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Arbuscular Mycorrhizal Fungi Alleviates Salt-Alkali Stress Damage on *Syneilesis aconitifolia*

Linlin Fang, Jiamei Xu and Chunxue Yang*

College of Landscape Architecture, Northeast Forestry University, Harbin, 150040, China

*Corresponding Author: Chunxue Yang. Email: senxiu99@163.com

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ABSTRACT

Syneilesis aconitifolia is a potential ground cover and decorative material in gardens, which exhibits a strong salt-alkali tolerance, and also has medicinal value. In this study, the arbuscular mycorrhizal (AM) fungi community in the soil surrounding *S. aconitifolia* roots in the Songnen saline-alkali grassland was used as the inoculation medium for a pot cultivation experiment. After normal culture for 90 days, NaCl and NaHCO₃ solutions were applied to subject plants to salt or alkali stress. Solution concentrations of 50, 100, and 200 mmol/L were applied for 10 days, and mycorrhizal colonization, biomass, relative water content (RWC), chlorophyll concentration, malondialdehyde (MDA) concentration, antioxidant system activity, and osmomodulator concentration were determined to identify the effects of AM fungi on root colonization status and salinity tolerance in *S. aconitifolia*. There were three key results. (1) Compared to the controls, the intensity and rate of colonization decreased under saline-alkali stress, and the adaptability of AM fungi under low concentration alkali stress was higher than that under salt stress. (2) The AM fungi could increase the biomass, RWC, and chlorophyll concentration, and decrease the MDA concentration of *S. aconitifolia* to some extent. With an increase in the salt or alkali solution concentration, AM fungi not only upregulated the activity of the antioxidant system, but also increased the concentration of osmotic regulatory substances. (3) A multivariate analysis of variance (ANOVA) and radar map analysis showed that the mechanisms of resistance to salt and alkali stress were not the same in *S. aconitifolia*. In the salt treatment, AM fungi mainly regulated salt stress through osmotic regulatory substances such as soluble sugars, soluble proteins, and proline. In the alkali treatment, AM fungi mainly regulated alkali stress through glutathione (GSH), soluble sugars, and MDA. The results showed that the colonization rate of *S. aconitifolia* under low concentration alkali stress was higher than that under salt stress, and the inoculation of AM fungi could significantly reduce the MDA concentration of *S. aconitifolia* plants under salinity and alkali stress, and improve the antioxidant enzyme activity and osmoregulatory substance accumulation, thereby improving the salinity tolerance of *S. aconitifolia*.

KEYWORDS

Physiological property; antioxidant system; osmotic regulation; abiotic stress; medicinal plant



1 Introduction

Soil salinization can restrict agricultural development and has become a global environmental problem [1]. As the rapid development of the global economy has intensified the demand for land, increasing attention has been given to saline-alkali soils, which account for 7% of the world's land area [2]. There are several mechanisms by which saline-alkali soil restricts plant growth. Saline-alkali stress will lead to an increase in soil osmotic pressure, resulting in plant roots being unable to absorb water and mineral elements from the soil, leading to osmotic stress. This will also produce a large amount of reactive oxygen species (ROS) in the plant, causing damage to the plant cell membrane system, and thereby affecting the physiological and biochemical reactions in the cells [3–5]. Alkaline soil can do more damage to plants than a saline soil because of its higher pH, which can affect the ion balance and nutrient absorption [6]. To overcome the harm caused by salinity-alkali stress, plants will also carry out a series of mechanistic adjustments to maintain normal growth, such as the accumulation of osmoregulatory substances, the enhancement of antioxidant systems, and the transport of numerous toxic ions to the vacuoles [7–10].

Arbuscular mycorrhizal (AM) fungi are widely found in a variety of natural environments and farmland ecosystems, including salt-soil habitats [11], alpine grasslands [12], and desert areas [13]. Arbuscular mycorrhizal (AM) fungi are soil's most important beneficial microorganisms and coexist with most plants worldwide [14,15]. The symbiosis between AM fungi and plants can increase the antioxidant enzyme activity and the content of osmoregulatory substances in host plants, thus enhancing the salt tolerance and drought tolerance of host plants [16,17]. At the same time, AM fungi also play an important role in regulating the nutrient elements of plants, improving the root crown ratio of host plants, and enhancing plant photosynthesis [18,19]. There are also abundant fungal resources in saline-alkali soils [20,21]. It has been reported that a mixed inoculation of AM fungi is more beneficial to host plants than a single inoculation of AM fungi, and local AM fungi have a more significant effect on the growth of host plants [22]. Therefore, in this study, we used AM fungi in the root perimeter soil of *S. aconitifolia* in Songnen salt-alkali grassland to inoculate *S. aconitifolia* in a pot experiment and investigate the effect of a local AM fungal inoculation on the host plants.

Syneilesis aconitifolia is a perennial herb of the Compositae family. It is widely used as a Chinese herbal medicine because it contains various active ingredients such as alkaloids and terpenoids, and the whole plant is considered to have medicinal properties [23–25]. It does not have a high medicinal value but is a commonly cultivated plant in urban landscaping because of its peculiar plant type and lush flowering ability. During the investigation of the saline-alkali grassland in Songnen, it was found that *S. aconitifolia* was unevenly distributed in the area, with dense vegetation and good growth state, which inferred that *S. aconitifolia* had a certain degree of salt tolerance. It was found that there were abundant AM fungi resources in the soil surrounding the root of *S. aconitifolia* in Songnen saline grassland, and they could form a good symbiotic relationship with *S. aconitifolia* [26]. However, there have been no studies of the growth status and physiological characteristics of *S. aconitifolia* under salt-alkali stress, and the symbiotic relationship between AM fungi and *S. aconitifolia* and the response of the AM fungus-*S. aconitifolia* symbiosis system to salt-alkali stress has not been determined. Therefore, this study took *S. aconitifolia* as the research object to study the effects of inoculation of AM fungi under different salt-alkali stress on the growth and physiological characteristics of *S. aconitifolia*, which provided data support for AM-*S. aconitifolia* symbiosis under salt-alkali stress, and provided a certain theoretical basis for the rational utilization of salt-alkali land resources.

2 Materials and Methods

2.1 Experimental Design

Experiments were conducted to investigate salt (NaCl) stress, alkali (NaHCO₃) stress, and AM fungal inoculation treatments. Based on the growth of *S. aconitifolia* plants in a preliminary experiment, the NaCl

and NaHCO_3 concentrations in the stress treatments were set to 0, 50, 100, and 200 mmol/L, with the 0 mmol/L treatment being just distilled water. Under salt and alkali stress conditions, colonized and non-colonized treatments were established, respectively. The colonization of AM fungus was achieved by applying a 30 g inoculation on the soil surface of each pot, which was mixed evenly with the surface soil to ensure full contact with the *S. aconitifolia* root system. For the treatments without colonization, 30 mL of inoculation filtrate was applied to each pot. Inoculation filtrate was obtained by mixing the inoculating agent and water in a ratio of 1:2, and then passing the mixture through a 400-mesh sieve [27]. There were 16 treatments with three replicates per treatment for a total of 140 seedlings. Ninety days after seedling migration, the plants were subjected to salt and alkali stress at the set concentrations, with 50 mL of NaCl and NaHCO_3 solution applied to each potted plant every day for 10 days (six groups of NaCl and NaHCO_3 solutions with different concentrations were set up, which were 50, 100 and 200 mmol/L, respectively, and the control group was treated with distilled water). *S. aconitifolia* growth indicators were measured after the stress treatment was completed.

2.2 Experimental Material

The *S. aconitifolia* plants used in the experiment were obtained from sowing and their seeds were purchased from Shuikong Pinglinmu Seed Department Store in Jiaxian County, Pingdingshan City, Henan Province, China. Seeds were disinfected before sowing. The treated seeds were placed in a glass Petri dish lined with double moistened filter paper. The Petri dish was placed into a constant temperature incubator to induce seed germination, with the temperature set to 18°C, humidity of 50%, and a 16:8 h light dark photoperiod. When the root system grew to approximately 1 cm after germination, the seeds were transferred to a pot with an AM fungal agent, with four seedlings in each pot. The AM fungal inoculation used for colonization was the peri-soil of *S. aconitifolia* roots collected from Songnen salt-alkali grassland, and it included AM fungal hypha and spores. The spore density was about 30/g. At the same time, we used morphological methods to identify the spores in the inoculant and found that the *Glomus* accounted for the largest number, and *Septoglomus deserticola* was the dominant species [26]. The collected soil surrounding the *S. aconitifolia* root was sifted for 20-mesh sieve, and then the sifted peri-soil of *S. aconitifolia* roots was used as the AM fungal inoculation, applied 30 g of inoculation to the surface of each pot of soil, and evenly mixed the fungus with the surface soil to make full contact between the fungus and the root system of *S. aconitifolia*. The size of the cultivated pot was 14 cm in diameter and 15 cm in height. The cultivation substrate was mixed according to a volume ratio of grass charcoal soil and vermiculite of 1:1 and sterilized at 121°C for 2 h before use. After the seedlings were moved, the plants were placed in the flower experimental nursery of Northeast Forestry University for cultivation.

2.3 Determination of Plant Root Colonization

After the salt and alkali stress treatments, the plants were separated from the soil, with care taken to avoid the root system during the separation process. After the root system was separated, rinsed with tap water, and the surface moisture was absorbed, the root segments were cut to about 1 cm with scissors, soaked in FAA fixative solution, and labeled and stored at 4°C. According to Phillips' method [28], the root system was boiled and stained. After staining, 30 root segments were randomly selected from each pot, placed on a glass slide dripped with glycerol lactate, covered with a coverslip, and placed under an optical microscope (Axio Scope A1, Zeiss, Jena, Germany) to observe the hypha, arbuscular, and vesicles. Photographs were also taken. The mycorrhizal colonization (%) and intensity of structural colonization (%) were determined using MYCOCALC software. The mycorrhizal colonization (%) indicated the proportion of roots containing mycorrhizal structures to the entire root system. Intensity of structural colonization (%) indicated the intensity of AM fungal formation throughout the root system.

2.4 Determination of Biomass and Water Content

Complete plants with uniform growth under the different NaCl and NaHCO₃ treatments were selected and separated from the soil for the determination of biomass and water status. They were also used for the determination of other physiological indicators after cutting away the aboveground parts and removing surface impurities. Each indicator was measured three times. The fresh weight (Wf), dry weight (Wd), and saturated weight (Wt) of the plants were accurately weighed by an electronic balance. The relative water content (RWC) and water saturation deficit (WSD) of the plants were calculated according to the method proposed by Liu et al. [29]: $RWC = [(WF - WD)/(Wt - WD)] \times 100\%$, $WSD = 1 - RWC$. The physiological and biochemical indicators of *S. aconitifolia* leaves under stress were determined by the method of Wang et al. [30].

2.5 Determination of Chlorophyll and Osmoregulatory Substance Concentration

After stress treatments, samples were taken from 16 treated plants, and the leaves of the same plants were removed with sterilised scissors, 0.5 g of leaves for each repeated stress treatment, where fresh leaves were determined for the concentration of osmomodulators (proline, soluble sugars, and soluble proteins), chlorophyll and MDA. The remaining samples are wrapped in tin foil and stored at ultra-low temperature (−80°C). The concentration of chlorophyll and osmoregulatory substances (soluble sugar, soluble protein and proline) were determined according to the method described by Wang et al. [30]. The chlorophyll a (Chl a) and chlorophyll b (Chl b) concentrations in fresh leaves were assessed spectrophotometrically. The concentration of proline, soluble protein, and soluble sugar were determined by the acid ninhydrin method, Coomassie brilliant blue G-250 staining, and anthrone colorimetry method.

2.6 Determination of Malondialdehyde Concentration and Antioxidant Enzymes Activity

Plant leaves treated with different salt and alkali stress were clipped with scissors, and 0.5 g of leaves were repeatedly used for each stress treatment. The concentration of malondialdehyde was determined according to the method described by Wang et al. [30], and antioxidant enzymes activity of the plants were calculated according to the method proposed by Liu et al. [29]. The MDA concentration was determined by the thiobarbituric acid (TBA) method. The glutathione (GSH) concentration was reduced by 2,3,4,6-tetradeuterio-5-nitrobenzoic acid (TDNB), the superoxide dismutase (SOD) activity was determined by the NBT method, the activity of peroxidase (POD) was determined by the guaiacol method and the activity of catalase (CAT) was determined by the permanganate titration.

2.7 Analysis of Data

A one-way analysis of variance (ANOVA) was conducted to analyze all results using the SPSS 25.0 statistical analysis package. All data were presented as the mean ± standard deviation (SD) of the three independent replications, and Duncan's multiple range test was applied with the level of significance fixed at $p < 0.05$. Under the conditions of saline-alkali stress and AM fungus inoculation, we used a two-way ANOVA to analyze the physiological indexes of *S. aconitifolia*, and to confirm under which treatment the optimal growth performance was achieved. Excel was used for all calculations, and the results were expressed as the mean ± standard error (SE). Results were presented in histograms using SigmaPlot 14.0 and a radar chart was constructed using gene cloud drawing.

3 Results

3.1 Colonization Characteristics

After 10 days of stress, colonized structures such as hyphae, vesicles, and arbuscules were observed in the root system of *S. aconitifolia* (Supplementary Fig. S1). With an increase in the salt or alkali stressor concentration, the colonization rate showed different degrees of decline, with the lowest colonization rate under the 200 mmol/L treatment. There was no significant difference in the colonization rate between the

salt and alkali treatments ($p > 0.05$). The intensity and rate of colonization were the same under alkali stress but displayed a trend of first decreasing and then increasing under salt stress, with the lowest colonization rate under the 100 mmol/L treatment (Fig. 1). Under salt stress, the intensity and rate of colonization had a strong correlation ($p < 0.05$) with the Wf and Wd, and the concentration of chlorophyll a and total chlorophyll of *S. aconitifolia* (Table 1). Compared with salt stress, the intensity and rate of colonization were strongly correlated with the Wf, Wd, RWC, WSD, the concentration of chlorophyll a, chlorophyll b, and total chlorophyll of *S. aconitifolia* under alkali stress ($p < 0.01$).

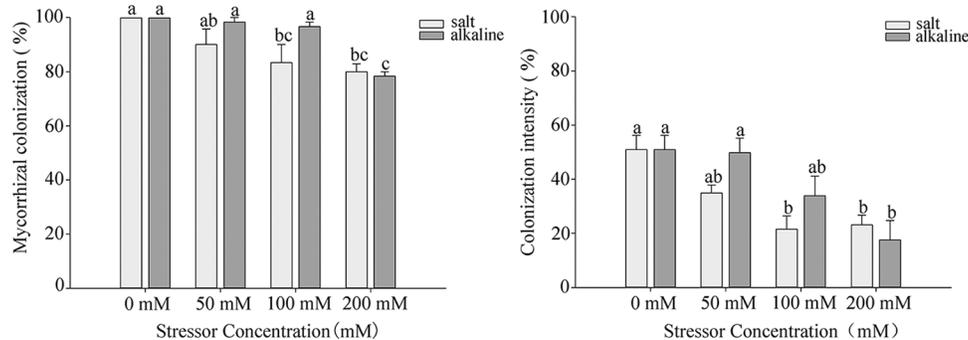


Figure 1: Effects of AM fungi on the colonization rate and colonization intensity of *S. aconitifolia* root under salt-alkali stress

Note: Values in the figure are mean \pm standard error, and different lowercase letters indicate significant differences among different treatment groups ($p < 0.05$). +AM indicates inoculation of AM fungi, and -AM indicates non-inoculation of AM fungi.

Table 1: Correlation between growth characteristics of *S. aconitifolia* and AMF colonization

	Fresh weight (g)	Dry weight (g)	RWC (%)	WSD (%)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Salt							
Colonization rate	0.701*	0.619*	0.459	-0.459	0.799**	0.554	0.788**
Colonization intensity	0.760**	0.746**	0.514	-0.514	0.853**	0.505	0.828**
Alkali							
Colonization rate	0.814 **	0.827**	0.861**	-0.861**	0.872**	0.770**	0.869**
Colonization intensity	0.833**	0.799**	0.824**	-0.824**	0.794**	0.745**	0.803**

Note: RWC: relative water content, WSD: water saturation deficit, *: $p < 0.05$, **: $p < 0.01$.

3.2 Growth Characteristics of *S. aconitifolia*

Under the same salt or alkali stress conditions, the Wf and Wd of inoculated *S. aconitifolia* plants were higher than those of the corresponding non-inoculated plants (Fig. 2). With an increase in the stress concentration, the Wf and Wd of inoculated and non-inoculated plants initially decreased. The Wd and Wf of non-inoculated and inoculated plants decreased by 64.8%, 34.2%, 57.5%, and 43.3%, respectively,

compared with the control under 200 mmol/L salt stress, and decreased by 62.5%, 42.3%, 60.2%, and 51.3%, respectively, compared with the control under the corresponding alkali stress. Compared with the non-inoculated plants, the RWC of inoculated plants was significantly increased under all concentrations in the salt and alkali treatments. The RWC of inoculated and non-inoculated plants decreased with an increase in the salt concentration, with the maximum value at 50 mmol/L. With an increase in the alkali concentration, the RWC of *S. aconitifolia* plants decreased gradually for inoculated and non-inoculated plants. The minimum value was observed at 200 mmol/L (Table 2).

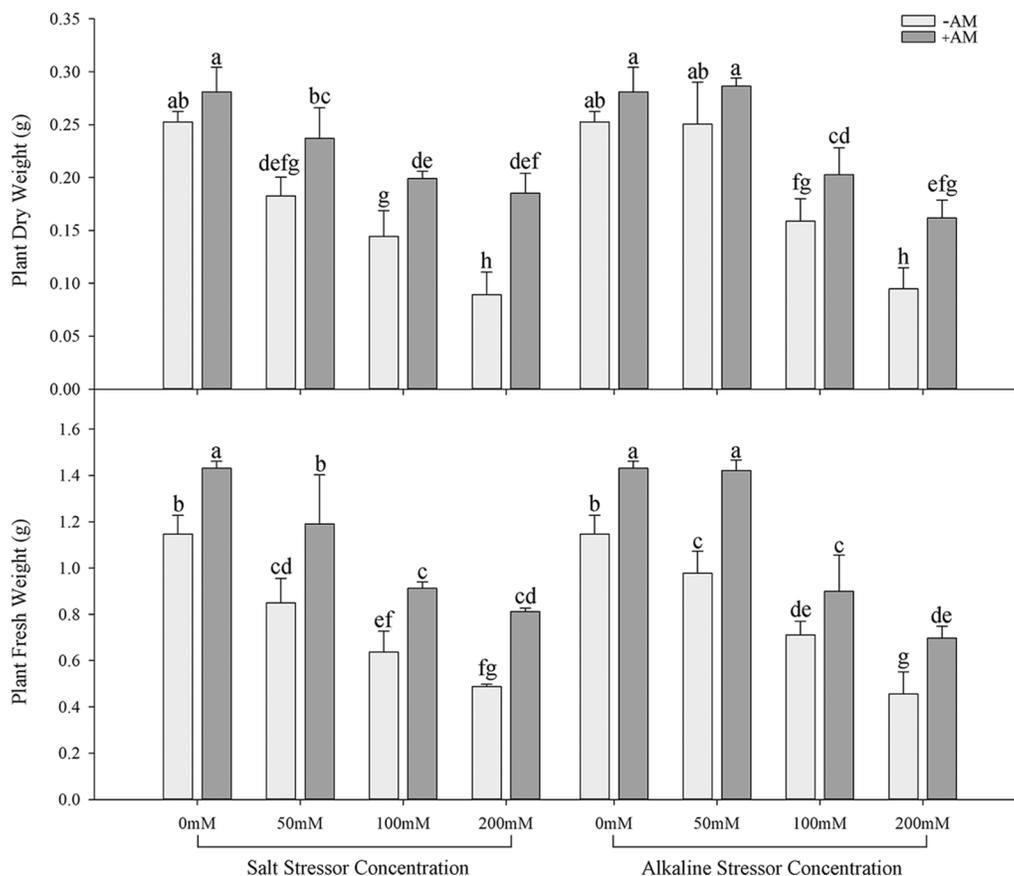


Figure 2: Effects of AM fungi on dry and fresh weight of *S. aconitifolia*

Note: Values in the figure are mean \pm standard error, and different lowercase letters indicate significant differences among different treatment groups ($p < 0.05$). +AM indicates the inoculation of AM fungi, and -AM indicates non-inoculation of AM fungi.

Compared with the non-inoculated treatment, the concentration of chlorophyll a and total chlorophyll of *S. aconitifolia* were higher in the inoculated treatment, but the concentration of chlorophyll b was decreased under alkali stress. With an increase of stress concentration, the concentration of chlorophyll a, chlorophyll b, and total chlorophyll displayed a trend of constant decline. The concentration of chlorophyll a and total chlorophyll in the leaves of inoculated plants were significantly higher than those in the control, while the concentration of chlorophyll b was not significantly different from the control. The two-way ANOVA showed that salt stress had significant effects on the concentration of chlorophyll a, chlorophyll b, and total chlorophyll of *S. aconitifolia* ($p < 0.01$), while alkali stress had a significant effect on the concentration of chlorophyll a and total chlorophyll ($p < 0.01$) (Table 3).

Table 2: Correlation between growth characteristics of *S. aconitifolia* and AMF colonization

Stress	Concentration (mmol/L)	AM fungi	RWC (%)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
NaCl	0	-	91.797 ± 0.703bc	1.419 ± 0.031b	0.156 ± 0.018ab	1.574 ± 0.014b
		+	93.843 ± 0.743ab	1.574 ± 0.050a	0.200 ± 0.041a	1.774 ± 0.009a
	50	-	92.443 ± 1.543bc	1.205 ± 0.032c	0.125 ± 0.027bc	1.330 ± 0.028d
		+	94.781 ± 1.498a	1.272 ± 0.043c	0.191 ± 0.015a	1.463 ± 0.029c
	100	-	87.787 ± 1.211d	1.033 ± 0.046d	0.115 ± 0.036bc	1.149 ± 0.011f
		+	91.708 ± 1.147c	1.100 ± 0.038d	0.155 ± 0.018ab	1.256 ± 0.033e
200	-	85.229 ± 1.120e	0.743 ± 0.047e	0.090 ± 0.032c	0.833 ± 0.035h	
	+	91.294 ± 0.857c	0.912 ± 0.047f	0.099 ± 0.018c	1.011 ± 0.029g	
NaHCO ₃	0	-	91.797 ± 0.703b	1.419 ± 0.031b	0.156 ± 0.018b	1.574 ± 0.014b
		+	93.843 ± 0.743a	1.574 ± 0.050a	0.200 ± 0.041a	1.774 ± 0.009a
	50	-	90.111 ± 0.662c	1.123 ± 0.033d	0.155 ± 0.028b	1.278 ± 0.010d
		+	92.636 ± 0.449ab	1.416 ± 0.007b	0.193 ± 0.013ab	1.609 ± 0.019b
	100	-	87.170 ± 0.486d	0.794 ± 0.012e	0.101 ± 0.019c	0.895 ± 0.014f
		+	90.119 ± 1.644c	1.301 ± 0.012c	0.098 ± 0.023c	1.400 ± 0.032c
200	-	85.031 ± 0.969e	0.520 ± 0.027f	0.079 ± 0.016cd	0.600 ± 0.012g	
	+	87.477 ± 1.045d	1.095 ± 0.033d	0.054 ± 0.008d	1.149 ± 0.037e	

Note: Values in the table are mean ± standard error, different lowercase letters indicate significant differences among different treatment groups ($p < 0.05$), +: inoculated AM, -: non-inoculated AM, RWC: relative water content.

Table 3: Multifactor analysis of physiological indices and salt, alkali, and AM of *S. aconitifolia*

Variate	Salt						Alkali					
	+AM		Salt × -AM		Salt × +AM		+AM		Alkali × -AM		Alkali × +AM	
	F	p	F	p	F	p	F	p	F	p	F	p
Fresh weight (g)	61.542	0.000	52.474	0.000	0.157	0.924	69.399	0.000	92.625	0.000	2.479	0.098
Dry weight (g)	50.793	0.000	45.984	0.000	2.921	0.066	22.736	0.000	55.956	0.000	0.827	0.498
RWC (%)	59.325	0.000	29.354	0.000	3.904	0.029	44.754	0.000	61.301	0.000	0.247	0.862
Chlorophyll a (mg/g)	44.540	0.000	269.172	0.000	2.591	0.089	1055.062	0.000	628.796	0.000	67.345	0.000
Chlorophyll b (mg/g)	12.841	0.002	10.444	0.000	1.102	0.377	2.156	0.161	35.394	0.000	3.224	0.051
Total chlorophyll (mg/g)	220.984	0.000	927.515	0.000	4.118	0.024	2175.637	0.000	1681.960	0.000	89.672	0.000
MDA (μ mol/g)	64.076	0.000	6.933	0.003	1.479	0.258	89.149	0.000	280.664	0.000	25.515	0.000
Proline (mg/g)	2556.743	0.000	641.371	0.000	3046.455	0.000	12.634	0.003	448.861	0.000	184.083	0.000
Soluble sugar (mg/g)	2709.774	0.000	2206.868	0.000	367.556	0.000	2569.907	0.000	3509.764	0.000	489.664	0.000
Soluble protein (mg/g)	90.427	0.000	286.129	0.000	21.901	0.000	12.273	0.003	133.162	0.000	12.494	0.000
SOD (U/mg)	157.338	0.000	51.494	0.000	44.922	0.000	27.039	0.000	191.480	0.000	25.646	0.000
POD (U/min)	4.835	0.043	36.161	0.000	2.329	0.113	13.968	0.002	31.751	0.000	3.304	0.047
CAT (U/mg)	190.536	0.000	272.181	0.000	32.043	0.000	18.605	0.001	61.049	0.000	5.777	0.007
GSH (U/mg)	591.394	0.000	280.145	0.000	140.187	0.000	409.191	0.000	38.250	0.000	2.685	0.082

Note: MDA: Malondialdehyde, SOD: Superoxide dismutase, POD: Peroxidase, CAT: Catalase, GSH: Reduced glutathione, RWC: relative water content, +: inoculated AM, -: non-inoculated AM.

3.3 Effects of AM Fungi on the Osmotic Regulator and Cell Membrane System of *S. aconitifolia* under Saline-Alkali Stress

Inoculation with AM fungi increased the soluble sugar concentration in the leaves of *S. aconitifolia* compared with the non-inoculated treatment (Table 4). With the increase in the stress concentration, the soluble protein concentration of *S. aconitifolia* leaves in the non-inoculated treatment displayed a trend of first increasing and then decreasing. The changes in the proline concentration differed from those of the soluble sugar and soluble protein concentrations. When the concentration was 0 mmol/L, the proline concentration in non-inoculated plants was 3.42 times that of inoculated plants. Under the same salt or alkali stress conditions (Fig. 3), the MDA concentration of inoculated *S. aconitifolia* plants was lower than that of the corresponding non-inoculated plants. With an increase in the salt or alkali concentrations, the MDA concentration in inoculated and non-inoculated plants continuously increased. However, the MDA concentration of inoculated and non-inoculated plants increased less than that under alkali stress. Table 3 shows that the interaction between AM fungi and alkali had a significant effect on MDA concentration ($p < 0.01$), while the interaction between AM fungus and salt had no significant effect on MDA concentration ($p > 0.05$).

Table 4: Effects of AM fungi on the concentration of total osmotic regulator in *S. aconitifolia* under saline-alkali stress

Stress	Concentration (mmol/L)	AM fungi	Soluble sugar (mg/g)	Soluble protein (mg/g)	Proline (mg/g)
NaCl	0	-	8.248 ± 0.160f	4.387 ± 0.169f	0.979 ± 0.015b
		+	8.495 ± 0.106f	5.900 ± 0.396e	0.286 ± 0.015e
	50	-	10.763 ± 0.153e	6.823 ± 0.318d	0.311 ± 0.019e
		+	16.193 ± 0.155b	8.094 ± 0.365c	1.204 ± 0.015a
	100	-	14.192 ± 0.185d	10.016 ± 0.430b	0.470 ± 0.011d
		+	16.650 ± 0.224a	9.662 ± 0.370b	1.214 ± 0.018a
	200	-	10.505 ± 0.145e	8.652 ± 0.303c	0.840 ± 0.016c
		+	15.623 ± 0.068c	11.394 ± 0.233a	1.207 ± 0.017a
NaHCO ₃	0	-	8.248 ± 0.160e	4.387 ± 0.169f	0.979 ± 0.015b
		+	8.495 ± 0.106e	5.900 ± 0.396d	0.286 ± 0.015e
	50	-	10.795 ± 0.136d	5.236 ± 0.209e	0.237 ± 0.028e
		+	18.469 ± 0.261c	6.156 ± 0.321d	0.525 ± 0.056d
	100	-	11.068 ± 0.286d	7.252 ± 0.317c	0.465 ± 0.037d
		+	18.832 ± 0.316c	7.559 ± 0.357c	0.683 ± 0.048c
	200	-	20.031 ± 0.235b	9.047 ± 0.512a	1.216 ± 0.051a
		+	22.066 ± 0.078a	8.257 ± 0.328b	1.169 ± 0.049a

Note: Values in the table are mean ± standard error, different lowercase letters indicate significant differences among different treatment groups ($p < 0.05$), +: inoculated AM, -: non-inoculated AM.

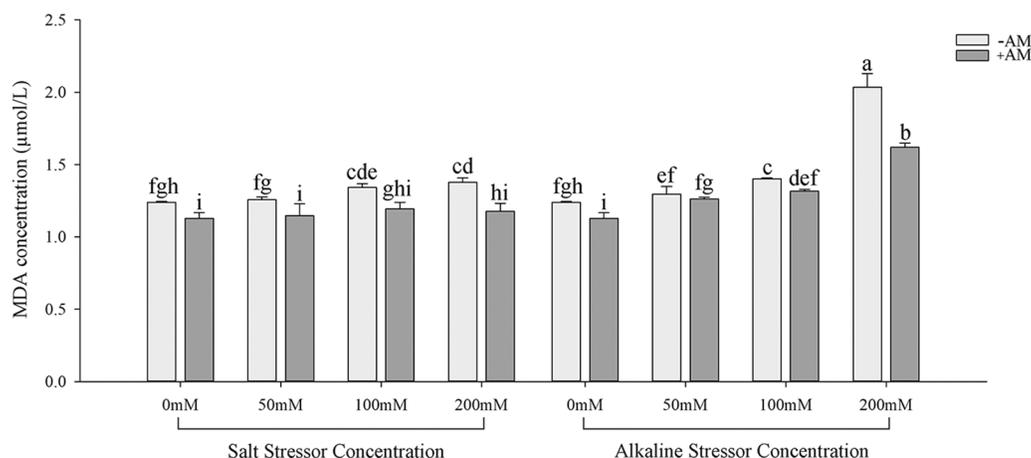


Figure 3: Effects of AM fungi on MDA of *S. aconitifolia*

Note: Values in the figure are mean \pm standard error, and different lowercase letters indicate significant differences among different treatment groups ($p < 0.05$). +AM indicates inoculation of AM fungi, and -AM indicates the non-inoculation of AM fungi.

3.4 Antioxidant System

With an increase in the NaHCO_3 concentration, the POD, SOD, and CAT activities in leaves first increased and then decreased regardless of whether they were inoculated, while GSH activity continuously increased (Table 5). The POD, SOD, and CAT activities reached a maximum at 100 mmol/L. Among them, POD, SOD and CAT were the maximum at 100 mM, and the activities of POD, SOD, and CAT of the inoculated plants increased by 0.15 times, 1.37 times and 0.88 times compared with the control group. GSH was maximized at 200 mM, and the GSH activity of the inoculated plants increased by 0.24 times compared with the control group. When the NaCl concentration increased, the changes in POD, SOD, CAT, and GSH activities differed, but the POD and CAT activities of inoculated plants were higher than those of non-inoculated plants. The POD activity reached a maximum under the 200 mmol/L treatment, with an increase of 0.13 times compared with the control group. The CAT activity reached a maximum under the 100 mM treatment, with an increase of 4.64 times compared with the control group.

Table 5: Effects of AM fungi on the antioxidant system of *S. aconitifolia* under saline-alkali stress

Stress	Concentration (mmol/L)	AM fungi	POD (U/min)	SOD (U/mg)	CAT (U/mg)	GSH (U/mg)
NaCl	0	-	250.151 \pm 4.465c	42.667 \pm 14.224e	2.228 \pm 0.335e	79.295 \pm 1.504f
		+	244.331 \pm 1.875cd	57.667 \pm 6.110de	5.013 \pm 0.425d	128.881 \pm 2.133d
	50	-	231.115 \pm 2.868e	81.667 \pm 12.055c	12.811 \pm 0.335c	99.088 \pm 7.749e
		+	267.463 \pm 3.357b	73.667 \pm 6.110cd	22.805 \pm 1.179b	196.758 \pm 6.051bc
	100	-	241.197 \pm 2.793d	90.333 \pm 6.429bc	12.563 \pm 3.602c	107.662 \pm 7.701e
		+	275.670 \pm 4.403a	115.333 \pm 12.503a	28.282 \pm 0.419a	203.872 \pm 4.381ab
	200	-	265.655 \pm 4.896b	106.667 \pm 13.650ab	1.547 \pm 0.375e	207.844 \pm 6.204a
		+	276.317 \pm 3.881a	114.667 \pm 13.650a	4.023 \pm 0.142de	189.113 \pm 5.978c
NaHCO_3	0	-	250.151 \pm 4.465c	42.667 \pm 14.224e	2.228 \pm 0.335e	79.295 \pm 1.504f
		+	244.331 \pm 1.875d	57.667 \pm 6.110de	5.013 \pm 0.425c	128.881 \pm 2.133c
	50	-	260.017 \pm 2.346b	72.000 \pm 12.288cd	3.837 \pm 1.543cd	99.610 \pm 5.726e
		+	263.666 \pm 2.618b	75.000 \pm 7.550cd	6.560 \pm 1.043b	134.071 \pm 5.391c

(Continued)

Table 5 (continued)

Stress	Concentration (mmol/L)	AM fungi	POD (U/min)	SOD (U/mg)	CAT (U/mg)	GSH (U/mg)
100		–	277.180 ± 3.981a	92.667 ± 17.214bc	7.953 ± 1.260ab	104.245 ± 8.682de
		+	281.093 ± 2.109a	136.667 ± 11.240a	9.438 ± 0.511a	146.534 ± 4.685b
200		–	225.975 ± 4.334e	88.333 ± 5.508bc	2.692 ± 0.609de	111.194 ± 5.150d
		+	250.997 ± 2.142c	99.667 ± 15.948b	1.826 ± 0.268e	160.340 ± 5.767a

Note: Values in the table are mean ± standard error, different lowercase letters indicate significant differences among different treatment groups ($p < 0.05$), +: inoculated AM, -: non-inoculated AM, SOD: Superoxide dismutase, POD: Peroxidase, CAT: Catalase, GSH: Reduced glutathione.

4 Discussion and Conclusion

Soil salinization not only inhibited plant growth but also affected AM fungal germination and colonization [31]. With an increase in the salt or alkali concentration, the intensity and rate of colonization of AM fungi displayed a decreasing trend, because the excessive Na^+ and Cl^- concentration in the soil affected the growth of plant roots, hindered the uptake of water and nutrients by the roots, reduced the germination of AM fungal spores and other propagules, inhibited the elongation of germinated hyphae in the soil, and reduced the chance of AM fungi contacting plant roots [32]. Previous studies showed that inoculation with saline-alkali tolerant AM fungi was more effective than inoculation with non-saline-alkaline tolerant AM fungi [33]. This was confirmed in this study. With an increase in the salt or alkali concentration, the rate and intensity of *S. aconitifolia* colonization displayed a downward but not significant trend. We also found that AM fungi isolated from the *S. aconitifolia* root enclosure were more suitable for low-concentration alkali stress than salt stress, which differed from the results of previous studies [34]. This could be because the symbionts formed by AM fungi isolated from the *S. aconitifolia* root enclosure were better able to adapt to alkali stress.

The seedling period is one of the most important and sensitive stages in the entire life history of plants [35], and it is important to assess salt or alkali stress at this stage to clarify the response of plant physiological states and identify the mechanism of saline-alkali stress tolerance [36]. Excessive NaCl and NaHCO_3 can affect the Wf and Wd of plants, and the high pH of NaHCO_3 is particularly harmful to plants. Therefore, the Wf and Wd of plants under alkali stress was lower than under salt stress [37,38]. With an increase in the salt or alkali concentration, the Wf and Wd of *S. aconitifolia* decreased to varying degrees, but when the concentration was less than 200 mmol/L, the plant weight in the alkali treatment group was higher than that in the salt treatment group. The weight of the inoculated plants was higher than that of the non-inoculated plants, indicating that the inoculated *S. aconitifolia* had an improved salinity tolerance, which agreed with the results of previous studies [39]. However, under alkali stress, the inoculated group was also able to significantly increase the Wf and Wd of *S. aconitifolia*, which differed from previous studies [40]. This could be because the *S. aconitifolia* was more adaptable to alkali stress than salt stress, and AM fungi were more likely to establish symbionts with the *S. aconitifolia* to repair the damage caused by adversity in a low alkali concentration environment.

Under salt or alkali stress, plants respond to osmotic stress by adjusting their own water content [41]. In this study, the RWC of *S. aconitifolia* was affected by salt and alkali, with alkaline conditions having the greater impact (Fig. 4). Under the alkali treatment, the absorption of water by *S. aconitifolia* was reduced and the synthesis of other osmotic regulatory substances, such as soluble sugars, soluble proteins, and proline, was promoted to resist stress. The study also found that inoculation with AM fungus significantly increased the RWC of *S. aconitifolia*, which indicated that AM fungi could form a mycorrhizal network around the root system and enhance the absorption of water by roots. The concentration of Chlorophyll is one of the most sensitive physiological indicators of abiotic stress in plants because it has an important

role in photosynthesis [42,43]. The chlorophyll concentration continuously decreases with an increase in chlorophyll degrading enzyme activity, which is a normal physiological stress response when plants are subjected to salt or alkali stress [44]. In this study, inoculation with AM fungi increased the concentration of chlorophyll a, chlorophyll b, and total chlorophyll in *S. aconitifolia*, indicating that the symbiosis of AM fungi and *S. aconitifolia* increased the uptake of Mg^{2+} by roots and also regulated the formation of hormones in plants to promote the synthesis of chlorophyll.

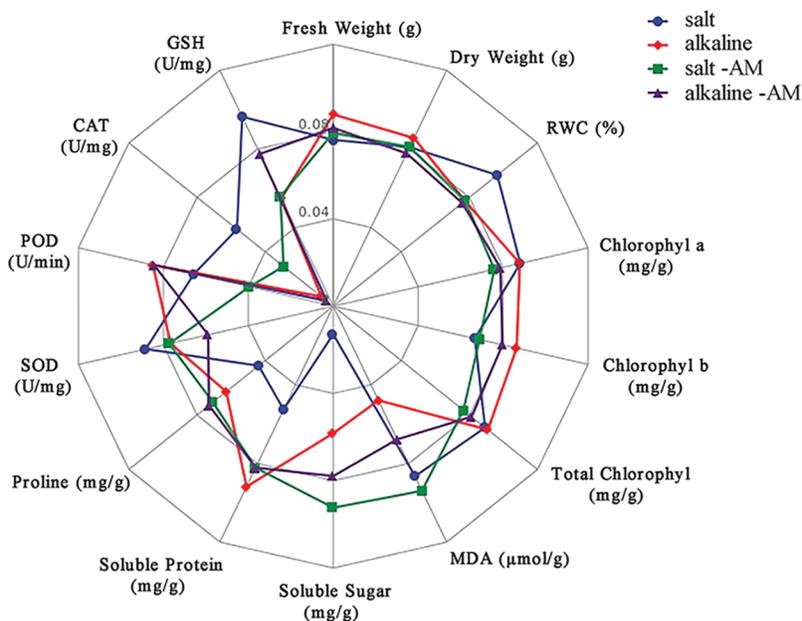


Figure 4: Radar map of *S. aconitifolia* response to different stresses

Note: The blue line indicates salt treatment, the red is alkali treatment, the green line indicates salt + AM fungal treatment, and the purple line indicates alkali + AM fungal treatment. MDA: malondialdehyde, SOD: superoxide dismutase, POD: peroxidase, CAT: catalase, GSH: reduced glutathione, RWC: relative water content, WSD: water saturation deficit.

Many ROS substances are produced in plants under salt-alkali stress, leading to membrane peroxidation [45–47]. Malondialdehyde is a product of lipid peroxidation, and the MDA concentration of *S. aconitifolia* could indicate the degree of damage to the cell membrane. The MDA concentration under the same concentration treatments was higher under alkali stress than salt stress, which indicated that the high pH was also damaging to the membrane system due to osmotic stress. Inoculation with AM fungi significantly reduced the MDA concentration and alleviated the damage to the saline base of the plant cell membrane system, which was consistent with previous research [45]. Plants enhance their salt and alkali tolerance by regulating their antioxidant and osmotic regulation systems [30,48]. The antioxidant system eliminates ROS in plants by improving the SOD, CAT, and POD activities and reducing the GSH activity [30,49,50]. The osmotic regulation system maintains the intracellular osmotic pressure and water content by synthesizing proline, soluble sugars, and soluble proteins [51,52]. In this study, *S. aconitifolia* depended more on proline and soluble protein for osmotic regulation under alkali stress and more on SOD and GSH for maintaining an antioxidant system under salt stress. This indicated that the stress resistance mechanism of *S. aconitifolia* differed under different types of stress. Inoculation with AM fungi increased the contribution of proline, soluble sugar, and soluble protein in the osmotic regulation system of *S. aconitifolia* under salt stress, and increased the contribution of GSH in the antioxidant system of *S. aconitifolia* under alkali stress. This indicated that AM fungi and *S. aconitifolia* were

symbionts that were involved in the synthesis of organic matter. The regulatory effect of AM fungi differed under the different types of stress.

In summary, this study proved that the physiological functions of *S. aconitifolia* are reduced to varying degrees under salt or alkali stress, and inoculation with local AM fungi could improve osmotic regulation, the concentration of chlorophyll, and the antioxidant system of *S. aconitifolia*, ultimately improving the salinity tolerance. *S. aconitifolia* was found to regulate itself differently in response to different types of stress. Under salt stress, the *S. aconitifolia* upregulated the antioxidant system to reduce the role of osmotic regulatory substances. Under alkali stress, *S. aconitifolia* upregulated the effect of osmotic regulatory substances and reduced the effect of the ascorbic acid-GSH circulatory system. The results provided the corresponding theoretical basis for the symbiotic system formed between the local AM fungi community and host plants to tolerate saline-alkaline conditions. However, due to experimental limitations, this could not be fully verified. In future studies, the response mechanism of *S. aconitifolia* to saline-alkali stress should be explored at the microscopic level through isotope labeling and molecular biology techniques.

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