

Cadmium Affect Root Growth in Japonica Rice (*Oryza sativa* L.) Negatively Interacting with JA at Bud Stage

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Research Article

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Abstract

To explore the role of jasmonic acid in seed germination of rice under Cd stress, the growth indices of JJ818 and NJ9108 under the bud stage were measured for identifying study the molecular mechanism of variety difference in cadmium tolerance. The tolerant variety JJ818 and the sensitive variety NJ9108 were treated under $150 \mu\text{mol}\cdot\text{L}^{-1}$ Cd concentration, methyl jasmonate (MeJA), and Ibuprofen (IBU) alone or in combination. The germination rate, root length, bud length, soluble protein content, soluble sugar content, and sugar components, α -amylase activity, and its related gene expression were measured. The results showed that the root length of the two rice materials were significantly inhibited during germination under the Cd treatment, however, the germination rate and shoot length had little affected. MeJA decreased the germination rate, root length and shoot length, and the contents of soluble sugar and sugar components and α -amylase activity as well. However, IBU treatment showed the opposite trend. Cd treatment also decreased the expression of the α Amy and *RL 1*, and the accompanying changes of up-regulated the gene expression of *JIOsPR10*, *OsLOX1*, and *OsLOX4* related to synthesizing jasmonic acid(JA). The results showed that JA negatively regulates the cadmium of rice and the genes related to the synthesis and response of JA were related to the variety difference in cadmium tolerance at the bud stage. Compared with the NJ9108, JJ818 was insensitive to jasmonic acid.

Introduction

Rice (*Oryza sativa* L.) is a staple food crop for more than 50 percent of the world's population(Wang et al., 2021a). With the intensification of the economy and industrialization, heavy metal pollution has seriously affected food security(Huang et al., 2021). Among them, cadmium (Cd) is one of the serious pollution elements, which has strong mobility and is easy to be enriched by crops including rice, and then directly or indirectly endangers human health through the food chain(Liu et al., 2020). Therefore, reducing the adverse effects of Cd on rice production is an important strategy to ensure people's food security. A systematic study on the Cd tolerance mechanism of rice will provide an effective means to reduce Cd stress in rice.

With the decrease of the rural population, the proportion of simplified cultivation in modern rice planting is increasing, and the area of direct seeding of rice is increasing year by year(Chen et al., 2021). Low germination rate and inconsistent germination rates are the main trouble for this cultivating way, resulting in low rice yield(Ali et al., 2020). As the starting point of the plant life process, seed germination is the earliest and most vulnerable growth stage for rice growth under Cd stress conditions(Xu et al., 2021). How to alleviate the impact of cadmium pollution during this stage is a key problem that needs to be solved urgently in the production of rice under simplified cultivation(Wang et al., 2020).

There has been much research on the response or tolerance of rice to cadmium(Wang et al., 2021d, Yang et al., 2021a, Meng et al., 2015), but there are few studies on the effect of the seed germination stage of rice. The process of rice seed has been studied deeply (Nie et al., 2020, Liu et al., 2019, Chen et al., 2019), which provides useful clues to understand the cadmium tolerance at its bud stage. As the starting point of crop plant growth, the bud stage is a sensitive period for plant hormones(Yang et al., 2012), including auxin (IAA)(Cui et al., 2021, Chhun et al., 2003), gibberellin (GAs)(Li et al., 2019), abscisic acid (ABA)(Wang et al., 2021b), and ethylene (ETH) (Hu et al., 2017). Previous studies have shown that they play an important role in seed germination individually or jointly(Yin et al., 2011, Pan et al., 2020, Zhang et al., 2021a, Lv et al., 2021). Jasmonic acid (JA) is a new class of the important lipid hormones in plants and plays an important role in plant resistance to biotic and abiotic stresses through JA synthesis and its signal transduction(Li and Li, 2019a). JA may realize its signal transduction process

through the ubiquitination of the inhibitor protein, JAZ (Jasmonate ZIM-domain)(Song et al., 2015b). It has been observed that JA can participate in the response of plants to heavy metal stress, including copper and potassium stress in plants(Li et al., 2013, Li et al., 2017), and also affect iron uptake(Cui et al., 2018). Previous studies on the effects of JA on rice growth under Cd stress mainly focus on the seedling stage(Yang et al., 2021b)and its interaction with other plant hormones(Li et al., 2021a). At the seedling stage of rice, JA inhibits the gene expression of MYC2 through its direct binding to the promoters of PLT1 and PLT2 under cadmium stress, thereby inhibiting the growth of the main root(Chen et al., 2011). Whether JA was involved in the early germination of rice seeds and the growth of plants is a scientific issue worthy of further study. Therefore, this study intends to screen japonica rice varieties under cadmium stress conditions through the performance of rice shoot growth indexes. Then, two rice varieties with different Cd tolerance were treated with methyl jasmonate (MeJA) or Ibuprofen (IBU). The growth index, endogenous soluble protein content, soluble sugar content and sugar composition, α -amylase activity and its related gene expression, root elongation gene, and JA-related gene expression were analyzed under $150 \mu\text{mol}\cdot\text{L}^{-1}$ Cd concentration. The results showed that JA negatively regulates the cadmium of rice and the genes related to the synthesis and response of JA were related to the variety difference in cadmium tolerance at the bud stage. The study will be helpful to reveal the mechanism of JA participating in the tolerance of rice to Cd stress at the germination stage.

1 Material And Methods

1.1 Test material

There were six rice (*Oryza sativa* L.) materials used in this study, namely, high expression maize C_4 -PEPC transgenic rice (PC), Japanese japonica rice Kitaake (WT), Jinjing 818 (JJ818), Nanjing 9108 (NJ9108), K37 and 73119. Among them, PC used Japanese japonica rice Kitaake as the receptor to introduce the complete maize C_4 -PEPC gene into rice production; wild type rice WT(Li et al., 2021b). JJ818 is a japonica conventional rice variety bred by Tianjin Rice Research Institute with japonica rice 9618 and japonica 1007(Fei et al., 2018). NJ9108 was bred by the Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, crossing Wuxiangjing 14 and Guandong 194 in 2009(Wang et al., 2013). It is a late-maturing medium japonica rice lines, K37 and 73119, which were provided by the Institute of Food Crops, Jiangsu Academy of Agricultural Sciences.

1.2 Treatments

The seeds with the same size and full particles harvested in the same year were selected and disinfected with 75% ethanol for 15 min and then rinsed with sterile water. The seeds were placed in a petri dish containing 40 mL sterile water and then placed in a 30°C incubator under dark conditions. The tested materials were treated after 24 h. Each treatment selected 30 seeds in a petri dish, then placed in light and dark conditions for $30^\circ\text{C}/14\text{h}$ (light), $25^\circ\text{C}/10\text{h}$ (dark) culture. Set the following 7 treatments: A.CK (Ultrapure water); B.Cd(+ $150 \mu\text{mol}\cdot\text{L}^{-1}$ Cd); C.IBU(+ $10 \mu\text{mol}\cdot\text{L}^{-1}$ IBU); D.MeJA (+ $0.5 \mu\text{mol}\cdot\text{L}^{-1}$ MeJA); E.Cd + IBU (+ $150 \mu\text{mol}\cdot\text{L}^{-1}$ Cd + $10 \mu\text{mol}\cdot\text{L}^{-1}$ IBU); F. Cd + MeJA(+ $150 \mu\text{mol}\cdot\text{L}^{-1}$ Cd + $0.5 \mu\text{mol}\cdot\text{L}^{-1}$ MeJA); G.Cd + IBU + MeJA(+ $150 \mu\text{mol}\cdot\text{L}^{-1}$ Cd + $10 \mu\text{mol}\cdot\text{L}^{-1}$ IBU + $0.5 \mu\text{mol}\cdot\text{L}^{-1}$ MeJA). Each treatment was repeated at least three times independently. The germinated rice seeds of different treatment times were frozen in liquid nitrogen and stored in a -80°C refrigerator for measuring the physiological indexes and the gene relative expression.

1.3 Determination items and methods

1.3.1 Determination of seed growth phenotype

Samples were taken every 24 hours, and 100 seeds from each treatment were selected for germination rate, shoot length, and root length determination, repeated three times, and selected 5 seeds for photography. The germination rate was calculated according to the standard that the shoots grew longer than 5 mm.

1.3.2 Determination of total soluble sugar

Reference anthrone colorimetric determination of total soluble sugar content (Somani et al., 1987). The seeds were dried in a 10°C oven for 15 min and then adjusted to 70°C overnight. After grinding the seeds, 50 mg samples were weighed and transferred into a 10 mL scale centrifuge tube, and 4 mL 80% ethanol was added. After stirring in a water bath for 40 min, the supernatant was centrifuged, and the residue was extracted twice with 80% ethanol, and the supernatant was combined. 10 mg activated carbon was added to the supernatant, decolorized at 80°C for 30 min, and then the volume was fixed to 10 mL with 80% ethanol. After filtration, the filtrate was determined. Take 1 mL of the above sugar extract, transfer into a clean test tube, add 5 mL anthrone reagent mixture, boiling water bath heating 10 min, remove cooling, and then use spectrophotometer (wavelength 625 nm) absorbance (OD value). The sugar content in the filtrate was calculated according to the standard curve, and the sugar content in the sample was further calculated.

1.3.3 Determination of sugar content

Method for determination of sucrose, glucose, and fructose in reference literature (Ambavaram et al., 2014). The sucrose standard solution was diluted with an 80% ethanol gradient (0, 10, 20, 40, 60, 80, and 100 $\mu\text{g}\cdot\text{mL}^{-1}$). 0.4 mL solution was added with 200 μL 2 $\text{mol}\cdot\text{L}^{-1}$ NaOH and boiled at 100°C for 5 min. After cooling, 2.8 mL 30% hydrochloric acid and 0.8 mL 0.1% resorcinol were added. After shaking, the solution was bathed at 80°C for 10 min. After cooling, the OD value was measured at 480 nm, and then the sucrose content in the sample was calculated. Glucose standard solution was diluted with 80% ethanol (0, 15, 30, 50, 75, 100, 150 and 200 $\mu\text{g}\cdot\text{mL}^{-1}$). Eight test tubes were added with 4 mL enzyme preparation and placed in a 30°C water bath. After the temperature of enzyme solution in the tube reached equilibrium, 2 mL gradient diluted glucose solution was added, shaken well, and kept for 5 min. Then 8 mL 10 $\text{mol}\cdot\text{L}^{-1}$ sulfuric acid was added to terminate the reaction. When the enzyme solution temperature was balanced with room temperature, the OD value at 460 nm was measured, and then the glucose content in the sample was calculated. Fructose solution was diluted with 80% ethanol gradient (0, 15, 30, 50, 75, 100, 150 and 200 $\mu\text{g}\cdot\text{mL}^{-1}$). Eight test tubes were taken and 1 mL of fructose solution with gradient dilution was added, and then 2 mL of 30% hydrochloric acid, 1 mL of 0.1% resorcinol, and 1 mL of H_2O were added and shaken. Reaction at 80°C in a water bath.

1.3.4 Determination of α -amylase activity

Determination of α -amylase activity by iodine colorimetry (Juliano, 1971). When starch with known concentration was used as substrate and excess was added, iodine solution was added to combine with unhydrolyzed starch to form a blue complex. According to the absorbance, the amount of hydrolyzed starch could be calculated to calculate the activity of α -amylase.

1.3.5 Determination of total protein content

Determination of soluble protein content by Coomassie brilliant blue G-250 method (Bradford, 1976). With the standard bovine serum protein concentration as the standard curve, 2 mL dye reagent was added to mix immediately, and the OD value at 595 nm was determined by spectrophotometer. 2 mL distilled water and 2 mL dye reagent were used as the blank control. Protein concentration was calculated by standard curve.

1.3.6 Total RNA extraction and qRT-PCR

RNA was extracted using FastPure® Plant Total RNA Isolation Kit (Polysaccharides & Polyphenolics-rich) (Nanjing Novatar). Reverse transcription was performed using HiScript III RT SuperMix for qPCR(+ gDNA wiper) kit (Nanjing Novozan). qRT-PCR was performed using ChamQ® SYBR® qPCR Master Mix kit (Nanjing Novozan). Primers were designed by Primer 5.0 (Table 1).

Table 1
Primer sequence

Gene	Forward primer(5'-3')	Reverse primer(5'-3')	Accessing number
<i>Actin</i>	CCCTCTTCATCGGTATGGA	TTGATCTTCATGCTGCTTGG	<i>Os10g0510000</i>
<i>OsAmy1A</i>	TTTCGGTCCTCATCGTCCTCC	TCCACGACTCCCAGTTGAATC	<i>Os08g0473900</i>
<i>OsAmy1C</i>	TGGTATCGATCAGAAACCGGC	GTCCGACCTTCGTGATGACC	<i>Os02g0765600</i>
<i>OsAmy3C</i>	AAGCATTCCACCACAATGAGC	AGGAAGTTGTACCACCCACC	<i>Os08g0191433</i>
<i>OsAmy3E</i>	TCACCCTGTGTTGTGTCGTT	AAACTTGTACCACCCGCCTT	<i>Os04g0624600</i>
<i>RL1</i>	CCCGCCAAATCGTCCTGTGTATG	ATCCAGCTTCACTCATCTCAACAAGG	<i>Os08g0525500</i>
<i>OsLOX1</i>	TACCACTACGGCGGCTACTTCC	CTCCTCCTCCTTGTTCCTCCAC	<i>Os02g0194700</i>
<i>OsLOX4</i>	CTGTGATGGAGCCGTTTCGTGATC	GGGCGTTGATGGTCATCGTGTC	<i>Os03g0700400</i>
<i>JIOsPR10</i>	CGCTTACAATAAATCGTCATCATGCC	TTCTCGCTGCTCACTTCTCAATCAC	<i>Os03g0300400</i>
Actin: actin; Amy: α-amylase gene; <i>RL1</i> : Root elongation gene; LOX: lipoxxygenase synthesis gene; <i>JIOsPR10</i> : Jasmonic acid-induced disease-related protein gene			

1.4 Data analysis

GraphPad Prism 8.0 software was used for plotting. The qRT-PCR data were analyzed using the $2^{-\Delta\Delta Ct}$ method. Cluster analysis ($P < 0.05$), one-way ANOVA ($P < 0.05$), and correlation analysis ($P < 0.05$) were performed using SPSS 25.0 software.

2 Results

2.1 Screening and identification of cadmium tolerance in rice varieties

Due to the lack of research on cadmium treatment in rice bud, this paper first referred to the screening method of rice seedling stage and set up different concentrations of cadmium treatment (Yang et al., 2017). In this study, the seedling length, root length, and dry weight of each rice material and treatment were measured when the rice grew to the 5-leaf stage (Table 2). The ratio of different Cd concentrations of the same variety to its CK treatment was

used as a parameter for the difference of cadmium tolerance varieties. WARD clustering analysis (Euclidean distance as the measurement standard) was used to cluster the 26 sets with a threshold of 7 as the clustering standard (Fig. 1) and were divided into 4 types (the average value of specific parameters is shown in Table 3). The growth of rice at bud stage was similar to that of the untreated control, which was classified as Type 1, accounting for 30.7% of the tested materials. Type 2 was inhibition of seedling length and dry weight growth, accounting for 30.7% of the tested materials. Type 3 was an inhibition of dry weight growth, accounting for 19.2% of the tested materials. Type 4 was inhibition of seedling length, root length, and dry weight growth, accounting for 19.2% of the tested sets. Based on the comprehensive analysis results, JJ818 was finally selected as the Cd tolerant variety, NJ9108 was the sensitive one, under $150 \mu\text{mol}\cdot\text{L}^{-1}$ Cd treatment, which was further studied on the Cd tolerant molecular mechanism at the bud stage.

Table 2
Screening growth phenotype data of varieties

index	concentration	PC	WT	JJ818	NJ9108	73119	K37
Seedling length(cm)	0 $\mu\text{mol}\cdot\text{L}^{-1}$	14.12 \pm 1.20a	13.18 \pm 0.53a	12.71 \pm 0.56a	17.34 \pm 0.50a	13.56 \pm 0.88a	12.90 \pm 0.37a
	50 $\mu\text{mol}\cdot\text{L}^{-1}$	12.92 \pm 0.47b	11.16 \pm 0.36b	8.52 \pm 0.44b	12.11 \pm 0.36b	10.48 \pm 0.30c	9.18 \pm 0.75b
	100 $\mu\text{mol}\cdot\text{L}^{-1}$	12.24 \pm 0.45b	11.96 \pm 1.05b	8.76 \pm 0.27b	12.22 \pm 1.38b	11.52 \pm 0.79b	8.36 \pm 0.64c
	150 $\mu\text{mol}\cdot\text{L}^{-1}$	12.53 \pm 0.47b	11.90 \pm 0.53b	8.24 \pm 0.70b	11.88 \pm 1.01c	11.36 \pm 1.45b	8.20 \pm 1.28c
	200 $\mu\text{mol}\cdot\text{L}^{-1}$	11.38 \pm 0.82c	11.08 \pm 0.80c	8.48 \pm 0.27b	11.84 \pm 0.43c	11.50 \pm 0.62b	8.80 \pm 0.79c
root length(cm)	0 $\mu\text{mol}\cdot\text{L}^{-1}$	8.04 \pm 0.55a	7.78 \pm 0.70b	7.88 \pm 0.31b	10.22 \pm 1.81a	6.52 \pm 0.44b	6.84 \pm 0.60a
	50 $\mu\text{mol}\cdot\text{L}^{-1}$	8.12 \pm 0.31a	8.84 \pm 0.44a	8.56 \pm 1.00a	6.75 \pm 0.83b	6.22 \pm 0.54b	6.92 \pm 0.39a
	100 $\mu\text{mol}\cdot\text{L}^{-1}$	8.32 \pm 0.79a	7.84 \pm 0.87b	6.06 \pm 0.21c	6.40 \pm 0.82b	7.82 \pm 0.60a	5.54 \pm 0.89b
	150 $\mu\text{mol}\cdot\text{L}^{-1}$	8.59 \pm 0.85a	7.74 \pm 0.56b	6.30 \pm 0.71c	6.66 \pm 0.59b	7.26 \pm 1.21a	5.66 \pm 0.74b
	200 $\mu\text{mol}\cdot\text{L}^{-1}$	8.54 \pm 0.43a	6.90 \pm 0.58c	5.80 \pm 0.36d	6.32 \pm 0.47b	6.95 \pm 0.77b	6.90 \pm 0.73a
dry weight(g)	0 $\mu\text{mol}\cdot\text{L}^{-1}$	0.050 \pm 0.002a	0.051 \pm 0.005a	0.040 \pm 0.005a	0.036 \pm 0.004a	0.054 \pm 0.003a	0.052 \pm 0.004a
	50 $\mu\text{mol}\cdot\text{L}^{-1}$	0.042 \pm 0.004b	0.031 \pm 0.002b	0.027 \pm 0.005b	0.025 \pm 0.003b	0.043 \pm 0.004b	0.039 \pm 0.004b
	100 $\mu\text{mol}\cdot\text{L}^{-1}$	0.029 \pm 0.002c	0.031 \pm 0.003b	0.025 \pm 0.003b	0.021 \pm 0.002b	0.039 \pm 0.003b	0.033 \pm 0.003b
	150 $\mu\text{mol}\cdot\text{L}^{-1}$	0.026 \pm 0.003c	0.028 \pm 0.002b	0.023 \pm 0.003b	0.020 \pm 0.002b	0.041 \pm 0.006b	0.021 \pm 0.002c
	200 $\mu\text{mol}\cdot\text{L}^{-1}$	0.018 \pm 0.004d	0.023 \pm 0.002c	0.018 \pm 0.002b	0.019 \pm 0.003b	0.034 \pm 0.005c	0.022 \pm 0.002c

Table 3
Cluster analysis on the ratio of growth phenotype indexes of tested rice

Type	Set	The name of material and treatment	Seedling length ratio (%)	Root length ratio (%)	Dry weight ratio (%)
1	8	JJ818 + 0 PC + 0 K37 + 0 WT + 0 PC + 50 WT + 150	103.36 ± 13.28	101.54 ± 3.98	94.96 ± 9.68
2	8	K37 + 50 JJ818 + 50 PC + 100 WT + 50 WT + 100 WT + 100 WT + 200	80.92 ± 8.25	106.20 ± 7.83	67.05 ± 7.48
3	5	PC + 200 K37 + 150 K37 + 200 WT + 200 JJ818 + 150	86.33 ± 13.72	91.70 ± 11.43	44.24 ± 8.06
4	5	K37 + 100 JJ818 + 100 JJ818 + 200 NJ9108 + 100 NJ9108 + 150	67.90 ± 2.18	71.86 ± 7.78	56.89 ± 7.17

2.2 Effects of JA on seed germination rate, shoot length, and root length of rice varieties under Cd treatment

Cd treatment significantly reduced the root growth of JJ818 and NJ9108 seeds but had relatively little effect on germination rate and shoot length (Fig. 2A and 2B). The application of JA significantly reduced the germination rate (Fig. 2C, 2D), shoot length (Fig. 2E, 2F), and root length (Fig. 2G, 2H) of two rice varieties. The JA inhibitors, IBU, could alleviate the inhibitory effect of Cd stress on the germination of rate in two rice seeds. Among them, the inhibitory effect of MeJA treatment on the shoot length of NJ9108 was significantly higher than that of JJ818. Cd + IBU treatment had a better-alleviating effect on the root growth of JJ818 than that of NJ9108. It can be seen that JA significantly inhibited the germination of rice seeds under Cd stress, the effect on root length was greater than germination rate and shoot length, and there are differences between rice varieties.

2.3 Effect of JA on soluble sugar content in seeds of rice varieties under Cd treatment

Accumulation of osmotic adjustment substances is an effective way for plants to tolerant to adversity (Wang et al., 2017). The soluble sugar content of JJ818 showed a fluctuating upward trend before 96 h (Fig. 3A). Cd treatment significantly reduced the soluble sugar content at each time point and reached the peak at 48 h. After Cd treatment, the peak was delayed to 60 h, and the fluctuation of soluble sugar content slowed down. The soluble sugar content of NJ9108 showed a fluctuating upward trend before 120 h, and it first increased (36 h) and then decreased (96 h) within 120 h after Cd treatment, and the content was lower than that of CK treatment.

The contents of sucrose (Fig. 3B, 3C), fructose (Fig. 3D, 3E), and glucose (Fig. 3F, 3G) in JJ818 and NJ9108 seeds were further determined at 24 h and 48 h, exhibiting an increase at 24 h and a decrease at 48 h during germination, and the differences among treatments were significant. After Cd treatment, sucrose and glucose content decreased at 24 h and 48 h, while fructose content did not change much. Cd + MeJA treatment further reduced the contents of sucrose and glucose, and the glucose content had the lowest in these treatments at 24 h and returned to the same level as Cd treatment at 48 h, but slightly lower than this level. After increasing IBU treatment, there was little difference in various sugar components at Cd + IBU treatment. The results showed that Cd treatment could decrease the content of sucrose and glucose within 48 h, and the content of sucrose and glucose would be further reduced by Cd + MeJA treatment, and the content of glucose would be significantly reduced within 24 h, and JJ818 decreased more than NJ9108. Cd treatment might affect the energy required for seed germination by reducing the content of glucose, thereby further affecting seed germination. And JA treatment further reduced glucose content.

2.4 Effects of JA on the soluble protein content in seeds of rice varieties under Cd treatment

Soluble proteins are important osmotic regulators and nutrients, and their increase and accumulation can improve the water retention ability of cells (Song et al., 2015a). The results showed that the soluble protein (Fig. 4A) showed a fluctuating upward trend with CK and Cd treatment. Among them, the soluble protein content of Cd treatment was lower than that of CK treatment.

Further application of methyl jasmonate and its synthetic inhibitor treatment, analysed the change of soluble protein content during the germination of rice seeds for 24 h (Fig. 4B) and 48 h (Fig. 4C) with different treatment. The results showed that the soluble protein content decreased slowly at 48 h with CK and Cd treatment, and tended to be stable. The soluble protein content of JJ818 was than that of NJ9108 in 48h by CK treatment. After Cd treatment, the soluble protein content. The soluble protein content of NJ9108 under Cd + IBU treatment could be restored to the CK treatment level, and the difference was not significant with JJ818. The soluble protein content under Cd + MeJA treatment increased as compared with that under Cd treatment alone. It can be seen that Cd treatment inhibited the accumulation of osmotic adjustment substances in the tested seeds.

2.5 Effects of JA on α -amylase activity in seeds of rice varieties under Cd treatment

The α -amylase activity (Fig. 5A) of the two rice seeds reached a peak at 48 h, and increased first and then decreased at 72 h. The peak value after Cd treatment was delayed to 60 h, which was consistent with the trends of soluble sugar content, and the α -amylase activity after Cd treatment was lower than that of the CK within 60 h. It can be seen that Cd treatment can reduce the activity of α -amylase during seed germination, and the inhibition of NJ9108 was slightly higher than that of JJ818.

The effects of methyl jasmonate and its synthetic inhibitors on the activity of α amylase in tested seeds were further determined at 24 h (Fig. 5B) and 48 h (Fig. 5C). The results showed that α -amylase activity of two rice seeds increased at 48 h, which was consistent with the previous results (Fig. 5A). The activity of α -amylase was inhibited by Cd and MeJA treatment respectively. The activity of α -amylase further decreased at Cd + MeJA treatment, and the difference was significant in two rice seeds by CK treatment. After Cd + IBU treatment, the activity of α -amylase could be restored within 48 h. It can be seen that JA can inhibit seed germination by reducing the activity of α -amylase in the seeds of two rice varieties, and NJ9108 was more sensitive to JA than JJ818 was, which was also similar to its changes of soluble sugar content.

2.6 Effects of JA on the expression of JA-related genes in seeds of rice varieties under Cd treatment

OsLOX1 and *OsLOX4* are important genes in JA biosynthesis in plants (Hua et al., 2021). *OsLOX1* (Figs. 6A and 6B) increased significantly with time in the seeds of JJ818 at 24 h and tended to be stable in the seeds of NJ9108. *OsLOX4* (Figs. 6C, 6D) decreased first and then increased with time, and there was no significant difference in two rice seeds. After Cd and MeJA treatment, the expression of the two genes in NJ9108 could be significantly up-regulated, but those of JJ818 were significantly different compared with CK treatment. After Cd + IBU treatment, the expression level on JJ818 was down-regulated to CK level.

JIOsPR10 is a disease-related gene induced by JA (Wu et al., 2016). The expression of *JIOsPR10* tended to fluctuation change in JJ818 seeds, decreased first, and then increased over time, but increased significantly over time in NJ9108 (Fig. 8E and Fig. 8F). After Cd, MeJA, and Cd + MeJA treatments, the expression of *JIOsPR10* was up-regulated, and IBU and Cd + IBU treatments could restore the expression of *JIOsPR10* to a certain extent. It can

be seen that Cd can up-regulate the expression of genes related to JA synthesis, thereby might be helpful to increase the content of JA. After JA treatment, the expression of the *JIOsPR10* gene can also be up-regulated in the tested seeds, and the expression level of NJ9108 is much higher than that of JJ818.

2.7 Effects of JA on the expression of α -amylase-related genes in tested seeds under Cd treatment

α -amylase is the key enzyme to determine the germination characteristics of rice. It has been confirmed that α -amylase is encoded by 11 genes in rice(Liao et al., 2010), of which *OsAmy1/3* is the key genes, especially in the process of rice seed germination(Li et al., 2021c). The expression level of the *OsAmy1/3* gene in NJ9108 was much higher than those in JJ818 at 24 h (Fig. 7). The expression pattern of JJ818 seeds decreased first and then increased within 24 h, while the expression pattern of NJ9108 seeds increased with time (Fig. 7A, 7C, and 7G). Cd treatment could all down-regulate the levels of *OsAmy1A*, *OsAmy1C*, *OsAmy3C*, and *OsAmy3E* by Cd treatment in 24 h. *OsAmy3C* varied greatly in JJ818 seeds. The differences in the expression of the four genes in MeJA treatments were not significant. In NJ9108 seeds, MeJA and Cd + MeJA treatment also significantly decreased the expression of four genes. Compared with CK treatment, Cd treatment significantly inhibited the expression of four genes in two rice seeds, and the expression levels were consistent with the growth phenotype. In conclusion, Cd can affect seed germination by down-regulating the expression of α -amylase-related genes. Under Cd stress, the effect of MeJA on the α -amylase activity of JJ818 seeds was lower than that of NJ9108, and the inhibition of IBU to restore jasmonic acid changed higher than it in JJ818, indicating that NJ9108 was more sensitive to MeJA.

2.8 Effects of JA on the expression of genes related to root growth in tested seeds under Cd stress

RL1 is an important gene related to radicle growth in rice seed roots(Wu et al., 2020). From Figs. 8A and 8B, it can be seen that *RL1* showed an upward trend with time in CK and IBU treatments of the two tested seeds at 24 h. The expression of this gene under Cd + MeJA treatment was inhibited. In JJ818 seeds, MeJA, Cd + MeJA, and Cd + IBU + MeJA treatments compared with NJ9108 maintained a low expression state at 24 h, while in NJ9108 seeds, it showed a trend of decreasing first and then increasing, which was contrary to the radicle growth of the tested seeds. It may be that MeJA treatment inhibits the growth of seed roots by decreasing the level of *RL1*. NJ9108 significantly up-regulated the level of *RL1* under Cd + IBU treatment, which also indicated that NJ9108 was a MeJA sensitive variety.

2.9 Correlation of the indexes of seeds under Cd stress

Figure 9A shows that there is a correlation between each index of JJ818, glucose content and α -amylase activity were significantly positively correlated ($P < 0.05$), *OsAmy3C* and *OsAmy3E* were significantly positively correlated, *OsLOX1* and *OsLOX4* were significantly positively correlated, *OsAmy1A* and *OsAmy1C*, *OsAmy3C*, *OsAmy3E*, *RL1* were significantly positively correlated, and *JIOsPR10* was significantly negatively correlated with α -amylase activity and glucose. In Fig. 9B, *OsAmy3C* of NJ9108 was significantly positively correlated with *OsAmy1C* and *OsAmy3E*, and its α -amylase activity was significantly positively correlated with *OsAmy1A*. *OsLOX1* was significantly negatively correlated with *OsAmy3C* and *OsAmy3E*, and *OsLOX1* was significantly negatively correlated with *RL1* and α -amylase activity. It can be seen that JA content negatively regulates cadmium tolerance at the bud stage of rice, but the relationship between the two varieties with different tolerance differences and JA synthesis-related genes is different.

3 Discussion

Previous research showed that Cd inhibits the germination of rice seeds by inhibiting the length of seed roots, activities of amylase, germination index, root number, and the antioxidant enzyme activities as well (Wang et al., 2019, Liu et al., 2021b). External application of melatonin(Liu et al., 2016), hydrogen peroxide(Yang et al., 2018), Ca^{2+} , Mg^{2+} (Teng et al., 2020)can alleviate the inhibition of germination rate and the growth of root length. Studies have shown that the tolerance of rice to cadmium is different among varieties and Indica rice varieties treated by the concentration of cadmium was only $10 \mu\text{mol}\cdot\text{L}^{-1}$ (Liu et al., 2021a). In this paper, there were also differences in Cd tolerance in japonica rice, and the concentration range of Cd tolerance was high to $150 \mu\text{mol}\cdot\text{L}^{-1}$, which was significantly higher than that in indica rice. Under $150 \mu\text{mol}\cdot\text{L}^{-1}$ Cd concentration, the growth of seed roots of the tested materials was also significantly reduced and JA negatively regulated this process. Compared with NJ9108, JJ818 had less inhibition of root growth under Cd treatment, which was closely related to the expression of *RL1*.

Amylase plays an important role in life(Akbari et al., 2015), which hydrolyzes the α -1,4- glycosidic bond and α -1,6- glycosidic bond in starch molecules to obtain products such as glucose, oligosaccharide, or dextrin(Wang and Copeland, 2015). According to the hydrolysis products, it can be divided into α , β , and γ amylases(Luo and Wei, 2018). Among them, α -amylase can rapidly hydrolyze the stored starch during seed germination as the energy source for growth(Nakata et al., 2017). In this paper, $150 \mu\text{mol}\cdot\text{L}^{-1}$ Cd treatment reduced the activity of α -amylase in the seeds of the tested materials, which showed significant inhibition of seed germination. It is worth noting that the content of total soluble sugar in seeds was also significantly reduced, in which glucose was more severely inhibited. The synthesis and decomposition of carbohydrates promote the whole process of plant growth and development and regulate gene expression as signal factors and interact with other genes, hormones, and defense signals(Feng et al., 2021).In the process of sugar synthesis and decomposition, α -amylase is a key enzyme involved, which can change the intracellular sugar level to meet the energy required for plant growth(Damaris et al., 2019). Previous studies have shown that the expression of wheat cell cycle protein *ZmCycb1* depends on sugar content, and the effect of glucose on it is greater than that of sucrose(Lara-Nunez et al., 2021). Our results also showed that the α -amylase related genes *OsAmy1A*, *OsAmy1C*, *OsAmy3C*, *OsAmy3E* were significantly inhibited after Cd and MeJA treatment alone or in combination, indicating that JA might negatively regulate the germination process of rice via sugar under Cd stress.

Jasmonic acid, as a plant hormone and signal molecule related to injury, widely exists in plants and participates in the plant life process(Xu and Yan, 2005). Activation of the JA synthesis pathway is essential for stress signal transduction and amplification(Li et al., 2002). Its biosynthesis is generated by α -linolenic acid (18:3) through the octadecane pathway. Lipoxygenase (LOX), allene oxide synthase (AOS), allene oxide synthase (AOS), and allene oxide cyclase (AOC) are involved in the initial steps of JA biosynthesis(Wasternack and Hause, 2013). Jasmonic acid plays a role under drought(Wang et al., 2021c), salt(He et al., 2021, Xiang et al., 2021), cold(Ding et al., 2021, Gao et al., 2021)and heavy metal(Zhang et al., 2021b)stress. MeJA may respond to Cd stress by increasing activities of antioxidant enzymes and contents of antioxidant substances in tomato seedlings(Wei et al., 2021); MeJA regulates rice root growth at the seedling stage(Shao, 2015). JA is also involved in the germination process of rice seeds(Toda et al., 2013). This study further showed that JA was found to be involved in the germination of rice seeds under Cd stress, and it had a significant inhibitory effect on the germination rate, shoot, and root growth, and the inhibition of root was the most significant. Furthermore, Cd treatment could increase the levels of *OsLOX1*, *OsLOX4*, and *JIOsPR10*, as well in rice at bud stage, indicating that the Cd tolerance among rice varieties might be related to JA. How Cd inhibits the expression of α -amylase-related genes via JA remains to be studied. From the

perspective of plant hormones and soluble sugars, this study revealed that Cd inhibited seed germination by affecting the level of JA, which enriched the understanding of rice seed germination under Cd stress and will help to enrich the relevant information of JA in plant stress response.

4 Conclusions

In summary, the tolerance of cadmium stress at the bud stage was different among japonica rice varieties. JA has a negative effect on rice in this process under the Cd treatment, with the increase of the level of *OsLOX1* and *OsLOX4*. In addition, JA activated the levels of *JIOsPR10* and affected the expression of the α -amylase gene and *RL1*, which ultimately affected the germination rate and radicle growth of rice. Cadmium-sensitive rice variety NJ9108 was sensitive to JA.

Declarations

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Competing Interests

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

Author Contributions

Dr. Xia. Li designed research and drafted the entire manuscript. Mr. Bohan Wu performed research and analyzed data. Material preparation were performed by Dr. Xia Li, Mr. Fangquan Wang and Dr. Jie Yang. Data collection were performed by Mr. Bohan Wu, Miss Zifan Zeng and Miss Yingying Li. Analysis were performed by Mr. Bohan Wu and Dr. Xia Li. The first draft of the manuscript was written by Bohan Wu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Cluster analysis of growth phenotypic index ratio of tested rice

Figure 2

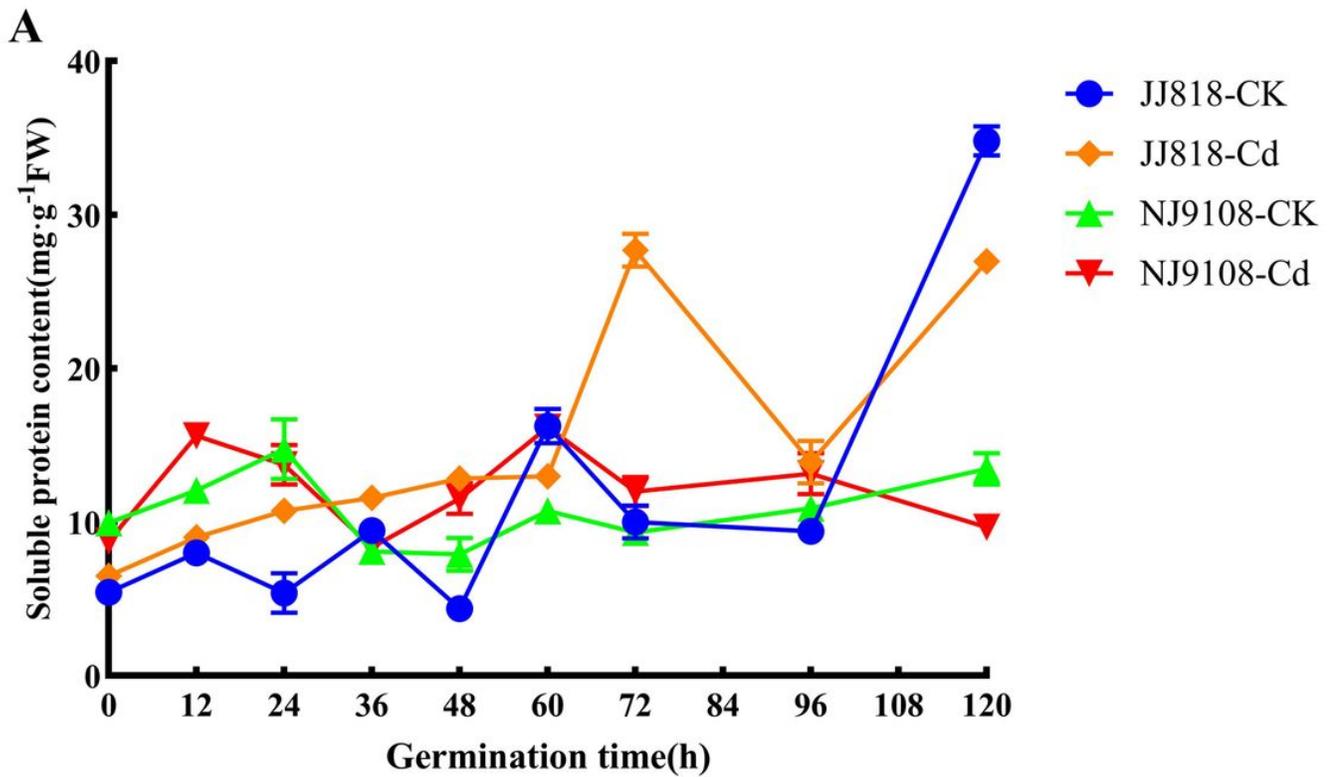
Effects of different treatments on phenotype of rice seed germination

(A), (B) growth phenotype photos ; (C), (D) germination rate ; (E), (F) shoot length ; (G), (H) root length. Bars = 1 cm

Figure 3

Effect of JA on soluble sugar content in seeds of rice varieties with different Cd tolerance

(A) 120 h soluble sugar content ; (B) 24 h sucrose content ; (C) 48 h sucrose content ; (D) 24 h fructose content ; (E) Fructose content at 48 h ; (F) 24 h glucose content ; (G) 48 h glucose content ; significant differences in lowercase letters ($P < 0.05$)



B

Figure 4

Effects of different jasmonic acid treatments on the soluble protein content of tested seeds under Cd stress

(A) 120 h soluble protein content ; (B) 24 h soluble protein content ; (C) 48 h soluble protein content ; significant differences in lowercase letters ($P < 0.05$)

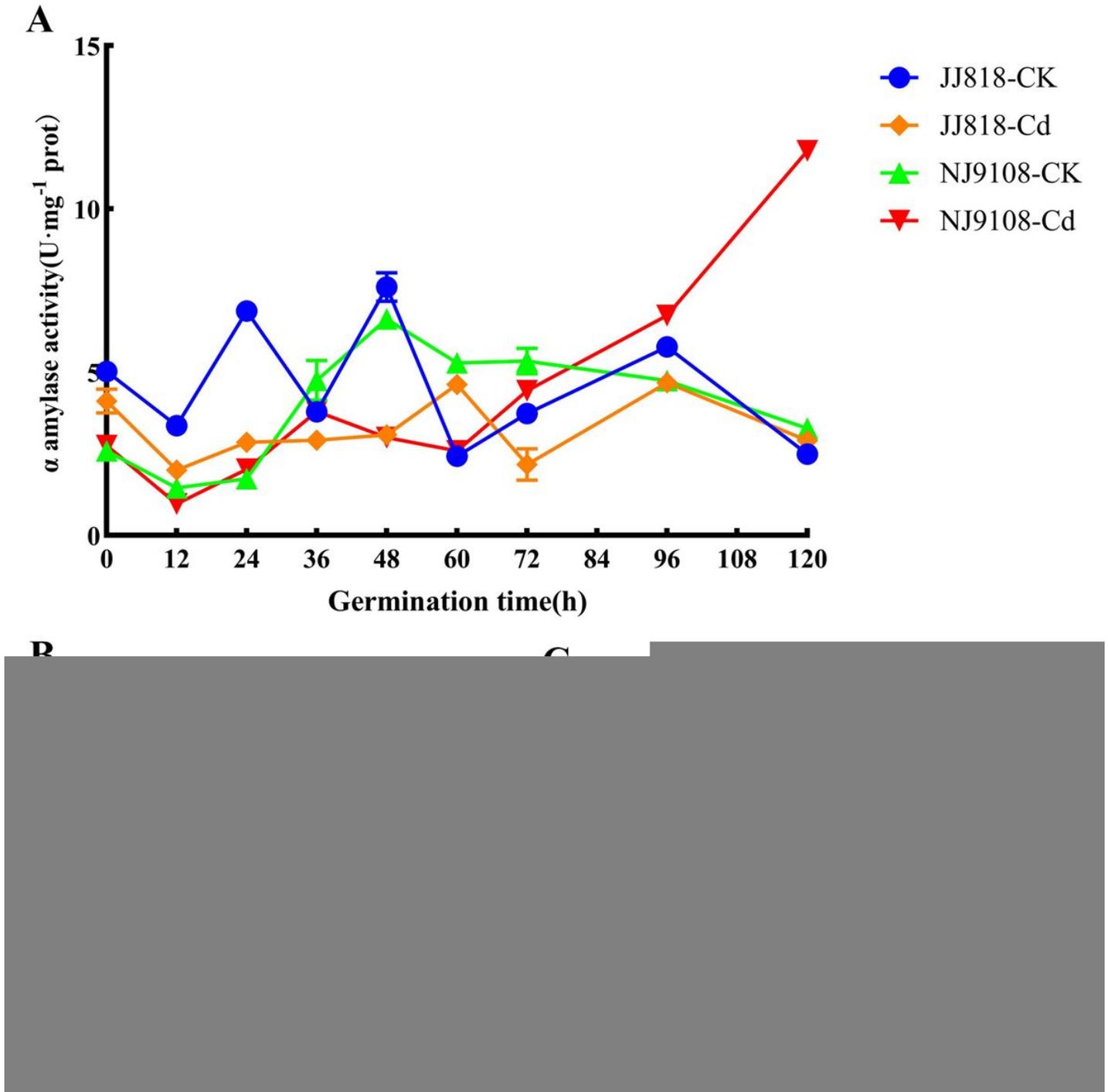


Figure 5

Effects of different jasmonic acid treatments on α -amylase activity of tested seeds under Cd stress

(A) 120 h α amylase activity content (B) 24 h α amylase activity content ; (C) α amylase activity content at 48 h ; significant differences in lowercase letters ($P < 0.05$)

Figure 6

Effects of different JA treatments on JA-related gene expression in tested seeds under Cd stress

(A), (B) *OsLOX1* relative expression ; (C), (D) *OsLOX4* relative expression ; (E), (F) *JlOsPR10* relative expression ; significant differences in lowercase letters ($P < 0.05$)

Figure 7

Effects of different JA treatments on the expression of α -amylase-related genes in tested seeds under Cd stress

(A), (B) *OsAmy1A* relative expression ; (C), (D) *OsAmy1C* relative expression ; (E), (F) *OsAmy3C* relative expression ; (G), (H) *OsAmy3E* relative expression ; significant differences in lowercase letters ($P < 0.05$)

Figure 8

Effect of different jasmonic acid treatments on expression of genes related to radicle elongation of test seeds under Cd stress

(A) *RL1* relative expression of JJ818 (B) *RL1* relative expression of NJ9108; different lowercase letters indicate a significant difference ($P < 0.05$)

Figure 9

Correlation of different jasmonic acid treatments on seed indexes under Cd stress

(A) Correlation of JJ818 indicators (B) Correlation of NJ9108 indicators. * $P < 0.05$; ** $P < 0.01$. Glu : glucose ; fru : fructose ; suc : sucrose ; sp : soluble protein ; α AMY : α amylase.