EFFECTS OF SALINE-ALKALI STRESS ON GROWTH AND PHYSIOLOGY OF *AMORPHA FRUTICOSA* L. SEEDLINGS

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Abstract. This study determined the effects of salt stress (NaCl) and alkali stress (Na_2CO_3) on the growth and physiology of *Amorpha fruticosa* seedlings. The results showed that salt and alkali stress inhibited the growth of root, stem and leaf, but the appropriate concentration of salt and alkali stress could stimulate root elongation. Salt stress and alkali stress resulted in the decrease of photosynthetic capacity of seedlings. The degree of Na^+ absorption by the roots is relatively low, and a large amount of Na^+ absorbed into the body is separated in the stem and total petiole. The concentration K^+, Ca^{2+} and Mg^{2+} in diverse plant organs significantly changed under both stresses while concentration of K^+, Ca^{2+} and Mg^{2+} in leaflets did not change significantly. In addition, accumulation of Cl^- under salt stress was not obvious. Soluble sugar accumulation in roots and lobules remarkably increased under both stresses. The distribution of soluble sugar in each vegetative organ was changed. The absorption of degree of harmful ions was relatively low, and more harmful ions were accumulated in the stem and total petiole. This was the response strategy of the seedlings to adapt to salt and alkali stress and it provides a theoretical basis for planting *Amorpha fruticosa* in areas with relatively low salinization degree.
Keywords: *Amorpha fruticosa, photosynthetic pigments, germination, seedling, growth, physiology*

Introduction

Salinity and alkaline stresses are serious threat to crop productivity and global food security (Alenazi et al., 2024; Gajjar et al., 2023). The extent of salinity and alkalinity

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stresses is continuously increasing which is a serious concern, thus appropriate measures must be taken to tackle these stresses (Trotti et al., 2024). Salt and alkaline stress negatively affects plant growth and development by impairing photosynthesis, leaf water relations and increasing reactive oxygen species (ROS) production (Ntanasi et al., 2023). Most of studies around the globe mostly focused on the above-ground organs (stems and leaves) of adult seedlings, however, limited studies are conducted to determine the effect of salinity and alkaline stress on adaptation strategies of various above and underground vegetative organs. At present, the comparative studies on the tolerance of above-ground and underground plant organs to salt stress have different results, and the studies show that the effects of salt stress on different plant organs vary according to different plant species. For example, under NaCl salt stress, the dry weight and fresh weight of sacsaoul increased with increasing salt concentration (Abuduwaili et al., 2015), while the biomass of the stems and leaves of areca nut decreased significantly, but the biomass of the roots did not decrease significantly, and even increased significantly at low concentrations (da Silva et al., 2008). By comparing the physiological changes of sheep roots, rhizostems, stems and leaves under salt-alkali stress, it was indicated that the root system of *Leymus chinensis* had the best salt tolerance among all organs, while the rhizostems had the worst salt tolerance (Hameed et al., 2011).

In addition, effect of salt stress could be different on different growth stages, plant organs and osmoregulation effects (Jia et al., 2019). Osmoregulatory substances are different in different species. For example, under NaCl salt stress, halophyte plants such as paspalum mainly resist stress by synthesizing organic matter such as soluble sugar (Lee et al., 2008; Lokhande and Suprasanna, 2012), while in plants such as prickly thistle and wheat, inorganic ions such as Cl^- play a major role in osmoregulation (Benlloch-González et al., 2005; Ghavami and Ramin, 2008). Different organs react physiologically differently to alkali and salt stress. For instance, the antioxidant activity patterns of sunflower and cornflower stems, leaves, and roots differed under saline stress (Alamer et al., 2022; Naveed et al., 2021). Certain plants store a lot of Na^+ in their roots, where it is much more concentrated than in their stems and leaves (Saqib et al., 2005). Conversely, areca nut leaves have a higher Na^+ content than their roots (da Silva et al., 2008). The impacts of salt and alkaline stress on growth and physiology of vegetative organs, especially the physiological response of vegetative organs under stress, are still unclear. Therefore, we determined the effects of NaCl and Na_2CO_3 stress on the growth of various vegetative organs (root, stem, total petiole and leaflet), physiological traits and ions accumulation of *Amorpha fruticosa* seedlings.

Materials and methods

Experimental materials and stress conditions

The seeds of *Amorpha fruticosa* were collected from the natural mountains in Dongliao County. The experiment was conducted at Northeast Normal University China. Before the experiment, amorpha seeds with uniform size and full grain were selected, soaked in a constant temperature hot water bath at 60℃ for 20 minutes before sowing, disinfected with 0.1% $KMnO_4$ solution for 5 minutes, then washed with water. Seeds were sown in a plastic flowerpot with a diameter of 20 cm and filled with washed fine river sand. After planting, place the pot outdoors for artificial protection from rain. After emergence, Hoagland nutrient solution was used once a day from 17:00 to 17:30. The ingredients of Hoagland nutrient solution include: Ca^{2+} (5.00 mM), Mg^{2+} (2.00 mM), K^+

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(6.04 mM), EDTA-Fe^{2+} (22.2 μM), Mn^{2+}(6.72 μM), Cu^{2+}(3.16 μM), Zn^{2+} (0.765 μM), SO_4^{2-} (2.10 mM), $H_2PO_4^-$ (1.00) mM), H_3BO_3(46.3 μM), H_2MoO_4 (0.556 μM), NO_3^-(15.04 mM) (Hothem et al., 2003). The seedlings were thinned in time, and 10 seedlings were fixed in each pot.

Stress conditions and treatment: neutral salt NaCl and basic salt Na_2CO_3 were used to simulate salt stress and alkali stress, and Hoagland nutrient solution was used to prepare simulated stress solutions with different concentration gradients, NaCl concentrations were 0, 50, 100, 200 mM, and Na_2CO_3 concentrations were 0, 10, 20, 30 mM, respectively.

Following four weeks of growth, 28 pots of uniformly growing *Amorpha fruticosa* seedlings were chosen at random and split into seven groups. One group was the control group, and the other six groups were used for stress treatment, that is, each treatment was repeated 4 times. From 16:30 to 17:30 every day, each pot was given 500 ml of the corresponding stress solution, and the control group was only given 'Hoagland' nutrient solution for continuous treatment for 3 weeks.

Measurement of growth index

The growth indexes included survival rate, plant height, root length, underground and above-ground biomass. On the second day after the last stress treatment, the survival rate (survival rate = number of surviving plants/total number of plants 100%) and plant height of each treatment were investigated respectively. After that, the seedlings were taken out, washed with distilled water. The roots, stems, leaves and total petiole were partially opened, and the root length, plant height and total petiole length were measured. The leaves were immediately dried at 105℃ for 15 minutes for green treatment, and then the roots, stems, leaves and total petioles were baked in the oven at 70℃ to constant weight, and the dry weight of each part was weighed respectively. After drying, the roots, stems, leaves and total petioles were crushed and ground, screened by an 80-mesh sieve, and used to measure the physiological indexes of inorganic ions and organic solutes.

Determination of photosynthetic indexes and photosynthetic pigment content

Photosynthetic indexes and photosynthetic pigments were measured before sampling. Functional leaves were selected for each basin, that is, 12 leaves were measured for each treatment. Red and blue LED light source was used to measure the leaves' transpiration rate, stomatal conductance, intercellular CO_2 concentration, and net photosynthetic rate (LI-6800XT, Li-Cor, Inc, Lincoln, NE, USA). Extraction of photosynthetic pigments: fresh leaves were taken, accurately weighed 0.02 g, leaves were cut and put into a centrifuge tube, then added 5 ml extraction reagent (prepared by mixing 80% acetone reagent, ultra-pure water and anhydrous ethanol according to the ratio of volume 4:1:5), shade them with black cloth, placed them in a dark place for 2 days, and fully extracted the chlorophyll to be measured. The concentration of photosynthetic pigments was determined by Determination of photosynthetic pigment concentration with a 752 N visible light spectrophotometer produced by Shanghai Youke: the chlorophyll extract was poured into the colorimetric dish, and the absorbance was determined by the extraction reagent as the control, and then the absorbance value (OD) was determined at the wavelength of 470 nm, 645 nm and 663 nm, respectively to determine chlorophyll a, chlorophyll b and carotenoid contents.

The calculation formula is as follows, respectively:

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$$C_a = 12.72 \times OD_{663} - 2.59 \times OD_{645} \quad \text{(Eq.1)}$$

$$C_b = 22.88 \times OD_{645} - 4.67 \times OD_{663} \quad \text{(Eq.2)}$$

$$C_{ar} = (1000 \times OD_{470} - 2.05 \times C_a - 104 \times C_b) \div 229 \quad \text{(Eq.3)}$$

Photosynthetic pigment content (mg/gFW) count:

$$Photosynthetic pigment content = \frac{C \times V \times n}{W \times 1000} \quad \text{(Eq.4)}$$

where C is the chlorophyll concentration (u g/ml); V is the total volume of the extract (ml); n is the dilution of the extract; W is the sampled fresh weight (g).

Determination of inorganic ions

Each dry sample was weighed at 0.05 g, placed in a centrifuge tube, added with 10 ml deionized water, and soaked in a boiling water bath for 1 h. Inorganic ions include cations and anions, cations are Na^+, K^+ and free Ca^{2+}, Mg^{2+}, anions are Cl^-, NO_3^-, SO_4^{2-} and $H_2PO_4^-$. Cations were determined by atomic absorption spectrophotometer (TAS-990, Purkinje General, Beijing). The anions were determined by an ion chromatography system (model DX-300, DIONEX, Sunnyvale, USA) under the following conditions: the ion exchange column model AS4A-SC, the conductivity detector model CDM-II, and the mobile phase $Na_2CO_3/NaHCO_3$ = 1.7/1.8 mM.

Determination of soluble sugar

Soluble sugar was determined by H_2SO_4 anthrone colorimetric method, and glucose was used as the standard sample. OD values were obtained at 620 nm wavelength. The calculation formula is as follows:

$$\text{Soluble sugar content (mg/g. DL)} = C \times V / W / 1000$$

where V is the total volume of extracted liquid (ml); W is the sample amount; and C is the concentration of soluble sugar as determined by the standard curve (ug/ml).

Statistical analysis of data

One-way analysis of variance was utilized to perform statistical analysis using SPSS26.0 (SPSS Inc., Chicago, IL, USA). The significance of variations between various NaCl concentrations and studied traits was determined with LSD test.

Experimental results

Survival ability of saline-stressed seedlings

The NaCl and Na_2CO_3 stress did not have any effect on the survival rate of the purple spike locust seedlings, which reached 100%.

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Growth of subsurface and above-ground vegetative organs

The growth of root, stem and leaf of *Amorpha fruticosa* under NaCl and Na_2CO_3 stress is shown in *Table 1*. There was not significant different among both level of salt stress for root length. However, root was significantly decreased under 200 mM salinity stress. Under the stress of Na_2CO_3, the root length at the concentration of 10 mM was higher than control ($P < 0.05$), and the root length had no significant change when the concentration was higher than that of the control. The plant height decreased gradually with increasing salts concentration ($P < 0.05$). The plant height at 200 mM NaCl was 31.9 cm, 0.72 times that of the control. At 30 mM Na_2CO_3, the plant height was 25.3 cm, which was only 0.57 times that of the control. The results indicated that there was non-significant difference among treatments for number of leaves. The number of leaflets decreased significantly with the increase of stress intensity ($P < 0.05$). With an increase in NaCl and Na_2CO_3 stress intensity, the total petiole length gradually decreased and it was short as compared to control ($P < 0.05$). As the intensity of the NaCl and Na_2CO_3 stresses increased, the ratio of root length also reduced. The ratio was significantly higher than the control ($P < 0.05$) at concentrations of NaCl greater than 100 mM. It was also significantly higher under Na_2CO_3 stress ($P < 0.05$).

Table 1. *Effects of NaCl stress and Na_2CO_3 stress on root length, shoot length, compound leaf number, and lobule number Amorpha fruticosa seedlings*

Stress content ($mmolL^{-1}$)		Root length (cm)	Plant height (cm)	Compound leaf number	Number of leaflets	Primary petiole length	Root/shoot
Salt stress NaCl	0	13.7 ± 0.3ab	44.5 ± 0.9d	11.5 ± 0.3ab	17.2 ± 0.3c	12.0 ± 0.3c	0.31 ± 0.01a
	50	14.1 ± 0.3b	40.4 ± 0.8c	12.0 ± 0.3b	16.3 ± 0.3bc	11.5 ± 0.2c	0.36 ± 0.01a
	100	15.6 ± 0.4c	36.6 ± 0.5b	11.6 ± 0.2ab	15.6 ± 0.4ab	10.4 ± 0.2b	0.43 ± 0.01b
	200	13.0 ± 0.3a	31.9 ± 0.5a	11.0 ± 0.2a	14.9 ± 0.4a	9.6 ± 0.2a	0.41 ± 0.01b
Alkaline stress Na_2CO_3	0	13.7 ± 0.3a	44.5 ± 0.9d	11.5 ± 0.3b	17.2 ± 0.3c	12.0 ± 0.3d	0.31 ± 0.01a
	10	15.2 ± 0.5b	34.0 ± 0.8c	11.0 ± 0.3b	14.5 ± 0.4b	9.4 ± 0.3c	0.46 ± 0.02b
	20	13.8 ± 0.4a	29.1 ± 0.8b	9.5 ± 0.2a	13.3 ± 0.4a	8.3 ± 0.3b	0.48 ± 0.01b
	30	13.6 ± 0.4a	25.3 ± 0.6a	9.3 ± 0.3a	12.5 ± 0.4a	7.2 ± 0.2a	0.55 ± 0.02c

The values are means (±SE) of quadruplicate samples. Different letter indicates significant difference at $P < 0.05$

Underground and above-ground biomass

Both underground biomass and above-ground biomass decreased with increasing salts concentration ($P < 0.05$). The biomass of stem, leaf and total petiole of aboveground part also decreased with increasing concentration of both stresses ($P < 0.05$). Although the biomass of underground and above ground decreased with the increase of stress intensity, the ratio of underground-to-underground biomass had different changes. Under NaCl stress, the ratio first decreased and then increased, and the ratio was significantly lower than the control when NaCl concentration was lower than 100 mM ($P < 0.05$). Under Na_2CO_3 stress, the ratio of subsurface to above-ground

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biomass gradually grew as the level of stress increased and was noticeably greater than the control ($P < 0.05$). The leaf biomass of the control treatment occupied 55.6% of the above biomass. Under NaCl stress, the proportion of lobular biomass on land area was 57.0%, 58.1% and 61.3%, respectively. Under Na_2CO_3 stress, the proportion of leaf biomass on land area was 57.0%, 58.8% and 59.5%, respectively (*Table 2*).

Table 2. *Effects of NaCl stress and Na_2CO_3 stress on underground biomass and above ground biomass of Amorpha fruticosa seedling (g)*

Stress content ($mmolL^{-1}$)		Underground biomass	Above-ground biomass				Underground/ above-ground
		Root	Total	Stemp	Lobule	Primary petiole	
Salt stress NaCl	0	3.19 ± 0.13c	10.76 ± 0.26c	3.63 ± 0.13d	5.98 ± 0.22c	1.15 ± 0.04b	0.23 ± 0.01b
	50	2.46 ± 0.12b	9.35 ± 0.25b	2.99 ± 0.05c	5.32 ± 0.21bc	1.04 ± 0.04b	0.21 ± 0.01a
	100	1.98 ± 0.15a	7.88 ± 0.53a	2.52 ± 0.17b	4.58 ± 0.30ab	0.77 ± 0.08a	0.20 ± 0.01a
	200	2.08 ± 0.10ba	6.84 ± 0.27a	1.96 ± 0.07a	4.19 ± 0.18a	0.69 ± 0.03a	0.23 ± 0.01b
Alkaline stress Na_2CO_3	0	3.19 ± 0.13b	10.76 ± 0.26c	3.63 ± 0.13c	5.98 ± 0.22c	1.15 ± 0.04c	0.23 ± 0.01a
	10	2.37 ± 0.33a	7.27 ± 1.01b	2.45 ± 0.52b	4.14 ± 0.46b	0.67 ± 0.10b	0.25 ± 0.02ab
	20	2.22 ± 0.08a	5.77 ± 0.43ab	1.83 ± 0.19ab	3.39 ± 0.19ab	0.54 ± 0.07ab	0.28 ± 0.01b
	30	1.87 ± 0.08a	4.46 ± 0.40a	1.41 ± 0.14a	2.66 ± 0.22a	0.40 ± 0.04a	0.30 ± 0.02b

The values are means (±SE) of quadruplicate samples. Different letter indicates significant difference at $P < 0.05$

Photosynthetic pigment content in leaves

The contents of carotenoids, chlorophyll a, and chlorophyll b under NaCl and Na_2CO_3 stress progressively decreased with an increase in stress intensity. When compared to the control, the contents of the three photosynthetic pigments under NaCl stress did not significantly differ. However, when NaCl concentration was 50 mM, the contents of the three photosynthetic pigments were all higher than that of the control, indicating that the formation of photosynthetic pigments in leaves could be promoted under this concentration. The contents of carotenoid and chlorophyll b were not significantly different from the control when the concentration of Na_2CO_3 was 10 mM; however, when the concentration was greater, the contents of both carotenoid and chlorophyll b were significantly lower ($P < 0.05$) than the control. According to these results, it can be inferred that when the concentration of Na_2CO_3 is higher than 10 mM, the synthesis of chlorophyll is inhibited, or the decomposition of chlorophyll is accelerated.

Among the three photosynthetic pigments, the content of chlorophyll accounted for the largest proportion, and the content of chlorophyll a under NaCl and Na_2CO_3 stress accounted for about 65% of the content of photosynthetic pigments, which had no significant difference from the control treatment (*Table 3*). As the stress level increased, the ratio of chlorophyll a to chlorophyll b content significantly dropped. At 10 mM it was higher as compared to control while at 30 mM it was significantly lower than control (*Table 3*).

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Table 3. *Effects of NaCl and Na_2CO_3 stress on the photosynthetic pigment content of Amorpha fruticosa seedlings (mg $g^{-1}FW$)*

Stress content ($mmolL^{-1}$)		Chlorophyll a	Chlorophyll b	Carotenoid	Chlorophyll a + b	Chlorophyll a/b
Salt stress NaCl	0	27.02 ± 3.46a	8.26 ± 1.13a	5.78 ± 0.77a	35.28 ± 4.59a	3.28 ± 0.04a
	50	30.77 ± 2.48a	9.13 ± 0.71a	6.44 ± 0.48a	39.90 ± 3.18a	3.37 ± 0.02ab
	100	27.31 ± 1.33a	7.82 ± 0.38a	5.83 ± 0.25a	35.13 ± 1.70a	3.49 ± 0.04b
	200	25.01 ± 1.47a	7.56 ± 0.41a	5.38 ± 0.31a	32.56 ± 1.86a	3.31 ± 0.06a
Alkaline stress Na_2CO_3	0	27.02 ± 3.46c	8.26 ± 1.13b	5.78 ± 0.77b	35.28 ± 4.59c	3.28 ± 0.04ab
	10	23.71 ± 1.29bc	7.13 ± 0.32b	5.21 ± 0.18b	30.83 ± 1.60bc	3.32 ± 0.05b
	20	20.33 ± 1.26b	6.21 ± 0.45ab	4.56 ± 0.22ab	26.54 ± 1.71ab	3.28 ± 0.04ab
	30	14.06 ± 0.88a	4.43 ± 0.23a	3.31 ± 0.25a	18.49 ± 1.11a	3.17 ± 0.03a

The values are means (±SE) of quadruplicate samples. Different letter indicates significant difference at $P < 0.05$

Photosynthetic index

Under NaCl stress, the net photosynthetic rate of each treatment was 0.68, 0.65 and 0.51 times of the control, the transpiration rate was 0.57, 0.48 and 0.36 times of the control, and the stomatal conductance was 0.51, 0.49 and 0.33 times of the control, respectively. Under Na_2CO_3 stress, the net photosynthetic rate, transpiration rate and stomatal conductance were 0.45, 0.29, 0.21 times of the control, 0.37, 0.36, 0.19 times of the control, and 0.38, 0.30, 0.22 times of the control (*Table 4*). The distribution of intercellular CO_2 concentration was different under salt and alkali stress, and it gradually decreased with the increase of NaCl stress concentration, and was significantly lower than that of the control ($P < 0.05$), and each treatment was 0.86, 0.85, 0.73 times of the control, respectively. Na_2CO_3 concentration was higher in salt stress and it was 0.98, 1.08, 1.12 times than the control (*Table 4*).

Table 4. *Effects of NaCl and Na_2CO_3 stress on photosynthetic indexes of Amorpha fruticosa seedlings*

Stress content ($mmolL^{-1}$)		P_N ($\mu mol\ m^{-2}S^{-1}$)	E ($mmol\ m^{-2}S^{-1}$)	G_S ($mol\ m^{-2}S^{-1}$)	Ci ($\mu mol\ m^{-2}S^{-1}$)
Salt stress NaCl	0	7.48 ± 0.64b	25.13 ± 0.38d	0.29 ± 0.021b	204.96 ± 9.24b
	50	4.25 ± 0.12a	17.06 ± 0.19c	0.15 ± 0.003a	175.68 ± 3.82ab
	100	3.61 ± 0.28a	16.39 ± 0.83bc	0.14 ± 0.009a	174.99 ± 8.28ab
	200	2.69 ± 0.08a	12.78 ± 0.44a	0.10 ± 0.004a	149.73 ± 3.04a
Alkaline stress Na_2CO_3	0	7.48 ± 0.64c	25.13 ± 0.38d	0.29 ± 0.021c	204.96 ± 9.24ab
	10	2.74 ± 0.06b	11.31 ± 0.21c	0.11 ± 0.002b	200.45 ± 4.06a
	20	2.73 ± 0.13b	7.27 ± 0.27b	0.09 ± 0.004ab	221.92 ± 8.53bc
	30	1.44 ± 0.10a	5.24 ± 0.27a	0.06 ± 0.005a	230.44 ± 4.21c

The values are means (±SE) of quadruplicate samples. Different letter indicates significant difference at $P < 0.05$

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Inorganic ion content

Figure 1 shows the changes of cation content in *Amorpha fruticosa* seedlings under NaCl and Na_2CO_3 stress. Under NaCl stress, Na^+ contents in roots, stems, total petioles and lobules increased with the increase of stress intensity, and were significantly higher than those in the control ($P < 0.05$, *Figure 1*). When NaCl concentration was 200 mM, the Na^+ contents in roots, and stems were significantly higher than that in roots and lobules.

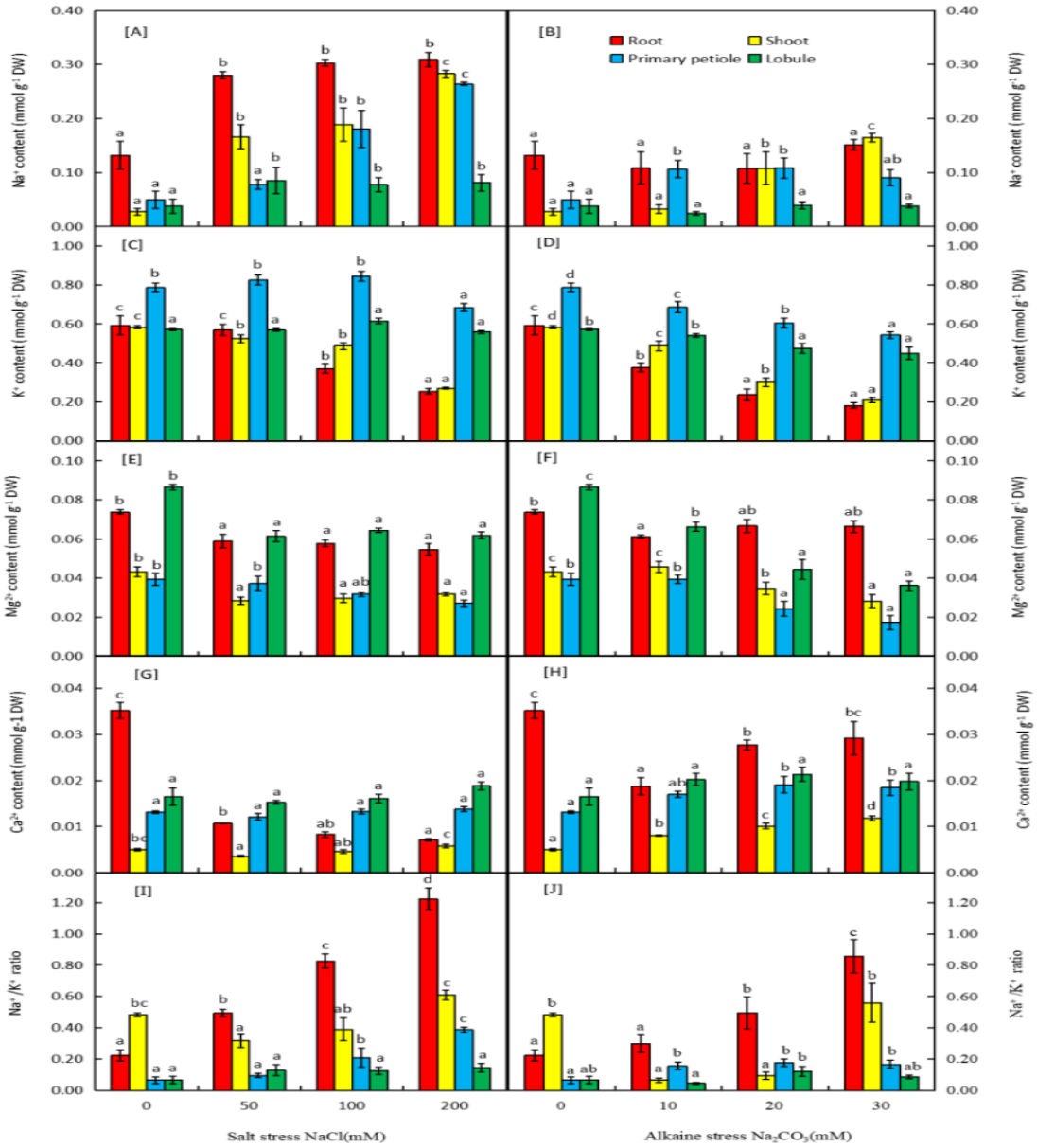

Figure 1. *Effect of NaCl and Na_2CO_3 stress on positive ion of Amorpha fruticosa seedling. The data represent the means (±SE) of three replications and significant difference at $P < 0.05$ is indicated by a different letter*

Na^+ concentration in plant root and lobules did not change under Na_2CO_3 stress. As stress intensity increased, Na^+ content in the stems was increased. At 20 mM ($P < 0.05$),

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it was significantly higher than the controls, and at its highest, it was 6.02 times that of the control. The total petiole's Na^+ content increased and then decreased as stress intensity increased. All treatments showed a significant difference from the control ($P < 0.05$), with the highest Na^+ content being 2.19 times that of the control (*Figure 1*).

The distribution pattern of Na^+ content in all organs of control treatment was root > total petiole > lobule > stem, and the Na^+ contents in root, stem and total petiole were 3.48, 0.72 and 1.31 times of that in lobule, respectively. Under NaCl stress, the distribution pattern of Na^+ content in all organs was different from that of the control, that is, root > stem > total petiole > lobule. When NaCl concentration was 200 mM, the Na^+ content in root, stem and total petiole was 3.81, 3.49 and 3.25 times of that in lobule, respectively, all of which were higher than the control level. Under Na_2CO_3 stress, the distribution of Na^+ content in different organs was different under different treatments, but the content of Na^+ in leaflets was lower than that in roots, stems and total petioles. When the concentration of Na_2CO_3 was 30 mM, the content of Na^+ in roots, stems and total petioles was 4.00, 4.35 and 2.38 times of that in leaflets, respectively, which was also higher than the control level.

The increase in salts concentration decreased the K^+ concentration in root and stem. Under NaCl stress, the K^+ content of total petiole and lobule had no significant change, and the K^+ content of total petiole was significantly lower than that of control at 200 mM ($P < 0.05$). Under Na_2CO_3 stress, the K^+ content of total petiole and lobule gradually decreased with the increase of stress intensity, and was significantly lower than that of control ($P < 0.05$). When NaCl concentration was 200 mM, the K^+ content in roots, stems, petioles and lobules was 0.43, 0.46, 0.87 and 0.98 times that of the control, respectively. When the concentration of Na_2CO_3 was 30 mM, the content of K^+ in roots, stems, petioles and leaflets was 0.31, 0.36, 0.69 and 0.79 times that of the control, respectively.

The change of free Mg^{2+} content is shown in *Figure 1*. Under NaCl stress, the free Mg^{2+} content of roots, stems and leaflets had no significant change. The content of free Mg^{2+} in root under Na_2CO_3 stress had no significant change, but it was lower than the control ($P < 0.05$). The free Mg^{2+} content in stem, total petiole and leaflet gradually decreased with the increase of stress intensity. When the concentration of Na_2CO_3 was higher than 20 mM, the content in stem and total petiole was lower as compared to control. When NaCl concentration was 200 mM, the free Mg^{2+} content in roots, stems, petioles and lobules was 0.74, 0.74, 0.71 and 0.69 times that of the control, respectively. When the concentration of Na_2CO_3 was 30 mM, the free Mg^{2+} content in roots, stems, petioles and lobules was 0.90, 0.65, 0.44 and 0.42 times that of the control, respectively.

When roots were under NaCl stress, their free Ca^{2+} content steadily dropped as stress levels increased and was noticeably lower than in the control group ($P < 0.05$). As stress intensity increased, the amount of free Ca^{2+} in the stems increased gradually and was noticeably higher than in the control group at 200 mM NaCl concentration. In comparison to the control, there was no discernible change in the total petiole and lobule's free Ca^{2+} content. Under Na_2CO_3 stress, the amount of free Ca^{2+} in the roots and stems gradually increased as the stress intensity increased. There was no significant change in the content of free Ca^{2+} in total petiole and leaves, but the content of total petiole was significantly higher than that of control ($P < 0.05$), and the content of leaves was not significantly different from that of control. When NaCl concentration was 200 mM, the content of free Ca^{2+} in roots, stems, petioles and lobules was 0.20, 1.44, 1.05 and 1.14 times that of the control, respectively. When the concentration of Na_2CO_3

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was 30 mM, the content of free Ca^{2+} in roots, stems, petioles and lobules was 0.83, 2.35, 1.40 and 1.20 times that of the control, respectively.

Under NaCl stress, the Na^+/K^+ ratios of stems, roots, total petioles, and lobules increased gradually as the stress intensity increased. The concentration of NaCl was lower in 100 mM and Na^+/K^+ ratio in stem was lower as compared to control. The NaCl concentration > 100 mM resulted in maximum Na^+/K^+ ratio of the entire petiole (*Figure 1*). *Figure 2* shows the changes of anion content in *Amorpha fruticosa* seedlings under NaCl and Na_2CO_3 stress. Under NaCl stress, there was no significant change in root Cl^- content (*Figure 2*), which did not change with the increase of Cl^- content in the environment, but was significantly higher than that in the control ($P < 0.05$). The Cl^- content of stem, total petiole and lobule was gradually significantly increased with the increase of stress intensity. Under Na_2CO_3 stress, the Cl^- content of roots, total petioles and lobules gradually decreased with the increase of stress intensity, the Cl^- content of total petioles and lobules was significantly lower than that of the control ($P < 0.05$), and the Cl- content of roots was significantly lower than that of the control when the concentration of Na_2CO_3 was higher than 20 mM ($P < 0.05$). There was no significant change in Cl^- content in stems, but it was significantly lower than that in control ($P < 0.05$). When NaCl concentration was 200 mM, the Cl^- content in roots, stems, petioles and lobules was 5.24, 6.00, 7.53 and 3.51 times that of the control, respectively. When the concentration of Na_2CO_3 was 30 mM, the Cl^- content of root, stem, total petiole and lobule was 0.36, 0.49, 0.43 and 0.34 times of that of control, respectively.

Figure 2 shows the change of NO_3^- content. Under NaCl stress, NO_3^- content of root was significantly higher than that of control when NaCl concentration was lower than 100 mM ($P < 0.05$). There was no significant difference in NO_3^- content between stem and control when NaCl concentration was lower than 100 mM, but significantly lower than control at 200 mM ($P < 0.05$). The NO_3^- content of stem, total petiole and leaflet had no significant changes, but was significantly lower than that of control ($P < 0.05$). When NaCl concentration was 200 mM, NO_3^- content in root, stem, total petiole and leaflet was 0.83, 0.44, 0.25 and 0.49 times of that in control, respectively. When the concentration of Na_2CO_3 was 30 mM, the NO_3^- content of root, stem, total petiole and leaflet was 0.47, 0.29, 0.18 and 0.44 times of that of control, respectively.

As stress intensity increased, the H_2PO_4-content in the stems gradually decreased. At 50 mM ($P < 0.05$), it was significantly higher than the control; at 100 mM, there was no significant difference with the control; and at 200 mM, it was significantly lower than the control. H_2PO_4- content did not significantly differ between the control and total petiole. As stress intensity increased, lobules' H_2PO_4-content gradually dropped and was noticeably lower than that of the control group ($P < 0.05$).

As stress intensity increased, the $H_2PO_4^-$content in stems, petioles, and leaflets gradually decreased and was significantly lower than that of the control group ($P < 0.05$). The $H_2PO_4^-$content in the roots, stems, petioles, and lobules was 2.36, 0.84, 0.92, and 0.81 times that of the control at a 200 mM concentration of NaCl, respectively. The SO_4^{2-} content of the root, total petiole, and lobule under NaCl stress significantly decreased ($P < 0.05$) and decreased gradually with increasing stress intensity.

As stress intensity increased, the SO_4^{2-} content of the entire petiole gradually decreased but remained significantly higher than that of the control group ($P < 0.05$). The SO_4^{2-} content in the roots, stems, petioles, and lobules was 0.41, 1.32, 0.40, and 0.36 times that of the control at a 200 mM concentration of NaCl, respectively.

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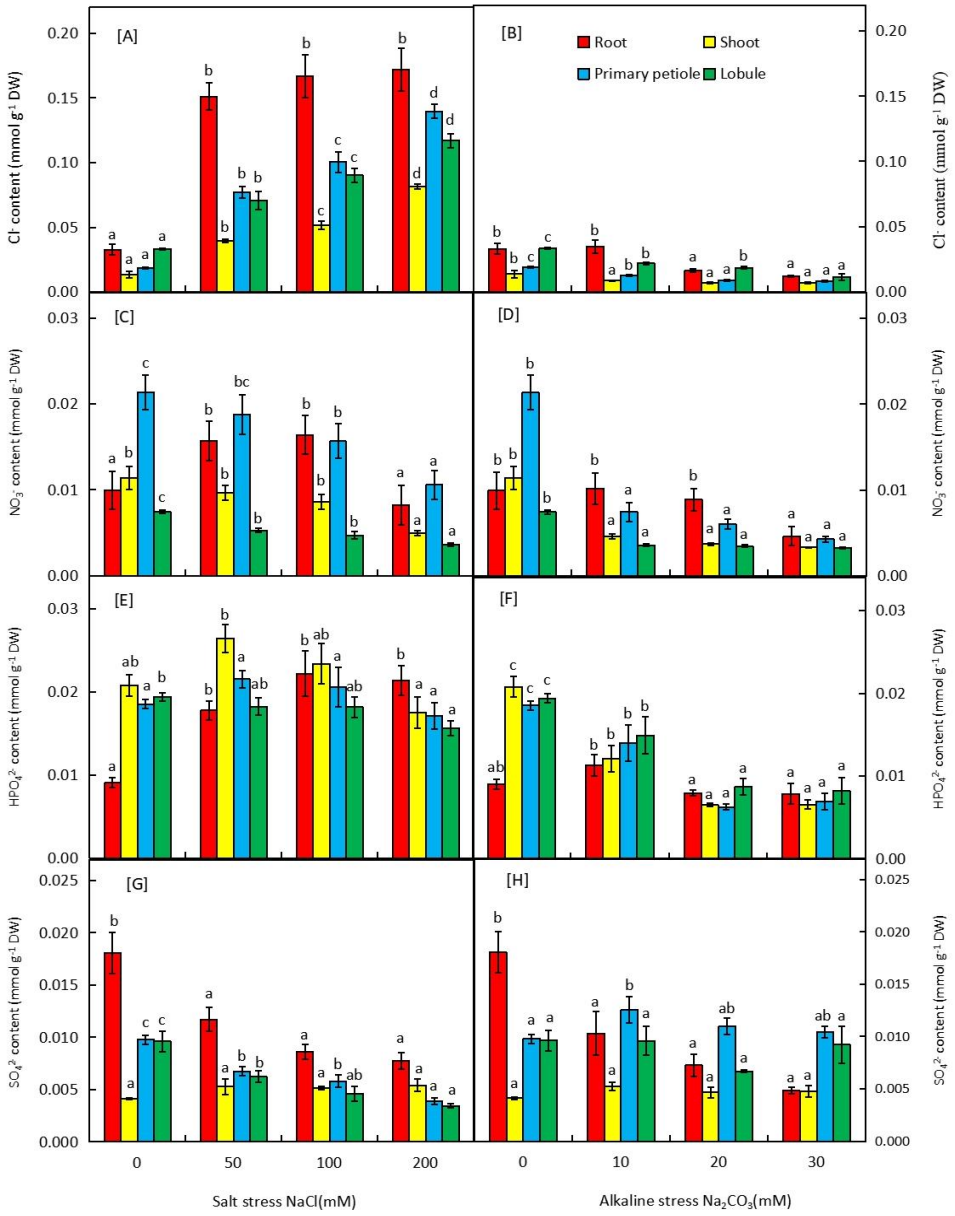

Figure 2. *Effect of NaCl and Na_2CO_3 stress on negative ion of Amorpha fruticosa seedling. The data represent the means (±SE) of three replications and significant difference at $P < 0.05$ is indicated by a different letter*

Soluble sugar content

With an increase in stress intensity, the soluble sugar content of the lobule and total petiole increased gradually (*Figure 3*). The soluble sugar in petiole was higher at 200 mM than control and as the level of stress increased, the soluble sugar content of the entire petiole and lobule grew gradually and was considerably higher than that of the control group ($P < 0.05$). Soluble sugar content in roots, stems, petioles, and lobules was 2.83, 0.66, 1.20, and 1.43 times that of the control at 200 mM NaCl concentration, respectively. The soluble sugar content increased more in the roots, stems, petioles, and lobules at 30 mM concentration of Na_2CO_3, as measured by 4.43, 1.67, 1.47, and 1.31 times higher than that of the control.

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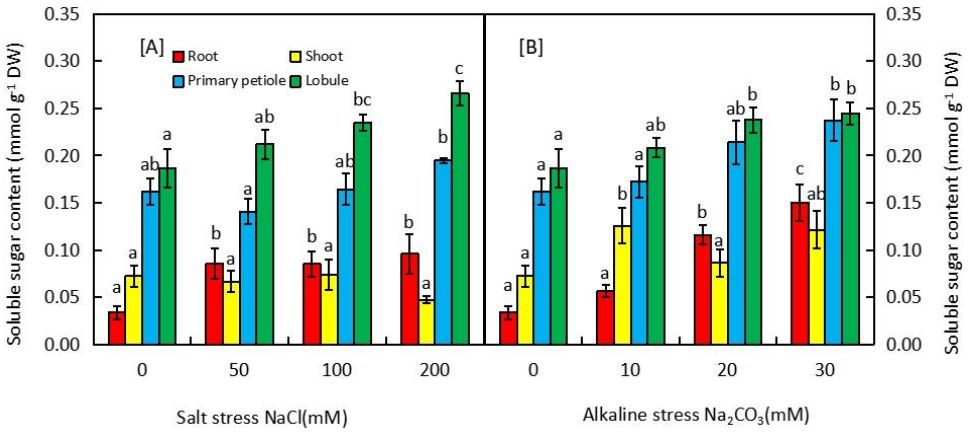

Figure 3. *Effect of NaCl and Na_2CO_3 stress soluble sugar of Amorpha fruticosa seedling. The data represent the means (±SE) of three replications and significant difference at $P < 0.05$ is indicated by a different letter*

Discussion

Effects of salt and alkali stress on seedling growth

Salt and alkali stress had different effects on root, stem and leaf growth of *Amorpha fruticosa* seedlings. The root length was significantly increased under proper concentration of salt stress (less than 100 mM NaCl) ($P < 0.05$), but significantly decreased under high concentration of salt stress (200 mM NaCl) ($P < 0.05$). There was no difference in root growth between alkali stress and control. The plant height decreased significantly under salt and alkali stress ($P < 0.05$). The ratio of root length to plant height increased to different degrees due to the changes of root length and plant height.

The number of compound leaves, the number of leaflets and the total petiole length decreased significantly under salt stress ($P < 0.05$), which indicated that the growth of compound leaves was inhibited to some extent. The main reason for the inhibition of plant growth by high salt concentration is the decrease in photosynthetic area of leaves (Parida et al., 2004), and the effect on the growth of complex leaves of *Amorpha fruticosa* seedlings may be the direct reason for the inhibition of the growth of *Amorpha fruticosa* by salt and alkali stress.

Salt and alkali stress significantly affected the accumulation of underground and above-ground biomass ($P < 0.05$), and the biomass of root, stem, leaflet and total petiole was negatively correlated with stress intensity. It can be seen that under high salt (200 mM NaCl) and alkali stress, the root of *Amorpha fruticosa* seedlings has a strategy of preferential growth, which is consistent with the conclusion that nutrients and more photosynthetic products are used for root growth under stress conditions (Malambane et al., 2023). The proportion of biomass occupied by lobular biomass under salt and alkali stress was significantly higher than that under control, and was positively correlated with stress intensity, indicating that above-ground biomass accumulation was preferred to lobular growth under salt and alkali stress. In summary, the rule of biomass accumulation under salt-alkali stress is as follows: underground and above-ground accumulation is preferred to root growth, and above-ground biomass accumulation is preferred to leaflet growth, which can ensure the absorption of water and mineral nutrients by roots, ensure the photosynthetic physiological activities of leaves, and

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facilitate the accumulation of photosynthetic products, so as to better adapt to salt-alkali stress environment. The root system is an important bridge connecting the material exchange between the soil and the above-ground part of the plant, and a strong root system is the foundation for supporting and nurturing the vigorous and prosperous crown. As an organ that absorbs water, mineral nutrients and physiologically active compounds, the number, size, biomass and physiological status of roots directly affect the tolerance strength of plants to environmental stress (Yousfi et al., 2010). The root system has important metabolic functions and can synthesize amino acids, organic acids, plant hormones, alkaloids and other secondary metabolites, which play an important role in plant stress resistance (Khare et al., 2020). Leaf is the place of photosynthesis, is an important organ of primary production. Therefore, it can be said that the biomass accumulation rule of *Amorpha fruticosa* seedlings is a good embodiment of its adaptation to salt and alkali stress.

Effects of salt and alkali stress on photosynthetic pigments and photosynthetic capacity

Chlorophyll a content in leaves of *Amorpha fruticosa* seedlings was higher, accounting for 65.81% of photosynthetic pigment content. However, chlorophyll a content was significantly decreased under alkali stress ($P < 0.05$), and chlorophyll b and carotenoids were also significantly decreased when Na_2CO_3 concentration was higher than 10 mM ($P < 0.05$). The reason may be that the root absorbs CO_3^{2-} and forms insoluble matter with Mg^{2+}, and Mg is an important component of chlorophyll, so it inhibits the synthesis of chlorophyll or accelerates the decomposition of chlorophyll.

Plant growth and productivity are directly influenced by their photosynthetic capacity, and plant growth can be directly reflected in the net photosynthetic rate. Plant growth is positively correlated with photosynthetic efficiency and salt tolerance (Penella et al., 2016). Under stress conditions, stomatal conductance and transpiration rate of some plants decreased with the increase of stress (Hasanuzzaman et al., 2023; Sharipova et al., 2022). Under normal circumstances, the value of stomatal conductance affects the level of CO_2 content in leaves, and the photosynthetic rate of plants is also affected. A high value of stomatal conductance can increase the assimilation rate of CO_2, and thus increase the production efficiency and output of plants (Hasanuzzaman et al., 2023). In salt-stressed environments, salt ions enter various parts of leaves with transpiration and destroy physiological functions of leaves to varying degrees (Abobatta, 2020). In addition to the decrease of transpiration rate and stomatal conductance, the decrease of net photosynthetic rate is also related to the decrease of photosynthetic area caused by stress inhibition of compound leaf growth (as previously mentioned), which ultimately leads to the decrease of photosynthetic productivity. Under salt stress, intercellular CO_2 concentration was significantly lower than that of control ($P < 0.05$), and its changing trend was similar to that of biomass accumulation. This is different from the conclusion that the higher CO_2 content in leaves can increase the assimilation rate of CO_2, and thus make plants produce higher biomass (Flexas et al., 2002), indicating that alkali stress may cause the damage of photosynthetic system function.

Effects of salt and alkali stress on inorganic ions

Na^+ in the cytoplasm can be excreted from the cells through the K^+/Na^+ exchange of vacuoles. The roots of salt-tolerant plants can reject the absorption of Na^+, so that Na^+ is

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excluded from the body of the plant, thus reducing the concentration of Na^+ in the plant, or the Na^+ absorbed into the plant can be separated in some special parts to avoid damage to the plant. Therefore, Na^+/K^+ in the leaves of salt-tolerant plants is low (Akter and Oue, 2018). More Na^+ was accumulated in stems and total petioles, thus keeping the content of Na^+ in roots and leaflets relatively stable, so that roots and leaflets would not be affected by the ionic toxicity caused by excessive Na^+, which was conducive to maintaining the absorption function of roots and the photosynthetic function of leaves. The changes of Na^+ content in all organs under alkali stress were not very regular, but the overall trend was that the Na^+ content in roots and lobules did not change significantly, while the Na^+ content in stems and total petioles increased with the increase of stress intensity. In conclusion, a large amount of Na^+ was accumulated in stems and total petioles under alkali stress, while the content of Na^+ in roots and lobules remained relatively stable, and the content of Na^+ in lobules was lower than that in other organs. It has also been reported that the phloem of the leaf, the phloem of the petiole and the pith of the *Amorpha fruticosa* have a salt-accumulating effect (Zhao et al., 2022). These results indicated that the accumulation of salt in special parts of the body is an important physiological response of amorpha to resist salt stress.

The free Mg^{2+} content of total petiole was negatively correlated with stress intensity. The decrease of free Mg^{2+} content in total petiole might contribute to the relative stability of free Mg^{2+} content in other organs, especially in lobules, which is conducive to the stability of chlorophyll content and biosynthesis. The change in the content and distribution of Mg^{2+} may be an important condition for maintaining the control level of chlorophyll content under salt stress (as previously mentioned). The experimental results showed that free Mg^{2+} content and chlorophyll content were positively correlated under salt and alkali stress, so it could be inferred that chlorophyll content and free Mg^{2+} content was directly related under salt and alkali stress. Therefore, free Mg^{2+} not only plays an imperative role in osmo-regulation but also maintains higher chlorophyll concentration under salt stress. Calcium is essential for plants as it involves in signaling perception, transmission and improves plant tolerance against stress conditions (Kour et al., 2023). The change trend of free Ca^{2+} content in root was completely different under salt stress and alkali stress. The contents of stem and total petiole were different under different stresses, but there was no significant difference in the content of free Ca^{2+} in lobules. It can be seen that Ca^{2+} plays a certain role in maintaining the normal physiological function of the leaves.

When plants are stressed by salt and alkali, they often accumulate inorganic anions to regulate ion balance and the stability of intracellular environment, and inorganic ions mainly include Cl^-, NO_3^-, SO_4^{2-} and $H_2PO_4^-$ (Guo et al., 2009; Liu and Shi, 2010). Some studies have shown that the distribution of Cl^- among different tissues is significantly uneven (Yong et al., 2004). In this experiment, the contents of various anions in various vegetative organs of *Amorpha fruticosa* were different under salt stress and alkali stress. It shows that the degree of Cl^- absorption of root is relatively low, keeping relatively stable Cl^- content can not only regulate the osmotic balance, but also prevent the excessive absorption of Cl^- and interfere with the ion balance. The content of Cl^- in root, stem, total petiole and lobule was decreased under alkali stress, and was significantly lower than that in control ($P < 0.05$). It can be seen that Cl^- does not play a role in osmotic regulation under alkali stress. Under salt-alkali stress, only the root responds when the salt stress is lower than 100 mM, indicating that NO_3^- has little effect on osmotic regulation under salt-alkali stress. The SO_4^{2-} content of the stem and small

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leaves did not change significantly, and the SO_4^{2-} content of the total leaf handle was significantly higher ($P < 0.05$), Therefore, SO_4^{2-} on saline stress is not very important.

Saline stress on soluble sugar

Soluble sugar is not only an effective osmotic agent in the plant, but also the carbon and energy source of other organic solute synthesis, and also can buffer the concentration of inorganic ions in the cell, which is a protective effect of the enzyme. Other studies have shown that the accumulation of soluble sugar in salt stress is associated with the significant positive correlation of the Na^+ concentration. Similar results were obtained in this experiment, but the accumulation of soluble sugar in different nutritional organs under salt pressure was not consistent. The soluble sugar content in leaves under control was higher while, soluble sugar content of various organs such as root, leaves and stem were higher in stress conditions. Therefore, soluble sugar is not only the organic solute that regulates the osmotic equilibrium of the seedling root and the small leaf, but also provides more energy and carbon storage sources for the growth of the root, thereby enhancing the resistance of the root.

Conclusion

Salt stress reduced photosynthetic efficiency, and seedling growth, however, K_+, Ca^{2+} and Mg^{2+} content of the small leaves did not change significantly which is beneficial to the normal physiological function of the small leaves. Further, absorption degree of harmful ions was relatively low, more harmful ions were accumulated in the stem and total petiole. Thus, this study provided theoretical basis for planting *Amorpha fruticosa* in areas with relatively low salinization degree.

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