MORPHO-PHYSIOLOGICAL RESPONSES OF AN ALUMINUM-STRESSED RICE VARIETY 'LIANGYOUPEI 9'

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Abstract

This study explored the influence of aluminum (Al) stress on the growth of 'Liangyoupei 9 (LYP9)' rice variety. Seedlings were cultured under treatment with different concentrations of Al (0, 100, 500, and 1000 µmol/L; pH4.5) for 7 days and analyzed for their root Al accumulation, dehydrogenase activity, root H_2O_2 accumulation, antioxidant enzyme activity, and other relevant morphological indices. Results showed no significant differences between the rice seedlings treated with different concentrations of Al and those treated with the control, but the root length and relative root growth of the former decreased. Treatment with 100 µmol/L Al significantly led to the increase of fresh/dry weight and chlorophyll content of the rice seedlings. The increase in fresh weight and dry weight positively correlated with the increase in chlorophyll content. By contrast, treatment with 500 and 1000 µmol/L Al exerted inhibitory effects at different degrees. Hematoxylin staining showed that Al mainly accumulated at the apical meristematic and maturation zones, and its accumulation increased with increasing Al concentration. Treatment with 100 µmol/L Al stress significantly increased the root dehydrogenase activity of the rice seedlings, whereas treatment with 500 and 1000 µmol/L Al significantly decreased this parameter. Diaminobenzidine staining showed that Al stress induced root H_2O_2 accumulation in the rice seedlings and that the output of H_2O_2 demonstrated a dose-effect relationship with Al concentration. The activities of root superoxide dismutase(SOD), peroxidase (POD), and catalase (CAT) initially increased and subsequently decreased. However, the activities of these enzymes significantly decreased under 1000 µmol/L Al stress. Treatment with 100 µmol/L Al did not significantly influence the malondialdehyde (MDA) content in the roots, whereas treatment with 500 and 1000 µmol/L Al significantly increased this parameter. These findings show that the LYP9 rice variety has certain resistance to Al stress. However, this resistance is inhibited at Al^{3+} concentrations exceeding 500 µmol/L, and the inhibitory effect increases along with the concentration.

Key words: Rice, Aluminum stress, Anti-oxidation system, Aluminum tolerance.

Introduction

Soil acidification is considered to be a primary cause of soil degradation, which leads to loss of mineral elements, further decreasing soil fertility and finally affecting crop growth (Roem & Berendse, 2000). Acidic soil is mainly distributed in tropic, subtropic, and temperate areas across the world. In China acidic soil is mainly distributed in the southern tropic and subtropic areas, i.e., Yunnan and Guizhou areas, covering an area of about 2.04×10^8 hm^2 (Yi, 2006). Aluminum (Al) toxicity in acidic soil is a major factor affecting the yield and quality of crops (Kochian, 1995). Al exists in various forms in soil. In acidic conditions (pH<5.0), Al mainly exists in the form of Al^{3+} in the soil solution. At high pH values, $Al(OH)^{2+}$ and $Al(OH)^{z+}$ are the main forms of Al. Moreover, Al exists in the form of solid $Al(OH)^3$ when the soil solution is close to neutral and in the form of $Al(OH)^{4-}$ or aluminate when the solution is alkaline(Xu & Ji, 1998). An Al solution that is partially neutralized by strong basicity may produce a toxic form, i.e., Al_{13}, a type of ionic Al (Xu & Ji, 1998). Inorganic types of ionic Al, including Al^{3+}, $(AlOH)^{2+}$, and $Al(OH)^{2+}$, exert the most significant pernicious effect on plant roots(Azmat & Hasan, 2008).

Rice is a key grain crop worldwide. Approximately 13% of rice is planted in acidic soil across the world (Uexküll & Mutert, 1995). *Indica* and *Japonica* are two major subvarieties of rice. *Indica* is mainly distributed in tropic and subtropic areas, whereas *Japonica* is mainly distributed in temperate areas (Zhang *et al.*, 1992). In the highly weathered acidic soil, Al is a major stress factor that restricts rice growth and mineral element absorption, resulting in reduced crop yield (Fageria & Carvalho, 1982). Researchers observed that rice Al toxicity mainly occurs in dry acidic soil (Tanaka *et al.*, 1966). Rice root growth is significantly inhibited by Al toxicity (Xue *et al.*, 2009). It's reported that rice roots grow short and thick under Al stress, root tips appear like fishhooks, and aboveground tiller number is small. The fresh weight of aboveground parts and roots decreases significantly with increasing Al concentration, and this effect is more obvious in Al-sensitive varieties than in Al-tolerant varieties (Miftahudin *et al.*, 2007). Many researchers have analyzed the mechanisms underlying physiological Al tolerance at the tissue level, biochemical Al tolerance at the cell level, and genetic Al tolerance at the gene level, but a consensus on these mechanisms has yet to be reached (Liu *et al.*, 2007; Xue *et al.*, 2009; Wang *et al.*, 2014; Mei *et al.*, 2018). The Al tolerance capacity differs among growth periods of the same plant and among varieties of the same plant (Miftahudin *et al.*, 2007).

'Liangyoupei 9 (LYP9)' rice is cultivated through a two-line hybrid with Pei'ai 64S as the female parent and 9311 as the male parent(Liu *et al.*, 2015). This rice variety

features a higher chlorophyll content and photosynthetic rate than the hybrid rice Shanyou 63 that was popularized in large areas, and its photo-inhibition of photosynthesis is low at noon of sunny days (Guo *et al.*, 2013). Under photo-oxidation, the chlorophyll content of LYP9 decreases slightly because of its tolerance to this condition (Guo *et al.*, 2007). Although many studies have focused on LYP9 its response to Al stress remains unknown to date. Thus, the present study analyzed the Al tolerance of LYP9.

Materials and Methods

Cultivation and Al stress treatment of rice seedlings: LYP9 hybrid rice seeds of the same size and intact shape were selected. After the seeds were sterilized with 0.1% $HgCl_2$ for 15min, they were rinsed three to five times with distilled water, placed in a culture basin that contained perlite, added with the appropriate amount of water, and then placed at 25°C for germination. After germination, the seedlings were placed in a sunlight greenhouse for cultivation under a day/night temperature of 26/22°C, a photoperiod of 16h/8h, and a relative humidity of 70%. The seedlings were placed in Hoagland nutrient solution to grow for 7 days. Then, the seedlings that grew well and homogenously were selected for treatment as follows: control group (100 μmol/L $CaCl_2$, pH4.5) and 100, 500, and 1000 μmol/L$AlCl_3$ treatment groups (all contained 100 μmol/L $CaCl_2$, pH4.5) for 72h. The treatment of each concentration included15 rice seedlings and was repeated three times.

Measurement of relative root growth (RRG), fresh weight, and dry weight: The rice seedlings were divided into two groups, i.e., control group (0 μmol/L Al^{3+}) and treatment group (100, 500, and 1000 μmol/L Al^{3+}), to measure the main root length at 72h of treatment. To obtain the RRG, we deducted the original length from the root length measurement at 72h and then divided the elongation during the treatment period by the elongation of the control group during the same period. Rulers were used to measure the plant height and root length. The water on the surface of all the selected plants was dried with absorbent paper. Then, the plants were weighed on an electronic balance to determine their fresh weight. Fresh plants were placed in a 105°C oven for 10 min and then dried at 65°C to a constant weight.

Measurement of physiological indicators: The Al^{3+} distribution at the root tip was measured by hematoxylin staining (Polle *et al.*, 1978), and the Al^{3+} content at the root tip was measured by eriochrome cyanine R method (Qiu, 1989). Superoxide dismutase (SOD)activity wasmeasured by the NBT-illumination method, peroxidase (POD) activity by the guaiacol method, catalase (CAT) activity by the ultraviolet absorption method, malondialdehyde (MDA) by the thiobarbituric acid method, root activity by the TTC method, and chlorophyll content by the 80% acetone extraction method. The root H_2O_2 amount was determined in situ by the diaminobenzidine (DAB) staining method(Trujillo *et al.*, 2004).

Data processing and statistical analysis: Data measurement was conducted three times, and the results are expressed as the mean value± standard deviation (mean ± SD). The significance of difference and relevance was analyzed using IBM SPSS Statistics 19 (LSD, $\alpha=0.05$). Data processing was conducted with Microsoft Excel 2010.

Results

Effect of Al stress on the morphological indicators of rice seedling: As shown in Table 1, treatment with Al at 100, 500, and 1000 μmol/L significantly reduced the rice seedling RRG by 23.0%, 37.0%, and 50.0%, respectively. The treatment also significantly decreased root length, and this effect positively correlated with the change in RRG. Compared with the control group, the 100, 500, and 1000 μmol/L Al treatment groups decreased in plant height by 1.0%, 2.3%, and 2.9%, respectively, but the difference was not significant. This result indicates that Al stress has a minimal effect on the aboveground parts of rice. Treatment with 100 μmol/L Al significantly increased the fresh and dry weights of the rice seedlings, whereas treatment with 500 and 1000 μmol/L Al significantly decreased their fresh weight and dry weight by 6.3% and 7.4%, respectively, but the difference was not significant. Compared with that in the control group, the chlorophyll content in the 100 μmol/L Al treatment group was significantly higher, that in the 500 μmol/L Al treatment group was not significantly different, and that in the 1000 μmol/L Al treatment group was lower. Correlation analysis showed that the fresh and dry weights of the rice seedlings were significantly positively correlated with chlorophyll content (Table 2). This result indicates that a low Al concentration increases the fresh and dry weights of rice seedlings by increasing leaf chlorophyll content and promoting photosynthesis.

Root Al distribution and root activity changes in rice seedlings under Al stress: As shown in Fig. 1A, hematoxylin staining revealed the close relationship of the accumulation degree of Al at the root tip to the Al concentration in the soil solution where the roots were located. A few Al were distributed at the meristematic and elongation zones of the rice root tip after treatment with 100 μmol/L Al, whereas many Al were distributed at the meristematic and maturation zones of the rice root tip after treatment with 500 and 1000 μmol/L Al. As shown in Fig. 2, the content of Al at the root tip of the rice seedlings significantly increased with increasing Al processing concentration. The Al stress may also change root activities. TTC staining showed the intensity of root activity, i.e., the darker the red is, the stronger the root activity. As shown in Fig. 1B, the TTC staining of the seedling roots initially increased and then decreased with increasing Al processing concentration. Fig.2 also shows that the 100 μmol/L Al treatment significantly promoted root dehydrogenase activity, whereas the 500 and 1000 μmol/L Al treatments inhibited this activity. Correlation analysis showed that the root activity negatively correlated with the Al content at the root tip, indicating that the accumulation of Al may reduce the activity.

Table 1. RRG, root length and other growth indicators of rice seedlings under Al stress.

Al	RRG	RL /(cm)	PH /(cm)	FW /(g)	DW /(g)	ChlC/ (mg/g)
0	1.00±0.00Aa	25.77±0.85Aa	27.47±1.01Aa	8.78±0.03Bb	0.95±0.04Bb	2.97±0.13Bb
100	0.77±0.08Bb	19.85±0.91Bb	27.19±1.47Aa	10.02±0.15Aa	1.51±0.15Aa	3.68±0.17Aa
500	0.63±0.06BCb	16.74±1.25Bc	26.84±0.76Aa	7.56±0.27Cc	0.89±0.08Bb	2.99±0.17Bb
1000	0.50±0.05Cc	12.69±0.99Cd	26.66±1.75Aa	7.01±0.18Cc	0.88±0.12Bb	2.05±0.13Bc

Note: Letters in the table indicate significant differences at p<0.01 or p<0.05 level, respectively. RL, Root length; PH, Plant height; FW, Fresh weight; DW, Dry weight; ChlC, Chlorophyll content

Table 2. Correlation analysis of some growth indicators of rice seedlings under Al stress.

	RRG	PH	RL	FW	DW	ChlC
RRG	1	0.96**	1.00**	0.47	0.19	0.29
PH		1	0.95**	0.52	0.28	0.42
RL			1	0.43	0.23	0.34
FW				1	0.97*	0.91*
DW					1	0.93*
ChlC						1

Note: * and ** in the table indicate significant Pearson correlation at p<0.05 and p<0.01 level, respectively
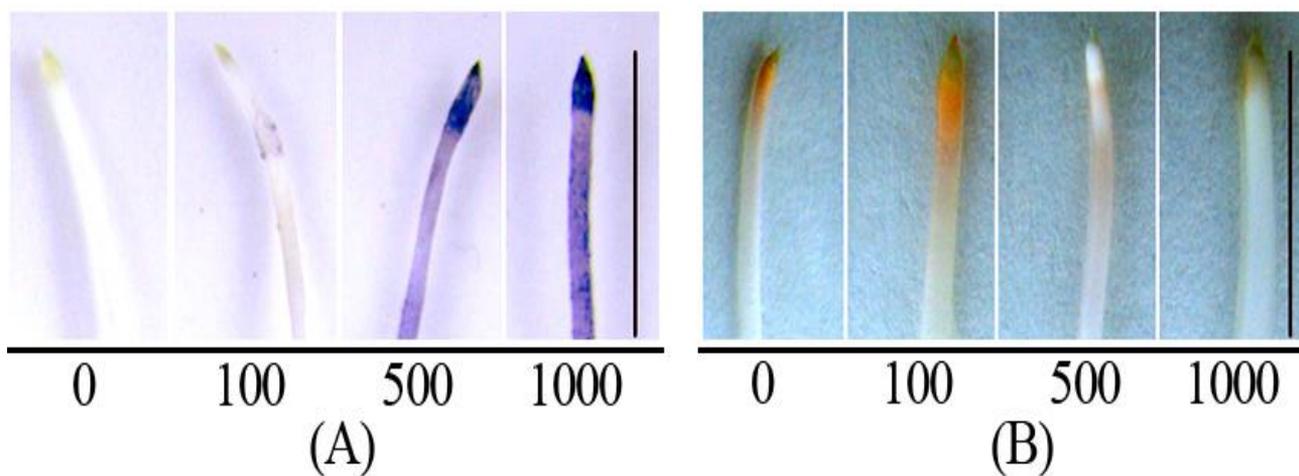

Fig. 1. Exhibition of root dehydrogenase activity and Al distribution in the root tips of rice seedlings under Al stress. Scale bar=0.5 cm. A, Al distribution displayed by Hematoxylin staining; B, Root dehydrogenase activity displayed by TTC staining
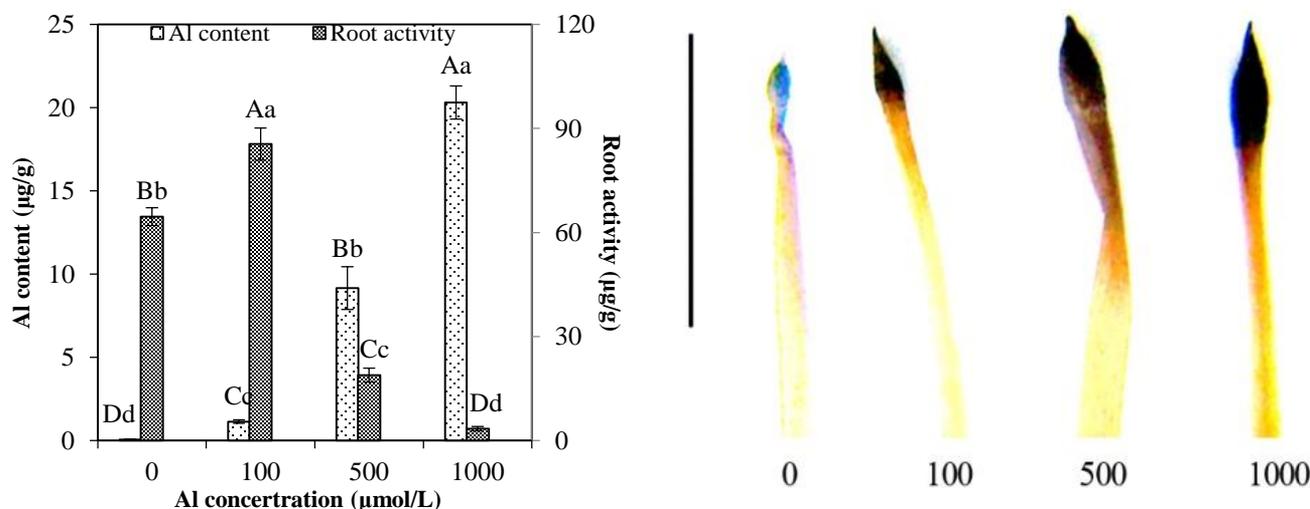

Fig. 2. Determination of Al content in the root tips and the root activity under Al stress.

Fig. 3. H$_2$O$_2$ accumulation in the root of rice seedlings demonstrated by DAB staining. Scale bar=1.0 cm.
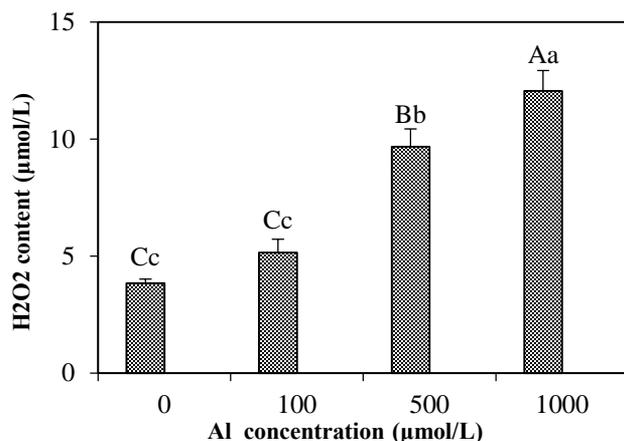
Fig. 4. H_2O_2 production in root of rice seedlings under Al stress.

Changes in H_2O_2 content of rice roots under Al stress:
As shown in Fig. 3, DAB staining indicated that the H_2O_2 yield was affected by the treatment with Al at different concentrations. With the increase in Al concentration, DAB staining became intense gradually; the most intense staining was observed in the 0–0.5cm meristematic zone, indicating that the H_2O_2 content was relatively high in this zone. Quantitative analysis showed that the H_2O_2 yield was not significantly higher in the 100 μmol/L Al-treated group than in the control group but was significantly higher in the 500 and 1000μmol/L Al-treated groups (Fig. 4). Correlation analysis showed that the H_2O_2 yield at the roots significantly positively correlated with the content

of Al at the root tip, i.e., the more Al that accumulated at the root tip, the greater the effect of oxidative stress on the roots. At the same time, the root activity significantly negatively correlated with the H_2O_2 yield at the roots. This result indicates that H_2O_2 accumulation is also a key factor contributing to the decrease in root activity.

Changes in antioxidant enzyme activity of rice roots under Al stress: As shown in Fig. 5, the antioxidant enzyme system of the rice seedling roots exhibited different responses to Al stress. SOD activity initially increased and then decreased with the increase of Al. The SOD activity of the rice seedling roots treated with 100 and 500 μmol/L Al significantly increased by 39.8% and 47.9%, respectively. Meanwhile, the SOD activity under 1000 μmol/L Al treatment was 12.5% lower than that of the control group, but the changes were not significant. Similarly, POD activity initially increased and then decreased with the increase of Al. Comparing with the control group, the POD activity under the 500 μmol/L Al treatment was significantly higher. CAT activity was significantly influenced by Al stress. The CAT activity of the rice seedling roots treated with 100 μmol/L Al significantly increased. Meanwhile, the CAT activity in the 500 μmol/L Al treatment group was not significantly lower than that in the control group. Treatment with 1000 μmol/L Al significantly inhibited CAT activity. Meanwhile, the root MDA content increased with increasing Al processing concentration. However, no significant difference in MDA content was found between the 100 μmol/L Al treatment group and the control group.
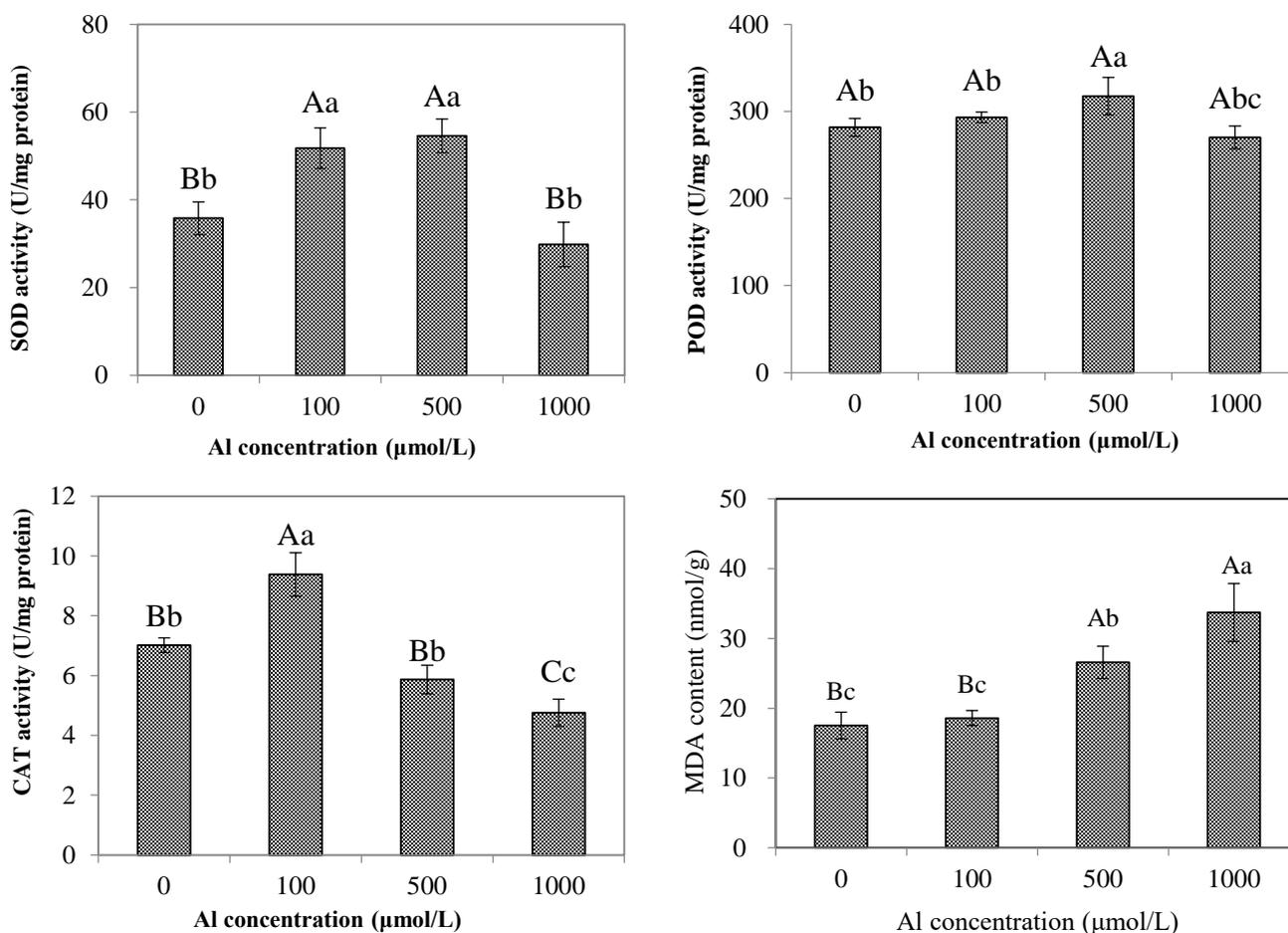

Fig. 5. Effects of different concentrations of Al on SOD, POD, CAT activities and MDA content in rice seedlings.

Table 3. Relationship of root antioxidant enzyme activities, Al content, dehydrogenase activity and H$_2$O$_2$ production of rice seedlings under Al stress.

	SOD	POD	CAT	MDA	Al content	Root activity	H$_2$O$_2$
SOD	1	0.88	0.54	-0.28	-0.46	0.31	-0.27
POD		1	0.93*	-0.74	-0.78	0.79	-0.35
CAT			1	-0.85	-0.83	0.95*	-0.79
MDA				1	0.98**	-0.93*	1.00**
Al content					1	-0.91*	0.99**
Root activity						1	-0.93*
H$_2$O$_2$							1

Note: * and ** in the table indicate significant Pearson correlation at p<0.05 and p<0.01 level, respectively

Discussion

Influence of Al stress on rice seedling roots and plant height: Bao *et al.*, (2015) found that Al inhibits the root length and number of root tips of rice varieties "Wuyunjing 7" and "Yangdao 6". Wang *et al.*, (2016) observed that 100 μmol/L Al stress significantly inhibits the root tip elongation and growth and reduces the plant biomass of "Nipponbare" rice. In the present study, the RRG of LYP9 gradually decreased with increasing Al concentration. This result indicates that the Al treatment can significantly inhibit the root elongation of LYP9 rice variety. Kang *et al.,* (2011) determined that Al stress can significantly inhibit the growth of the rice seedling parts above the ground. However, the results of the present study showed that the Al treatment did not significantly decrease rice seedling height possibly because different varieties of rice may exhibit different responses to Al stress. Overall, Al stress exerted a more significant effect on the underground parts than on the aboveground parts of rice seedlings (Table 3).

Al distribution at the root tip and changes in root dehydrogenase activity caused by Al stress: Inhibition of root elongation and growth is the typical symptom of Al toxicity, and inhibition of root growth is the result of the inhibitory effect on cell division(Meng *et al.*, 2009). Several studies indicated that the 2–5mm part of the root tip is the most sensitive area to Al toxicity(Yan *et al.*, 2009). In the present study, treatment with 500 and 1000 μmol/L Al caused a significant amount of Al to accumulate at the root cap and meristematic zone of the root tip, consistent with the findings of previous studies(Delhaize *et al.*, 1993; Liu & Xu, 2015). Moreover, a large amount of Al was distributed in the maturation zone of the root tip. Considering that the maturation zone is the major area for absorption of water and mineral nutrients, we speculated that it may also be the major area for plants to absorb Al from the soil. Li *et al.,* (2004) observed that the 100 μmol/L Al stress could increase the root dehydrogenase activity of Al-tolerant corn varieties. The root dehydrogenase activity of *Trichosantheskirilowii* gradually increases with increasing Al stress from 100μmol/L to 300, 500, and 1000 μmol/L(Zhou *et al.*, 2011). In the present study, the 100 μmol/L Al treatment significantly promoted the root dehydrogenase activity, whereas the 500 and 1000 μmol/L Al treatments inhibited this activity. This finding indicates that the root dehydrogenase activity of rice is sensitive to Al concentration. Correlation analysis revealed that the reduction of root dehydrogenase activity is closely related to the accumulation of Al at the root tip.

Influence of Al stress on the fresh weight, dry weight, and chlorophyll content of rice seedlings: It's reported that treatment with 100 μmol/L Al significantly inhibits the content of chlorophyll in rice(Wang *et al.*, 2016). In the present experiment, the 100 μmol/L Al treatment significantly increased the chlorophyll content of LYP9 rice variety, the 500 μmol/L Al treatment did not significantly influence this content, and the 1000 μmol/L Al treatment significantly reduced this content. This finding indicates that the rice variety is characterized by high Al tolerance. However, a high concentration of Al stress is detrimental to the chlorophyll synthesis of rice or results in chlorophyll degradation. The influence of Al treatment on the biomass of rice seedlings is dose dependent. The 100 μmol/L Al treatment can significantly increase of the fresh and dry weights of rice seedlings probably because an increase in chlorophyll content may promote the photosynthesis of leaves and accordingly facilitate the accumulation of organic matter. Under the 500 and 1000 μmol/L Al treatments, the fresh weight decreased significantly, whereas the dry weight did not significantly decrease. The root activity of the seedlings at the concentrations also significantly decreased. Thus, we speculated that Al decreases the fresh weight by inhibiting the absorption of water and mineral substances. The 100 μmol/L Al treatment inhibited the root elongation of LYP9 rice variety while increased the chlorophyll content. This finding indicates that the leaves and roots exhibit different responses to Al stress.

Influence of Al stress on root antioxidant enzyme activity and root H2O2 accumulation: Al-induced ROS burst and mitochondrial dysfunction are the key factors inhibiting the root growth of plants (Yamamoto *et al.*, 2002). When the plants are in stress, SOD, POD, CAT, and other protective enzyme systems could effectively remove the reactive oxygen produced by membrane lipid peroxidation, guard against the peroxidation of plant cell membrane, and accordingly reduce the damage to plant cells (Liu *et al.*, 2004). SOD can catalyze reactive oxygen ($O_2^{\bullet-}$) to produce O_2 and H_2O_2 and is a major projective

enzyme of the antioxidant defense system (Pagariya *et al.*, 2012). POD and CAT are also key anti-oxidation enzymes used to eliminate excess H_2O_2 in cells and maintain a low level of reactive oxygen (Liu *et al.*, 2012). It's observed that the root POD activity of the resistant rice variety *Azucena* increases under Al stress (Ma *et al.*, 2012). The POD and CAT activities of LYP9 rice roots under 500 μmol/L Al stress do not differ significantly from those of the control group, whereas those under 750 μmol/L Al stress increase significantly(Xie *et al.*, 2009). In the present study, the SOD and CAT activities increased after 100 μmol/L Al treatment, indicating that the oxidative stress of rice seedling root increased. By contrast, the SOD and CAT activities decreased after the 1000 μmol/L Al treatment. This finding indicates that the oxidative damage of the roots was aggravated. Within the range of concentration in this study, the POD activity initially increased and subsequently decreased, whereas the SOD and CAT activities showed a change opposite to that of the H_2O_2 level. This finding indicates that POD, SOD, and CAT participate in the removal of reactive oxygen. Correlation analysis revealed that the accumulation of many Al at the root tip may decrease root antioxidant enzyme activity and increase MDA content, which further lead to H_2O_2 burst and finally inhibit the root activity and affect the metabolic activity of root cells.

Conclusions

In summary, analysis of the effect of different concentrations of Al on LYP9 rice seedlings showed that the it has a certain tolerance to Al. Although the 100 μmol/L Al stress reduces RRG, it may strongly promote the chlorophyll content, fresh weight, and dry weight of the seedlings. Moreover, the 100 μmol/L Al treatment could significantly promote the root activity, improve the root SOD and CAT activities, and maintain relatively low levels of H_2O_2 and MDA at the roots. Thus, an appropriate concentration of Al stress could promote the *Indica*-type rice to adapt well to acidic soil.

Acknowledgments

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