2007; Han et al., 2019). Glutamate receptors
91 (GLRs) is the most widely distributed NSCCs in the roots and vegetative organs of
92 plant cell protoplasmic membrane (Ortiz-Ramírez et al., 2017). The activity of GLRs
93 channels is closely related to the absorption and transport of essential mineral ions
94 such as Fe^{2+} , Mn^{2+} and Ca^{2+} , as well as heavy metal ions such as Cd^{2+} and Cr^{2+}
95 (Zhang et al., 2023a; Li et al., 2019). The permeability of rice GLRs to essential metal
96 ions is higher than that of Cd ions, and the activity of GLRs in HCA rice is lower than
97 that in LCA rice, high Cd stress leads to the loss of GLRs selectivity, resulting in
98 excessive accumulation of Cd in rice (Zhang et al., 2023a). *OsGLR3* is involved in the
99 regulation of GLRs activity, and then affecting the absorption and transport of metal
100 ions in rice (Li et al., 2019; Ni et al., 2016; Chen et al., 2018). Signal molecules such
101 as Gly, Glu, and GSH can act as ligands to activate GLRs, Ca^{2+} influx is an important
102 manifestation of GLRs activation (Qi et al., 2006; Vatsa et al., 2011; Shao et al.,
103 2020). While, it is unclear whether exogenous Gly can regulate the expression level of
104 *OsGLR3* gene and increase GLRs activity, affecting the absorption and transport of
105 Cd in rice.

106 In addition, the phenomenon of excessive production of oxygen free radicals
107 (ROS) induced by Cd in rice is widespread (Gallego et al., 2012; Tripathi et al., 2012).
108 Studies have found that increasing the content of endogenous Glu significantly
109 increased the activity of antioxidant enzymes such as catalase (CAT) and superoxide
110 dismutase (SOD) in Cd stressed rice, significantly reduced the content of hydrogen

111 peroxide (H₂O₂) and superoxide anions (O₂^{·-}) in rice, and avoided oxidative damage
112 caused by membrane lipid peroxidation (Xue et al., 2023). Further exploration is
113 needed to determine whether Gly could enhance the antioxidant capacity of rice by
114 increasing endogenous Glu in rice.

115 With the rapid development of drones, foliar Cd reducing agents have been
116 widely used (Cui et al., 2023; Gao et al., 2020). Although Gly is soluble in water, its
117 dissolution rate is slow, so it often occurs that Gly cannot be completely dissolved
118 when sprayed with drones. Carboxymethylammonium chloride (CC) is an amino acid
119 ionic liquid with good water solubility, strong stability and green environmental
120 protection and rich in Gly ions (see **Scheme 1**). It has been reported that CC play a
121 role in Cd-contaminated soil leaching by chelating with Cd (Deng et al., 2023). And
122 the previous study has shown that exogenous application of CC effectively reduced
123 the accumulation of Cd in the rice seedlings (Liu et al., 2023). However, the
124 mechanism by which CC inhibits Cd uptake and transport in rice is not yet clear.
125 Therefore, hydroponic and field experiments in this study were conducted to learn the
126 effects of exogenous CC on Cd accumulation, chelating substances synthesis,
127 antioxidant capacity, amino acid synthesis and *OsGLR3* expression in rice under Cd
128 stress. Results showed that exogenous application of CC improved the tolerance to Cd
129 by enhancing the antioxidant capacity; inhibited the absorption, transport and
130 accumulation of Cd in rice by (1) promoting chelation, (2) increasing the GLRs
131 activity through upregulating the content of Glu, Gly, as well as the expression of
132 *OsGLR3.1-3.5*.



Scheme 1. Synthetic form of CC

133

134 2. Materials and methods

135 2.1 Culture and treatment methods

136 2.1.1 Field experiment

137 A random block with the double cropping rice paddy field (with each area of 2.5
 138 m×4 m=10 m², 0.53 mg kg⁻¹ Cd, 5.5 pH and 40.3 g kg⁻¹ organic matter) experiment
 139 was conducted in Xiangtan City, Hunan Province of China (N: 28°41′, E: 112° 52′).
 140 Rice seeds from indica conventional early rice (*Oryza sativa* L.) cultivar Z35 and
 141 indica conventional late rice cultivar Huazhan (widely cultivated rice varieties in
 142 Hunan province, high-Cd-accumulation rice) were selected.

143 Most of the Cd in rice grains comes from the re-transformation during the
 144 flowering to grain filling period of Cd stored in rice leaves and stems before flowering
 145 (Xue et al., 2019; Zhou et al., 2018). Spraying foliar regulators during flowering is an
 146 effective and environmentally friendly method to inhibit the transfer of Cd into grains.
 147 In the present study, at the flowering stage of rice, CC solution with different
 148 concentration (T0, T1, T2, T3 and T4 = 0, 0.20, 0.50, 0.80 and 1.20 mmol L⁻¹ CC)
 149 was sprayed, the volume of each treatment solution was 300 mL. The spray treatment
 150 was performed once a day for two consecutive days. Each treatment was triplicated.
 151 Field management was consistent with that of high-yield rice fields in Hunan (the
 152 details were described in Supplementary Material 1.1.1). After 5 days of spraying,
 153 fresh flag leaves were collected for ROS content determination. Mature grains were

154 collected for measuring the content of Cd, K, Mg, Ca, Fe, Mn, Zn and amino acids.

155 ***2.1.2 Hydroponic experiment***

156 Hydroponic experiments are easy to control for a single variable. Adding CC to
157 hydroponic nutrient solution ensures that rice could absorb different levels of CC to
158 further explore the mechanism of CC levels regulating Cd migration and transport in
159 rice seedlings. To avoid the impact of different growth seasons and cycles on the
160 experiment, Z35 (HCA rice) and another indica conventional early rice cultivar X24
161 (LCA rice) were selected. Pre experiment found that CC (0, 0.20, 0.50, 0.80 and 1.20
162 mmol L⁻¹) had no significant effect on the growth status, Ca content, Glu content, and
163 CAT activity of Z35 rice seedlings under non-Cd conductions, so this experiment
164 mainly focused on the mechanism of CC regulating Cd migration and transport in rice
165 under Cd stress. Uniform seedlings with three leaves were selected and placed into
166 1/10 Hoagland nutrient solution (detailed formulation was described in
167 Supplementary Material 1.1.2) containing 2.7 μmol L⁻¹ Cd and different levels of CC
168 (T0, T1, T2, T3 and T4 = 0, 0.20, 0.50, 0.80 and 1.20 mmol L⁻¹ CC) with 5.6-6.5 pH
169 for 7 days. Each treatment was triplicated. The treatment solution was changed every
170 2 days. The hydroponic experiment was conducted in the artificial climate room. The
171 conditions of the artificial climate room were as follows: 16 hours of day and 8 hours
172 of night, 105 μmol m⁻² s⁻¹ light intensity, 28 °C and 25 °C day and night temperature,
173 respectively, and 60% relative humidity.

174 After 7 days, the rice seedlings were soaked with 5 mmol·L⁻¹ CaCl₂ for 30
175 minutes to remove ions from the root surface, then the roots were repeatedly rinsed

176 with deionized water. The samples of rice seedlings were collected and divided into
177 shoots and roots (fresh and dry samples), some samples were wrapped in tin foil and
178 immediately stored in liquid nitrogen for RNA extraction. Dry samples were used to
179 determine Cd and amino acids content, fresh samples were used to determine
180 sulfhydryl compound content, reactive oxygen species levels, enzyme activities, and
181 gene expression.

182 ***2.2 Determination index and method***

183 ***2.2.1 Determination of Cd, K, Ca, Mg, Fe, Mn, and Zn content***

184 The Cd, K, Ca, Mg, Fe, Mn and Zn content was determined based on previous
185 studies (Xue et al., 2019; Yang et al., 2021). The digestion procedure was completed
186 on ED54 (LabTech, Beijing, China) using HNO₃-H₂O₂ wet-digestion. The
187 concentrations of Cd, K, Ca, Mg, Fe, Mn, and Zn in plant samples were determined
188 by inductively coupled plasma interfaced with mass spectrometry (ICP-MS, Agilent
189 7500a, USA). Detailed determination procedures were described in Supplementary
190 Material 1.2.1.

191 Quality assurance and quality control (QA/QC) are respectively expressed by
192 standard rice flour reference material (GBW(E)100350, Academy of State
193 Administration of Grain, SAAG) and blank digestion sample. The recovery rate was
194 90%-105% to ensure the accuracy and trustworthiness of data. Standard solutions
195 with K, Ca Mg and Fe (Cd, Mn and Zn) of 0~10000 (0~100) µg L⁻¹ were prepared
196 from the environmental calibration standard solution (Agilent technologies, Part #
197 5183-4688) to plot standard curves and to detect elements in digested liquor of

198 samples.

199 ***2.2.2 Determination of sulfhydryl compounds content***

200 The extraction and determination of sulfhydryl compounds were completed as
201 previously reported (Han et al., 2019). The sulfhydryl compounds in shoots and roots
202 of fresh rice seedling samples were extracted by 0.1% TFA (Trifluoroacetic acid)
203 extraction buffer. The obtained samples were analyzed by High Performance Liquid
204 Chromatography (Agilent Technologies, 1200 series, USA). Detailed determination
205 procedures were described in Supplementary Material 1.2.2.

206 The content of Cys, GSH and PC₂₋₄ in samples was calculated by external
207 standard method. Cys, GSH and PC₂₋₄ could exhibit different absorption peaks at
208 different times as the mobile phase flows. Standard solutions (Cys, GSH and PC₂₋₄)
209 with different concentrations using standard samples were prepared. Under the same
210 chromatographic conditions, the peak time and peak area of the standard solution
211 were recorded, and the standard curve was drawn according to the peak area and
212 corresponding concentration. The content was calculated by comparing the peak area
213 of the measured sample and the standard sample.

214 ***2.2.3 Determination of total amino acid content***

215 The total amino acid content in rice grains was determined according to previous
216 study (Yuan et al., 2020). According to the national food safety standards of China
217 (GB5009.124-2016), total amino acids analysis was carried out by High Performance
218 Liquid Chromatography (Agilent Technologies, 1200 series, USA). Detailed
219 determination procedures were described in Supplementary Material 1.2.3.

220 The content of total amino acids was calculated by comparing the peak area of
221 the measured sample and the standard sample.

222 ***2.2.4 Determination of ROS and enzyme activities***

223 For enzyme activity determination, shoots and roots of fresh rice samples were
224 separately ground with liquid nitrogen. The contents of H₂O₂ and O₂⁻ and the
225 activities of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR),
226 and glutathione synthetase (GSS) in shoots and roots of rice seedlings were
227 determined by kit method. All kits were purchased from Solarbio Science &
228 Technology Co., Ltd.

229 ***2.2.5 Computational quantum chemical analysis of complexes***

230 The molecular model of the complex of Cd and CC was realized by Gaussian 16
231 and Gaussview 6.1 software package.

232 ***2.2.6 Determination of transporter OsGLR3.1-3.5 expression***

233 RNA was extracted from the collected shoots and roots of fresh rice seedling
234 samples by OMEGA plant total RNA extraction kit. Complementary DNA (cDNA)
235 was prepared by reverse transcription using HiScript II Q RT SuperMix for qPCR
236 (+gDNA wiper) R223 kit. In the BioRad CFX96 management system, real-time
237 quantitative polymerase chain reaction (qRT-PCR) was used in ChamQ Universal
238 SYBR qPCR Master Mix (Vazyme Q711) to detect the expression level of the
239 *OsGLR3*. The relative expression level of *OsGLR3* gene was calculated by 2^{-ΔΔCt}
240 method (Livak et al., 2001). The amplification primer sequence is shown in Table S1.

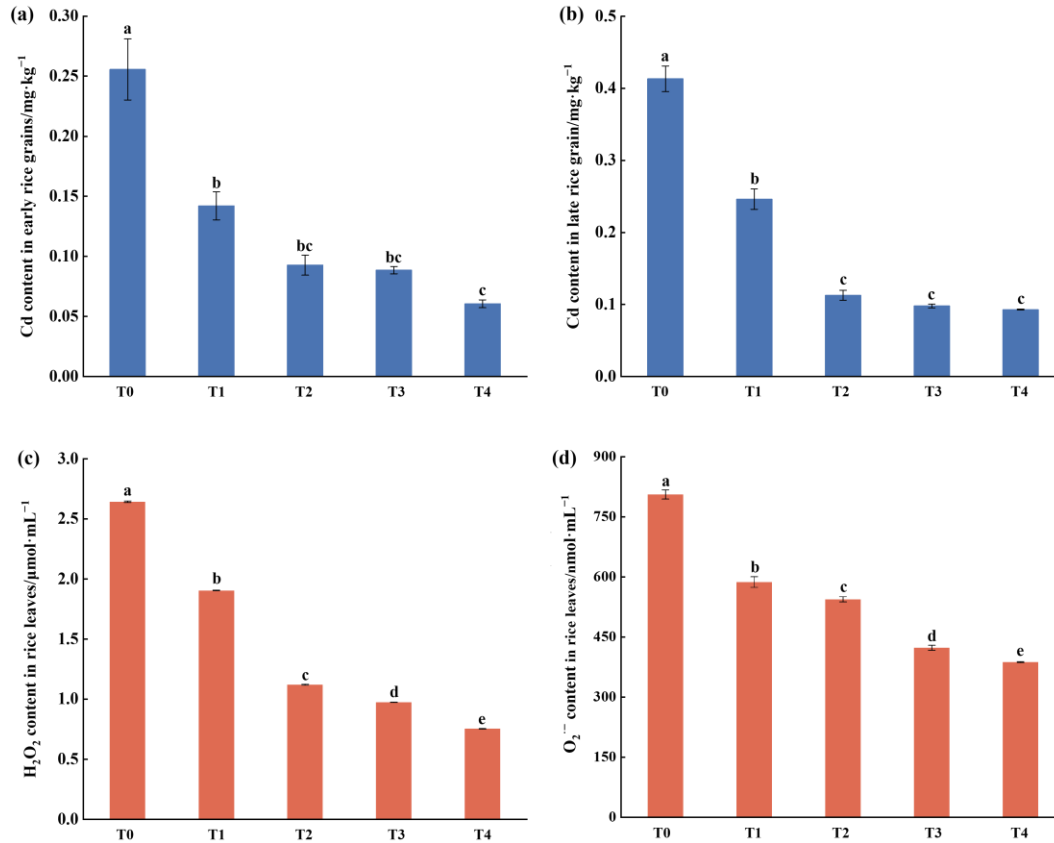
241 ***2.3 Statistical analysis***

242 All experimental data were mean values obtained from the results of three
243 repeated tests, and they were expressed as mean \pm standard deviation. To evaluate
244 differences between treatments, the used statistical tests were one-way analysis of
245 variance (ANOVA) and Duncan's test for multiple comparisons, at $p < 0.05$
246 significance level. Person's correlation analysis was used for correlation analysis.
247 Origin 2021 and Microsoft Excel 2021 were used to process the data and complete the
248 chart drawing. SPSS 26 software was used for statistical significance analysis of the
249 data.

250 **3. Results**

251 *3.1 Effects of spraying CC in field on Cd accumulation in rice grains and oxidative* 252 *damage in rice leaves*

253 Cd content in early rice grains and late rice grains was determined to investigate
254 the effect of spraying CC on Cd accumulation in rice grains in the field. As shown in
255 Fig.1, the Cd content in rice grains decreased significantly after the application of CC
256 at the flowering stage. Cd content in early rice grains was decreased by 44%-77%
257 upon application of 0.2-1.2 mmol L⁻¹ CC (Fig. 1 a). In the late rice grains Cd content
258 was decreased by 39%-78% with application of 0.2-1.2 mmol L⁻¹ of CC (Fig. 1 b). In
259 conclusion, the best spraying concentration to effectively reduce Cd accumulation in
260 rice grains was 1.2 mmol L⁻¹ under all conditions.



261 **Fig. 1** Effects of CC spraying on Cd content in filed early rice grains (a) and in filed late rice
 262 grains (b). Effects of CC spraying on H₂O₂ (c) and O₂⁻ (d) content in filed rice leaves. Different
 263 lowercase letters (a, b, c, d, e) indicate significant differences between treatments ($p < 0.05$).

264 Fig. 1c and Fig. 1d showed the content of H₂O₂ and O₂⁻ in field rice leaves after
 265 spraying CC on the leaf surface at the flowering stage. Compared with T0, the content
 266 of H₂O₂ and O₂⁻ decreased significantly after CC treatment. Upon addition of 1.2
 267 mmol·L⁻¹ CC, the content of H₂O₂ and O₂⁻ in rice leaves were decreased by 72% and
 268 31%, respectively.

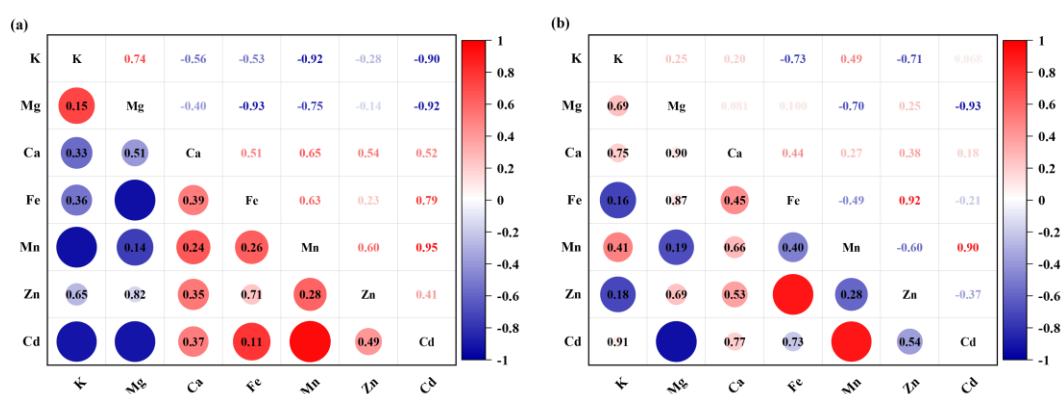
269 **3.2 Effects of spraying CC in field on essential elements content in rice grains**

270 The content of essential elements (K, Mg, Ca, Fe, Mn, and Zn) in early rice
 271 grains was determined after spraying CC at the flowering stage, as shown in Table S2.
 272 The content of K in rice grains was the highest, followed by Mg and Ca, while the

273 content of Zn was the lowest. Compared with T0, the content of Mg, Fe, and Zn was
 274 not modified significantly by CC. In contrast, 1.2 mmol L⁻¹ CC reduced Mn content
 275 by 35% and increased K and Ca contents by 12% and 21%, respectively.

276 As can be seen from Table S2, the content of essential elements in late rice grains
 277 followed the decreasing order: K > Mg > Ca > Zn. The content of Mg, Fe, Mn, and
 278 Zn in late rice grains had no significant change upon application of CC. Compared
 279 with T0, the K content in rice was decreased by 9% and the Ca content was increased
 280 by 12% by spraying 1.2 mmol L⁻¹ CC.

281 The effect of leaf-spraying CC on the linear correlation coefficient and
 282 significance of essential elements and in early rice grains and late rice grains was
 283 shown in Fig 2. In early rice grains, Cd content had a significant negative correlation
 284 with K (R = -0.90) and Mg (R = -0.92) and a significant positive correlation with Mn
 285 (R = 0.95). In late rice grains, Cd content was negatively correlated with Mg (R =
 286 -0.93) and positively correlated with Mn (R = 0.90).



287 **Fig. 2** Pearson's correlation analysis of Cd content and essential elements in filed early rice grains
 288 (a) and in filed late rice grains (b). The upper part of the table showed the correlation coefficient
 289 between different elements, and the lower part showed the p-value of significant correlation

290 between different elements ($p < 0.05$).

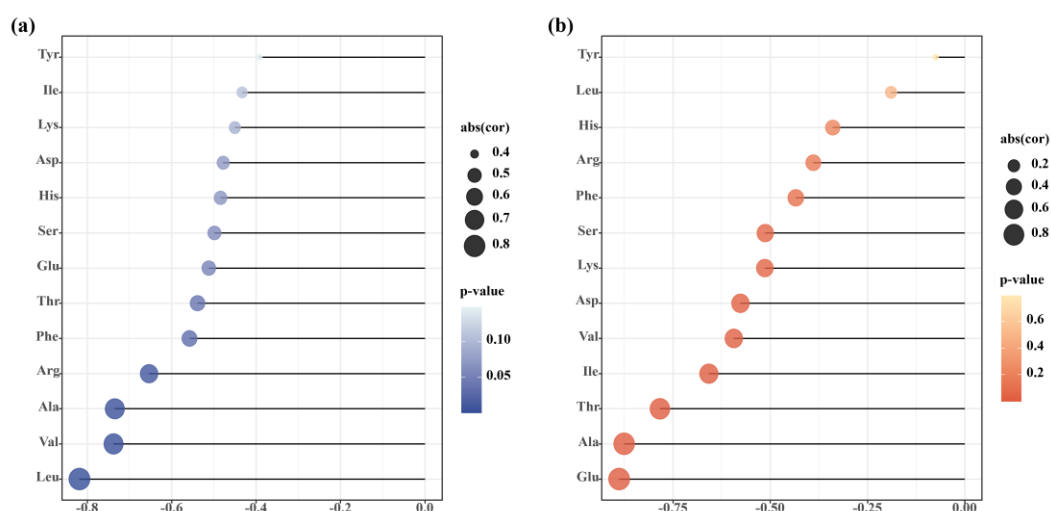
291 ***3.3 Effects of spraying CC in field on total amino acid content of rice grains***

292 Under Cd stress, the content of amino acids in rice grains was generally
293 increased by spraying CC at the flowering stage. The content of glutamate in early
294 rice grains was the highest with $10.89 \text{ g}\cdot\text{kg}^{-1}$ after the application of CC. Valine and
295 aspartic acid followed, while histidine content was the lowest. Compared with T0,
296 leaf spraying with $0.8 \text{ mmol}\cdot\text{L}^{-1}$ CC significantly increased the content of aspartic
297 acid (11%), glutamate (15%), threonine (68%), arginine (33%), alanine (27%),
298 tyrosine (25%), valine (20%), phenylalanine (18%), leucine (50%), and lysine (37%).
299 While the content of serine, histidine, and isoleucine had no significant change (Fig.
300 S1a and Fig. S1b).

301 The highest content of valine was $9.99 \text{ g}\cdot\text{kg}^{-1}$ in late rice grains, followed by
302 glutamate and aspartic acid, and the lowest content of histidine. Compared with T0,
303 leaf spraying with 1.2 mmol L^{-1} CC significantly increased the content of aspartic
304 acid (18%), glutamate (30%), threonine (103%), arginine (33%), alanine (81%),
305 valine (9%), phenylalanine (35%), isoleucine (91%), and lysine (39%). In particular,
306 the content of serine, histidine, and tyrosine did not change significantly (Fig. S1c and
307 Fig. S1d).

308 The correlation between different amino acids and Cd was different after
309 spraying CC (Fig. 3). Pearson's correlation analysis showed that Cd content in rice
310 grains was negatively correlated with amino acids content. In early rice grains, the
311 content of leucine, valine, alanine, arginine, phenylalanine and threonine was

312 significantly correlated with the content of Cd. Besides alanine, threonine and valine,
 313 glutamate, isoleucine and aspartic acid content in late rice was also significantly
 314 correlated with Cd content. The results suggested that amino acid synthesis might
 315 play a vital role in the reduction by CC of Cd accumulation in rice grains.

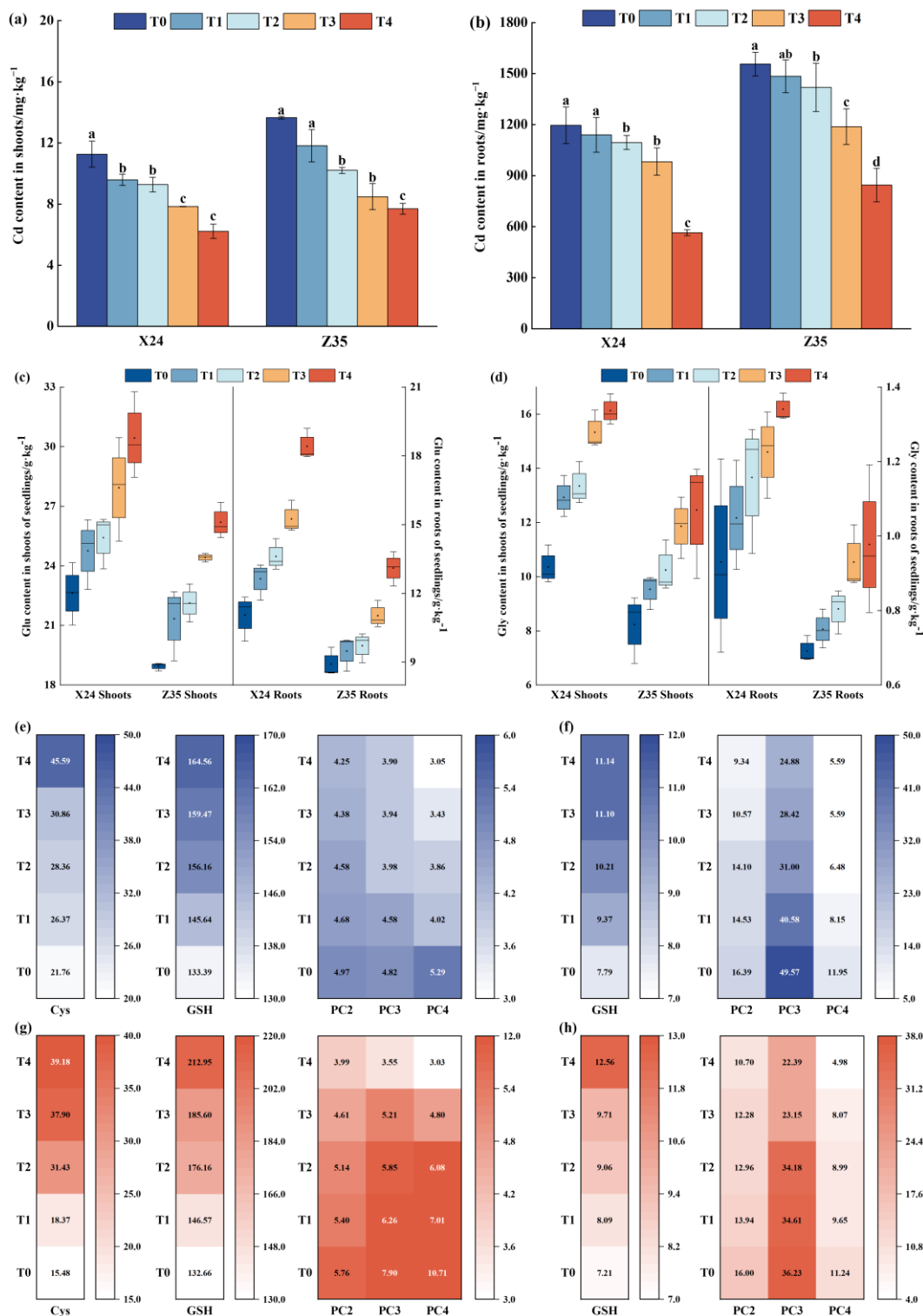


316 **Fig. 3** Pearson's correlation analysis of Cd content and amino acids content in filed early rice
 317 grains (a) and in filed late rice grains (b).

318 *3.4 Effects of CC application on Cd, Glu, Gly and sulphydryl compounds content in* 319 *hydroponic seedlings*

320 The effects of CC on Cd concentration in shoots and roots of hydroponic
 321 seedlings were shown in Fig. 4 a-b. The results showed that the application of 0.2-1.2
 322 mmol L⁻¹ CC significantly reduced the content of Cd in shoots and roots of
 323 hydroponic seedlings. The accumulation of Cd in shoots was much lower than that in
 324 roots. The highest inhibition efficiency on Cd accumulation in X24 and Z35 was
 325 observed upon addition of 1.2 mmol L⁻¹ of CC. By so doing, Cd content in shoots of
 326 X24 and Z35 hydroponic seedlings reduced by 45% and 44%, respectively.
 327 Correspondingly, the content of Cd in hydroponic seedling roots underwent

328 reductions by 53% (X24) and 46% (Z35).



329 **Fig. 4** Effects of CC on Cd content in shoots (a) and roots (b) of hydroponic seedlings, and Glu (c),
 330 Gly (d) content in hydroponic seedlings. Effects of CC on sulfhydryl compounds content in shoots
 331 (e) and roots (f) of X24 hydroponic seedlings and in shoots (g) and roots (h) of Z35 hydroponic

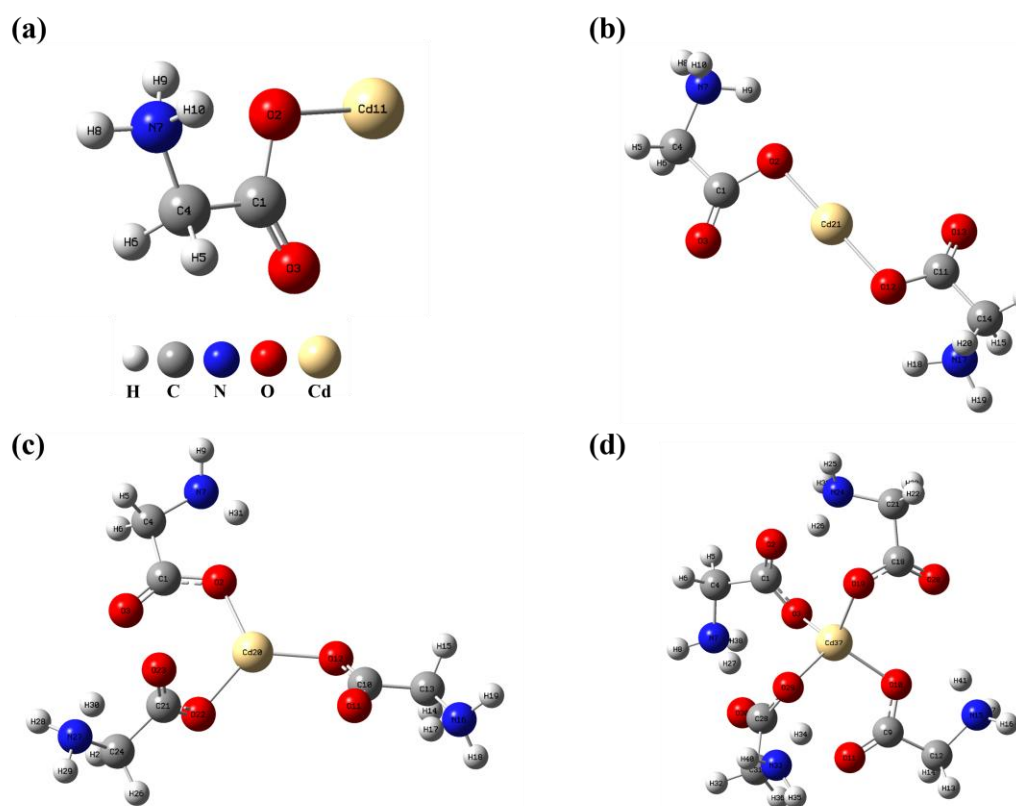
332 seedlings.

333 The content of Glu, Gly, Cys, GSH, and PCs (PC₂, PC₃, PC₄, where PC_n = (Glu-
334 Cys)_n-Gly) in the shoots and roots of hydroponic seedlings under Cd stress was
335 investigated (Fig. 4 c-h). Compared with the control, CC significantly increased the
336 content of Glu, Gly, Cys and GSH in X24 and Z35 hydroponic seedlings, while the
337 content of PC₂, PC₃, and PC₄ decreased. The content of PC₃ was higher than that of
338 PC₂ and PC₄. The highest modification in sulfhydryl compounds was observed upon
339 application of 1.2 mmol·L⁻¹ CC. In X24 hydroponic seedlings, the content of Glu,
340 Gly, GSH increased by 35%, 56%, 17% in shoots and by 67%, 44%, 43% in roots,
341 while PC₄ decreased by 27% in shoots and PC₃ decreased by 50% in roots. In Z35
342 hydroponic seedlings, the content of Glu, Gly, GSH increased by 38%, 51%, 17% in
343 shoots and by 47%, 41%, 43% in roots, while PC₄ decreased by 72% in roots and PC₃
344 by 38% in roots.

345 ***3.5 Molecular model of carboxymethylammonium and Cd complex***

346 The carboxyl group in CC may provide a site for the chelation of Cd. The
347 molecular models of carboxymethylammonium and Cd chelate were established and
348 optimized by Gaussian 16. The results showed that carboxymethylammonium can
349 form a complex through different chelation of O atom on the carboxylic group with
350 Cd (Fig. 5). The distance between the O atom of carboxymethylammonium and Cd
351 was shown in Table S3. When the complex was [Gly]⁺₃-Cd, the distances between O
352 and Cd were the shortest, which were 2.01 Å, 2.00 Å and 2.01 Å respectively. The

353 distance between O and Cd in $[\text{Gly}]_2^+-\text{Cd}$ and $[\text{Gly}]_4^+-\text{Cd}$ was 2.07 Å, while the
354 distance in $[\text{Gly}]^+-\text{Cd}$ was 2.03 Å.

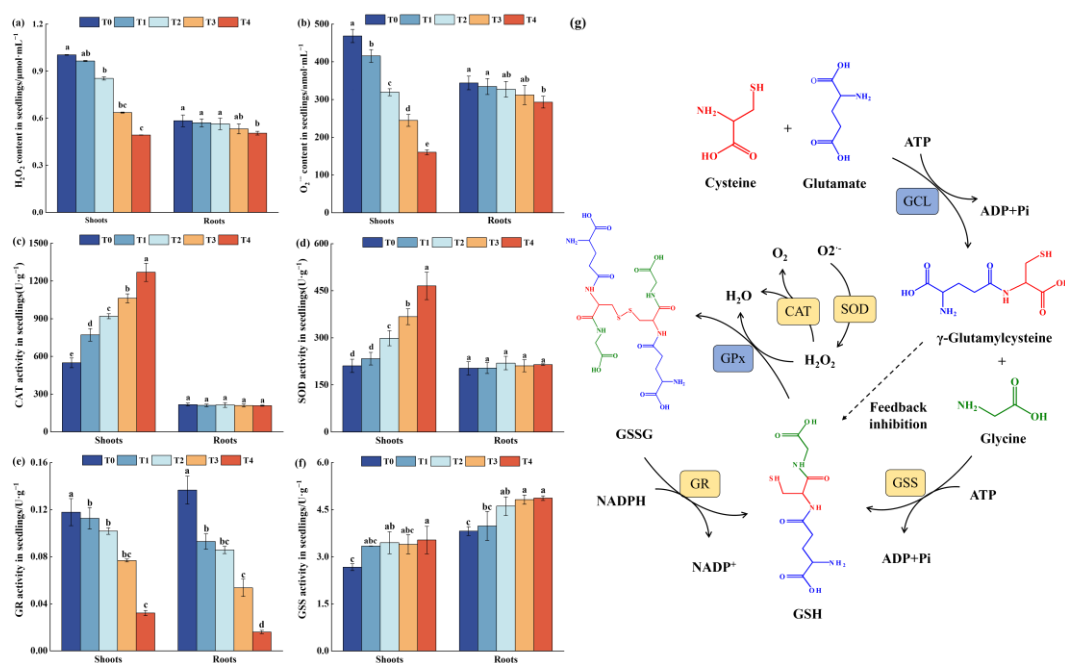


355 **Fig. 5** Different molecular geometric of carboxymethylammonium and Cd complexes, $[\text{Gly}]^+-\text{Cd}$
356 (a), $[\text{Gly}]_2^+-\text{Cd}$ (b), $[\text{Gly}]_3^+-\text{Cd}$ (c), $[\text{Gly}]_4^+-\text{Cd}$ (d).

357 **3.6 Effects of CC application on antioxidant system in hydroponic seedlings**

358 The change of Cd, Glu, Gly and sulfhydryl compounds content was similar in
359 X24 and Z35 hydroponic seedlings after CC application. Therefore, the Z35 was
360 selected (consistent with field trials) for further determination of antioxidant system
361 indicators. Exposure of plants to Cd can induce generation of ROS, which might
362 cause oxidative stress. Fig. 6 a and Fig. 6 b showed the H_2O_2 and $\text{O}_2^{\cdot-}$ content in
363 shoots and roots of hydroponic seedlings under Cd stress. In the presence of 2.7 μmol
364 L^{-1} Cd, the content of H_2O_2 and $\text{O}_2^{\cdot-}$ in shoots and roots of hydroponic seedlings was

365 the highest but they decreased upon application of CC. In the presence of 1.2 mmol
 366 L⁻¹ of CC, H₂O₂ in hydroponic seedlings decreased by 51% (shoots) and 13% (roots),
 367 while O₂⁻ decreased by 66% (shoots) and 15% (roots).



368 **Fig. 6** Effects of CC on H₂O₂ (a), O₂⁻ (b) content and CAT (c), SOD (d), GR (e), GSS (f)
 369 activities in Z35 hydroponic seedlings and the biological processes involved in these enzymes (g).
 370 Different lowercase letters (a, b, c, d, e) indicate significant differences between treatments
 371 (*p*<0.05).

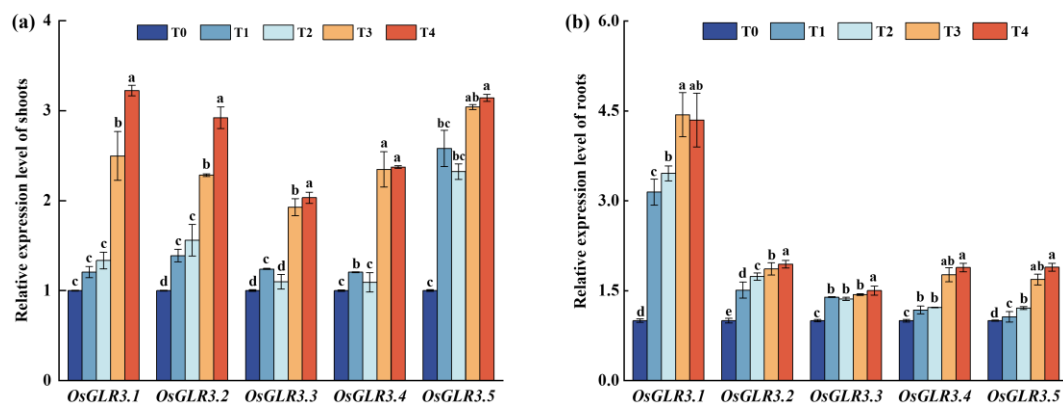
372 The effects of different concentrations of CC on the activities of SOD, CAT, GR,
 373 and GSS in hydroponic seedlings under Cd stress were also studied (Fig. 6 c-f).
 374 Compared with T0, CAT activity was increased by 41-131%, SOD activity was
 375 increased by 11-121%, GR activity was decreased by 4.5-73%, and GSS activity was
 376 increased by 25-32% in shoots of hydroponic seedlings after CC application. At the
 377 same time, the GR activity in roots of hydroponic seedling decreased by 32-88%, and
 378 the GSS activity increased by 4.3-27%. In all previous cases the highest increase or

379 decrease was observed upon application of 1.2 mmol L⁻¹ CC, but the application of
 380 this compound did not modify significantly the activities of SOD and CAT in the roots
 381 of hydroponic seedling ($p>0.05$).

382 In conclusion, the application of CC obviously inhibited the generation of
 383 reactive oxygen species and promoted the activities of antioxidant enzymes, thereby
 384 alleviating the oxidative stress in hydroponic seedlings under Cd stress.

385 *3.7 Effects of CC application on expression of OsGLR3.1-3.5 in shoots and roots of* 386 *hydroponic seedlings*

387 The effects of CC on the expression of *OsGLR3.1-3.5* in Z35 hydroponic
 388 seedlings were shown in Fig. 7. With the application of different concentrations of CC,
 389 the relative expression levels of *OsGLR3.1-3.5* isogenies were significantly increased.
 390 In particular, with 1.2 mmol·L⁻¹ CC, the relative expression level of *OsGLR3.1-3.5* in
 391 shoots increased by, respectively, 222%, 192%, 103%, 137%, and 214%. The
 392 expression level in roots system increased by 435%, 194%, 150%, 189%, and 189%,
 393 respectively.



394 **Fig. 7** Effects of CC on the relative expression of *OsGLR3.1-3.5* in shoots (a) and roots (b) of Z35
 395 hydroponic seedlings. Different lowercase letters (a, b, c, d) indicate significant differences

396 between treatments ($p < 0.05$).

397 **4. Discussion**

398 ***4.1 Exogenous CC promoted the chelation to reduce Cd bioactivity in rice by*** 399 ***increasing chelates such as Gly, Glu and GSH***

400 Rice cytoplasm contains abundant organic ligands that can chelate Cd to reduce
401 the bioactivity of Cd (Zhang et al., 2009; Ricachenevsky et al., 2018; Hazama et al.,
402 2015). Among them, Glu could form stable chelates with Cd through carboxyl oxygen
403 atoms (Xue et al., 2022). Gly could generate Glu by transferring amino groups to α -
404 Ketoglutaric acid. Glu and Gly are precursors for the synthesis of GSH and PCs
405 (Zhang et al., 2008; Hasan et al., 2016). GSH and PC₂ form chelates or complexes
406 with Cd through the S atom on sulfhydryl group and fix Cd in vacuoles (Xue et al.,
407 2023; Hasanuzzaman et al., 2017; Noctor et al., 2012). In the present study, CC
408 significantly increased the content of Gly, Glu and GSH in rice, which was conducive
409 to Cd chelation and decreased Cd activity. CC significantly decreased PCs content in
410 rice, indicating that CC alleviated the induction effect of high Cd stress on the
411 synthesis of PCs. The quantum computational chemistry was used to clarify that the
412 Gly radical provided by CC could form various complexes with Cd through carboxyl
413 oxygen atoms. These indicated that exogenous CC promoted the chelation to reduce
414 Cd bioactivity in rice by increasing chelates such as Gly, Glu and GSH.

415 ***4.2 Exogenous CC improved the tolerance of rice to Cd by enhancing the*** 416 ***antioxidant capacity***

417 As biological functional macromolecular proteins, CAT and SOD are key

418 enzymes in rice for clearing ROS such as H₂O₂ and O₂^{·-} (Pandey et al., 2016). Amino
419 acids are the basic unit of protein composition (Yang et al., 2022). Cd stress
420 significantly reduced the amino acid content in rice, thereby interfering with protein
421 synthesis (Yuan et al., 2020; Zhao et al., 2020); It also attacked the amino acid
422 residues of antioxidant enzymes to destroy their structure and reduce enzyme activity
423 (Xue et al., 2023). Therefore, ensuring the normal metabolism of amino acids is a
424 prerequisite for increasing the quantity and activity of antioxidant enzymes, and is the
425 key to enhancing the antioxidant capacity of rice. In the present study, CC
426 significantly alleviated the interference of Cd stress on amino acid metabolism and
427 significantly increased the synthesis of amino acids, thereby increasing the bioactivity
428 of CAT and SOD, and clearing Cd-induced excess H₂O₂ and O₂^{·-}.

429 The antioxidant defense system not only includes enzymatic systems, but also
430 non-enzymatic systems (Li et al., 2014; Gill et al., 2010). As an important indicator in
431 non-enzymatic systems, GSH is a ubiquitous Cys-containing tripeptide (γ -Glu-Cys-
432 Gly) that functions as an antioxidant in redox control in plants (Teng et al., 2024). Glu,
433 Cys and Gly are the precursors for the synthesis of GSH (Cao et al., 2018). In the
434 present study, the increase of Glu, Cys and Gly significantly promoted the increase of
435 GSH, which further promoted the reduction of ROS.

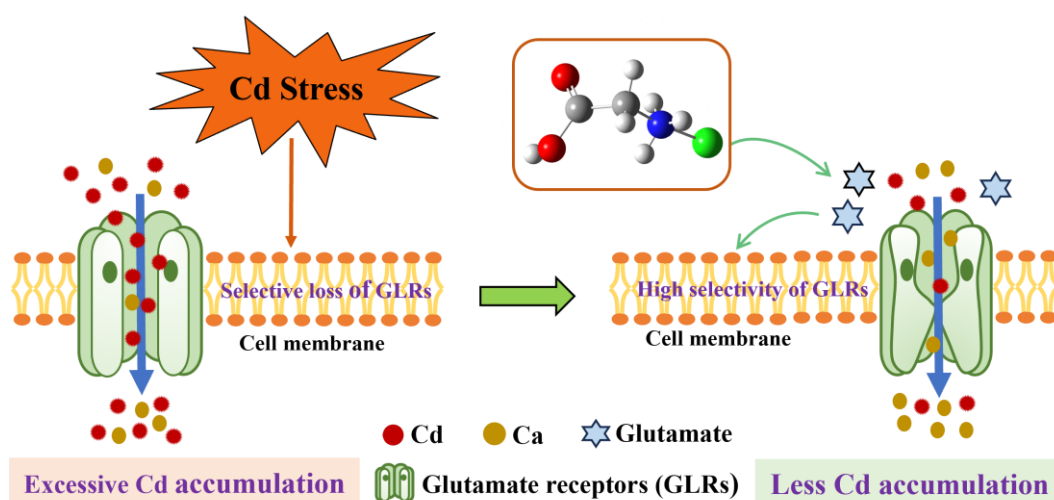
436 ***4.3 Exogenous CC inhibited the absorption and transport of Cd in rice by***
437 ***increasing GLRs activity through upregulating the synthesis of Glu, Gly and the***
438 ***expression of OsGLR3.1-3.5***

439 As the most widely distributed NSCCs in the plant cell protoplasmic membrane,

440 GLRs play an important role in mineral elements uptake and transport, for example,
441 essential elements such as Ca^{2+} , Mg^{2+} , K^+ , NH_4^+ , Zn^{2+} and Mn^{2+} , and heavy metal
442 such as Cd^{2+} (Gangwar et al., 2021; Zhang et al., 2023a). The transport rate of
443 beneficial elements by GLRs is significantly higher than that of harmful elements, and
444 the recognition and screening ability of GLRs for beneficial and harmful elements
445 depends on its activity (Zhang et al., 2023a; Zhang et al., 2023b). Reports have shown
446 that the ratio of Cd transported by GLRs to essential mineral elements transported by
447 GLRs in LCA rice with high activity of GLRs is much lower than that in HCA rice
448 with low activity of GLRs. GLRs for permeation of $\text{Ca}^{2+}/\text{Mn}^{2+}/\text{Fe}^{2+}/\text{Zn}^{2+}$ over Cd^{2+} in
449 LCA rice is much higher than that in HCA rice. High Cd stress leads to the loss of
450 GLRs selectivity, resulting in excessive accumulation of Cd in rice (Zhang et al.,
451 2023a; Zhang et al., 2023b). These indicated that increasing the activity of GLRs can
452 promote beneficial elements but inhibit Cd uptake and transport in rice.

453 Signal molecules such as Gly, Glu, and GSH can act as ligands to activate GLRs,
454 and triggers long-distance calcium-based plant defense signals during injury defense,
455 Ca^{2+} influx is an important manifestation of GLRs activation (Simon et al., 2023;
456 Toyota et al., 2018). *OsGLR3* is also involved in the regulation of GLRs activity,
457 studies have shown that the expression level of *OsGLR3* family genes (*OsGLR3.1-3.5*)
458 is negatively correlated with Cd content and positively correlated with the activity of
459 GLRs in plants (Zhou et al., 2023; Zhang et al., 2023a; Zhang et al., 2023b). In this
460 study, CC significantly increased the content of Gly, Glu, GSH, Ca, and the relative
461 expression level of *OsGLR3.1-3.5* in rice, all of which were induced to be

462 downregulated by Cd under single Cd treatment. These results indicate that CC not
 463 only increased the competitive effect of Ca on Cd, but also increased the activity of
 464 GLRs in rice. It was further proved that exogenous CC inhibited the absorption,
 465 transport and accumulation of Cd in rice by increasing GLRs activity through
 466 upregulating the synthesis of Glu, Gly and the expression of *OsGLR3.1-3.5* (Fig. 8).



467 **Fig. 8** Effects of CC on glutamate receptors selectivity in rice cell membrane.

468 5. Conclusion

469 Field experiments showed that CC spraying could effectively reduce Cd content
 470 in rice grains, significantly increase Ca content and amino acids content in rice grains,
 471 and reduce the oxidative damage of Cd in rice leaves. Hydroponic experiments further
 472 verified the inhibitory effect of CC on Cd accumulation in rice seedlings. Exogenous
 473 application of CC significantly increased the content of Glu, Gly and GSH, the
 474 activities of CAT and POD, and the expression levels of *OsGLR3.1-3.5* in hydroponic
 475 seedlings. CC also provided Gly radicals that can be chelated with Cd through
 476 carboxyl oxygen atoms. In summary exogenous application of CC can improve the
 477 tolerance of rice to Cd by enhancing antioxidant capacity. Moreover, by promoting

478 chelation and up-regulating Glu, Gly content and *OsGLR3.1-3.5* expression, GLRs
479 activity was increased, and Cd absorption, transport and accumulation were inhibited.

480

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491 **References**

- 492 Cao, Z.Z., Qin, M.L., Lin, X.Y., Zhu, Z.W., Chen, M.X., 2018. Sulfur supply reduces
493 cadmium uptake and translocation in rice grains (*Oryza sativa* L.) by enhancing
494 iron plaque formation, cadmium chelation and vacuolar sequestration. *Environ.*
495 *Pollut.* 238, 76–84.
- 496 Chen, D., Ye, X.Z., Zhang, Q., Xiao, W.D., Ni, Z.Y., Yang, L., Zhao, S.P., Hu, J.,
497 Gao, N., Huang, M.J., 2020. The effect of sepiolite application on rice Cd uptake
498 – A two-year field study in Southern China. *J. Environ. Manage.* 254, 109788.

499 Chen, J.G., Zou, W.L., Meng, L.J., Fan, X.R., Xu, G.H., Ye, G.Y., 2019. Advances in
500 the uptake and transport mechanisms and qtls mapping of cadmium in rice. *Int. J.*
501 *Mol. Sci.* 20(14), 3417.

502 Chen, X.H., Ouyang, Y., Fan, Y., Qiu, B., Zhang, G.P., Zeng, F.R., 2018. The
503 pathway of transmembrane cadmium influx via calcium-permeable channels and
504 its spatial characteristics along rice root. *J. Exp. Bot.* 69(21), 5279-5291.

505 Cui, H., Tang, S.T., Huang, S.Q., Lei, L.D., Jiang, Z.M., Li, L., Wei, S.Q., 2022.
506 Simultaneous mitigation of arsenic and cadmium accumulation in rice grains by
507 foliar inhibitor with ZIF-8@Ge-132. *Sci. Total Environ.* 860: 160307.

508 Demidchik, V., Maathuis, F.J.M., 2007. Physiological roles of nonselective cation
509 channels in plants: from salt stress to signalling and development. *New Phytol.*
510 175(3), 387-404.

511 Deng, Y., Wang, S., Beadham, I., Gao, X., Ji, M.M, Wang, G., Zhang, C.B., Ruan,
512 W.Q., 2023. Effect of soil washing with an amino-acid-derived ionic liquid on
513 the properties of cd-contaminated paddy soil. *Toxics.* 11(3), 288.

514 Feng, X.M., Han, L., Chao, D.Y., Liu, Y., Zhang, Y.J., Wang, R.G., Guo, J.K., Feng,
515 R.W., Xu, Y.M., Ding, Y.Z., Huang, B.Y, Zhang, G.L., 2017. Ionomics and
516 transcriptomic analysis provides new insight into the distribution and transport of
517 cadmium and arsenic in rice. *J. Hazard. Mater.* 331, 246-256.

518 Gallego, S.M., Pena, L.B., Barcia, R.A., Azpilicueta, C.E., Iannone, M.F., Rosales,
519 E.P., Zawoznik, M.S., Groppa, M.D., Benavides, M.P., 2012. Unravelling

520 cadmium toxicity and tolerance in plants: insight into regulatory mechanisms.
521 Environ. Exp. Bot. 83, 33–46.

522 Gangwar, S.P, Green, M.N, Michard, E., Simon, A.A., Feijó, J.A, Sobolevsky, A.I.,
523 2021. Structure of the arabidopsis glutamate receptor-like channel glr3.2 ligand-
524 binding domain. Structure. 29(2), 161-169.

525 Gao, M.L., Xu, Y.L., Chang, X.P., Dong, Y.M, Song, Z.G, 2020. Effects of foliar
526 application of graphene oxide on cadmium uptake by lettuce. J. Hazard. Mater.
527 398, 122859.

528 Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in
529 abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48(12), 909-930.

530 Han, X.X., Zhang, C.B., Wang, C.R., Huang, Y.C., Liu, Z.Q., 2019. Gadolinium
531 inhibits cadmium transport by blocking non-selective cation channels in rice
532 seedlings. Ecotoxicol. Environ. Saf. 179 (15), 160-166.

533 Hasan, M.K., Liu, C.C., Wang, F.N., Ahammed, G.J., Zhou, J., Xu, M., Yu, J.Q., Xia,
534 X.J., 2016. Glutathione-mediated regulation of nitric oxide, S-nitrosothiol and
535 redox homeostasis confers cadmium tolerance by inducing transcription factors
536 and stress response genes in tomato. Chemosphere. 161, 536-545.

537 Hasanuzzaman, M., Nahar, K., Anee, T.I., Fujita, M., 2017. Glutathione in plants:
538 biosynthesis and physiological role in environmental stress tolerance. Physiol.
539 Mol. Biol. Plants. 23(2), 249-268.

540 Hazama, K., Nagata, S., Fujimori, T., Yanagisawa, S., Yoneyama, T., 2015.
541 Concentrations of metals and potential metal-binding compounds and speciation

542 of Cd, Zn and Cu in phloem and xylem saps from castor bean plants (*Ricinus*
543 *communis*) treated with four levels of cadmium. *Physiol. Plant.* 154(2), 243-255.

544 Ismael, M.A., Elyamine, A.M., Moussa, M.G., Cai, M.M., Zhao, X.H., Hu, C.X.,
545 2019. Cadmium in plants: uptake, toxicity, and its interactions with selenium
546 fertilizers. *Metallomics.* 11(2), 255-277.

547 Jiang, M., Jiang, J., Li, S., Li, M., Tan, Y.Y., Song, S.Y., Shu, Q.Y., Huang, J.Z.,
548 2020. Glutamate alleviates cadmium toxicity in rice via suppressing cadmium
549 uptake and translocation. *J. Hazard. Mater.* 384, 121319.

550 Jing, H.A., Yang, W.T., Chen, Y.L., Yang, L.Y., Zhou, H., Yang, Y., Zhao, Z.J., Wu,
551 P., Zia-ur-Rehman, M., 2023. Exploring the mechanism of Cd uptake and
552 translocation in rice: Future perspectives of rice safety. *Sci. Total Environ.* 897,
553 165369.

554 Kulsum, P.G.P.S., Khanam, R., Das, S., Nayak, A.K., Tack, F.M.G., Meers, E.,
555 Vithanage, M., Shahid, M., Kumar, A., Chakraborty, S., Bhattacharya, T.,
556 Biswas, J.K., 2023. A state-of-the-art review on cadmium uptake, toxicity, and
557 tolerance in rice: From physiological response to remediation process. *Environ.*
558 *Res.* 220, 115098.

559 Li, H., Liu, Y., Zeng, G., Zhou, L., Wang, X., Wang, X.Q., Wang, C.L., Hu, X.J., Xu,
560 W.H., 2014. Enhanced efficiency of cadmium removal by *Boehmeria nivea* (L.)
561 *Gaud.* in the presence of exogenous citric and oxalic acids. *J. Environ. Sci.*
562 26(12), 2508-2516.

563 Li, H., Luo, N., Li, Y.W., Cai, Q.Y., Li, H.Y., Mo, C.H., Wong, M.H., 2017.
564 Cadmium in rice: Transport mechanisms, influencing factors, and minimizing
565 measures. *Environ. Pollut.* 224, 622-630.

566 Li, Y.H., Yu, X.Z., Mo, L.Y., Lin, Y.J., Zhang, Q., 2019. Involvement of glutamate
567 receptors in regulating calcium influx in rice seedlings under Cr exposure.
568 *Ecotoxicology.* 28 (6), 650-657.

569 Liao, H.S., Chung, Y.H., Hsieh, M.H., 2022. Glutamate: A multifunctional amino
570 acid in plants. *Plant Science*, 318: 111238.

571 Ni, J., Yu, Z.M., Du, G.K., Zhang, Y.Y., Taylor, J.L., Shen C.J., Xu, J., Liu, X.Y.,
572 Wang, Y.F., Wu, Y.R., 2016. Heterologous expression and functional analysis of
573 rice GLUTAMATE RECEPTOR-LIKE family indicates its role in glutamate
574 triggered calcium flux in rice roots. *Rice.* 9(1), 9.

575 Noctor, G., Mhamdi, A., Chaouch, S., Han, Y., Neukermans, J., Marquez-Garcia, B.,
576 Queval, G. Foyer, C. H., 2012. Glutathione in plants: an integrated overview.
577 *Plant Cell Environ.* 35(2), 454-484.

578 Ortiz-Ramírez, C., Michard, E., Simon, A.A., Damineli, D.S.C., Hernández-Coronado,
579 M., Becker, J.D., Feijó, J.A., 2017. Glutamate RECEPTOR-LIKE channels are
580 essential for chemotaxis and reproduction in mosses. *Nature.* 549(7670), 91-95.

581 Pál, M., Janda, T., Szalai, G., 2018. Interactions between plant hormones and thiol-
582 related heavy metal chelators. *Plant Growth Regul.* 85(2), 173-185.

583 Pandey, P., Srivastava, R.K., Rajpoot, R., Rani, A., Pandey, A.K., Dubey, R.S., 2016.
584 Water deficit and aluminum interactive effects on generation of reactive oxygen

585 species and responses of antioxidative enzymes in the seedlings of two rice
586 cultivars differing in stress tolerance. *Environ. Sci. Pollut. Res.* 23(2), 1516-1528.

587 Qi, Z., Stephens, N.R., Spalding, E.P., 2006. Calcium entry mediated by GLR3.3, an
588 *Arabidopsis* glutamate receptor with a broad agonist profile. *Plant Physiol.*
589 142(3), 963-971.

590 Ricachenevsky, F.K., de Araujo, A.T., Fett, J.P., Sperotto, R.A., 2018. You shall not
591 pass: root vacuoles as a symplastic checkpoint for metal translocation to shoots
592 and possible application to grain nutritional quality. *Front. Plant Sci.* 9, 412.

593 Sasaki, A., Yamaji, N., Yokosho, K., Ma, J.F., 2012. Nramp5 is a major transporter
594 responsible for manganese and cadmium uptake in rice. *The Plant cell.* 24(5),
595 2155-2167.

596 Shao, Q.L., Gao, Q.F., Lhamo, D., Zhang, H.S., Luan, S., 2020. Two glutamate- and
597 pH-regulated Ca^{2+} channels are required for systemic wound signaling in
598 *Arabidopsis*. *Sci. Signaling.* 13(640), eaba1453.

599 Shimo, H., Ishimaru, Y., An, G., Yamakawa, T., Nakanishi, H., Nishizawa, N.K.,
600 2011. Low cadmium (LCD), a novel gene related to cadmium tolerance and
601 accumulation in rice. *J. Exp. Bot.* 62(15), 5727-5734.

602 Simon, A.A., Navarro-Retamal, C., Feijó, J.A., 2023. Merging signaling with
603 structure: functions and mechanisms of plant glutamate receptor ion channels.
604 *Annu. Rev. Plant Biol.* 74, 415-452.

605 Tan, L.T., Qu, M.M., Zhu, Y.X., Peng, C., Wang, J.R., Gao, D.Y., Chen, C.Y., 2020.
606 ZINC TRANSPORTER5 and ZINC TRANSPORTER9 function synergistically
607 in zinc/cadmium uptake. *Plant Physiol.* 183(3), 1235-1249.

608 Teng, Y., Yang, Yan., Wang, Z.J., Guan, W.J., Liu, Y.T., Yu, H.Y., Zou, L.Y., 2024.
609 The cadmium tolerance enhancement through regulating glutathione conferred
610 by vacuolar compartmentalization in *Aspergillus sydowii*. *Chemosphere.* 352,
611 141500.

612 Toyota, M., Spencer, D., Sawai-Toyota, S., Wang, J.Q., Zhang, T., Koo, A.J, Howe,
613 G.A., Gilroy, S., 2018. Glutamate triggers long-distance, calcium-based plant
614 defense signaling. *Science*, 361(6407): 1112-1115.

615 Tripathi, P., Mishra, A., Dwivedi, S., Chakrabarty, D., Trivedi, P.K., Singh, R.P.,
616 Tripathi, R.D., 2012. Differential response of oxidative stress and thiol
617 metabolism in contrasting rice genotypes for arsenic tolerance. *Ecotoxicol.*
618 *Environ. Saf.* 79, 189-198.

619 Uruguchi, S., Kamiya, T., Sakamoto, T., Kasai, K., Sato, Y., Nagamura, Y., Yoshida,
620 A., Kyojuka, J., Ishikawa, S., Fujiwara, T., 2011. Low-affinity cation transporter
621 (OsLCT1) regulates cadmium transport into rice grains. *Proc. Natl. Acad. Sci. U.*
622 *S. A.* 108(52), 20959-20964.

623 Vatsa, P., Chiltz, A., Bourque, S., Wendehenne, D., Garcia-Brugger, A., Pugin, A.,
624 2011. Involvement of putative glutamate receptors in plant defence signaling and
625 NO production. *Biochimie.* 93(12), 2095-2101.

626 Wang, K.J., Yu, H.Y., Ye, D.H., Wang, Y.D., Zhang, X.Z., Huang, H.G., Zheng, Z.C.,
627 Li, T.X., 2021. The critical role of the shoot base in inhibiting cadmium transport
628 from root to shoot in a cadmium-safe rice line (*Oryza sativa* L.). *Sci. Total*
629 *Environ.* 765, 142710.

630 Wang, P., Chen, H.P., Kopittke, P.M., Zhao, F.J., 2019. Cadmium contamination in
631 agricultural soils of China and the impact on food safety. *Environ. Pollut.* 249,
632 1038-1048.

633 Xue, W.J., Zhang, C.B., Huang, Y.C., Wang, C.R., Zhang, X., Liu, Z.Q., 2022. Rice
634 organs concentrate cadmium by chelation of amino acids containing dicarboxyl
635 groups and enhance risks to human and environmental health in Cd-
636 contaminated areas. *J. Hazard. Mater.* 426, 128130.

637 Xue, W.J., Zhang, C.B., Wang, P.P., Wang, C.R., Huang, Y.C., Zhang, X., Liu, Z.Q.,
638 2019. Rice vegetative organs alleviate cadmium toxicity by altering the chemical
639 forms of cadmium and increasing the ratio of calcium to manganese. *Ecotoxicol.*
640 *Environ. Saf.* 184, 109640.

641 Xue, W.J., Zhang, X., Zhang, C.B., Wang, C.R., Huang, Y.C., Liu, Z.Q., 2023.
642 Mitigating the toxicity of reactive oxygen species induced by cadmium via
643 restoring citrate valve and improving the stability of enzyme structure in rice.
644 *Chemosphere.* 327: 138511.

645 Yan, H.L., Xu, W.X., Xie, J.Y., Gao, Y.W., Wu, L.L., Sun, L., Feng, L., Chen, X.,
646 Zhang, T., Dai, C.H., Li, T., Lin, X.N., Zhang, Z.Y., Wang, X.Q., Li, F.M., Zhu,
647 X.Y., Li, J.J., Li, Z.C., Chen, C.Y., Ma, M., Zhang, H.L., He, Z.Y., 2019.

648 Variation of a major facilitator superfamily gene contributes to differential
649 cadmium accumulation between rice subspecies. *Nat. Commun.* 10, 2562.

650 Yan, J.L., Wang, P.T., Wang, P., Yang, M., Lian, X.M., Tang, Z., Huang, C.F., Salt,
651 D.E., Zhao, F.J., 2016. A loss-of-function allele of *OsHMA3* associated with
652 high cadmium accumulation in shoots and grain of Japonica rice cultivars. *Plant*
653 *Cell Environ.* 39(9), 1941-1954.

654 Yang, J.C., Zhou, Y.J., Jiang, Y., 2022. Amino acids in rice grains and their
655 regulation by polyamines and phytohormones. *Plants.* 11(12), 1581.

656 Yang, X.R., Wang, C.R., Huang, Y.C., Liu, B., Liu, Z.Q., Huang, Y.Z., Cheng, L.L.,
657 Huang, Y.F., Zhang C.B., 2021. Foliar application of the sulfhydryl compound
658 2,3-dimercaptosuccinic acid inhibits cadmium, lead, and arsenic accumulation in
659 rice grains by promoting heavy metal immobilization in flag leaves. *Environ.*
660 *Pollut.* 285, 117355.

661 Yu, H.Y., Guo, J.Y., Li, Q., Zhang, X.Z., Huang, H.G., Huang, F., Yang, A.Q., Li,
662 T.X., 2020b. Characteristics of cadmium immobilization in the cell wall of root
663 in a cadmium-safe rice line (*Oryza sativa* L.). *Chemosphere.* 241, 125095.

664 Yu, H.Y., Wu, Y., Huang, H.G., Zhan, J., Wang, K.J., Li, T.X., 2020a. The
665 predominant role of pectin in binding Cd in the root cell wall of a high Cd
666 accumulating rice line (*Oryza sativa* L.). *Ecotoxicol. Environ. Saf.* 206, 111210.

667 Yuan, K., Wang, C.R., Zhang, C.B., Huang, Y.C., Wang, P.P., Liu, Z.Q., 2020. Rice
668 grains alleviate cadmium toxicity by expending glutamate and increasing
669 manganese in the cadmium contaminated farmland. *Environ. Pollut.* 262: 114236.

670 Zhang, C.H., Ge, Y., 2008. Response of glutathione and glutathione s-transferase in
671 rice seedlings exposed to cadmium stress. *Rice Science*.15(1), 73-76.

672 Zhang, J., Sun, W.C., Li, Z.J., Liang, Y.C., Song, A.L., 2009. Cadmium fate and
673 tolerance in rice cultivars. *Agron. Sustainable Dev.* 29(3), 483-490.

674 Zhang, X., Xue, W.J, Qi, L., Zhang, C.B., Wang, C.R., Huang, Y.C., Wang, Y.T.,
675 Peng, L.C., Liu, Z.Q., 2023b. Malic acid inhibits accumulation of cadmium, lead,
676 nickel and chromium by downregulation of *OsCESA* and up-regulation of
677 *OsGLR3* in rice plant. *Environmental pollution*, 341: 122934.

678 Zhang, X., Xue, W.J., Zhang, C.B., Wang, C.R., Huang, Y.C., Wang, Y.T., Peng,
679 L.C., Liu, Z.Q., 2023a. Cadmium pollution leads to selectivity loss of glutamate
680 receptor channels for permeation of $Ca^{2+}/Mn^{2+}/Fe^{2+}/Zn^{2+}$ over Cd^{2+} in rice plant.
681 *J. Hazard. Mater.* 452, 131342.

682 Zhao, Y.L., Zhang, C.B., Wang, C.R., Huang, Y.C., Liu, Z.Q., 2020. Increasing
683 phosphate inhibits cadmium uptake in plants and promotes synthesis of amino
684 acids in grains of rice. *Environ. Pollut.* 257: 113496.

685 Zhou, H., Zhu, W., Yang, W.T., Gu, J.F., Gao, Z.X., Chen, L.W., Du, W.Q., Zhang,
686 P., Peng, P.Q., Liao, B.H., 2018. Cadmium uptake, accumulation, and
687 remobilization in iron plaque and rice tissues at different growth stages. *Ecotox*
688 *Environ Safe*, 152, 91-97.

689 Zou, M.M., Zhou, S.L., Zhou, Y.J., Jia, Z.Y., Guo, T.W., Wang, J.X., 2021. Cadmium
690 pollution of soil-rice ecosystems in rice cultivation dominated regions in China:
691 A review. *Environ. Pollut.* 280, 116965.