Pak. J. Bot., 52(2): 419-426, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-2(20)

RESPONSE OF THE GROWTH OF *OPHIOPOGON JAPONICUS* AND ITS PHYSIOLOGICAL CHARACTERISTICS TO ALUMINUM STRESS

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Abstract

To explore aluminum (Al) tolerance in *Ophiopogon japonicus*, we used $AlCl_3$ at the concentrations of 0 (control), 10, 20, 30, 40, 50, and 60 mmol/L to treat *O. japonicas* for detecting the growth and physiological indexes of the plant under $AlCl_3$ stress. Compared with the control, fresh weight, dry weight, water content, contents of K, Mg, and Fe, activity of catalase in leaves and roots, Ca^{2+}content in roots, superoxide dismutase (SOD) activity, and root activity of *O. japonicus* roots showed a declining trend with the increase in the $AlCl_3$ concentration. Meanwhile, the chlorophyll, Ca, and soluble protein (SP) contents of leaves, SOD activity and peroxidase (POD) activity of leaves and roots initially increased and then declined. However, the soluble sugar (SS) and Proline (Pro) contents of leaves slightly declined and then increased. The SS, SP, and Pro contents of roots, Al, free amino acid (FAA), superoxide anion ($O_2^{\bullet-}$), and MDA contents, and plasma membrane permeability of leaves and roots showed a increasing trend. Therefore, low-concentration $AlCl_3$ stress inhibited the growth of*O. japonicus*, resulting in low water deficit and decline in root activity. Organic osmotica, such as SS, SP, Pro, and FAA, accumulated in leaves and roots, but no inorganic ions (including K, Ca, Mg, and Fe) were found. The antioxidant capacity decreased slightly, and the degree of oxidative damage was mild. High-concentration $AlCl_3$ stress seriously inhibited the growth of *O. japonicus*, leading to a remarkable reduction in water deficit and root activity. Organic small-molecule osmotica further accumulated in the plant, but inorganic ion deficit was aggravated. The antioxidant capacity decreased, whereas the degree of oxidative damage increased. Comprehensive analysis demonstrated that *O. japonicus* could endure $AlCl_3$ stress ≤30mmol/L.

Key words: *Ophiopogon japonicus*; Al stress; Osmotica; Oxidation resistance; Physiological property

Introducation

Al is the most abundant metallic element in the Earth's crust, accounting for 8% of the total mass of the Earth's crust. In neutral or alkaline soil solution, the main forms of Al are insoluble silicate and oxides, which are harmless to plant growth. However, in acid (pH<5.0) soil, Al in the fixed state is easily activated to form soluble Al (e.g.,Al^{3+}), which is harmful to plants (Ma & Furukawa, 2003). At present, the application of acid chemical fertilizers and the frequent occurrence of acid rain and acid fog cause excessive acidification, thereby releasing a large amount of $Al^{3+}$from the soil, which can cause major toxic effects on soil, plants, and ecosystem (Rout *et al*., 2001; Ma & Furukawa, 2003; Larsen *et al*., 1997). According to statistics, more than 50% of arable land in the world contains acidic soil, which is widely distributed (Barcelo & Poschenrieder, 2002). Soil acidification is an important problem in turf establishment and maintenance (Murray & Foy, 1978). Previous studies reported that Al toxicity seriously restricts the growth of turf grass in acid soil (Yan & Liu 2008; Zhang *et al*., 2015; Chen *et al*., 2011; Foy & Murray,1998a; Foy & Murray,1998b; Huang *et al*., 2017; Yan *et al*., 2010; Chu *et al*., 2012; Rengel & Robinson, 1989). Therefore, selecting and planting Al-tolerant turf grass in the greening of acidic Al-toxic soil is one of the effective ways used to improve the quality of turf grass.

Ophiopogon japonicus, also called Ophiopogon (Radix ophiopogonis), is a perennial evergreen herb of the genus Ophiopogon Ker, Liliaceae, with strong stress resistance. It is heliophilous, shade-tolerant, and easy to reproduce, with low requirements for the growing environment (Liu *et al*., 2016). Pruning, irrigating, and fertilizing after planting are unnecessary. Hence, the cost of planting and conservation is very low (Zhang, 2003). *O. japonicas* is considered the first choice of herbaceous ornamental leaf cover plants in the landscaping and greening of areas with poor soil and low light. Several studies have analyzed the response of *O. japonicas* to acid rain in terms of growth and physiological characteristics (Liu *et al*., 2011), SO_2 (Yang *et al*., 2017), lead pollution (Xiong *et al*., 2010), salt stress (Liu *et al*., 2016), and other adverse situations. However, Al tolerance of *O. japonicus* in acid soil is rarely reported. For this reason, potted *O. japonicas* was treated by using different concentrations of $AlCl_3$ to explore the growth changes in leaves and roots, the content of osmotica, antioxidant capacity, and other aspects. Results of this study can lay the foundation for further research on Al-tolerant capacity and Al-tolerant mechanism of *O. japonicus*. This study also provides a reference for the rational selection of *O. japonicus* for greening in shady areas of acidic Al-toxic soil, saving conservation costs and increasing the diversity of turf resources.

Materials and Method

Cultivation of *O. japonicus* and treatment by $AlCl_3$: *O. japonicus* was from the plant division on *O. japonicus* turf in the Western Campus of Anhui Science and Technology University. *O. japonicus* plants of similar growing trend were selected on March 5, 2017. The plants were moved to plastic basins with equal amounts of clean sand (15 cm high, diameter 20cm), with five plants in each basin. This study involved a total of 70 basins. The plants were watered with tap water and then shaded with a sunshade net. The sunshade net was removed 10 days later, and the plants were cultivated outdoors under natural light. Complete Hoagland nutrient solution was used to irrigate and cultivate plants under similar management measures. On May 10, $AlCl_3$ stress treatment was conducted. $AlCl_3$ concentrations were 0 (control), 10, 20, 30, 40, 50, and 60 $mmol \cdot L^{-1}$, resulting in seven treatments with 10 basins for each treatment. The corresponding treatment solution was prepared with complete Hoagland nutrient solution. To

guarantee stable $AlCl_3$ concentration, complete Hoagland nutrient solution in a given concentration was employed to irrigate once every day, with 300 mL of irrigation amount per basin. About 200 mL of treatment solution flowed out. On June 1, the relevant indexes were determined. For each treatment, the determination of each index was repeated three times.

Determination method: Fresh weight, dry weight, water content, chlorophyll content, and root activity were determined according to the relevant literature (Liu & Zhao, 2005a). Spectrophotometry of Eriochrome Cyanine R was conducted to determine the Al content (Chen *et al*., 1993). The contents of K, Ca, Mg, Fe, soluble sugar (SS), soluble protein (SP), free amino acid (FAA), proline (Pro), $O_2^{\bullet-}$, and malondialdehyde (MDA); plasma membrane permeability; and superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activity were determined according to the relevant literature (Liu & Zhao, 2005b; Zhang *et al*., 2018).

Data processing: The original data measured in Excel 2010 software test were used. DPSv7.0 was employed for analysis and multiple comparisons. The test results are represented by the mean ± standard deviation (mean ± SD).

Results and Analysis

Influence of $AlCl_3$stress on fresh weight, dry weight, Al content, water content, chlorophyll content, and root activity of *O. Japonicus*: Compared with the control group, the fresh weight and dry weight of *O. japonicus* leaves and roots under $AlCl_3$stress showed a declining trend. The fresh and dry weights of *O. japonicus* leaves and roots under $AlCl_3$stress significantly decreased by 14.96%-44.40%, 6.85%-49.25%, 13.23%-31.43%, and 5.12%-39.41%, respectively, and the descending extent of fresh weight of roots was the maximum reduction observed (Fig. 1).

In leaves and roots of control plants, Al contents were 1.23and 7.64 mg/g DW, respectively. Under 10–60 mmol/L $AlCl_3$ stress, Al contents in leaves and roots significantly increased by 0.98–4.38 and 0.42–1.59 times, respectively. Al contents in leaves were lower than those in roots under the same treatment. Under $AlCl_3$ stress, water contents in leaves and roots declined. Compared with the control, the water contents in leaves and roots significantly decreased by 0.72%–8.31% and 0.41%–4.22%, respectively. Under treatment with the same concentration, the water content in leaves was lower than that in roots, whereas the descending extent of the water content in leaves was higher than that in roots with rising $AlCl_3$concentration. Compared with the control, the chlorophyll content under 10mmol/L $AlCl_3$stress was higher than that of the control by 6.62%, but the difference was not significant. Under 20–60mmol/L $AlCl_3$stress, the chlorophyll content was significantly lower than that of the control by 10.87%–57.32%. Under $AlCl_3$stress, root activity decreased by 6.39%–63.60% compared with the control. Under 10mmol/L $AlCl_3$stress, the difference in root activity compared with the control was not significant, but that under 20–60mmol/L $AlCl_3$stresswas significant (Fig. 2).

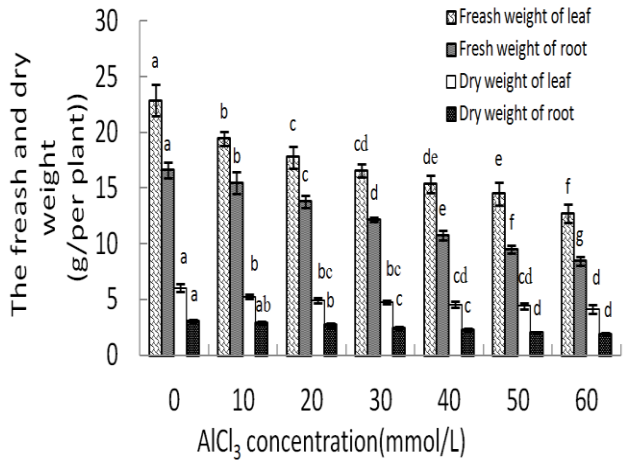

Fig. 1. Changes in the fresh and dry weight under $AlCl_3$ stress. Note: Different lowercase letters in the same column indicate a significant difference at $p<0.05$. Similarly hereinafter

Influence of $AlCl_3$ stress on K, Ca, Mg, and Fe contents in *O. Japonicus*: Under $AlCl_3$ stress, the K content in leaves and roots significantly decreased by 6.43%–39.98% and 3.05%–41.73%, respectively, compared with the control. Under the same treatment, the K content was higher in leaves than in roots. Under 10mmol/L $AlCl_3$ stress, the Ca content in leaves was higher than that of the control by 0.88%, but the difference was not significant. Under 20–60 mmol/L $AlCl_3$ stress, the Ca content was reduced by 3.33%–16.74% compared with the control. Under 10–60 mmol/L $AlCl_3$ stress, the Ca content in roots was reduced by 16.39%–72.24% compared with the control. Under $AlCl_3$ stress, the Mg content in leaves and roots decreased by 5.10%–46.27% and 14.57%–50.09%, respectively, compared with the control, and the Fe content was significantly reduced by 9.66%–50.28% and 9.35%–49.95%, respectively. Under the same treatment, the Mg and Fe contents were lower in leaves than in roots (Fig. 3).

Influence of $AlCl_3$stress on SS, SP, FAA, and Procontents of *O. Japonicus*: Under 10mmol/L $AlCl_3$ stress, the SS content of *O. japonicus* leaves slightly decreased by 2.74% compared with that of the control, but the difference was insignificant. Under 20–60mmol/L $AlCl_3$ stress, the SS content was 10.95%–114.94% higher than that of the control. The SS content in roots under $AlCl_3$ stress increased by 20.44%–341.20% compared with that of the control group. Under 20–50mmol/L$AlCl_3$stress, the SP content in leaves was13.29%–44.35% higher than that of the control. The SP content in leaves under 60mmol/L $AlCl_3$ stress was lower than that under 50mmol/L stress but still 22.72% higher than that of the control, with a significant difference. Under $AlCl_3$stress, the SP content in roots increased by 6.34%–49.34% compared with the control, whereas the FAA contents in leaves and roots were 6.72%–54.09% and 14.67%–293.33% higher than the corresponding control, with a significant difference. Under 10mmol/L $AlCl_3$stress, the Pro content in leaves was 5.89% lower than that of the control, but the difference was insignificant. Under 20–60mmol/L $AlCl_3$stress, the Pro content was 5.30%–75.62% higher than that of the control. Under 10–60 mmol/L $AlCl_3$stress, the Pro content in roots significantly increased by 27.35%–245.74% compared with the control (Fig. 4).

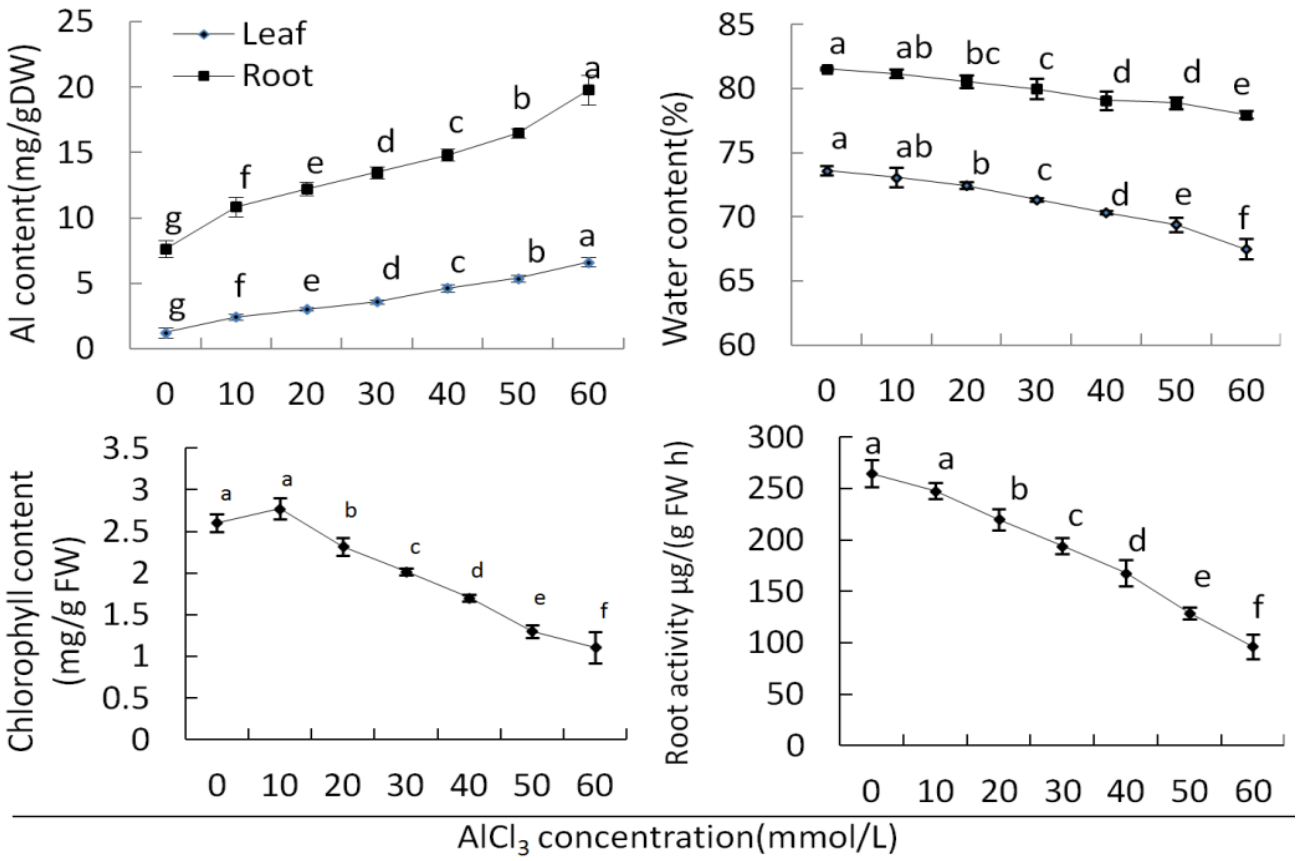

Fig. 2. Changes in Al content, water content, chlorophyll content, and root activity under $AlCl_3$ stress.

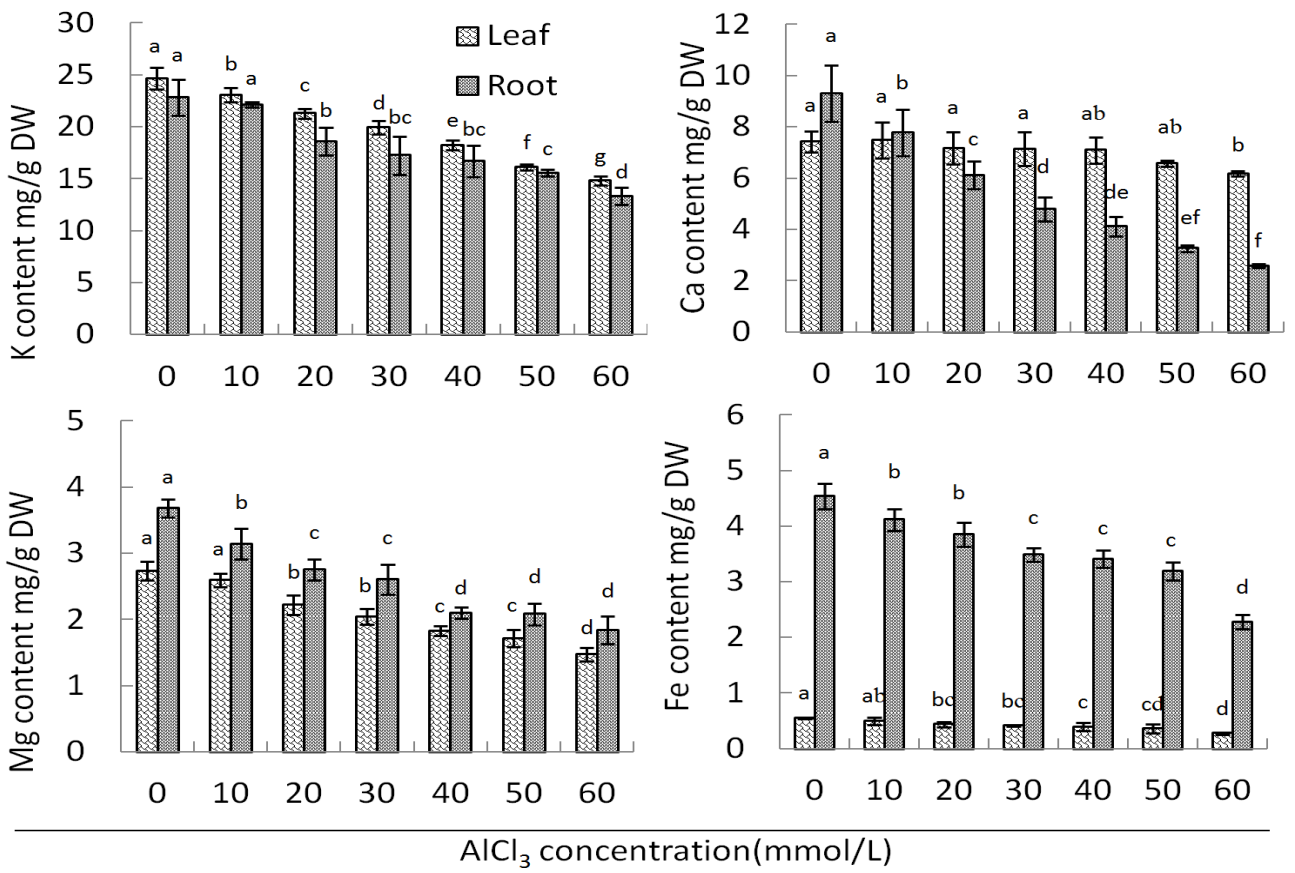

Fig. 3. Changes in K, Ca, Mg, and Fe contents in *O. japonicas* under $AlCl_3$ stress.

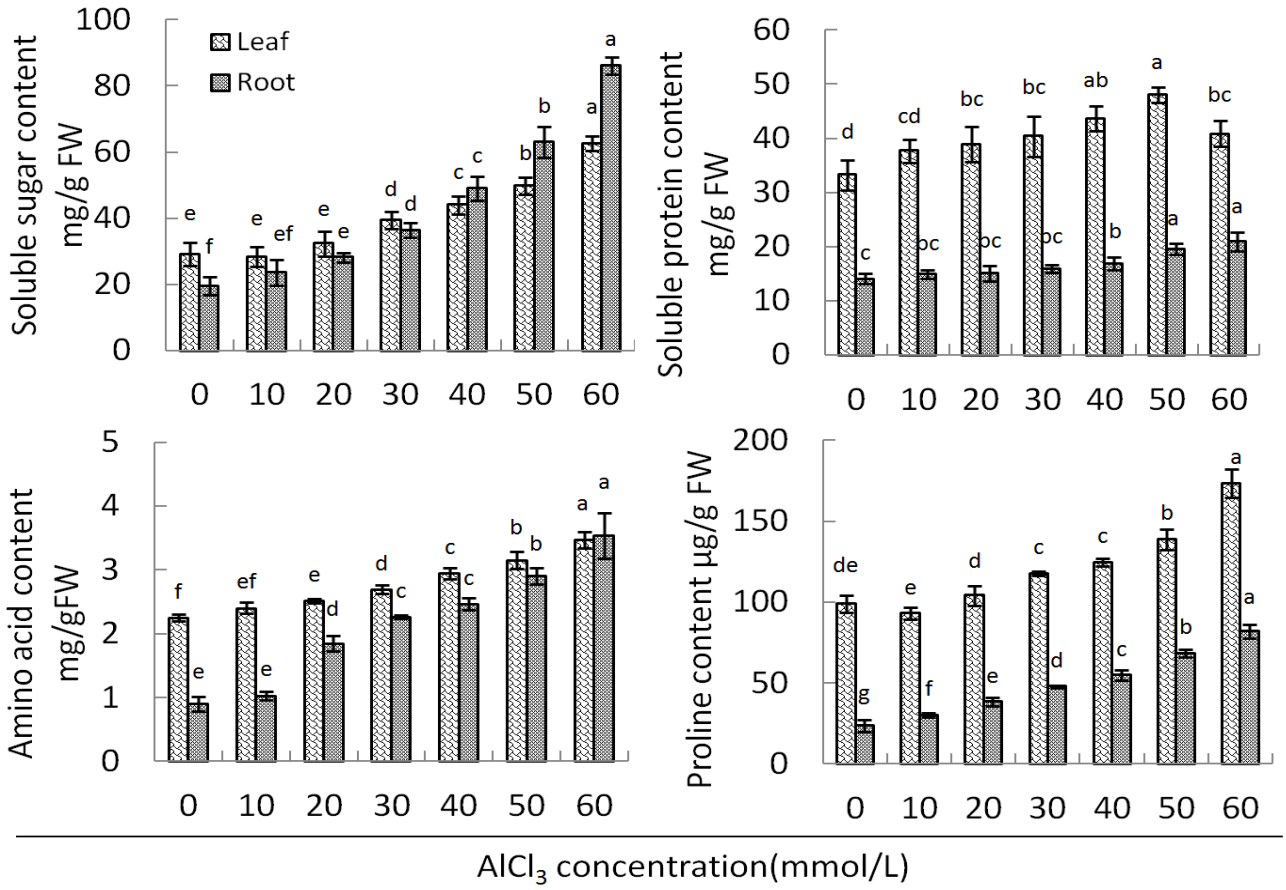

Fig. 4. Changes in the contents of soluble sugar, soluble protein, amino acid, and proline under $AlCl_3$ stress.

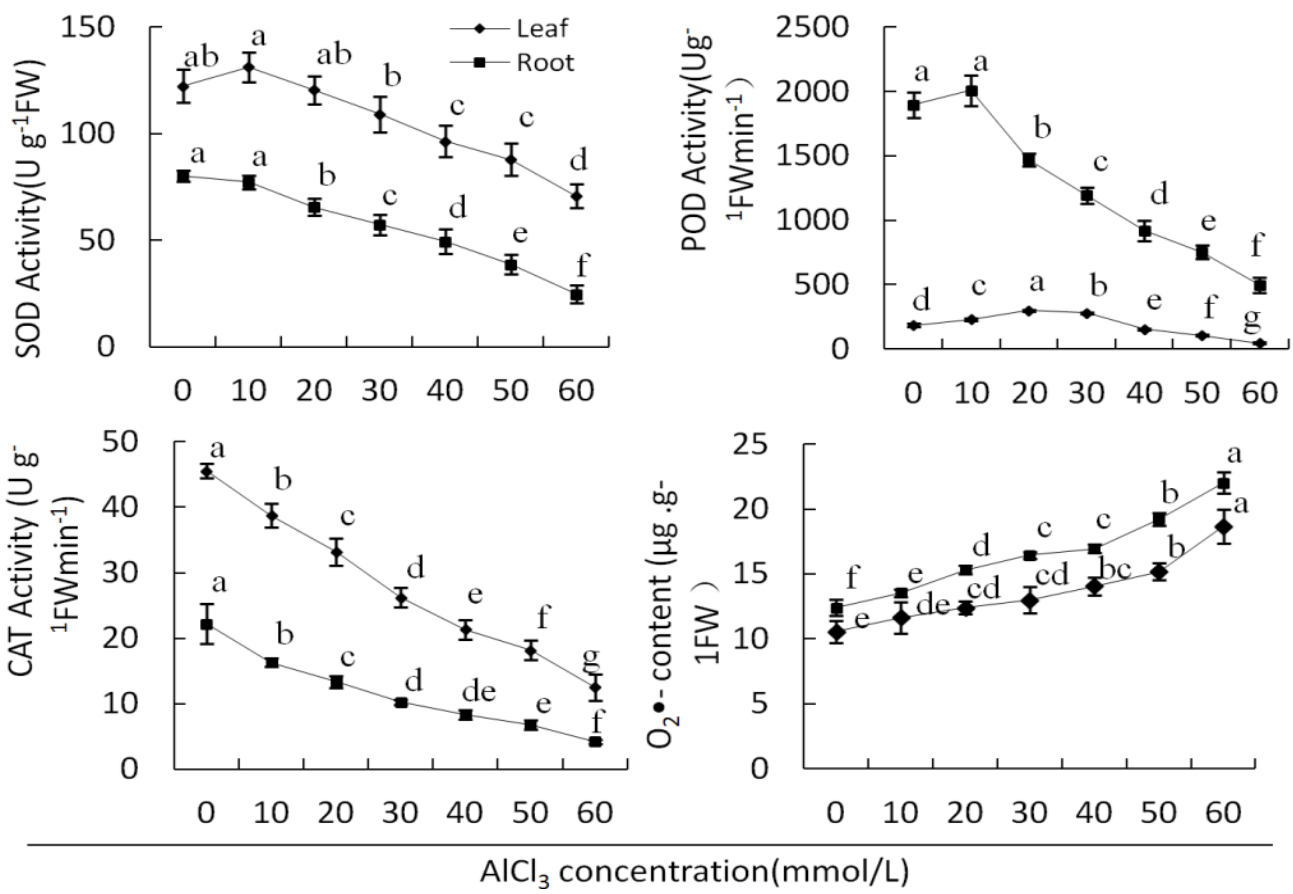

Fig. 5. Changes in the activities of SOD, POD, and CAT and $O_2^{\bullet-}$ content of *O. japonicas* under $AlCl_3$ stress.

Influence of $AlCl_3$ stress on SOD, POD, and CAT activity;$O_2^{\bullet-}$content; plasma membrane permeability; and MDA content of *O. Japonicus*: Compared with the control, under 10mmol/L $AlCl_3$ stress, SOD activity in leaves increased by 7.25%, but the difference was not significant. Under 20–60 mmol/L $AlCl_3$ stress, SOD activity decreased by 1.44%–42.20%.Thus, SOD activity of roots decreased by 3.54%–69.10% compared with the control under $AlCl_3$stress.Under 10mmol/L $AlCl_3$stress, the difference in SOD activity in roots from the control was not significant, but SOD activity was significantly lower than that of the control under 20–60 mmol/L $AlCl_3$. Under the same treatment, SOD activity of leaves was higher than that of roots. Under $AlCl_3$ stress, POD activity in leaves initially increased and then decreased; under 10, 20, and 30mmol/L $AlCl_3$ stress, POD activity in leaves was 25.69%, 63.56%, and 52.31% higher than that of the control, respectively. Under 40–60 mmol/L $AlCl_3$ stress, POD activity was reduced by 15.81–75.30% compared with the control. Under 10mmol/L $AlCl_3$ stress, POD activity in roots was 5.99% higher than that of the control, and the difference was insignificant. Under 20–60 mmol/L $AlCl_3$ stress, POD activity in roots was reduced by 22.37–73.73% compared with the control; under the same treatment, POD activity in leaves was lower than that in roots. Under $AlCl_3$ stress, CAT activity in leaves and roots showed a declining trend. Compared with the control, CAT activity significantly decreased by 14.96–72.55% and 26.60–81.13%, respectively; under the same treatment, CAT activity in leaves was higher than that in roots (Fig. 5).

Compared with the control, the $O_2^{\bullet-}$ content in leaves and roots showed an increasing trend; it significantly increased by 9.99%–76.49% and 9.15%–77.63%, respectively. Under the same treatment, the$O_2^{\bullet-}$ content in leaves was lower than that in roots (Fig. 5). Under $AlCl_3$ stress, plasma membrane permeability in leaves and roots showed an increasing trend; they significantly increased by 5.39–51.16% and 20.24–168.49% respectively. Under the same treatment, plasma membrane permeability of leaves was lower than that of roots. Compared with the control, under Al stress, MDA contents in leaves and roots increased by 7.76–64.94% and 36.76–136.50%, respectively. In the control, the MDA content in leaves was higher than that in roots. However, under the same concentration of $AlCl_3$ stress, the MDA content in leaves was smaller than that in roots (Fig. 6).

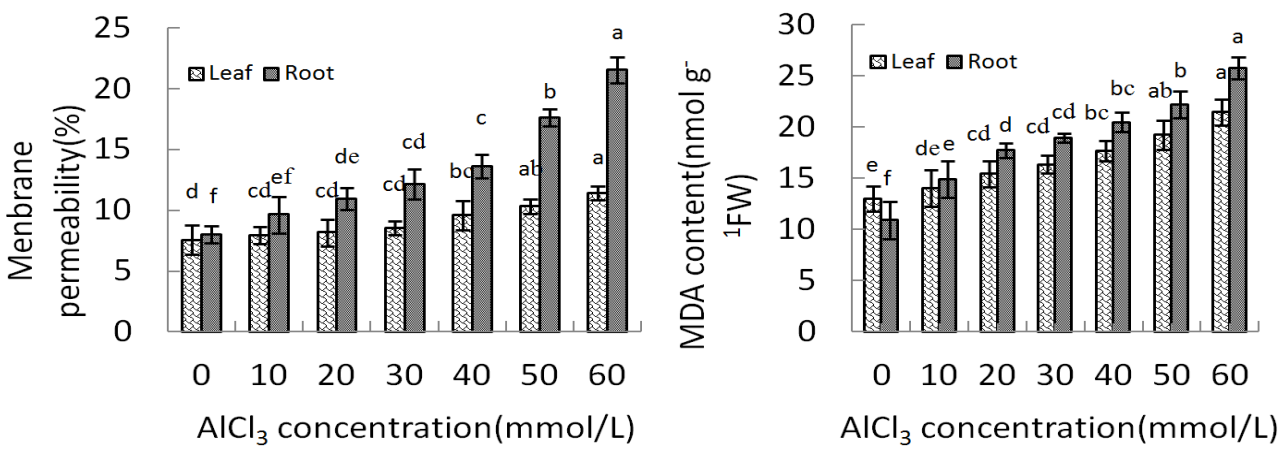

Fig. 6. Changes in membrane permeability and MDA content of *O.japonicus* under $AlCl_3$ stress.

Discussion

Biomass can directly reflect plant growth, and it is an important indicator for identifying Al tolerance of plants (Lin *et al*., 2001). Chlorophyll is the main photosynthetic pigment involved in the absorption, transfer, and transformation of light energy. Its content can reflect the photosynthetic capacity of plants to some extent (Li *et al*., 2018). Root activity reflects the roots metabolic state**,** indicating the ability of roots to actively absorb water and mineral nutrients. It is also closely related to Al tolerance of plants (Ma & Furukawa, 2003; Wang *et al*., 2006). In this experiment, the changes in Al content, fresh weight, dry weight, water content in leaves and roots; chlorophyll content, and root activity of *O. japonicus* under Al stress were analyzed. (1) Under Al stress, Al accumulated in leaves and roots, and the accumulation in leaves was lower than that in roots. (2) Under low-concentration Al stress, the inhibition of root growth was lower than that of leaf growth; under high-concentration Al stress, the inhibition of root growth was higher than that of leaf growth.(3) Under Al stress, water from leaves and roots was lost, and water deficit in leaves was higher than that in roots. (4) Under 10mmol/L $AlCl_3$ stress, the photosynthetic capacity increased slightly, and the ability of active absorption of water and minerals decreased slightly. Its capacity to resist Al stress also decreased. Therefore, the growth of leaves and roots was still inhibited. Under 20–60mmol/L $AlCl_3$ stress, the chlorophyll content and root activity decreased significantly, resulting in a significant decrease in photosynthetic capacity and root absorptive capacity. As a result, its growth was aggravated by the degree of inhibition.

K, Ca, Mg, and Fe are essential nutrients in plants, which are involved in regulating the activities of various enzymes in cells. They are advantageous to maintain ion concentration balance, colloid stability, and charge balance of the protoplasm. Ca, Mg, and Fe are also components of some structural substances (such as calcium pectin) and functional substances (such as chlorophyll, and cytochrome oxidase) in cells. Ca is the

second messenger in the cell, and it plays an important role in the Ca signaling pathway, participates in the regulation of plant responses to stress, and triggers cascade reactions associated with the enhancement of resistance (Reddy & Reddy, 2004). In this experiment, first, the growth of leaves and roots and the changes in Al, K, Ca, Mg, and Fe contents showed that Al stress inhibited the active absorption of roots to K, Ca, Mg, and Fe and the transfer from roots to leaves (except that the Ca content was slightly higher than that of the control under10mmol/L $AlCl_3$), which caused the lack of these nutrients in leaves and roots. Some studies have shown that Al^{3+}can bind with pectic substances from root hair cells to compete for the adsorption sites of K^+, Ca^{2+}, and Mg^{2+} on the cell membrane and inhibit the absorption effect; Al^{3+}also interferes with the transformation from $Fe^{3+}$to Fe^{2+}, inducing iron deficiency (Yan & Liu, 2008).Al also reduces the absorption of annual ryegrass to K^+ (Rengel & Robinson, 1989), and Al^{3+} affects the absorption of 10 kinds of warm-season turf grass to Ca (Baldwin *et al.*, 2005). This experimental result was consistent with the previous study results. Second, Al stress can also affect the physiological metabolism regulated by K, Ca, Mg, and Fe in leaves and roots, ion concentration balance, colloid stability, charge balance to be disturbed, and synthesis and action of some structural and functional substances. Third, Al may restrict Ca balance in root cells and other symplastic structures, such as calmodulin CaM (Ma, 2000). This phenomenon may disturb the response to Al stress induced by the Ca signaling pathway. Thus, the above three factors will lead to the inhibited growth of leaves and roots of *O. japonicus*.

A comparison of the increasing extents of SS, SP, FAA, and Pro contents in leaves and roots under Al stress revealed that the accumulation capacity of SP in leaves was stronger than that in roots, and the accumulation capacities of SS, FAA, and Pro in roots were stronger than those in leaves. Therefore, under Al stress, organic osmotica of small molecular accumulated in leaves and roots differed in types, accumulation amount, and concentration. Under adverse stress, a large amount of osmoregulation substances accumulate in plants, including SS, SP, FAA, and Pro, to maintain moisture and osmotic balance (Mahajan & Tuteja, 2005). SS can provide energy and carbon sources for the synthesis of other osmoregulators under adverse stress (Choudhary *et al*., 2011). Other studies also reported that SP production induced by plants under stress is beneficial to avoid damage to nucleic acids and other substances, thereby maintaining the normal metabolism of plant cells (Ashraf & Foolad, 2007). Floyd & Nagy (1984) found that Pro is not only an important osmotica but also an important antioxidant; it functions in defense against membrane lipid peroxidation injury, and it is closely related to scavenging reactive oxygen by plants. In this paper, the changes in SS, SP, FAA, and Pro contents in leaves and roots of *O. japonicus* under Al stress showed that these four kinds of soluble organic small-molecule osmotica accumulated in leaves and roots under Al stress. Its physiological function may include maintaining moisture and osmotic balance and providing energy and carbon sources for the synthesis of other organic osmotica to avoid the damage of Al to nucleic acids and prevent the oxidative damage caused by Al toxicity. These physiological functions all comprised the stress physiological response to alleviate Al toxicity. This physiological response was at the expense of reducing normal growth.

Under favorable conditions, reactive oxygen species (ROS) in plants are maintained in a dynamic balance to be continuously produced and removed. However, under adverse stress, the dynamic balance is disturbed, resulting in elevated ROS levels and oxidative damage. Stress-resistant plants can eliminate or reduce ROS accumulation by enhancing or maintaining the activity of protective enzymes to avoid or mitigate the damage of oxidative stress to plants (Xu, 2008). Compared with the control, under 10mmol/L$AlCl_3$stress, SOD and POD activity were enhanced in leaves and roots. Although CAT activity was slightly weakened, it could clean ROS and lead to small oxidative damage. This finding coincided with the result that the $O_2^{\bullet-}$content in leaves and roots, plasma membrane permeability, and MDA content were low. Under 20–30mmol/L$AlCl_3$ treatment, POD activity in leaves was continuously enhanced. SOD activity in leaves and roots and POD activity in roots were greatly reduced. By contrast, CAT activity in leaves and roots was continuously weakened. Therefore, oxidative damage could aggravate, which was verified by the increase in the $O_2^{\bullet-}$ content in leaves and roots, plasma membrane permeability, and MDA content. Under 40–60mmol/L$AlCl_3$treatment, SOD, POD, and CAT activities in leaves and roots sharply weakened, whereas the $O_2^{\bullet-}$ content, plasma membrane permeability, and MDA content sharply increased. Oxidative damage and membrane peroxidation also increased sharply. Thus, $AlCl_3$stress leading to oxidative damage to O.*japonicus* was the main reason for its inhibited growth.

SOD and CAT activities in leaves were stronger than those in roots, whereas POD activity in leaves was weaker than that in roots. This result indicated that ROS was cleaned by SOD and CAT activity in leaves but by POD in roots. Therefore, the expression types and abilities of antioxidant enzymes in *O. japonicas* differed in leaves and roots. Under $AlCl_3$stress, the $O_2^{\bullet-}$content, plasma membrane permeability, and MDA content in leaves were lower than those in roots. Thus, oxidative damage of leaves was smaller than that of roots mainly because fleshy root was in direct contact with $AlCl_3$. Relevant research demonstrated that Al stress can activate encoding SOD and other multiple genes (Ezaki *et al*., 2000; Basu *et al*., 2001; Milla *et al*., 2002; Sivaguru *et al*., 2003). Therefore, the changes in SOD, POD, and CAT activities showed that low-concentration $AlCl_3$stress activated the gene expression of SOD in leaves and POD in leaves and roots but inhibited the expression of CAT. High-concentration $AlCl_3$stress inhibited the gene expression of SOD, POD, and CAT.

Under low-concentration of $AlCl_3$ stress, the growth of *O. japonicus* was minimally inhibited, which may be related to the increase in chlorophyll and the enhancement

of SOD and POD activities promoted by a small amount of Al in the cells. Under high-concentration $AlCl_3$stress, the accumulation of organic osmotica in leaves and roots increased further, but growth was significantly inhibited. This result may be due to the accumulation of Al in the cells, resulting in a sharp decline in root activity and antioxidant capacity, sharp increase in the deficit of moisture and essential nutrients, and further aggravation of oxidative damage. Comprehensive analysis showed that Al toxicity of *O. japonicus* increased gradually with the increase in Al concentration. Moreover, the physiological mechanism of Al toxicity differed because we used different concentrations of Al and its organs. *O. japonicus* can be planted in sandy soil with no more than 30mmol/L $AlCl_3$, and Al toxicity is aggravated when the concentration of Al exceeds this level.

Acknowledgements

This work was supported by the agricultural science and technology achievements transformation fund project of Anhui provincial science and technology department (1404032007) and the scientific and technological project of Anhui provincial science and technology department (1301031030). We thank the key research and development projects of Anhui science and technology department (1704a07020081), Science and technology project of Chuzhou Municipal Science and Technology Bureau of Anhui Province (201706) for their financial support. We also thank the national natural science foundation of Anhui province (KJ2016A172) and the youth talent major project of college (gxyq2017043).

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(Received for publication 4 June 2018)