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Physiological Response of Rhododendron under Alkali Stress and Screening of Alkali Tolerance Indexes

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Abstract. **This study explored the response mechanisms of rhododendrons to alkali stress and identified indicators for evaluating alkali tolerance, thus providing technical support for the rapid identification and assessment of alkali tolerance in rhododendrons and for the breeding of new alkali-tolerant cultivars. The 10-month-old cuttings of two rhododendron cultivars with contrasting alkaline tolerances were subjected to alkaline stress treatment using hydroponic cultivation. Changes in their growth performance along with physiological and biochemical parameters were systematically examined. Rhododendron growth was inhibited and biomass was significantly lower under alkali stress. The alkali-sensitive cultivar exhibited symptoms of alkali damage, such as leaf chlorosis and desiccation. Under high alkali stress, quinic acid in rhododendron roots decreased, which promoted the accumulation of citric and malic acids. Alkali stress resulted in a significant increase in root Na^+ content, while leaf Na^+ content in the *R.* Zihe cultivar remained stable and was not significantly different from that in the control. In contrast, leaf Na^+ content in the *R.* Kirin cultivar was significantly higher than that in the control following 5 days of high alkali stress. Alkali stress also led to a notable accumulation of malondialdehyde in rhododendron roots. Superoxide dismutase (SOD) activity in *R.* 'Kirin' decreased with increasing stress, whereas it increased significantly in *R.* 'Zihe', indicating intercultivar differences in SOD activity. The degrees of leaf chlorosis, degree of root browning, root biomass, significantly different leaf Na^+ content compared with that in the control, and the direction of change in SOD activity can be used as indicators when screening alkali-tolerant cultivars of rhododendron. In addition, significant differences in growth and physiological indicators were observed in the two cultivars under high-alkali conditions, thus making this condition suitable for screening alkaline tolerance.**

Salinity and alkalinity often occur in the same soil, and they are frequently treated as interrelated factors in natural saline–alkali soils. Salinity is caused by the accumulation of neutral salt, such as sodium chloride (NaCl) or sodium sulfate (Na_2SO_4), while alkalinity results from high concentrations of alkaline salt, such as sodium bicarbonate($NaHCO_3$) or sodium carbonate (Na_2CO_3) (Shi and Wang 2005). Unlike neutral salt stress, alkali stress imposes a dual burden: ion toxic (Na^+, CO_3^{2-}, HCO_3^-) coupled with high-pH-driven damage to cellular homeostasis, as documented in key physiological studies (Guo et al. 2015; Li et al. 2021). The high pH characteristic of alkaline soils leads to soil solidification and causes metal ions to precipitate, which seriously impedes plant mineral uptake and indirectly causes nutrient stress, resulting in metabolic dysfunction and the disruption of ionic balance (Guo et al. 2017). Notably, microenvironmental variables, including soil moisture, temperature fluctuations, and microbial activity, may further modulate nutrient acquisition efficiency, as evidenced by recent studies of edaphic stress interactions (Zhang et al. 2025). Roots serve as the primary site for alkaline stress perception and experience direct physiological disruptions under elevated soil pH conditions. This stressor compromises cellular pH homeostasis, diminishes root activity (Guo et al. 2020), and alters root architecture through suppression of meristematic activity. Collectively, these impairments constrain nutrient and water acquisition, thereby suppressing plant growth and development.

Alkaline stress shares critical injury pathways with salinity stress, including osmotic imbalance, ion toxicity, and reactive oxygen species (ROS) generation, while concurrently inflicting additional high pH-specific damage. This dual stress impairs plant water and nutrient acquisition through two primary mechanisms: osmotic restriction leads to a reduction in soil water potential, thereby limiting the hydraulic conductivity of roots (Ahmed et al. 2021); however, it causes nutrient immobilization, such as the enhanced precipitation of Fe^{3+}, PO_4^{3-}, and Zn^{2+} under high pH conditions, rendering these nutrients unavailable for uptake (White and Broadley 2022). Consequently, plant water and nutrient absorption are compromised. In response, plants use two complementary biochemical mechanisms. The first is the accumulation of osmoprotectants, exemplified by sugar beet, which significantly elevates leaf levels of proline and betaine to maintain cytoplasmic osmotic homeostasis (Zou et al. 2019). The second is pH homeostasis, which is regulated through the mediation of synthesized or secreted organic acids. For example, the synthesis of malic acid and citric acid stabilizes cytoplasmic pH by chelating toxic ions (Fang et al. 2021). Grape roots secrete oxalic acid under alkaline stress to dissolve precipitated phosphorus and iron (Xiang et al. 2019), and alkali-tolerant citrus cultivars enhance rhizosphere acidification by secreting citric acid (Wang et al. 2018).

Plants produce ROS through photosynthesis, respiration, and photorespiration, and they maintain the balance between the production and elimination of these damaging molecules under normal conditions. However, the accumulation of ROS under alkali stress damages plant cell membrane systems and inhibits plant growth (Sun et al. 2018). To protect themselves from oxidative stress, plants increase antioxidant activity and produce nonenzymatic antioxidants to eliminate excess ROS. For example, prior research has documented the activation of antioxidant system defenses in tea tree under both saline and alkaline conditions. Under alkaline stress, trees produced greater quantities of the antioxidant ascorbic acid (ASA). Superoxide dismutase (SOD) activity also increased significantly in alkaline conditions but not in saline soil (Wan et al. 2024). Other research has demonstrated that rice root cell oxidation can be severely damaged under alkali stress treatment, with the accumulation of large amounts of malondialdehyde (MDA), peroxide (H_2O_2), and superoxide ions (O^{2-}) stimulating significant increases in the activities of antioxidant enzymes, such as SOD, peroxidase, and catalase. In addition, the Na^+ content of old rice leaves increased under alkali stress, inducing ionic toxicity and accelerating the senescence of old leaves (Zhang et al. 2017).

Rhododendron belongs to the genus *Rhododendron* of the Ericaceae and represents one of the 10 traditional famous flowers in China. There are approximately 1047 species of rhododendrons in the world, with 603 (~57%) found in China. Rhododendron prefers acidic soil with pH ranging between 4.5

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and 6.0 (Kinsman 1999), and it is mostly distributed throughout southwest China because of climate and soil conditions. Soil alkalinity is one of the key factors that limits the cultivation of rhododendrons. Based on an analysis of the soil characteristics of rhododendron origin, some scholars have suggested that more than 80 species of rhododendron have the potential for alkali tolerance (Stephen 2018; Wang et al. 2018). However, these alkali-tolerant species are rarely cultivated in China, and their capacity to cope with alkaline conditions has not been evaluated.

It is widely acknowledged that soil quality must be improved to cultivate rhododendron in urban areas. However, this kind of soil modification is difficult, and many rhododendrons cultivated in these locations have problems, such as leaf yellowing, growth restriction, wilting, or even death following a period of growth. Problems, such as the scarcity of alkali-tolerant resources and the high cost and lack of sustainability of urban soil improvement, have seriously limited the cultivation of rhododendron in cities. Therefore, it is necessary to evaluate the alkali tolerance of rhododendron, select and breed alkali tolerant cultivars, and identify indicators of alkali tolerance.

Research has suggested that alkali stress inhibits seed germination and seedling growth in rhododendron (Liu et al. 2021) and leads to differences in ROS accumulation and peroxidative damage at different subcellular sites (Liu et al. 2020). In addition, exogenous β-aminobutyric acid treatment can improve photosynthesis in cuckoo leaves, enhance antioxidant enzyme activity, and alleviate membrane lipid peroxidation under $NaHCO_3$ stress (Xu et al. 2018). However, the effects of alkali stress on the accumulation and distribution of organic acids and nutrients in rhododendron have not been fully elucidated.

Plant adaptation to alkali stress is primarily manifested in morphological and physiological indicators. Variations in these indicators can yield divergent results, potentially leading to labor-intensive experimentation and complicating the interpretation of complex outcomes. Consequently, analytical screening to identify key diagnostic indicators is essential. Furthermore, using pot culture with rhododendron (a woody species) presents significant limitations for alkaline stress research. The inherently slow response to alkaline stress in potted systems compromises the stability of experimental results. Additionally, the requirement to extract the root system from the growth medium for sampling is problematic. Rhododendron species possess dense, fibrous root systems with fine, delicate roots, making extraction highly destructive to root integrity. This disruption inevitably compromises the accuracy of subsequent physiological measurements. To mitigate these constraints, this study used a hydroponic system within a controlled-climate growth chamber. This methodology accelerates the initiation of new roots and promotes healthy root system development in rhododendron. The system also allows for the timely adjustment of the pH of the stress solution. Collectively, these advantages substantially enhance the stability and precision of the research data. This study aimed to compare the differential physiological responses to alkaline stress between two rhododendron cultivars with contrasting alkaline tolerance under hydroponic conditions, elucidate the mechanisms underlying alkaline tolerance in rhododendron, and identify key tolerance indicators, thus establishing an approach for rapid screening and evaluation of alkaline tolerance to facilitate genetic improvement and breeding of new alkaline-tolerant cultivars.

Materials and Methods

Materials. The experiment was conducted in 2022 in a growth chamber at the Shanghai Botanical Garden Research Center (31°14′N, 121°29′E) using 10-month-old cuttings of two cultivars of rhododendron with different alkali tolerances, *Rhododendron* 'Zihe' and *Rhododendron* 'Kirin'. Hydroponic materials were provided by Shanghai Diting Agroforestry Company. The growth chamber was maintained at a temperature of 20 to 25 °C with humidity held at 40% to 50%. Seedlings received 14 to 16 h/d of natural light.

Experimental design. Cutting seedlings were placed in a hydroponic installation in a fixing basket, and a sponge was used to hold the plants in place. Seedlings were precultured for 15 d using half-strength Hoagland nutrient solution (components: potassium nitrate, monoammonium phosphate, sodium ferric EDTA, ferrous sulfate, boric acid, borax, manganese sulfate, copper sulfate, zinc sulfate, ammonium sulfate, and calcium nitrate; pH 5.02), which was replaced every 5 d. Following the emergence of a substantial number of newly formed white roots, two levels of alkaline stress were applied by supplementing the nutrient solution with a mixed alkaline salt solution (Na_2CO_3: $NaHCO_3$ = 1:9 M ratio) to achieve target pH levels of 7.35 and 8.80, respectively. The control treatment consisted of half-strength Hoagland nutrient solution (pH 5.02) without the addition of Na_2CO_3-$NaHCO_3$ buffer. The experiment comprised six treatments, each replicated three times, with 30 seedlings per replicate.

Sample collection and treatment

Biomass. After 20 d of alkali stress, the experiment was ended and photographs were taken to assess growth index values. After harvesting, the plant was cut from the rhizome. Then, the aboveground and belowground parts were dried in a ventilated oven at 105 °C for 30 min and dried at 80 °C until a constant dry weight was achieved. The aboveground dry weight and belowground dry weight were measured as biomass.

Organic acids. Fresh root samples (0.3 g) were collected at multiple time points (0, 6, 12, 24, 48, and 72 h) for each replicate under alkali stress. Two aliquots of methanol (1 mL, 70%) were added to samples during grinding. Then, the mixture was centrifuged to obtain the supernatant, which was concentrated by drying using a vacuum centrifuge (SPD2010-230; Thermo Fisher Scientific, Shanghai, China). Subsequently, 100 μL of 50% methanol was added to each tube, and the contents were thoroughly mixed; thereafter, tubes were centrifuged again to obtain 60 μL of detection solution. A qualitative analysis of organic acids in rhododendron roots was performed using an Acquity I class ultra-high-performance liquid chromatography system coupled with a VION ion mobility quadrupole time-of-flight mass spectrometer (Waters Corporation, Shanghai, China). Chromatography was conducted using a WATERS ACQUITY UPLC BEH C18 reverse-phase silica column (2.1 mm × 100 mm; 1.7 μm). Elution conditions were as follows: 0.1% formic acid in water for mobile phase A and 0.1% formic acid in acetonitrile for mobile phase B. The following elution gradient was used: 0 min, 5% B; 3 min, 20% B; 10 min, 100% B; 12 min, 100% B; 15 min, 95% B; and 19 min, 95% B. The injection volume was 1 μL, with a flow rate of 0.4 mL/min and column temperature set at 45 °C.

Mineral elements. Roots, stems, and leaves from plants subjected to alkali treatment were harvested on days 1, 3, 5, 10, and 15. Tissues were dried to a constant weight, thoroughly ground, and sieved. Mineral element concentrations were determined according to the National Forestry Administration (1999). Zinc content was quantified using graphite furnace atomic absorption spectrometry, while the concentrations of calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), potassium (K), and sodium (Na) were analyzed by inductively coupled plasma optical emission spectrometer (iCAP 7000; Thermo Fisher Scientific, Shanghai, China).

Malondialdehyde content and redox enzyme activity. Following 15 d of alkali stress, fresh root tissue (0.1 g) was collected from each replicate seeding. Both MDA content and SOD activity were quantified using commercial assay kits according to the manufacturers' instructions. Ferric reduction oxidase (FRO) activity was determined using the methodology described by Wang (2013).

Data analysis. Statistical analyses were conducted using SPSS 23.0 (IBM Corp., Armonk, NY, USA). Treatment means were compared via Duncan's multiple range tests at a significance threshold of $P < 0.05$. Data processing, visualization, and figure generation used Excel 2007 (Microsoft Corp., Redmond, WA, USA) and OriginPro 2021 (OriginLab Corp., Northampton, MA, USA). For analyses of organic acids and mineral elements, alkali stress levels and duration of stress exposure were treated as fixed factors, with experimental replicates designated as random effects. For all other physiological parameters, alkali stress levels and the two cultivars were modeled as fixed effects, while replicates were incorporated as random effects.

Results

Morphological observations of the two cultivars under alkali stress. Phenotypic changes observed in the two cultivars after

20 d under control conditions (pH 5.02) and low (pH 7.35) and high (pH 8.80) alkali stress are shown in Fig. 1. Under control conditions, both cultivars exhibited normal growth phenotypes characterized by intact root systems, fully expanded green leaves, and sustained leaf emergence. When exposed to alkali stress, distinct cultivar-specific responses were observed. Under low alkali stress, *R.* 'Kirin' displayed early symptoms of alkali injury, including partial leaf wilting (~33% of mature leaves) and complete cessation of new leaf development; in contrast, *R.* 'Zihe' maintained active growth with persistent leaf emergence. Under high alkali stress, *R.* 'Zihe' occasionally developed brown spots on its leaves, whereas *R.* 'Kirin' exhibited withered leaves in approximately three-quarters of the foliage. Notably, although both cultivars showed brown discoloration in root tissues under alkali stress, *R.* 'Kirin' manifested visible alkali injury symptoms under low alkali stress, including foliar wilting and arrested new leaf emergence. In contrast, *R.* 'Zihe' sustained new leaf production despite developing sporadic brown necrotic lesions on older leaves, demonstrating superior stress tolerance.

Biomass is a comprehensive representation of the impact of the external environment on plants. Biomass decreased with increasing alkali stress for both cultivars (Fig. 2). Compared with the control, whole-plant biomass decreased significantly in *R.* 'Kirin'; these values declined by 26.83% and 34.15% in the low and high alkali stress treatments, respectively. However, there were no significant differences between the low and high alkali stress treatments. Under different levels of alkali stress, the root biomass of *R.* 'Zihe' was basically unchanged, and only aboveground dry weight decreased, but it was not significantly different from that of the control.

Effect of alkali stress on organic acid synthesis A total of seven organic acids, quinic acid, citric acid, malic acid, shikimic acid, cis-Aconitic acid, ascorbic acid, and caffeic acid, were detected in the roots of the two cultivars (Table 1). Quinic acid was the most abundant organic acid in roots. Under alkaline stress, quinic acid content first increased before decreasing in *R.* 'Zihe', with accumulation observed after 12 h. In contrast, the quinic acid content of *R.* 'Kirin' decreased significantly over time, decreasing by 62.05% relative to the control (0 h) after 6 h in high alkaline stress. It is possible that alkaline salts inhibited the accumulation of quinic acid in rhododendron roots. Citric acid was the second most abundant organic acid following quinic acid. Under low alkali stress, the citric acid content in the roots of *R.* 'Zihe' did not vary significantly with increasing stress duration. However, the citric acid content in *R.* 'Zihe' roots began to increase significantly after 12 h of high alkali treatment and continued to increase with prolonged stress duration. At 72 h, citric acid content reached its maximum and was 1.45-times higher than that of the control (0 h). Under low alkali stress, the citric acid content in *R.* 'Kirin' remained less than 2000 $\mu g \cdot g^{-1}$, with no significant differences observed between different time points. After 48 h under high alkali treatment, the citric acid content of *R.* 'Kirin' roots began to increase significantly, increasing by 59.73% compared with that of the control (0 h). Under low alkali stress, there were no significant differences in the malic acid contents of *R.* 'Zihe' and *R.* 'Kirin' under different stress durations. However, when pH increased to 8.80, the malic acid content in *R.* 'Zihe' roots exhibited an initial increase followed by a decrease, with a significant increase of 91.65% after 6 h compared with the control (0 h). In contrast, the malic acid content of *R.* 'Kirin' roots increased significantly after 48 h of continuous stress and peaked at 2.07-times that of the control (0 h) at 72 h. Root aconitic acid content was not significantly different between different pH treatments, and aconitic acid levels were extremely low (nearly undetectable) after 48 h of high alkaline stress. The contents of cis-aconitic acid, ascorbic acid, and caffeic acid in rhododendron roots were all low, indicating their limited roles under alkali stress. Additionally, caffeic acid was primarily concentrated in the roots of *R.* 'Kirin' compared with other organic acids.

Effect of alkali stress on mineral element content. Changes in the mineral element content of different tissues in the two cultivars with different alkali tolerances are presented in Figs. 3 and 4. Following alkali stress treatment, the root Na^+ content in both cultivars increased significantly compared with that of the control. After 10 d of high alkali stress, the Na^+ contents in roots and stem of *R.* 'Zihe' increased by 245.32% and 141.92%, respectively, compared with those of the control. In contrast, leaf Na^+ content remained unchanged during alkali stress exposure. For *R.* 'Kirin', rhizome Na^+ content also spiked after 10 d of alkali stress; however, leaf Na^+ content showed an upward trend from the early stages of alkali stress and was significantly higher than that of the control after 5 d of high alkali stress. As shown in Fig. 5, the ratios of K^+ to Na^+ in different organs of *R.* 'Kirin' under alkali stress were all lower than those in the control. However, after 3 d of high alkali stress, the ratio of K^+ to Na^+ in the leaves of *R.* 'Zihe' exhibited a slight increase. After 5 d of high alkali stress, the ratio of K^+ to Na^+ in the leaves of *R.* 'Zihe' was significantly higher than that in the control (46.48%). High alkali stress resulted in a significant increase in root Mg content but a slight decrease in leaf content. Elemental Mg in *R.* 'Zihe' was primarily distributed in leaves during the first 5 d of alkali stress, while Mg in *R.* 'Kirin' was highest in roots, followed by that in stems and that in leaves. In both cultivars, the distribution of K^+ in plant organs under alkali stress was relatively uniform, whereas Ca accumulated mainly in leaves. After 5 d of high alkali stress, the Ca content of *R.* 'Zihe' was significantly higher (25.48%) than that in the control. When alkali stress time increased to 10 d, leaf Ca content decreased by 9.91% compared with that in control, but there were no significant differences in Ca content in *R.* 'Kirin' between treatment groups. Elemental Fe was mainly concentrated in rhododendron roots and increased with increasing alkali stress intensity. Root Fe content in *R.* 'Zihe' and *R.* 'Kirin' peaked after 10 d of high alkali stress and was significantly higher than that in the control (2.68-times and 2.19-times larger, respectively).

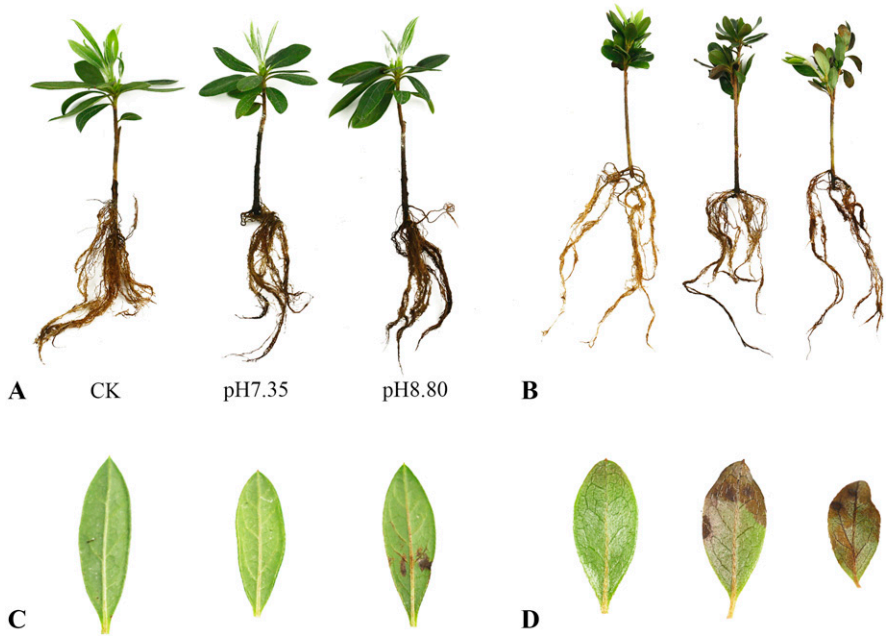

Fig. 1. Phenotypic changes under acidic (CK) and different alkali treatments (pH 7.35; pH 8.80) after 20 d of cultivation. Phenotypic changes in (**A**) *R.* 'Zihe' and (**B**) *R.* 'Kirin'. Phenotypic changes in leaves of (**C**) *R.* 'Zihe' and (**D**) *R.* 'Kirin'.

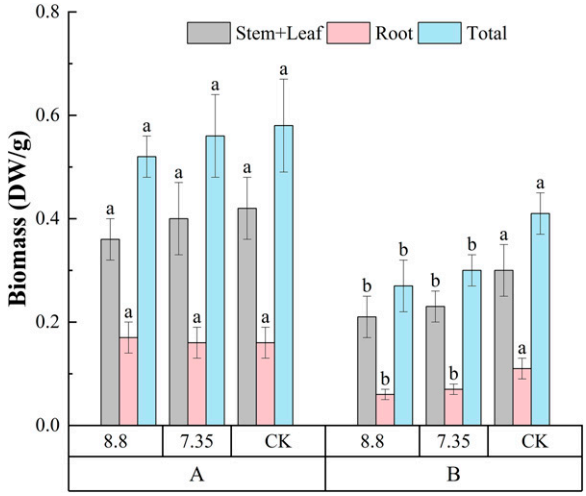

Fig. 2. Effects on the biomass of different parts of rhododendron under acidic (CK) and different alkali treatments (pH 7.35; pH 8.80). Biomass of different parts of (**A**) *R.* 'Zihe' and (**B**) *R.* 'Kirin'. Different lowercase letters indicate significant differences between treatments ($P < 0.05$). Bars indicate standard deviation (*SD*).

Effects of alkali stress on antioxidant enzymes and membrane lipid peroxidation. The end product of membrane lipid peroxidation is MDA, and changes in the MDA content serve as an important indicator of cell membrane damage (Mandhania et al. 2006). The results showed that the root MDA content tended to increase with increasing alkali stress in both cultivars (Fig. 6). Under alkali stress, the MDA content increased significantly, measuring 2.03-times higher in 'Kirin' than that in the control. However, the MDA content of *R.* 'Zihe' did not change significantly across alkali treatments.

The most important enzyme for protecting plants from the oxidative damage caused by free radicals is SOD, and its activity is closely related to a plant's ability to withstand alkalinity (Lu et al. 2024). Alkali stress was correlated with different trends in SOD activity in the two cultivars (Fig. 6). In *R.* 'Zihe', SOD activity increased gradually with increasing stress, and it was significantly different (53.49%) between the control and high alkali stress treatment groups, whereas SOD activity declined in the roots of *R.* 'Kirin' under alkali stress. Under low alkali stress, SOD activity was significantly lower in the roots of *R.* 'Kirin' (−20.91%) and continued to increase with increasing stress intensity (−35.03%) relative to control.

Root FRO activity tended to increase in both cultivars under alkali stress. Under high alkali stress, FRO activity was significantly higher in *R.* 'Zihe' (22.26%) relative to control, but not in *R.* 'Kirin'. There was no significant difference between treatments in FRO activity in *R.* 'Kirin'.

Correlation analysis of indicators. Correlation analyses were performed for the two cultivars under different alkali treatments using 26 indicator values (Fig. 7). Except for a few weak correlations, most indicators were significantly correlated with one another, including aboveground and below-ground biomass, SOD activity, leaf Mg^{2+} and Ca^{2+} contents, and total leaf Fe content. Leaf Na^{+} content was significantly or extremely significantly and positively correlated with root and rhizome P content, stem and leaf Mg content, root, rhizome, and leaf K content, and root Ca content. It was significantly negatively correlated with aboveground and belowground dry weight, MDA content, and SOD activity.

Discussion

Alkali stress inhibited plant growth. The apparent morphology and growth status of plants can directly reflect the degree of plant adaptation to abiotic stress (Athah et al. 2008). As the main nutrient organ of plants, roots absorb and transport water and nutrients from the soil as well as synthesize a variety of organic compounds (Li et al. 2019). When plants are exposed to alkaline conditions, roots quickly sense stress, exchange substances with and obtain information from the cultivated environment, and adjust adaptively. At the same time, because the root system is directly exposed to the alkaline solution, it is generally damaged more extensively than other organs. The results showed that rhododendron root tips were brown after 24 h, similar to changes observed in cinnamon root phenotypes following 48 h of alkali stress (Han et al. 2023). However, overall root browning time varied with cultivar. *R.* 'Zihe' began to brown after 5 d of alkali stress, and *R.* 'Kirin' began to brown after 3 d. In addition, leaf dehydration and yellowing were the most common symptoms of alkali damage in the plants. After 20 d of cultivation, *R.* 'Kirin' leaves were severely wilted under low alkali stress, while *R.* 'Zihe' showed only slight browning under high alkali stress.

Alkali stress can inhibit root development, change root configuration, and reduce root biomass. In this study, the biomass of both rhododendron cultivars decreased with increasing alkali stress. This is consistent with conclusions reported previously by Turner et al. (2020), who found that alkali stress can inhibit growth and dry matter accumulation in rhododendron. The difference in this study is that the decreasing biomass of *R.* 'Kirin' under alkali stress was significantly different from that of the control, but the decrease in biomass of *R.* 'Zihe' was not obvious. These findings suggest that *R.* 'Zihe' tolerates alkali stress better than *R.* 'Kirin'. Therefore, changes in root biomass, root browning, and yellowing leaf phenotypes can serve as morphological indicators when screening for alkali tolerance. Root biomass and the degree of leaf yellowing can be measured following exposure to alkaline conditions (after 20 d of alkali stress in this experiment), but root browning must be assessed at the onset of alkali stress (within 5 d in this experiment).

Alkali stress promoted the synthesis and accumulation of organic acids. Plants generally respond to alkali stress by accumulating diverse organic compounds and inorganic ions in their tissues (Ma et al. 2017), and organic acids can help maintain intracellular pH and ionic balance (Shi et al. 2002). The results of this study show that the organic acid content (citric, malic, shikimic, and ascorbic acids) did not change significantly with increasing stress duration under the low alkali treatment, and differences with the control were not significant. In contrast, significant accumulation of organic acids occurred only under high alkali stress. Yan et al. (2023) reported similar findings for *Leymus chinensis*, which accumulated organic acids under heavy saline stress. Notably, the timing of malic acid and citric acid accumulation differed significantly between the two cultivars under high alkali stress. In *R.* 'Zihe', malic acid synthesis was initiated in roots within 6 h of stress onset, followed by citric acid accumulation starting at 12 h. Both responses occurred substantially earlier than in *R.* 'Kirin', where accumulation of both acids only began after 48 h of stress exposure. This early response of organic acid synthesis in *R.* 'Zihe' enabled rapid stabilization of the intracellular environment during the critical phase of stress. This phenomenon is consistent with previous findings (Dong 2018), where alkali-tolerant genotypes exhibited significant upregulation of the citrate synthase gene within 1 h of stress, while sensitive cultivars showed delayed expression. Malic acid accumulation depends on the activity of phosphoenolpyruvate carboxylase and nicotinamide adenine dinucleotide-dependent malate dehydrogenase, while citric acid synthesis is constrained by the flux through mitochondrial citrate synthase (Igamberdiev and Eprintsev 2016). These results suggest that cultivars with superior alkali tolerance, like *R.* 'Zihe', may possess enhanced metabolic flexibility. This allows them to rapidly reconfigure TCA cycle flux to adapt to stress conditions (Sweetlove et al. 2010). In contrast to the work by Dong (2018), the present study found that root quinic acid content gradually decreased with increasing pH, with the most significant decrease observed under high alkali stress relative to CK. This finding indicates that high alkali stress inhibited the accumulation of quinic acid.

Table 1. Effects of acidic (CK) and different alkali (pH 7.35; pH 8.80) treatments on organic acid accumulation in the roots of two cultivars of rhododendron after 72 h. Treatments are compared for a given species. Data are presented as means ± standard deviation. Different letters within the same species and column indicate significant differences ($P < 0.05$). ND = not detected.

			Organic acid content (ug·g^{-1})						
Cultivar	pH	Time/h	Quinic acid	Citric acid	Malic acid	Shikimic acid	cis-Aconitic acid	Ascorbic acid	Caffeic acid
R. Zihe	5.02	0	9.307 ± 0.307 bcd	2.764 ± 0.153 efg	1.376 ± 0.077 f	0.970 ± 0.028 abcd	0.789 ± 0.020 cdefg	0.574 ± 0.002 bc	ND
	(CK)	6	11.925 ± 0.587 a	2.742 ± 0.316 efg	2.219 ± 0.226 b	0.996 ± 0.018 a	1.000 ± 0.074 a	0.577 ± 0.003 bc	
		12	8.196 ± 1.605 cde	3.442 ± 0.468 bc	1.338 ± 0.068 f	0.976 ± 0.033 abc	0.770 ± 0.008 defg	0.567 ± 0.002 cd	
		24	11.086 ± 1.162 ab	2.842 ± 0.089 def	2.068 ± 0.079 bc	0.987 ± 0.013 ab	0.930 ± 0.151 ab	0.575 ± 0.002 bc	
		48	9.424 ± 2.598 bcd	1.957 ± 0.137 h	1.656 ± 0.290 def	0.984 ± 0.011ab	0.892 ± 0.112 abcde	0.577 ± 0.002 bc	
		72	11.067 ± 0.612 ab	2.488 ± 0.471 fgh	1.824 ± 0.240 cde	0.979 ± 0.024 abc	0.917 ± 0.075 abc	0.585 ± 0.004 b	
	7.35	6	9.961 ± 2.318 abc	2.588 ± 0.315 efg	1.852 ± 0.075 cd	0.974 ± 0.024 abc	0.933 ± 0.037 ab	0.577 ± 0.002 bc	
		12	10.034 ± 0.633 abc	2.251 ± 0.156 gh	1.532 ± 0.104 def	0.966 ± 0.009 abcde	0.848 ± 0.035 bcdef	0.574 ± 0.003 bc	
		24	6.992 ± 2.116 ef	2.404 ± 0.244 fgh	1.355 ± 0.117 f	0.935 ± 0.003 e	0.742 ± 0.043 fg	0.572 ± 0.002 bcd	
		48	9.770 ± 0.377 abcd	2.352 ± 0.103 fgh	1.607 ± 0.218 def	0.961 ± 0.004 bcde	0.882 ± 0.067 abcde	0.567 ± 0.000 cd	
		72	7.629 ± 1.175 de	2.477 ± 0.064 fgh	1.711 ± 0.310 cdef	0.949 ± 0.008 cde	0.821 ± 0.088 bcdefg	0.570 ± 0.001 cd	
	8.80	6	9.570 ± 0.585 bcd	2.490 ± 0.041 fgh	2.637 ± 0.245 a	0.966 ± 0.005 abcde	0.901 ± 0.018 abcd	0.604 ± 0.007 a	
		12	10.454 ± 0.116 abc	3.326 ± 0.477 cd	1.527 ± 0.181 def	0.958 ± 0.014 de	0.710 ± 0.029 g	0.568 ± 0.012 cd	
		24	5.405 ± 0.166 f	3.106 ± 0.209 cde	1.608 ± 0.379 def	0.940 ± 0.016 fgh	0.723 ± 0.064 fg	0.560 ± 0.018 de	
		48	2.264 ± 0.410 g	3.913 ± 0.433 ab	1.443 ± 0.182 ef	ND	0.699 ± 0.022 g	0.560 ± 0.001 de	
		72	2.079 ± 0.109 g	4.004 ± 0.311 a	1.330 ± 0.209 f		0.761 ± 0.092 efg	0.554 ± 0.010 e	
R. Kirin	5.02	0	13.787 ± 0.059 a	2.108 ± 0.205 ef	1.413 ± 0.075 fgh	1.031 ± 0.013 a	0.619 ± 0.006 cd	0.579 ± 0.002 a	0.862 ± 0.019 bc
	(CK)	6	10.184 ± 0.974 bc	2.803 ± 0.218 b	1.704 ± 0.038 cd	0.988 ± 0.005 b	0.602 ± 0.008 ef	0.565 ± 0.002 cd	0.786 ± 0.012 de
		12	9.535 ± 0.146 bc	2.263 ± 0.066 de	1.721 ± 0.149 cd	0.984 ± 0.008 bc	0.630 ± 0.015 abc	0.566 ± 0.004 cd	0.786 ± 0.007 de
		24	7.446 ± 0.691 d	1.861 ± 0.230 fg	1.447 ± 0.066 fgh	0.978 ± 0.008 bcd	0.606 ± 0.002 def	0.573 ± 0.002 b	0.759 ± 0.006 e
		48	9.133 ± 0.206 c	2.424 ± 0.116 cd	1.695 ± 0.093 cde	0.966 ± 0.005 e	0.640 ± 0.002 a	0.579 ± 0.003 a	0.773 ± 0.046 de
		72	7.446 ± 1.202 d	2.400 ± 0.080 cd	1.614 ± 0.101 def	0.952 ± 0.007 f	0.623 ± 0.014 bc	0.574 ± 0.005 b	0.861 ± 0.054 bc
	7.35	6	10.155 ± 0.356 bc	1.992 ± 0.112 efg	1.496 ± 0.072 efg	0.968 ± 0.004 de	0.602 ± 0.002 ef	0.579 ± 0.002 a	0.765 ± 0.032 e
		12	10.721 ± 1.110 b	1.916 ± 0.049 fg	1.525 ± 0.064 def	0.973 ± 0.010 cde	0.608 ± 0.009 de	0.570 ± 0.001 bc	0.837 ± 0.034 cd
		24	7.395 ± 0.371 d	1.957 ± 0.012 fg	1.314 ± 0.075 gh	0.940 ± 0.003 g	0.582 ± 0.004 gh	0.564 ± 0.000 de	0.968 ± 0.006 a
		48	7.047 ± 0.705 d	1.745 ± 0.053 g	1.602 ± 0.043 def	0.940 ± 0.004 g	0.592 ± 0.002 fg	0.568 ± 0.001 cd	0.909 ± 0.037 ab
		72	6.792 ± 1.037 de	1.822 ± 0.101 fg	1.443 ± 0.130 fgh	0.935 ± 0.000 gh	0.584 ± 0.011 gh	0.566 ± 0.005 cd	0.911 ± 0.018 ab
	8.80	6	5.232 ± 0.722 f	2.670 ± 0.251 bc	1.265 ± 0.026 h	0.930 ± 0.005 gh	0.580 ± 0.004 gh	0.559 ± 0.001 e	0.831 ± 0.068 cd
		12	10.440 ± 1.154 bc	2.040 ± 0.010 ef	1.822 ± 0.012 c	0.951 ± 0.007 f	0.586 ± 0.012 gh	0.566 ± 0.003 cd	0.838 ± 0.054 cd
		24	5.494 ± 1.383 ef	2.616 ± 0.105 bc	1.444 ± 0.024 fgh	0.934 ± 0.002 gh	0.575 ± 0.001 g	ND	0.735 ± 0.018 e
		48	4.637 ± 1.298 f	3.367 ± 0.208 a	2.503 ± 0.282 b	0.927 ± 0.001 gh	0.592 ± 0.012 fg		ND
		72	3.196 ± 0.095 g	3.377 ± 0.231 a	2.924 ± 0.160 a	ND	0.635 ± 0.004 ab		

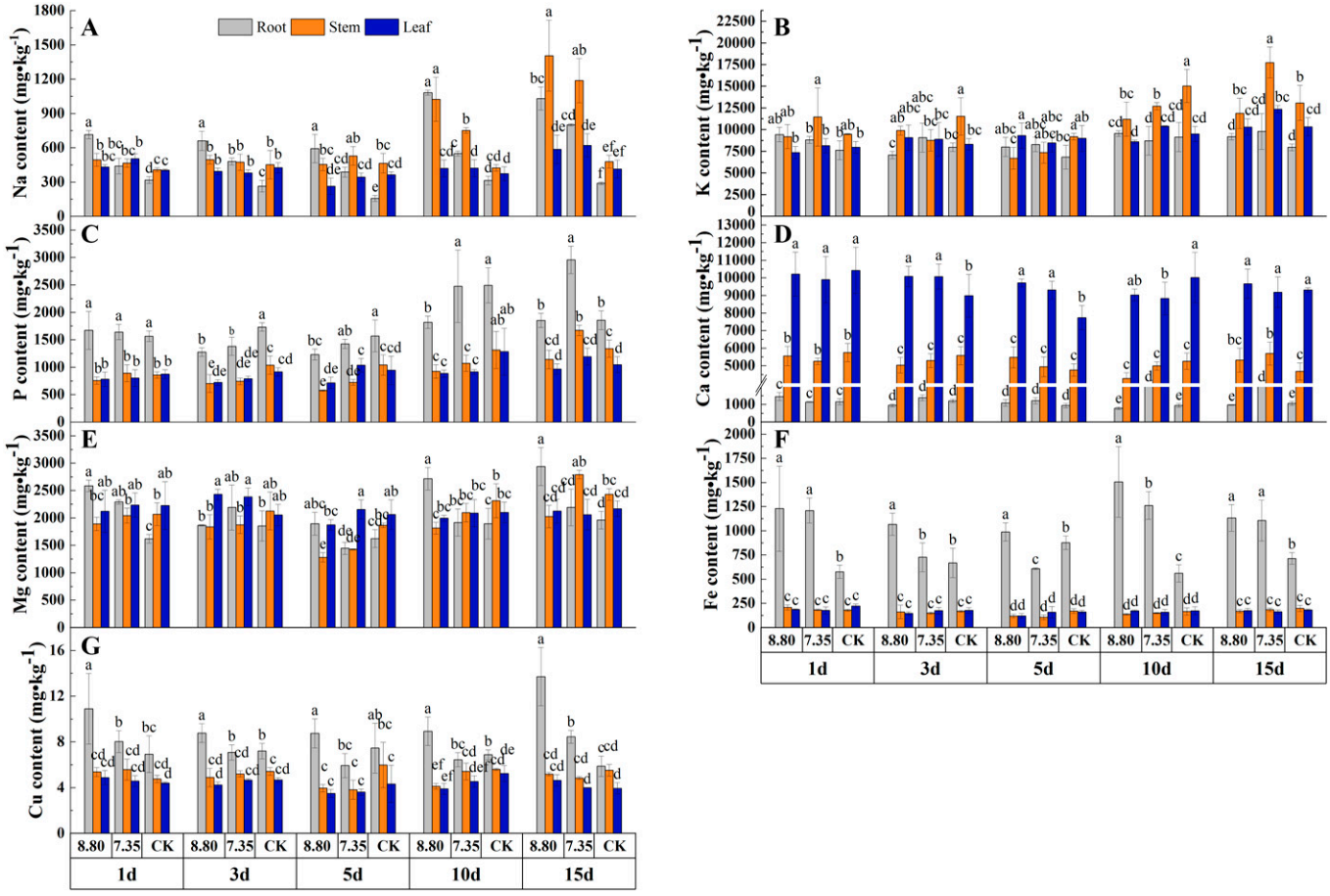

Fig. 3. Changes in mineral element content in different organs of *R.* 'Zihe' under acidic (CK) and alkali treatments (pH 7.35; pH 8.80) over 15 d of cultivation. (**A**) Sodium (Na) content. (**B**) Potassium (K) content. (**C**) Phosphorus (P) content. (**D**) Calcium (Ca) content. (**E**) Magnesium (Mg) content. (**F**) Iron (Fe) content. (**G**) Copper (Cu) content. Data are analyzed by days of alkali stress. Different lowercase letters under the same stress duration represent significant differences between treatments and tissues ($P < 0.05$). Bars indicate standard deviation.

This result is similar to observations of lower quininate content in rice leaves following salt treatment (Chang et al. 2019). Quinic and shikimic acid are often found together, and both have been shown to be involved in the biosynthesis of aromatic amino acids through the shikimic acid pathway (Shelden et al. 2016). Previous work (Ahmad and Prasad 2012) suggested that increasing cellular amino acid levels can protect cellular structure and reduce oxidative damage caused by salt and alkali stress. The decrease in amino acid content under alkali stress observed may be an important indicator of alkali stress, possibly because of its role as a precursor for amino acid synthesis, which provides more energy. The results showed that the accumulation of organic acids, mainly malic and citric acid, was significantly higher under alkaline conditions. Therefore, it is necessary to test rhododendron cultivars under only high alkali stress treatments when screening for alkali tolerance. In addition, malic acid and citric acid accumulated in the roots of both cultivars under alkali stress, with differences only in the timing and amount of accumulation. As such, whether malic acid and citric acid can be used as indicators when screening cultivars for alkali tolerance requires additional research.

Effect of alkali stress on mineral element content in different organs. Mineral elements are important for plants to conduct various metabolic activities. Studies have shown that salt stress induces both osmotic and ionic stress, which in turn causes secondary stresses in plants (Yang and Guo 2018). Under alkaline conditions, this is exacerbated by the stress induced by high pH. The high pH of the rhizosphere disrupts ionic balance in the surrounding soil, destroys root structure, and inhibits root absorption and nutrient element transport, thereby altering normal physiological metabolism (Yang et al. 2009).

When plants are exposed to saline–alkali stress, their root systems absorb large quantities of Na^+, thus hindering the uptake of other nutrients, potentially causing ionic imbalances and toxicity. Previous research suggested that plants can absorb and transport Na^+ to different areas, resisting stress damage via ion compartmentalization (Luo et al. 2021). Under alkaline stress, the alkali-tolerant cultivar *R.* 'Zihe' predominantly retained Na^+ in roots (root > stem > leaf), whereas the sensitive *R.* 'Kirin' exhibited abnormal Na^+ accumulation in leaves (> 600 $mg \cdot kg^{-1}$). This indicates that compartmentalization efficiency is critical for alkali tolerance. The efficient Na^+ accumulation in *R.* 'Zihe' roots relies on tonoplast NHX-type antiporters (Na^+/H^+ exchangers) whose activity is induced by saline–alkali stress (Zhang et al. 2020). These transporters use the vacuolar H^+ gradient (established by V-ATPase/V-PPase) to pump cytosolic Na^+ into vacuoles, reducing Na^+ translocation to shoots (Bassil et al. 2019). This aligns with Na^+ compartmentalization patterns observed in salt-tolerant Populus cultivars. The excessive Na^+ accumulation in *R.* 'Kirin' leaves reflects defects in cortical cell barrier function and dysregulation of xylem unloading control. Studies showed that HKT1-type transporters (e.g., AtHKT1;1) expressed in xylem parenchyma cells can intercept Na^+ during long-distance transport to shoots (Møller et al. 2009). Alkali-sensitive cultivars may lose control of Na^+ translocation because of impaired HKT function (Ren et al. 2018).

Salt stress and alkali stress could lead to competition between Na and K ions for entry into plant cells. Selective absorption of Na^+ and K^+ is affected by salt stress and alkali stress and disrupts ionic balance. Therefore, Na^+ and K^+ contents and Na^+/K^+ are key indicators for determining the salt tolerance and alkali tolerance of plants. *R.* 'Zihe' maintained a high leaf K^+/Na^+ ratio, preserving enzymatic activity and membrane stability, whereas this ratio significantly declined in *R.* 'Kirin' under intensified stress. This confirms that a high ratio of cytosolic K^+ to Na^+

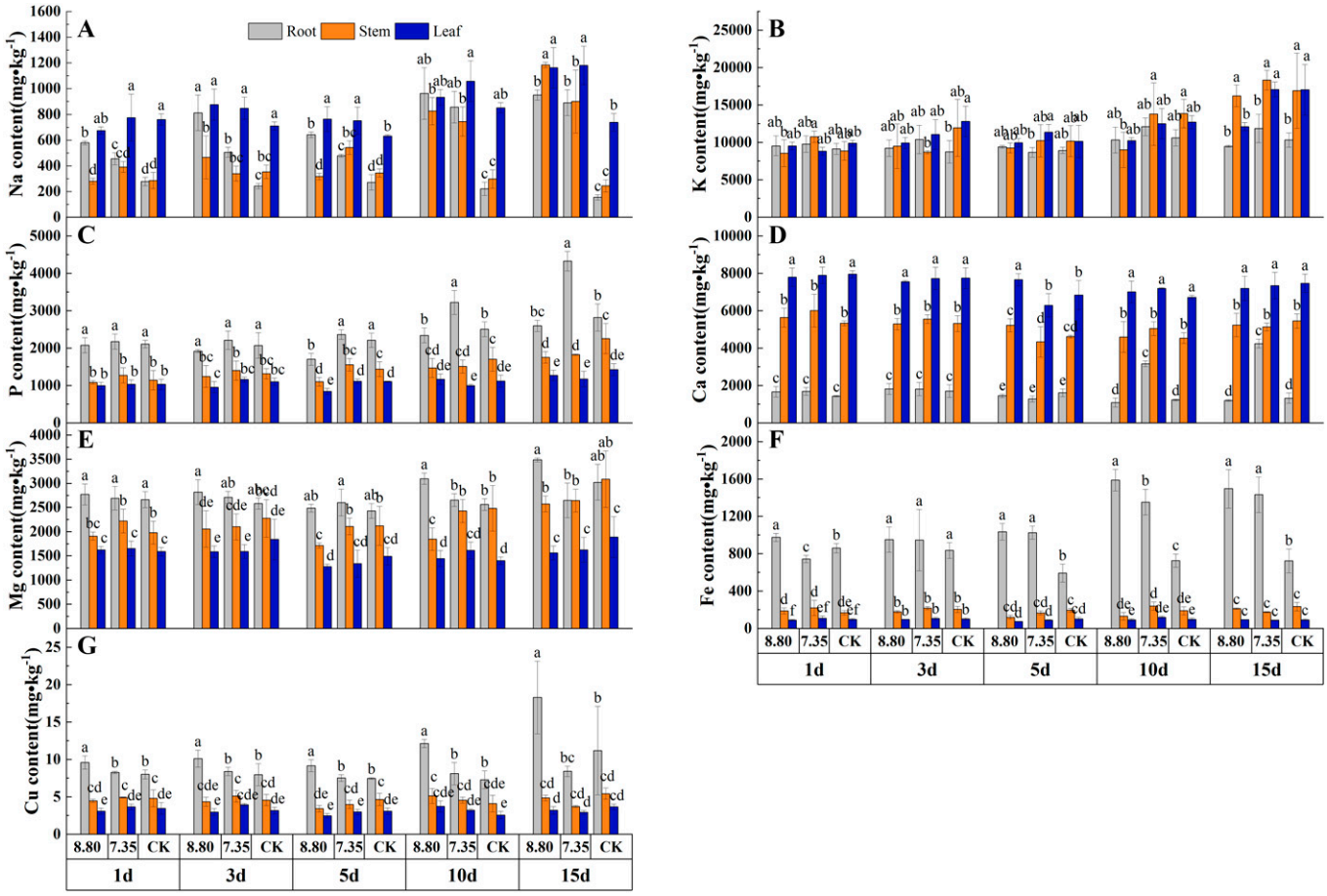

Fig. 4. Changes in the mineral element content in different organs of *R.* 'Kirin' under acidic (CK) and alkali treatments (pH 7.35; pH 8.80) over 15 d of cultivation. (**A**) Sodium (Na) content. (**B**) Potassium (K) content. (**C**) Phosphorus (P) content. (**D**) Calcium (Ca) content. (**E**) Magnesium (Mg) content. (**F**) Iron (Fe) content. (**G**) Copper (Cu) content. Data are analyzed by days of alkali stress. Different lowercase letters under the same stress duration represent significant differences between treatments and tissues ($P < 0.05$). Bars indicate standard deviation.

is necessary for plant growth and development (Hussain et al. 2021).

An essential meso-element that serves as an important regulator of plant growth is Ca. After 3 d of alkali stress, the leaf Ca content increased significantly in *R.* 'Zihe'. This indicates that the accumulation of Na^+ did not disrupt Ca^{2+} transport to leaves, thereby contributing to membrane stability and stress signaling (Hou et al. 2022). Moreover, leaf Ca^{2+} declined slightly in *R.* 'Kirin' under alkali stress.

As an important component of chlorophyll, Mg is key for normal plant growth and development. The preferential distribution of Mg^{2+} to leaves in *R.* 'Zihe' may underpin its sustained chlorophyll content and capacity for new leaf growth under stress, whereas

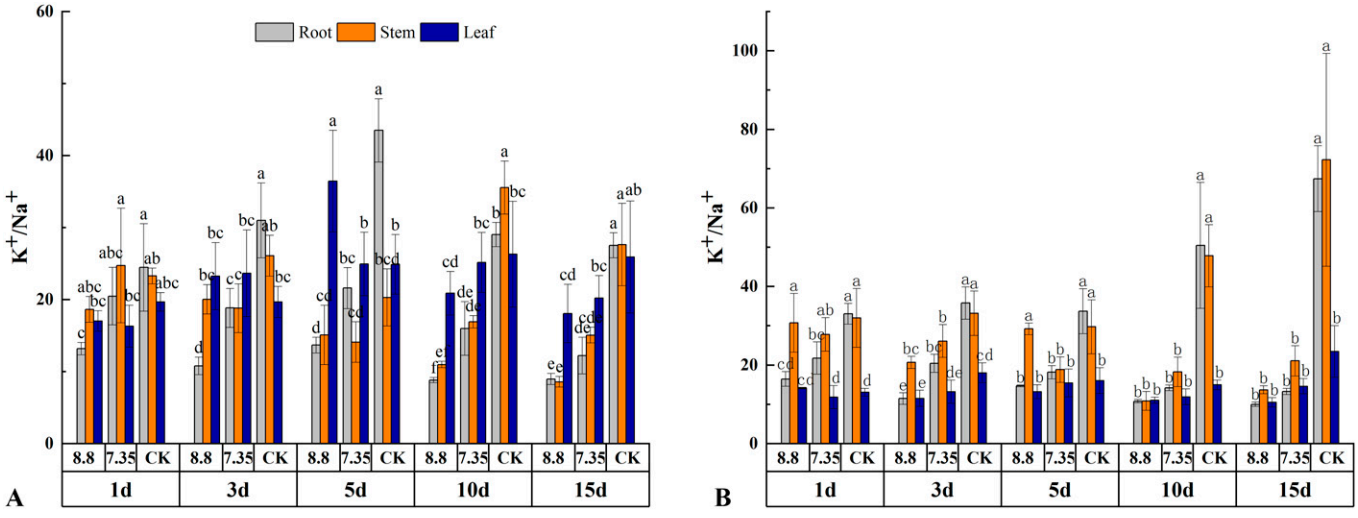

Fig. 5. Changes in K^+/Na^+ in different organs of rhododendron under acidic (CK) and alkali stress (pH 7.35; pH 8.80). (**A**) *R.* 'Zihe'. (**B**) *R.* 'Kirin'. Data are analyzed by days of alkali stress. Different lowercase letters under the same stress duration represent significant differences between treatments and tissues ($P < 0.05$). Bars indicate standard deviation.

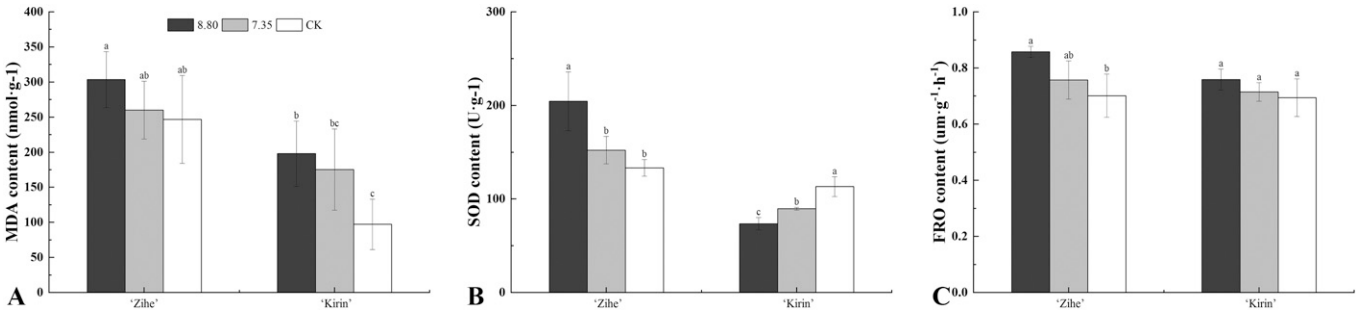

Fig. 6. Effects of alkali stress on malondialdehyde (MDA) (**A**) and the activities of superoxide dismutase (SOD) (**B**) and ferric reduction oxidase (FRO) (**C**) of two cultivars of rhododendron under acidic (CK) and alkali stress (pH 7.35; pH 8.80). Different lowercase letters represent significant differences between treatments ($P < 0.05$). Bars indicate standard deviation.

root Mg^{2+} accumulation in *R.* 'Kirin' suggests impaired translocation correlating with its growth inhibition.

The FRO activity represents the rate-limiting step for Fe absorption in nongraminaceous plants, and its regulation is also related to the size of the plant-available "Fe pool." Here, the total Fe content and root FRO activity increased with increasing pH under alkali stress in both cultivars. This is consistent with previously reported findings (Sun et al. 2005) and suggests that both higher pH and the accumulation of Fe in the root environment promote FRO activity. Notably, FRO activity was significantly different in *R.* 'Zihe' under high alkali stress, which is at odds with the observation that the total Fe content increased significantly in both cultivars. This paradox suggests that FRO activity may be regulated more by the size or bioavailability of the internal plant "Fe pool" rather than solely by external Fe availability or total root Fe (Moog and Bruggemann 1994). This observation underscores the complexity of Fe

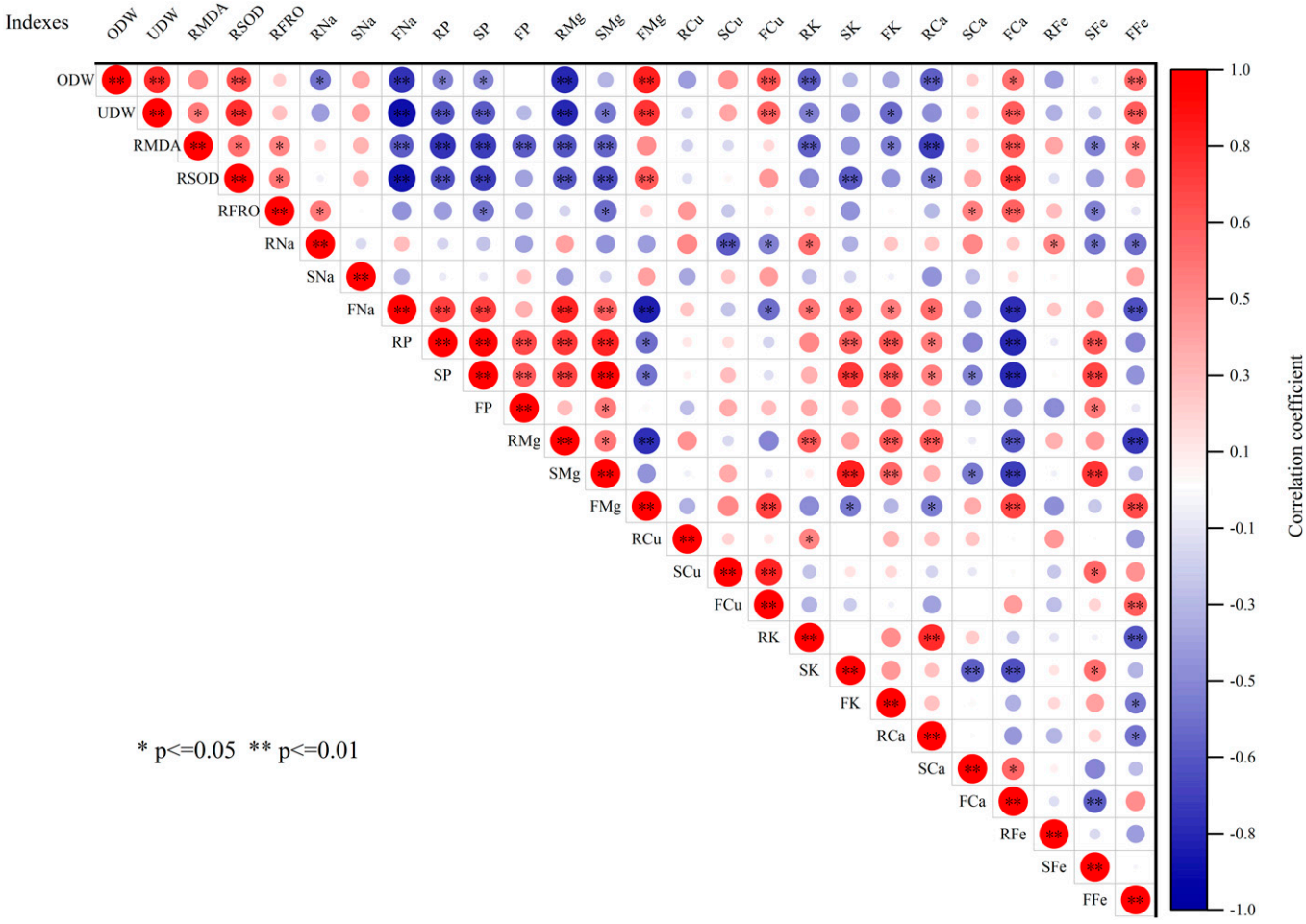

Fig. 7. Correlation analysis of alkalinity tolerance indexes among different rhododendron cultivars. The larger the circle, the greater the correlation coefficient. Red is positive and blue is negative. Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Na = sodium; P = phosphorus; FCa = Ca^{2+} in leaves; FCu = Cu^{2+} in leaves; FFe = total Fe content in leaves; FK = K^{+} in leaves; FMg = Mg^{2+} in leaves; FNa = Na^{+} in leaves; FP = P in leaves; FRO = ferric reduction oxidase; MDA = malondialdehyde; ODW = dry weight of aboveground biomass; RCa = Ca^{2+} in roots; RCu = Cu^{2+} in roots; RFe = total Fe content in roots; RFRO = root FRO activity; RK = K^{+} in roots; RMg = Mg^{2+} in roots; RMDA = root MDA content; RNa = Na^{+} in roots; RP = P in roots; RSOD = root SOD activity; SCa = Ca^{2+} in stems; SCu = Cu^{2+} in stems; SFe = total Fe content in stems; SK = K^{+} in stems; SMg = Mg^{2+} in stems; SNa = Na^{+} in stems; SOD = superoxide dismutase; SP = P in stems; UDW = dry weight of belowground biomass.

nutrition under alkali stress. It is hypothesized that FRO activity may be regulated, in part, by the bioavailable Fe in the plant's "Fe pool," but additional research is needed to determine whether there are differences in bioavailable Fe between the root systems of the two cultivars.

Alkali stress is essentially a mineral nutrition problem. An improved understanding of the mechanisms that underlie the uptake, distribution, and regulation of different ions in rhododendron and how plants maintain intracellular ion homeostasis via ion uptake and zonation is key to improving plant saline–alkali tolerance. Therefore, we conclude that differences in leaf Na^+ content relative to the control can serve as an important indicator for screening rhododendron cultivars for alkali tolerance. In this experiment, leaf Na^+ content in *R.* 'Kirin' was significantly different from the control after 5 d of high alkali stress, but it was not significantly different in *R.* 'Zihe'.

Effects of alkali stress on oxidoreductase activity. Alkali stress disrupts the dynamic balance between the production and elimination of ROS in plants. The accumulation of excessive quantities of ROS causes oxidative stress, which in turn produces MDA damage to cell membranes (Karuppanapandian et al. 2011). In this study, MDA accumulated in rhododendron roots following alkali stress treatments, and MDA in *R.* 'Kirin' roots was significantly higher under high alkali stress. In *R.* 'Zihe', it was not significantly different from the control. This is consistent with the conclusion by Hannachi and Van Labeke (2018), who reported that salt-sensitive cultivars of eggplant accumulate more MDA. This finding indicates that *R.* 'Kirin' was more sensitive to alkali stress and that its root cell membranes were readily damaged under high alkali stress. The oxidative stress induced by alkaline conditions causes rapid damage to plant cells, triggering the antioxidant system to neutralize ROS in cells and reduce their toxic concentrations. As the first line of defense in the antioxidant system, SOD protects cells from peroxidative damage by catalyzing the decomposition of superoxide radicals (Fink and Scandalios 2002). Here, SOD activity was significantly elevated in *R.* 'Zihe' roots under high alkaline stress, indicating that SOD was involved in oxygen scavenging to mitigate damage caused by high pH. However, SOD activity in *R.* 'Kirin' decreased significantly under low pH stress and continued to decrease continuously with increasing stress level. This suggests that the antioxidant enzyme system of *R.* 'Kirin' had been severely damaged and lost its internal balance and, thus, was unable to regulate levels of ROS. Jia et al. (2019) reported similar results in *Malus halliana* under alkali stress. Therefore, it is speculated that the high antioxidant defense capacity of the alkali-tolerant cultivar *R.* 'Zihe' under alkali stress can be attributed to the important role of SOD in alleviating membrane lipid peroxidation and reducing MDA accumulation. Changes in SOD activity can thus serve as a physiological indicator when screening for alkali tolerance.

Conclusion

A comprehensive analysis of differences in growth and physiological–biochemical responses between two rhododendron cultivars under alkali stress was used to identify five indicators suitable for evaluating rhododendron alkali tolerance: degree of leaf chlorosis, degree of root browning, root biomass, significantly different leaf Na^+ content relative to control, and the direction of change in SOD activity. These indicators reflect the responses of rhododendron growth, ion absorption and transport, and reactive oxygen scavenging capacity to alkali stress. Specifically, leaf chlorosis degree and SOD activity were measured after 15 d of alkali stress, and root biomass was measured at 20 d. Root browning was evaluated on the fifth day of exposure to alkali stress. Although some cultivars experienced stress under low alkali stress, differences in trait indicators between cultivars were not significant. Therefore, when selecting alkali-tolerant cultivars, only high alkaline stress (pH 8.80) needs to be considered.

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