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Genetic Variability among Egyptian Rice Genotypes (*Oryza sativa* L.) for Their Tolerance to Cadmium

W. F. Ghidan[1*], A. M. Elmoghazy[1], M. M. Yacout[2], M. Moussa[2] and A. E. Draz[1]

[1] *Rice Research and Training Center (RRTC), Field Crops Research Institute, Agricultural Research Center, 33717, Sakha, Kafr El-Sheikh, Egypt.*
[2] *Department of Genetics, Faculty of Agriculture, Alexandria University, Egypt.*

Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Heavy metals are significant environmental pollutants. Cadmium (Cd) is a toxic heavy metal and is also known as one of the major environmental pollutants. Therefore, study the germination ability, seedling growth performance and genetic variability of twelve Egyptian rice (*Oryza sativa* L.) genotypes in response to Cd stress.

Design: Twelve Egyptian rice genotypes are investigated for their tolerance to cadmium stress at seedling stage. Four cadmium chloride concentrations are applied *i.e.*, 0, 0.01, 0.02 and 0.04 mg/ml to the germinated rice seeds. Five traits are studied *i.e.*, germination percentage, germination index, root length, shoot length and root/shoot ratio.

Results: The results show that the most affected trait is root length in response to Cadmium stress, while germination percentage is the lowest affected trait. The studied rice genotypes show highly significant variability in their response to cadmium stress at seedling stage. The most tolerant genotypes are Giza 177 and Giza 178 for germination percentage, under cadmium stress. While, all studied Egyptian rice genotypes are highly sensitive to cadmium stress at high concentrations for all traits.

**Corresponding author: E-mail: w_ghidan@hotmail.com;*

Conclusion: It can be concluded that, highly genetic variability are observed among studied Egyptian rice genotypes for tolerance to cadmium stress. Moderate tolerance is observed for germination percentage trait, while the most sensitive trait to cadmium stress is root length.

Keywords: Cadmium tolerance; germination percentage; rice; root and shoot length; seedling.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important crops and is the staple food for nearly half the global population [1]. In Egypt, where rice is considered the second most important cereal crop after wheat [2], and also constitutes one of the country's main agricultural exports, the cultivated area with rice was 0.7 million hectares in 2013 with an average yield of 9.94 ton/ha, which is the highest productivity in the world [3].

Rice is a cereal foodstuff that forms an important part of the diet of many people worldwide. It is also known that people, especially those who take rice for daily energy are inevitably exposed to significant amounts of heavy metals because of fertilizers used in farms [4]. Rice is identified as the major source of cadmium (Cd) intake among the victims of itai-itai disease endemic in the Jinzu river basin in Japan in the mid-20th century [5]. Cadmium contamination of rice and its toxicity have also been reported in several populations in countries including China and Thailand [6,7].

Heavy metals are major environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The term ''heavy metals'' refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration [8]. Heavy metals such as Cu, Fe, Mn, Zn, Ni and As include elements with densities above 5 g cm^{-3}, but the term is extended to a vast range of metals and metalloids [9]. However, chemical properties of heavy metals are the most influencing factors compared to their density. Heavy metals include lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), chromium (Cr), arsenic (As), silver (Ag) and the platinum group elements. Environment can be defined as circumstances surrounding an organism or group of organisms especially the combination of external physical conditions that affect and influence the growth, development and survival of organisms [10]. Heavy metal accumulation in soil interrupts the normal functioning of soil ecosystems and plant growth [11,12].

Previous studies have reported that significant genotypic variations are detected in Cd, Ni and Pb concentrations of rice grains, indicating the possibility to reduce the concentrations of these heavy metals in grains through breeding approach [13]. Japonica brown rice varieties have the lowest average Cd and Pb uptake rates compared to the other two varieties namely, indica and hybrid [14].

A few metals, including Cu, Zn and Mn, are essential micronutrients required for a wide variety of physiological processes in plants [15]. Plants, like all other organisms, in order to maintain the concentration of essential metals within the physiological limits and to minimize the detrimental effects of nonessential metals, have evolved a complex network of homeostatic mechanisms that serve to control the uptake, accumulation, trafficking and detoxification of metals [16]. However, some plants can grow on soil contaminated with heavy metals and not only tolerate higher levels of metals but even hyper accumulate them [17]. This trait could be used in the process of phytoremediation to clean up contaminated soil and water [18]. However, these same metals can be toxic and inhibit growth of plants when present at excessive levels [19,20].

Cd, Ni and Pb are toxic pollutants of soils and are frequently accumulated by crops grown on soils polluted with such heavy metals. When crops are grown on heavy metal-polluted soils, these metals can enter the food chain with a significant potential to impair animal and human health [21]. The aim of this research is to study the germination ability, seedling growth performance and genetic variability of twelve Egyptian rice (*Oryza sativa* L.) genotypes in response to Cd stress.

2. MATERIALS AND METHODS

This experiment is conducted at Rice Research and Training Center (RRTC), Sakha, Kafr EL-Sheikh, Egypt and Laboratory of Genetics Department, Faculty of Agriculture, Alexandria University, during the growing seasons 2014.

Table 1. The studied twelve Egyptian rice genotypes and their pedigree and type

No	Genotypes	Pedigree	Type
1	Giza 177	Giza 171 / Yomji No.1 // Pi No.4	Japonica
2	Giza 178	Giza 175 / Milyang 49	Indica- japonica
3	Giza 179	IR 6269-12-1-2-1-1 / GZ 1368-5-5-4	Indica- japonica
4	Giza 181	IR 28 / IR 22	Indica
5	Giza 182	Giza 181/ IR 39422-163-1-2// Giza 181	Indica
6	Sakha 101	Giza 176 / Milyang 79	Japonica
7	Sakha 102	GZ 4096-7-1 / Giza 177	Japonica
8	Sakha 103	Giza 177 / Suweon 349	Japonica
9	Sakha 104	GZ 4096-8-1 / GZ 4100-9-1	Japonica
10	Sakha 105	GZ 5581-46-3 / GZ 4316-7-1-1	Japonica
11	Sakha 106	Giza 177 / Hexi 30	Japonica
12	Egyptian Yasmin	IR 262-43-8-1 / NAHNG SARN	Indica

2.1 Plant Material

The experiment included 12 different rice cultivars which are provided by initial seed increase of RRTC. Those cultivars are included 7 cultivars belong to Japonica type, 3 cultivars belong to Indicia type and 2 cultivars belong to Indicia-japonica type (above Table 1).

2.2 Treatments and Experimental Design

The experiment is arranged in factorial experiment in Randomized Complete Block Design (RCBD) with three replicates. Four different concentrations of cadmium chloride ($CdCl_2.2.5H_2O$) *i.e.* 0, 0.01, 0.02 and 0.04 mg/ml are applied. Fifty seeds of uniform size in each cultivar are allowed to germinate to primary roots of 2 mm length in a Petri dish containing a filter paper of 9 cm diameter; the Petri dishes are placed in a growth chamber for 7 days at 28±1℃, germinated seeds are selected and transferred to plastic pots (diameter 10 cm) containing quartz sand. Then, pots are transferred to the green house with day and night temperature of 25℃.

2.3 Studied Characteristics

After 14 days seedlings are selected from each replicate and then are evaluated as follows:

Germination Percentage (GP) is calculated according to the following equation [22]:

$$GP = \frac{\text{No. of germinated seeds}}{\text{Total No. of tested seeds}} \times 100$$

Germination Index (GI) is calculated according to the following equation described by [23]:

$$GI = \frac{\%\text{Germination in each treatment}}{\%\text{ Germination in the control}}$$

Root Length, RL (cm).
Shoot Length, SL (cm).
Root/Shoot Ratio, R/S.

2.4 Statistical Analysis

All obtained data are statistically analyzed according to the technique of analysis of variance (ANOVA) for the split-plot design [24], by using means of “MSTAT-C” computer software package. Least Significant Difference (LSD) method is used to test the differences between treatment means at 5% level of probability [25]. The comparison of the means is done by Duncan test at a probability level of 5 percent.

3. RESULTS AND DISCUSSION

A wide range of variability among genotypes, treatments and interactions are obtained for Germination Percentage (GP), Germination Index (GI), Root Length (RL), Shoot Length (SL) and Root/Shoot ratio (R/S) characteristics (Table 2).

Mean performances for germination percentage, germination index, root length, shoot length and root/shoot ratio are variable among studied genotypes. The results summarized in Table 3 shows highly significant differences ($P<0.05$) among the genotypes the cultivar Giza 177 shows high mean value (89.417%), while Giza 182 provides lowest mean value (71.50%) for germination percentage under cadmium stress. The analysis of variance indicates that germination percentage is significantly affected by cadmium application levels. As shown in Table 4, the untreated seedlings (control) record the high mean value of germination percentage (91.88%), while the application of cadmium with 0.04 mg/ml records the lowest mean for germination percentage (68.36%). The interactions between rice cultivars and Cd doses are highly significant for germination percentage. The results obtained as shown in Fig. 1A clearly indicates that the untreated cultivar Giza 179 gives the highest mean value (97.33%). Otherwise, the lowest mean value (28.76%) for germination percentage is obtained for the cultivar Sakha 102 germinated under 0.04 mg/ml Cd dosage. Many investigators find variations among rice cultivars in germination characters and seedling parameters [26,27,28].

Regarding Germination Index (GI), also, highly significant variations are calculated among studied rice genotypes, Giza 177 produced superior mean (0.991), while the lowest mean obtained from Giza 182 (0.757). Germination index is significantly affected by cadmium treatment (Table 3). The untreated seedlings (control) show the highly mean values of germination index, where the application of cadmium with 0.04 mg/ml shows the lowest mean values of germination index as shown in Table 4. The interaction between genotypes and Cd applications affect significantly germination index as shown in Fig. 1B. The results obtained reveal that the maximum mean value of germination index records from Giza 177 (1.007) with 0.01 mg/ml cadmium dosage. On other hand, the lowest mean of germination index (0.310) is obtained for the genotype Sakha 102 treated with 0.04 mg/ml cadmium dosage.

The root length varied significantly among cultivars and Cd application. The highest mean root length achieves from Sakha 102 (3.208 cm), where, Egyptian Yasmin rice genotype gives the lowest mean root length (1.842 cm) (Table 3). The results obtained demonstrate that, Cd applications are highly significant affected root length, since the control treatment provided the longest mean (7.297 cm), whilst the shortest mean value is (0.24 cm) obtained from 0.04 mg/ml cadmium dosage (Table 4). The rice genotypes and cadmium application interaction is highly significant for root length (Fig. 1C). The uppermost root length mean value is realized from genotype Sakha 102 (10.63 cm) with control. The change in root growth characteristics is probably due to the consequences of the direct exposure of the roots metal toxicity and preferential accumulation of metals in the emerging roots followed by slow mobility to the plant shoots [29,30]. An increase in the heavy metal concentration results in decreasing root length with stunted growth of roots. One of the explanations for roots to be more responsive to toxic metals in environment might be that roots are a specialized absorptive organ, which means that they are affected earlier and subjected to accumulation of more heavy metals than any of the other plant organs. This could be the main reason why root length is usually used as a measure for determining heavy metal tolerance of plants [31].

The highest mean values for shoot length are obtained for Sakha 106 (6.850 cm). Where, Giza 182 genotype shows the decreased mean shoot length (4.692 cm) as present in Table 3. The results obtained reveals that highly significant differences are recorded for shoot length in response to Cd application. The untreated seedlings (control) show the longest mean value of shoot length (9.667 cm), while the shortest shoot length mean value is 3.172 cm recorded for cadmium application of 0.04 mg/ml (Table 4). The rice genotypes and cadmium dosages interaction is highly significant for shoot length (Fig. 1D). The highest shoot length mean value is realized from genotypes Sakha 106 under control condition (12.33 cm). Meanwhile, the Egyptian Yasmin treated with 0.04 mg/ml Cd dosage donates the declined value 3.60 cm.

Table 2. Analyses of variance for Germination Percentage (GP), Germination Index (GI), Root Length (RL), Shoot Length (SL) and Root/Shoot ratio (R/S ratio) as affected by cadmium application

Source of variance	df	GP	GI	RL (cm)	SL (cm)	R/S ratio
Replication	2	0.44 ^{ns}	0.006 ^{ns}	0.057 ^{ns}	0.001 ^{ns}	9.34 ^{ns}
Genotypes	11	438.31 **	0.062 **	1.872 **	5.555 **	183.43 **
Treatments	3	3782.84 **	0.410 **	386.433 **	275.628 **	36730.48 **
Genotypes x treatments	33	284.76 **	0.038 **	2.575 **	2.094 **	266.64 **
Error	94	4.48	0.006	0.072	0.125	22.37

ns: Not significant; ***** *,* ****** *: Significant at 5% and 1% , respectively*

Table 3. Mean values of Germination Percentage (GP), Germination Index (GI), Root Length (RL), Shoot Length (SL) and Root/Shoot ratio (R/S ratio) for the studied twelve rice genotypes as affected by cadmium application

Genotypes	GP	GI	RL (cm)	SL (cm)	R/S ratio
Giza 177	89.417^{a}	0.991^{a}	2.625^{bc}	6.342^{b}	32.432^{de}
Giza 178	89.333^{a}	0.928^{ab}	2.275^{de}	5.425^{d}	38.373^{ab}
Giza 179	81.333^{d}	0.837^{c}	2.750^{b}	5.350^{d}	39.736^{a}
Giza 181	88.833^{a}	0.922^{ab}	2.042^{fg}	5.033^{e}	34.425^{bcd}
Giza 182	71.500^{f}	0.757^{d}	2.208^{ef}	4.692^{f}	38.025^{ab}
Sakha 101	86.417^{b}	0.951^{a}	2.750^{b}	6.267^{b}	35.193^{bcd}
Sakha 102	74.917^{e}	0.812^{cd}	3.208^{a}	6.142^{c}	36.867^{abc}
Sakha 103	75.583^{e}	0.957^{a}	2.608^{bc}	6.342^{b}	32.907^{cde}
Sakha 104	87.000^{b}	0.935^{ab}	2.575^{bc}	5.758^{c}	33.527^{cde}
Sakha 105	84.083^{c}	0.972^{a}	3.033^{a}	5.942^{c}	35.240^{bcd}
Sakha 106	84.500^{c}	0.851^{c}	2.442^{cd}	6.850^{a}	25.546^{f}
E. Yasmin	83.000^{cd}	0.877^{bc}	1.842^{g}	4.842^{ef}	30.148^{e}

Means followed by the same latter in each column are not significantly different by the least significant at p 0.05 according to Duncan's test

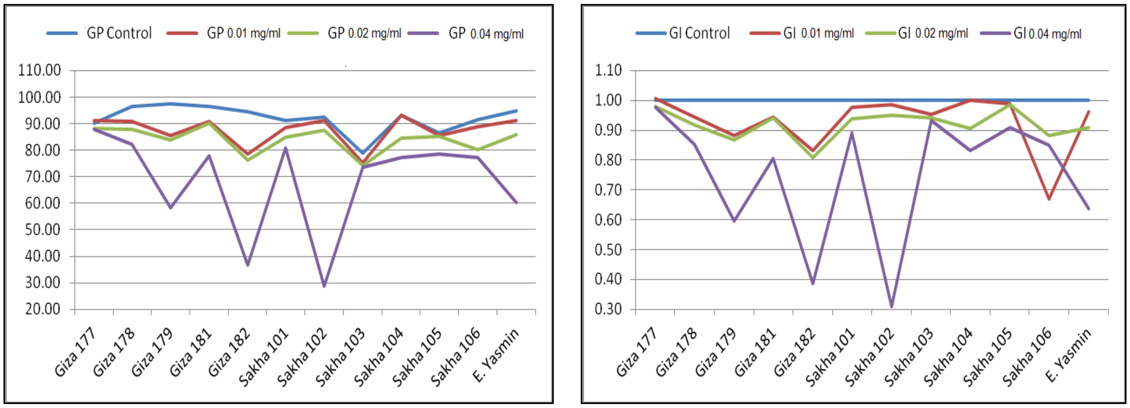
Table 4. Mean values for germination percentage, germination index, root length, shoot length and root/shoot ratio affected by cadmium application levels

Treatment	Germination percentage	Germination index	Root length	Shoot length	Root/shoot ratio
Control	91.889^{a}	1.000^{a}	7.297^{a}	9.667^{a}	75.449^{a}
0.01 mg/ml	87.528^{b}	0.929^{b}	2.053^{b}	4.878^{c}	43.863^{b}
0.02 mg/ml	84.194^{c}	0.919^{b}	0.533^{c}	5.278^{b}	10.506^{c}
0.04 mg/ml	68.361^{d}	0.749^{c}	0.236^{d}	3.172^{d}	7.654^{d}

Means followed by the same latter in each column are not significantly different by the least significant at p 0.05 according to Duncan's test

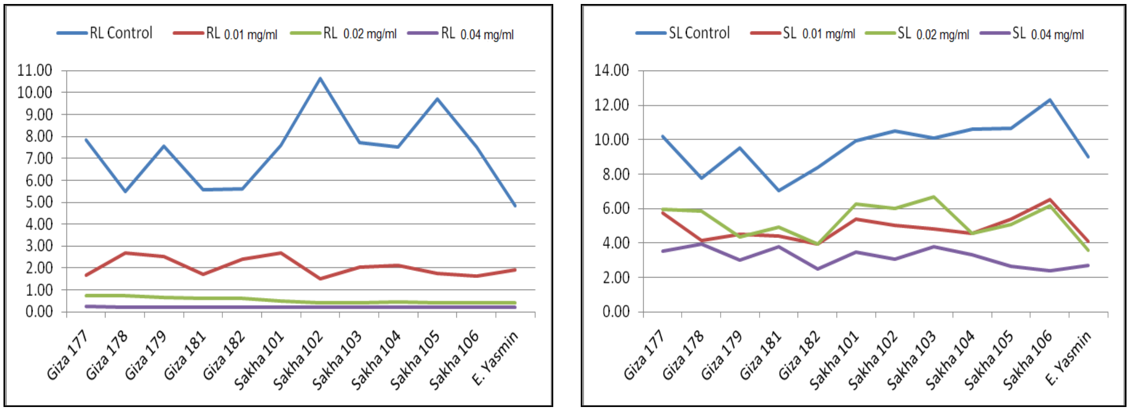
Generally, for root/shoot ratio, significant variations are detected among the studied genotypes (Table 3), the cultivar Giza 179 shows high mean value (39.736), while Sakha 106 variety provides decreased mean (25.546). As shown in Table 4, the untreated seedlings produce the highest mean of root/shoot ratio (75.449), while the application with 0.04 mg/ml cadmium shows the lowest mean for root/shoot ratio (7.654). The interaction between rice cultivars and Cd dosages significantly affects root/shoot ratio. The results show the highest mean (101.48) with Sakha 102 untreated seedlings (Fig. 1E). Otherwise, the lowest value (5.96) for root/shoot ratio obtains for cultivar Giza 178 with 0.04 mg/ml Cd dosage. Many studies have reported that plant seedlings respond quickly to a higher concentration of metals in terrestrial ecosystems by changing in their growth rates and root branching patterns compared to shoot growth [32,33,34,35].

The reduction percent of the studied traits in response to cadmium stress for the twelve Egyptian rice genotypes are presented at Table 5. The high cadmium dosage shows more reduced values than the low dosage for all studied traits and rice genotypes. The lowest affected trait is germination percentage since it records the lowest values of reduction percent for all genotypes. Meanwhile, root length is the most affected trait, since it shows the highest estimates for reduction percent for all genotypes. As discussed before that is could be due to earlier and direct exposure of roots to the stress. The tolerance of studied rice genotypes to cadmium stress is variable. Some genotypes showed moderately tolerance to low dosage of cadmium, but show high sensitivity at high dosages, such as, Giza 179, Giza 182, Sakha 105 and Sakha 106. The two genotypes Giza 177 and Giza 178 show moderately tolerance to high dosage of cadmium (0.04 mg/ml) for germination percentage trait. While, all studied Egyptian rice genotypes are highly sensitive to cadmium stress at high dosages for all studied traits.



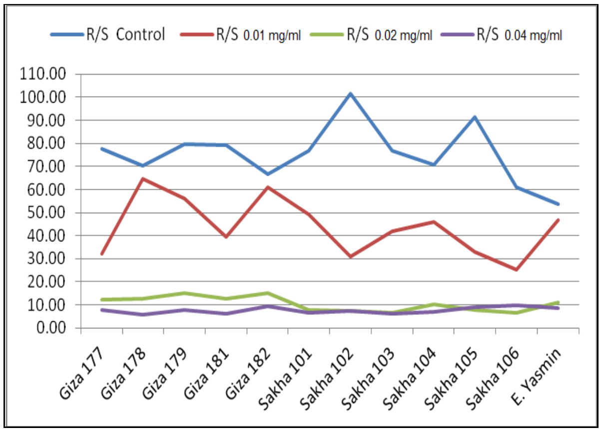
(A), Germination Percentage, GP

(B), Germination Index, GI



(C), Root Length, RL

(D), Shoot Length, SL



(E), Root/Shoot ratio, R/S

Fig. 1. Interaction between twelve rice genotypes and cadmium concentrations for germination percentage GP (A), germination index GI (B), root length RL (C), shoot length SL (D) and root/shoot ratio R/S (E)

Table 5. Reduction percent of the studied traits due to cadmium stress for the twelve Egyptian rice genotypes

Traits	**GP**			**GI**			**SL**			**RL**			**R/S ratio**		
genotypes	0.01	0.02	0.04	0.01	0.02	0.04	0.01	0.02	0.04	0.01	0.02	0.04	0.01	0.02	0.04
Giza 177	6.51	9.25	9.59	25.35	21.13	85.21	43.28	41.64	65.57	78.72	90.64	96.60	62.89	84.08	90.19
Giza 178	0.73	3.65	9.85	23.30	10.79	45.16	46.35	24.89	49.36	51.22	86.59	95.73	28.44	82.06	91.53
Giza 179	1.15	3.08	32.69	21.66	32.85	96.39	52.28	54.04	68.42	66.52	91.19	96.92	29.74	80.77	90.25
Giza 181	3.89	4.24	17.31	16.04	11.37	18.77	37.44	29.86	46.45	68.86	88.62	95.81	50.12	83.79	92.17
Giza 182	0.42	3.38	53.59	9.00	14.88	94.46	53.17	53.17	70.24	57.14	89.29	95.83	38.69	77.17	86.00
Sakha 101	2.56	6.59	10.99	32.65	15.84	20.96	45.64	36.91	65.10	64.91	93.42	96.93	35.63	89.58	91.20
Sakha 102	2.42	9.00	45.24	28.22	45.30	55.23	52.38	42.86	70.79	85.58	95.92	97.81	69.34	92.80	92.54
Sakha 103	1.11	11.44	18.86	47.78	65.53	67.92	52.15	33.99	62.71	73.71	94.40	96.98	45.29	91.53	91.83
Sakha 104	2.42	12.11	19.72	23.55	20.14	60.41	56.92	56.92	68.87	72.00	93.78	96.89	35.01	85.58	90.02
Sakha 105	0.72	2.17	14.80	7.77	20.14	62.19	49.69	52.50	75.00	81.85	95.89	97.60	63.93	91.35	90.26
Sakha 106	1.06	15.14	18.31	14.73	13.01	41.44	47.30	50.00	80.54	78.22	94.67	96.89	58.75	89.33	84.07
E. Yasmin	2.15	7.53	35.13	10.47	33.11	83.11	54.81	60.00	70.00	60.69	91.72	95.17	13.06	79.03	83.83

4. CONCLUSION

From the obtained results it can be concluded that, highly genetic variability are observed among studied Egyptian rice genotypes for tolerance to cadmium stress. Moderate tolerance is observed for germination percentage trait, while the most sensitive trait to cadmium stress is root length. Giza 177 and Giza 178 showed moderate tolerance to high dosage of cadmium for germination percentage trait. All studied Egyptian rice genotypes are highly sensitive to cadmium stress at high concentrations for all studied traits. The use of pesticides should be controlled in agriculture to avoid contamination of soil by heavy metals and thus its accumulation in plants such as rice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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