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Research Article

The effects of salinity stress on *Amorpha fruticosa* Linn. seed germination, physiological and biochemical mechanisms

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Abstract

Salinity stress is serious threat to crop productivity and globe food security. This study investigated the impact of NaCl (neutral salt) and basic salt (basic salt) on seed germination physiological and biochemical traits of *Amorpha fruticosa*. Salt stress had no significant effect on seed germination rate, however, alkali stress significantly decreased ($p \leq 0.05$) rate of germination. Both stresses also negatively affected the growth of radicle and germination ($P < 0.05$), and the effect of alkali stress was greater than that of salt stress. The concentration of K^+, Mg^{2+} and Na^+/K^+ in radicle and germ remained relatively stable, which was conducive to adapting to salt and alkali stress, but the concentration of K^+, Mg^{2+}, NO_3^-, $H_2PO_4^-$ and SO_4^{2-} changed differently under salt and alkali stress. Tartaric acid was the main component of the 8 organic acids, and the accumulation changes of each component were different under salt stress and base stress. Tartaric acid was accumulated in large quantities under salt stress, and the accumulation of other acids (citric, malic, acetic, oxalic, formic and lactic acids) were markedly enhanced under alkali stress ($P < 0.05$). Among the 16 free amino acids, arginine, alanine and threonine are the response solutes under salt stress, and glutamic acid and threonine are the response solutes under base stress. In In conclusion, proper concentration of salts can promote seed germination and radicle growth. Therefore, plant performance can be improved by soaking seeds in appropriate concentration of salts.

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Keywords: germination; nutrient homeostasis; soluble sugars; salt stress

Introduction

Soil salinity is one of the major abiotic stresses affecting the development of agriculture, animal husbandry and environmental ecology (Mustafa *et al.*, 2019; Cui *et al.*, 2024). Globally, 950 m ha are salt affected soils which accounts for 7.6% of total cultivated area. Among them, 20% of the agricultural cultivated land area is affected by salt stress (Mehra *et al.*, 2018). In the natural environment, the salt ions of saline-alkali soil mainly include Na^+, Ca^{2+}, Mg^{2+} and CO_3^{2-}, HCO_3^-, SO_4^{2-} and Cl^-. Soils with NaCl and Na_2SO_4 are called salt affected soils while soils with Na_2CO_3 and $NaHCO_3$ are called alkaline soil (Yu *et al.*, 2023). The neutral salts (NaCl and Na_2SO_4) are the most dominant natural salts and stress dominated by alkaline salts such as $NaHCO_3$ and Na_2CO_3 is called alkali stress (Bagayoko *et al.*, 2014). *A. fruticosa* has salt resistance structure and it also has good resistance against salt-alkali stress. *A. fruticosa* has saline-alkali resistance and drought resistance, and it can grow at salt concentration of 0.3-0.5%. The continuous planting can reduce the soil salt content in the tilled layer over the years and increase the soil organic matter (SOM). This indicates that *A. fruticosa* can improve the physical and chemical properties of saline soil (Wang *et al.*, 2023). Yan *et al.* (2008) used NaCl as a stress substance and Zou *et al.* (2011) used Na_2SO_4 as a stress substance to study the effects of salt on the growth and physiological indexes of *A. fruticosa* seedlings. Under NaCl solution stress, the stress intensity of NaCl on *A. fruticosa* seedlings gradually increased with the increase of stress concentration, and it had strong salt-resistant ability when NaCl stress was lower than 0.8%. When NaCl concentration reached 1.1%, the growth and development of seedlings were significantly inhibited, and leaf loss began to occur after 6 weeks of treatment. However, the branches remained fresh until the 9th week, indicating that the amorpha has a strong ability to resist salt. In order to adapt to NaCl stress environment, *A. fruticosa* may undergo moderate osmotic regulation by increasing the concentration of osmotic regulator. As for salt-alkali tolerance of *A. fruticosa*, growth and physiological changes at seedling stage were mostly studied under the salt NaCl, while salt tolerance at germination stage of *A. fruticosa* seed was not fully studied. Seed germination is more susceptible to salt damage than plant growth (Jouyban, 2012). Seed germination is a prerequisite for seedling formation, and seed germination in saline-alkali habitats is crucial to complete the whole life history of plants. Therefore, we hypothesized that appropriate concentration of salt and alkali stress can promote the seed germination and seedling growth, however, effects of both stresses can vary. This study was conducted to investigate the impact of salt and alkali stress on germination, seedling growth, physiological and biochemical traits of *A. fruticosa*.

Materials and Methods

Plant material and stress conditions

The seeds of *A. fruticosa* were collected from natural mountains of Dongliao County, Jilin Province. Salt and alkali stress was imposed by using NaCl and Na_2CO_3. The four different concentrations (50, 100, 150 and 200 mmol/L) of NaCl were prepared likewise, four different concentrations (10, 20, 30 and 40 mmol/L) of Na_2CO_3 according to treatments (Table 1). In control treatment deionized water was used.

Table 1. Different concentrations of NaCl and Na_2CO_3

Solution concentration（mmol/L）		pH
NaCl	50	6.4
	100	6.33
	150	6.21
	200	6.15
Na_2CO_3	10	10.81
	20	10.83
	30	10.85
	40	10.86

Before the experiment, the seeds were retreated and soaked in a constant temperature bath at 60 ℃ for 20 min. Further, 0.1% $KMnO_4$ solution was used to disinfect the seeds for five minutes, then rinsed with deionized water repeatedly, and then dried back naturally for experiment.

A. fruticosa seeds with full seeds and uniform size were selected, and 50 seeds were placed in each petri dish with a diameter of 15 cm. The petri dishes were filled with 0.4-0.5 cm thick quartz sand with uniform particles (screened with 20-mesh and 50-mesh screens, respectively), and each treatment solution was added to the saturated state. The petri dish was placed in a smart light incubator with 25 ℃ temperature, 12 h/d light time and 12000 LX illumination. Moreover, weighing method was used to replenish the evaporated water, and deionized water was used once a day in the morning and evening to maintain the concentration of each treatment solution.

Determination of seed germination and growth indexes

The seeds germination on every day was counted and seed germination potential (GP) was measured on 7^{th} day, on the other rate of germination (GR) was measured on 14^{th} day. Further, germination rate was measured with following equation: GR= (Ni/N) ×100%. In this equation Ni is number of germinated seeds on day i and N is number of test seeds.

Moreover, GP was measured with following equation: GP = Number of germinated seeds in specified days (3d)/total number of tested seeds ×100%. Additionally, germination index (GI) was determined with following method: GI=∑ (Gt/Dt).

In above equation Gt is number of germinated seeds on the t day and Dt is germination days corresponding to Gt. Further, vitality index (VI) was determined with this equation: VI=S×∑Gt/Dt. In this equation S is fresh weight of seedlings. Moreover, daily germination rate (DGR) was determined with following equation: DGR= $(W_{t+1}-W_t)$ /N. In this equation W_{t+1} is seed germination number on day t+1 while Wt is seed germination number on day t.

Determination of inorganic ion concentration

The sampling was done on the 14^{th} day of germination. We took 10 plants from each treatment, washed them repeatedly with deionized water to remove surface salt, and oven dried (70 ℃) and ground and sieved through 80-mesh for further testing. Each dry sample (0.5 g) was placed in a centrifuge tube, and 10 ml of water was added and boiled for 60 minutes. The extracted solution was used to determine various physiological indexes. The inorganic ions include cations and anions (Na^+, K^+, free Ca^{2+} and Mg^{2+}, $^+$, K^+, free Ca^{2+} and Mg^{2+}) were determined by atomic absorption spectrophotometer while Cl^-, NO_3^-, SO_4^{2-} and $H_2PO_4^-$ were measured by ion chromatography system.

Determination of soluble sugar concentration

Soluble sugar was determined by sulfuric acid (H_2SO_4) anthrone colorimetric method, and glucose was used as the standard sample. OD values were obtained at 620 nm wavelength and concentration of soluble sugars were determine with following formula: The calculation formula is as follows: Soluble sugar concentration (mg/ g DL) =C*V/W/1000

Where C is the concentration of soluble sugar measured from the standard curve (ug/ml); V is the total volume of extracted liquid (ml); W is the sample amount (g).

Determination of organic acid concentration

A dry sample of boiling water extract was used as the test material, and 0.22 um membrane was used for filtration before the test. The concentration of all the acids (tartaric acid, citric acid, malic acid, formic acid, lactic acid, acetic acid, succinic acid and oxalic acid) was determined with ion chromatography system. The total concentration of organic acids was determined by the sum of each component.

Determination of free amino acid concentration

The amino acid analyzer (SykamS-433D, Germany) was used to determine the concentration of amino acids. The dry protein was precipitated with sulfonyl salicylic acid, the amino acid was released by the complexed metal element ethylenediaminetetraacetic acid (EDTA), and the amino acid was separated by the separation column for color development with ninhydrin. The concentration of glutamic acid, arginine, serine, aspartic acid, histidine, valine, tyrosine, glycine, lysine, alanine, phenylalanine, proline, threonine, methionine, isoleucine and leucine were analyzed and determined. The sum of the contents of each amino acid component was the total free amino acid concentration.

Statistical analysis of data

The experimental data were statistically analyzed with analysis of variance (ANOVA) using SPSS26.0 (SPSS Inc.Chicago. IL. USA) software. The experimental results were expressed as "mean ± standard error", and multiple comparisons were made to separate the treatment means.

Results

Seed germination index

The results indicate that NaCl stress slow down germination process and a linear decrease in germination process was observed with increasing salts concentration (Figure 1 A). Under Na_2CO_3 stress, the germination process was basically similar to that of the control treatment due to the relatively low stress concentration (Figure 1 B). However, germination was promoted when the concentration was lower than 30 mm.

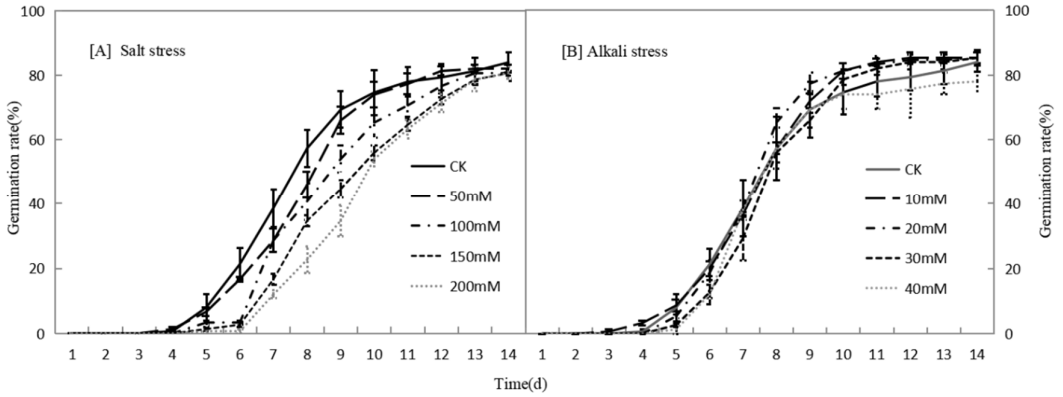

Figure 1. Germination process of *Amorpha fruticosa* seeds under salt and alkali stress

The results indicated that Na_2CO_3 stress (40 mM) significantly reduced the germination of *A. fruticosa* seeds. Under NaCl stress, GR and GR also significantly decreased with increasing salts concentration (Table 2). 100 mM salt stress decreased the germination potential by 50% as compared to control. Under Na_2CO_3 stress, germination potential and germination index had no significant changes compared with control treatment (Table 2).

Table 2. Effect of salt and alkali stress on germination indicator of *Amorpha fruticosa* seeds

Salt concentration		Germination rate	Germination energy	Germination index
Contrast	0	84.00±3.06a	38.67±8.82c	11.10±1.07c
Salt stress (NaCl)	50	82.00±1.16a	28.67±3.71bc	10.66±0.26bc
	100	80.67±2.40a	28.67±3.71bc	9.69±0.42abc
	150	80.67±0.67a	16.67±1.76ab	8.90±2.30ab
	200	80.67±1.76a	12.00±1.15a	8.43±0.43a
Alkaline stress (Na_2CO_3)	10	85.33±1.76b	36.67±0.67a	11.73±0.23a
	20	85.33±2.40b	38.67±.1.76a	11.51±0.22a
	30	85.33±2.41b	29.33±6.96a	10.76±0.48a
	40	78.00±3.06a	38.00±3.05a	10.21±0.55a

Note: The values indicating means (n=3) with ±SE and same letters shows non-significant different at P <0.05.

Radicle and germ growth

The increase in concentration of both salts, decreased the radicle (P < 0.01) (Figure 2 A, B). When NaCl concentration was 200 mM, the radicle length was 0.56 times and the germ length was 0.48 times of the control. When the concentration of Na_2CO_3 was 40 mM, the radicle length was 0.12 times and the germ length was 0.64 times of the control. Both stresses decreased the radicle and germination with increasing concentration. The biomass of radicle was significantly lower at 150 mM salt concentration under salt stress, similarly, alkaline stress also inhibited the radicle growth and reduced the radicle biomass.

The biomass ratio of radicle to germ gradually decreased with the increase of stress intensity. However, biomass ratio of radicle was significantly higher than that of the control when the concentration was less than 150 mM, indicating that NaCl can stimulate the growth of radicle (Figure 2 E). The length of radicle was significant decreased with increasing concentration of alkali stress and biomass ratio of radicle to germ was also considerably lower at 20 mM as compared to control (P< 0.01) (Figure 2 F).

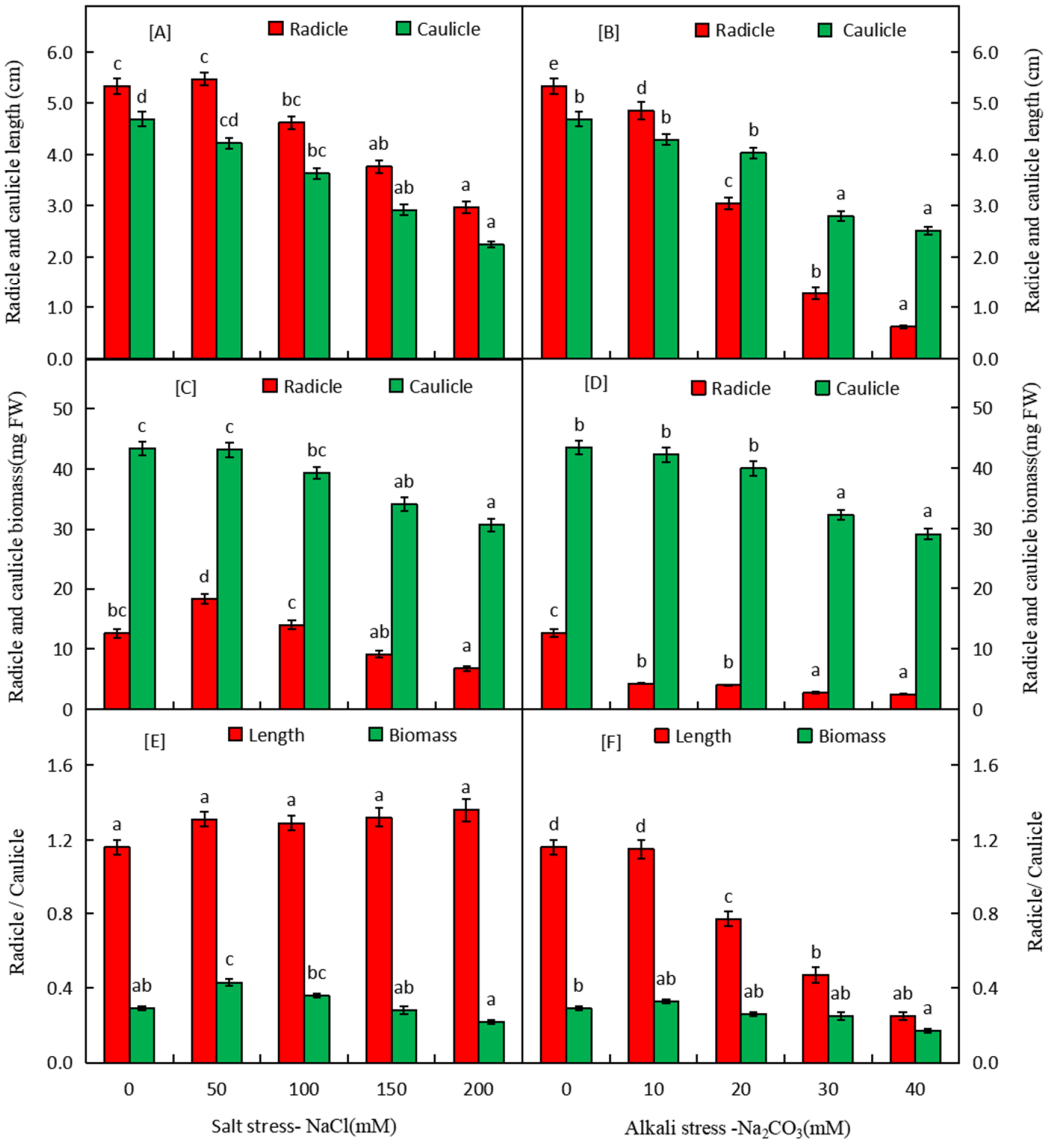

Figure 2. The impacts of alkali and salt stress on growth traits of *Amorpha fruticosa*
The values indicating means (n=3) with ±SE and same letters show non-significant difference at P <0.05.

Concentration of inorganic ions in radicle and germ

The concentration Na^+ was significantly increased under both salts as compared to control (Figure 3 A, B).

NaCl stress had no effect on K^+ concentration, which was not different from the control (Figure 3 C). There was no difference in K^+ concentration under Na_2CO_3 stress, but it was significantly decreased compared with the control, and the K^+ concentration was about 0.75 times of the control (P<0.05, Figure 3 D).

The concentration Mg^{2+} was increased under NaCl stress as compared to control, while there was no change in Mg^{2+} concentration under Na_2CO_3 (P< 0.05). The ratio of Na^+/K^+ also showed an increasing trend with increasing concentration of both salts (Figure 3 G, H). The maximum concentration of Na^+/K^+ ratio was recorded at 100 mM NaCl while in case of alkaline stress maximum Na^+/K^+ ratio was reported at 20 mM Na_2CO

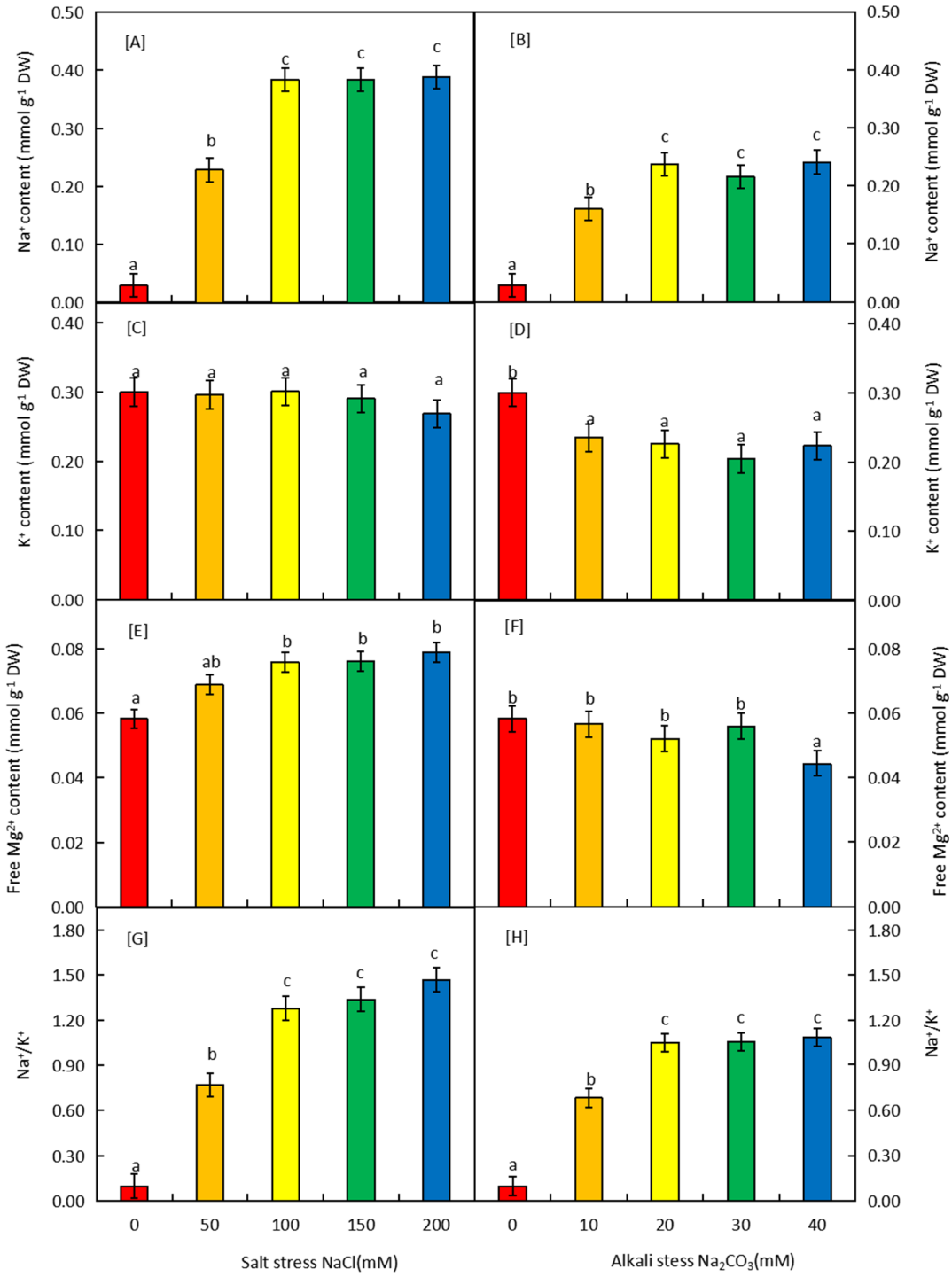

Figure 3. Effect of salt and alkali stress ionic concentration of *Amorpha fruticosa*
The values indicating means (n=3) with ±SE and same letters show non-significant different at P <0.05.

The concentration of Cl^- under NaCl and Na_2CO_3 stress was significantly higher than that of control (Figure 4). (P <0.05). Under NaCl stress, due to the high concentration of Cl^- in the stress environment, the concentration of Cl^- reached 7.16 $mmolg^{-1}$(DW) at 200 mM. Under Na_2CO_3 stress, Cl^- concentration reached the highest value of 0.52 $mmolg^{-1}$(DW) at 20 mM. The increasing concentration of NaCl firstly increased the NO_3^- concentration and then decreased, and reached the highest value of 0.26 mmol g^{-1}(DW) when NaCl concentration was 100 and 150 mM (Figure 4 C). NO_3^- concentration gradually decreased with the increase of Na_2CO_3 stress intensity, and was significantly lower than that of control when the concentration was higher than 30 mM (P <0.05) (Figure 4 D). The concentration of $H_2PO_4^-$ also decreased with an increase in concentration of NaCl salts. Conversely, concentration of $H_2PO_4^-$ was markedly increased content under Na_2CO_3 stress as compared to control (Figure 4 F).

The SO_4^{2-} concentration gradually decreased with the increase of NaCl stress intensity, and was significantly lower than that of the control (Figure 4 G). $H_2PO_4^-$ concentration first increased and then decreased under Na_2CO_3 stress, but it was significantly lower than that of control only at 40 mM (P <0.05) (Figure 4 H).

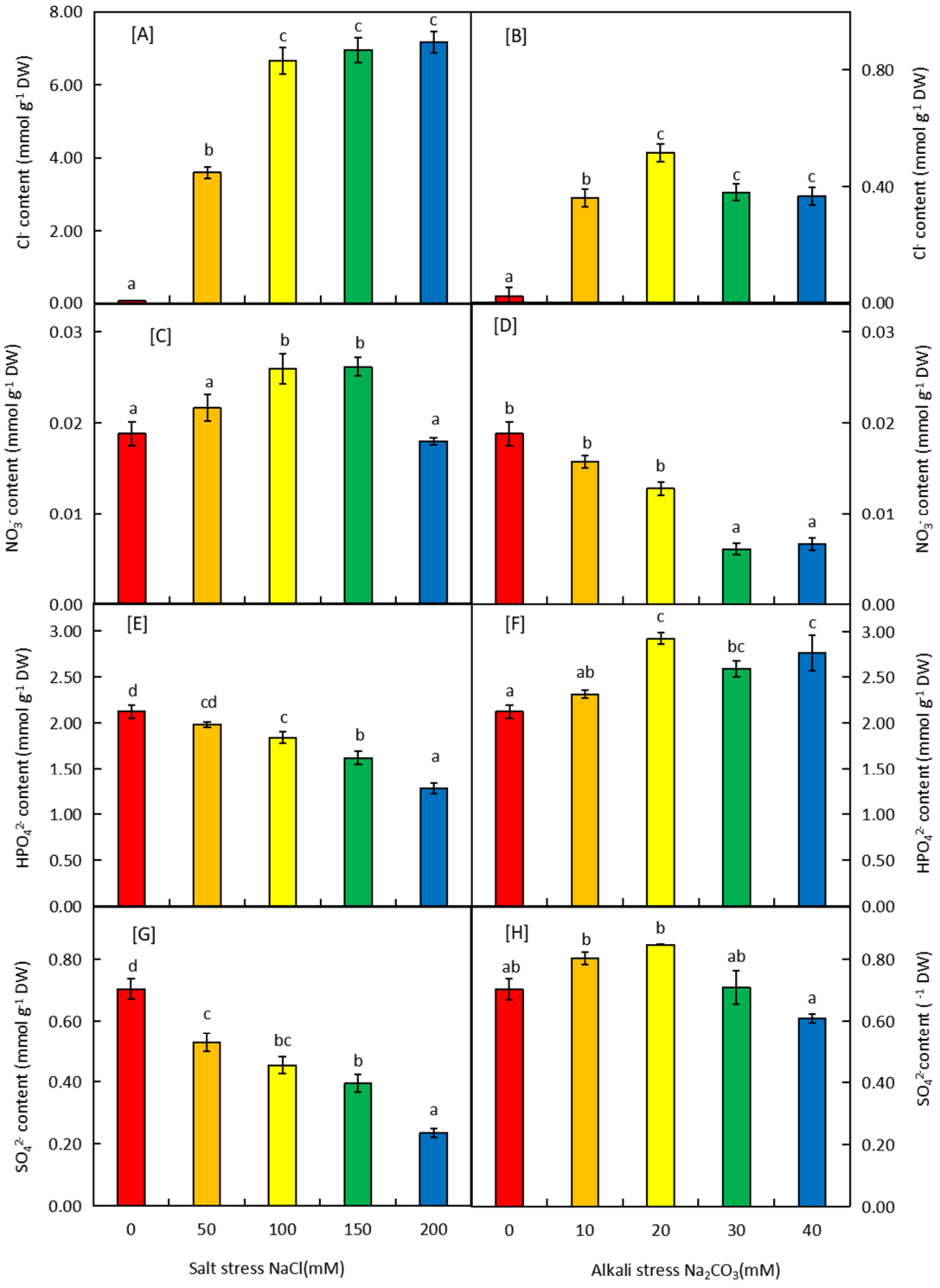

Figure 4. Effect of salt and alkali stress on ionic concentration of *Amorpha fruticosa*
The values indicating means (n=3) with ±SE and same letters show non-significant difference at P <0.05.

Soluble sugar concentration *of radicle and germ*

The concentration of soluble sugars increased with increase in concentration NaCl salts (P <0.05) (Figure 5A). In case of alkaline salts, the concentration of soluble sugars firstly increased then it showed a decline trend and higher concentration of soluble sugars (Figure 5B).

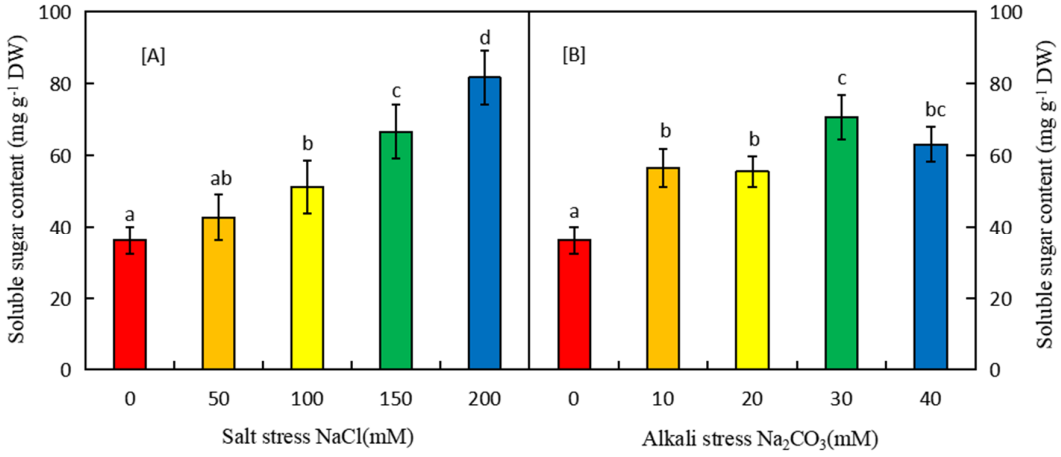

Figure 5. Effect of salt and alkalic stress on soluble sugar concentration of radicle and caulicle of *Amorpha fruticosa*

The values indicating means= (n=3) with ±SE and same letters show non-significant difference at P <0.05.

Radicle germ organic acid concentration

The organic acids firstly showed an increasing trend with increase in salts concentration (NaCl and Na_2CO_3) and then showed a decreasing trend. Under 100 mM of NaCl and 20 mM of Na_2CO_3, the total organic acid concentration reached to the highest value (Figure 6).

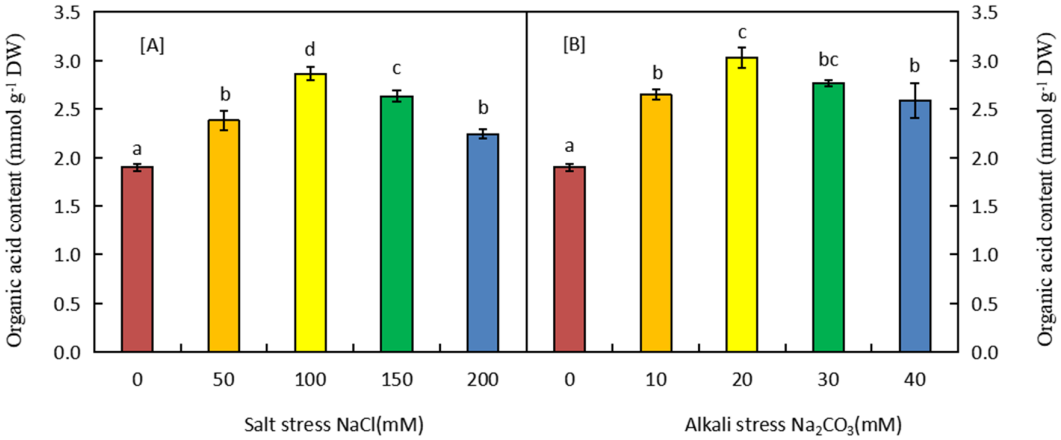

Figure 6. Effect of salt and alkalic stress on total organic acid of *Amorpha fruticosa*

The values indicating means (n=3) with ±SE and same letters show non-significant difference at P <0.05

The concentration of each organic acid under NaCl and Na_2CO_3 stress is shown in Figure 7. In the control treatment, the concentration of tartaric acid, citric acid, malic acid and acetic acid above 0.2 mmol g^{-1} (DW). The concentration of tartaric acid was the highest, which was 0.69 $mmolg^{-1}$(DW) in the control treatment, accounting for 36.34% of the total concentration of organic acids (Figure 7).

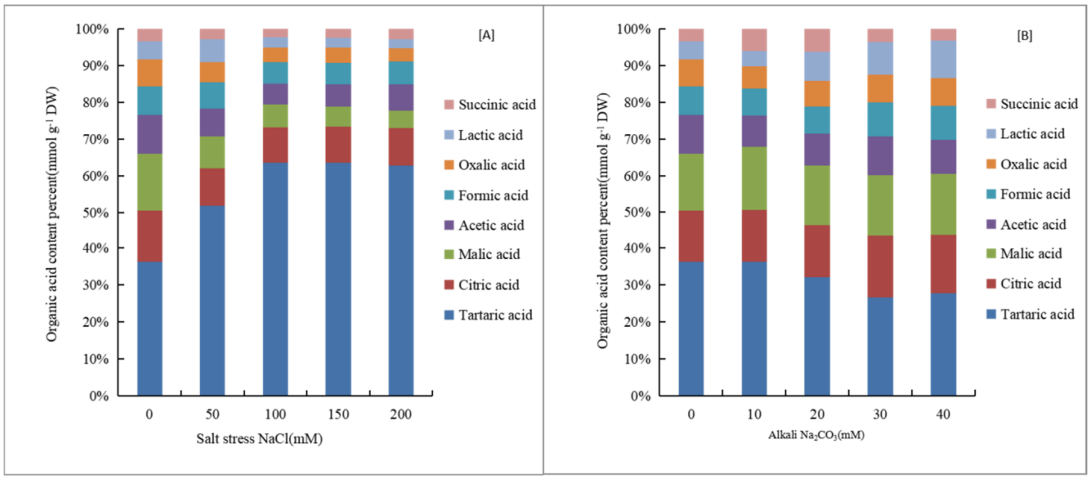

Figure 7. Effect of salt and alkali stresses on percentage of organic acids

The concentration of tartaric acid was firstly increased with increasing NaCl and Na_2CO_3 stress intensity, and then it showed the decreasing trend. The maximum concentration of tartaric acid was reached maximum value at 100 mM NaCl salt while under Na_2CO_3 stress, the maximum concentration of tartaric acid was recorded at 20 mM as compared to control (Figure 8 B). NaCl salt had a non-significant impact on concentration of citric acid, while Na_2CO_3 stress showed a positive effect of citric acid concentration. The concentration of malic acid was decreased with increasing NaCl concentration as compared to control (Figure 8 E, G). Under Na_2CO_3 stress, the concentration of malic acid and acetic acid first increased and then decreased with the increase of stress intensity.

Oxalic acid concentration gradually decreased with the increase of NaCl stress intensity, and was significantly lower than that of the control treatment (P <0.05). The concentration of oxalic acid at 200 mM was 0.08 mmol g^{-1}(DW), 0.59 times that of the control treatment (Figure 9 A). The oxalic acid concentration under Na_2CO_3 stress was significantly higher than that in the control treatment (P <0.05), and the highest concentration was 0.21 mmol g^{-1}(DW) at 20 mM, (Figure 9 B). Formic acids and lactic also showed a decreasing with increasing NaCl concentration and it reached to minimum values at 200 mM (P <0.05) (Figure 9 C). Conversely, concentration of formic acid was increased with increasing under Na_2CO_3 stress, and reached to maximum value at 20 mM Na_2CO_3 stress (Figure 9 D). Salt stress showed no effect on succinic acid concentration, however, under Na_2CO_3 stress succinic acid concentration firstly increased with increasing salt concentration and then decreased. The maximum succinic acid concentration was recorded at 20 mM, it was 3.04 times as compared to control.

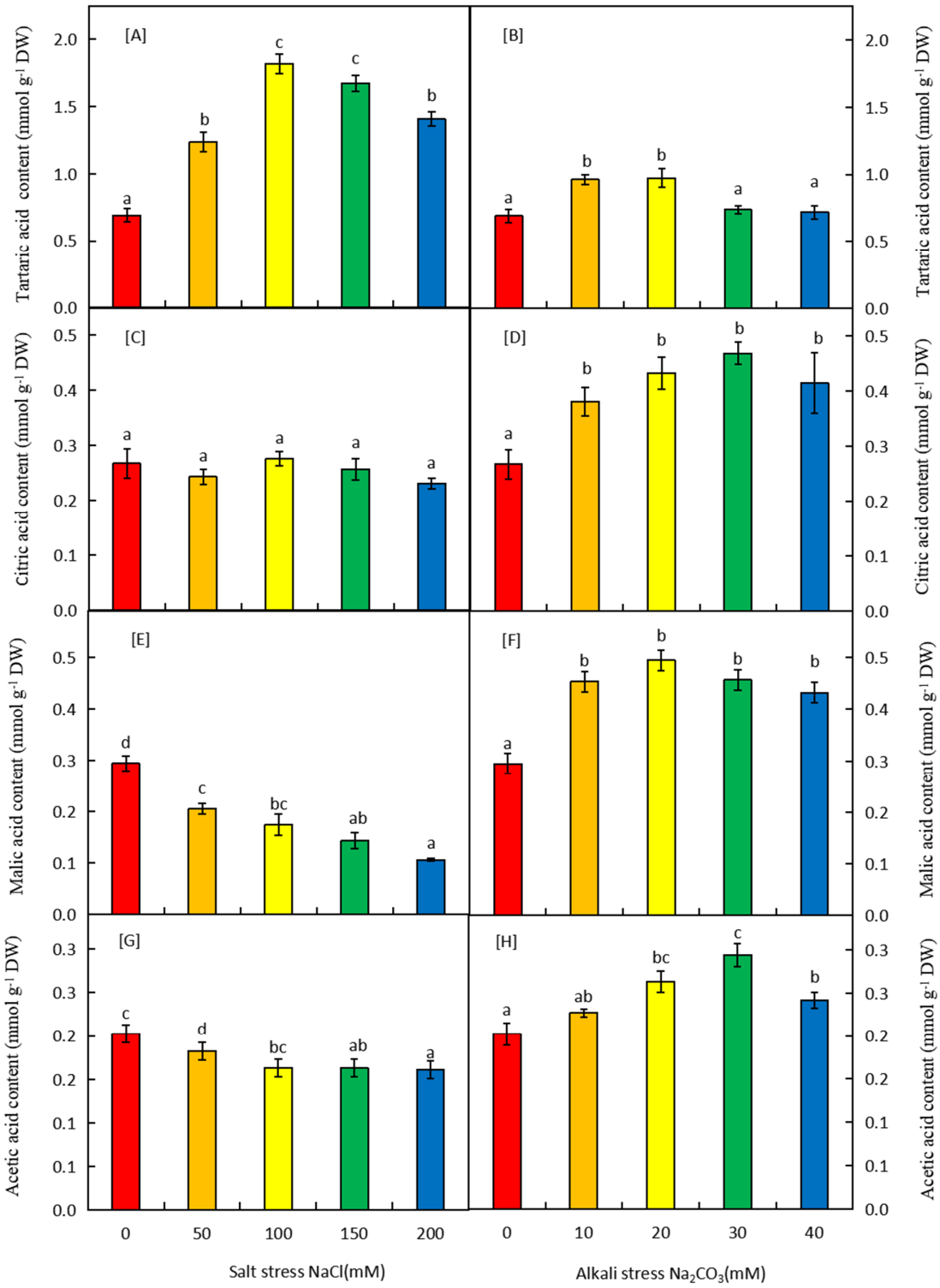

Figure 8. Effect of salt and alkali stress on each organic acids' concentration of radicle and caulicle of *Amorpha fruticosa*

The values indicating means (n=3) with ±SE and same letters show non-significant difference at P <0.05.

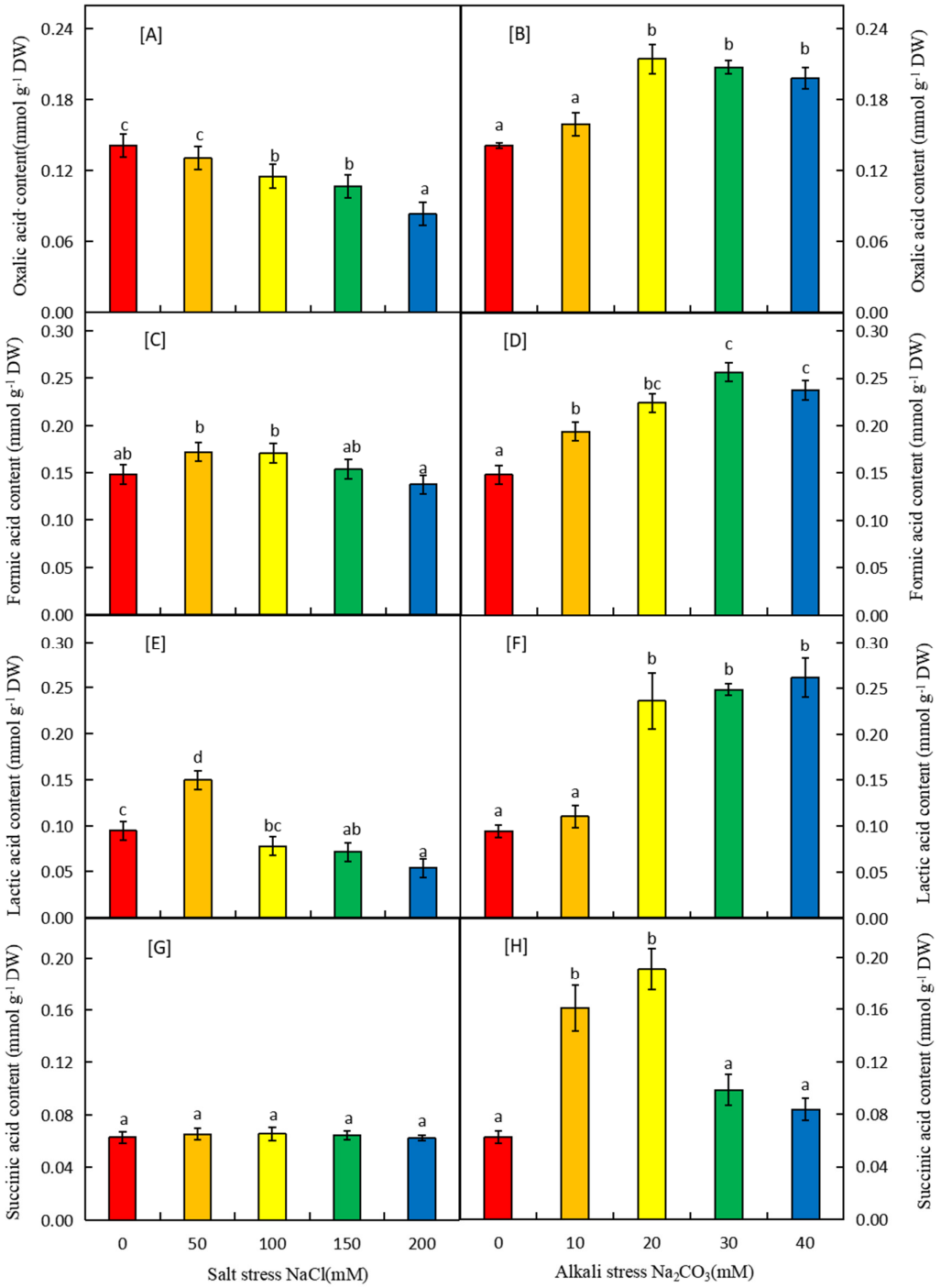

Figure 9. Effect of salt and alkali stress on each organic acids' concentration of radicle and caulicle of *Amorpha fruticosa*

The values indicating means (n=3) with ±SE and same letters show non-significant difference at P <0.05.

Concentration of free amino acids in radicle and germ

The changes of free amino acid concentration under salt and alkali stress are shown in Table 3. A total of 16 free amino acids were detected, which were glutamic acid, arginine, serine, aspartic acid, histidine, valine,

tyrosine, glycine, lysine, alanine, phenylalanine, proline, threonine, methionine, isoleucine and leucine. The concentration of free glutamic acid, arginine and serine was the highest, accounting for 46.98% of the total concentration of free amino acids in the control treatment (Table 3)

Both stresses reduced the concentration of free amino acids (FAA). The concentration of arginine, alanine and threonine was higher control while concentration of glutamic acid and glycine was higher at 50 mM. Under Na_2CO_3 stress, the concentration of glutamic acid and threonine was higher as compared to control and arginine concentration was higher at 10 mM while proline concentration was higher at 20 mM as compared to control.

Table 3. Effect of NaCl and Na_2CO_3 stress on free amino acid of *Amorpha fruticosa* seedling (μmol g^{-1} DW)

Stress content (mmol L^{-1})		0	50	100	150	200
NaCl	Total	102.48±7.09b	101.50±8.27b	95.46±6.41a	95.24±8.50a	94.52±10.06a
	Glutamic acid	18.61±2.11	19.91±3.02	18.40±2.36	17.81±1.15	16.97±1.32
	Arginine	16.41±1.78	24.80±3.02	22.38±3.21	22.29±2.38	25.11±2.81
	Serine	13.12±0.97	12.15±1.43	12.86±1.07	13.05±1.46	13.06±0.89
	Aspartic acid	8.63±0.72	6.68±0.69	6.47±0.76	6.09±0.48	5.81±0.66
	Histidine	8.17±1.01	5.86±0.52	5.75±0.52	5.95±0.63	5.27±0.44
	Valine	5.59±0.61	4.10±0.11	3.50±0.27	3.79±0.24	3.60±0.55
	Tyrosine	3.95±0.19	2.72±0.09	2.69±0.08	2.76±0.16	2.75±0.22
	Glycine	3.88±0.48	4.10±0.24	3.90±0.33	3.28±0.40	3.29±0.26
	Lysine	3.84±0.41	3.34±0.24	2.74±0.27	2.69±0.19	2.87±.23
	Alanine	3.84±0.33	4.35±0.39	4.49±0.47	4.15±0.51	4.12±.34
	Phenylalanine	3.30±0.31	2.36±0.25	2.23±0.21	2.58±0.25	2.44±0.27
	Proline	3.09±0.34	2.85±0.19	2.61±0.28	2.59±0.24	2.54±0.20
	Threonine	2.77±0.26	3.52±0.17	3.32±0.24	3.28±0.35	3.04±0.31
	Methionine	2.48±0.23	1.51±0.11	1.18±0.16	1.15±0.11	0.92±0.08
	Isoleucine	2.72±0.24	1.72±0.19	1.27±0.09	1.96±0.15	1.33±0.11
	Leucine	2.08±0.21	1.53±0.17	1.67±0.12	1.82±0.16	1.40±0.13
Na_2CO_3	Total	102.48±7.09c	91.88±6.37b	90.13±9.93b	90.38±6.26b	86.01±8.02a
	Glutamic acid	18.61±2.11	18.82±1.27	19.43±1.83	22.04±2.91	21.38±1.34
	Arginine	16.41±1.78	21.27±2.46	16.22±1.51	15.67±2.41	13.62±1.76
	Serine	13.12±0.97	11.62±0.85	11.74±1.34	11.68±0.75	11.76±1.62
	Aspartic acid	8.63±0.72	5.67±0.37	6.29±0.58	5.72±0.57	6.47±0.43
	Histidine	8.17±1.01	4.79±0.22	4.80±0.35	5.09±0.61	4.27±0.26
	Valine	5.59±0.61	3.82±0.41	3.76±0.27	3.60±0.40	3.63±033
	Tyrosine	3.95±0.19	2.47±0.12	3.16±0.16	2.99±0.08	4.05±0.30
	Glycine	3.88±0.48	2.81±0.34	3.93±0.37	3.89±0.19	3.13±0.21
	Lysine	3.84±0.41	2.76±0.30	2.63±0.27	2.57±0.35	1.81±0.17
	Alanine	3.84±0.33	4.38±0.48	3.71±0.40	4.06±0.38	3.05±0.31
	Phenylalanine	3.30±0.31	2.87±0.29	2.75±0.30	2.05±0.19	2.68±0.29
	Proline	3.09±0.34	3.76±0.34	3.73±0.32	2.96±0.28	2.65±0.27
	Threonine	2.77±0.26	2.94±0.28	3.00±0.25	3.05±0.31	2.82±0.25
	Methionine	2.48±0.23	1.86±0.20	1.62±0.15	1.73±0.18	1.52±0.18
	Isoleucine	2.72±0.24	0.92±0.10	1.41±0.11	1.73±0.16	1.69±0.19
	Leucine	2.08±0.21	1.12±0.11	1.95±0.22	1.55±.016	1.48±0.14

Discussion

Seed germination is the most sensitive stage to salts. In the saline environment cations and anions are dissolved which increase their absorption by seed. This allows the entry of toxic ions to seed embryo which in turn reduce the water absorption and resulting in reduction in seed germination. The salt concentrations within 200 mM had no significant impact on germination rate of the seeds of smut. Likewise, germination rate of the seeds decreased significantly when the Na_2CO_3 stress concentration was reached 40 mM (P <0.05). The results indicated that the seed had strong germination ability in saline-alkali habitat, indicating that the seed had strong saline-alkali tolerance.

Both stresses significantly reduced the radicle growth. Though lower salts concentration stimulated the radicle growth and germination. The growth status of radicle and germ will directly affect the growth momentum and production of plants in the long term after seedling. For some salt-tolerant plants (such as *Chinensis sinensis*), salt can stimulate the growth of their radicle and germ, which is also an important reason for the survival of salt-tolerant plants in a suitable salt-alkali environment (Zhou *et al.*, 2023). Under NaCl stress, the length ratio of radicle to germ had no significant change and was slightly higher than that of the control. Therefore, in the actual production process, soaking the seeds with appropriate concentration of salt solution can promote the growth of radicle and germ and improve the planting effect.

Saline-alkali stress induced ionic and osmotic stress suppress the plant growth and development (Guo *et al.*, 2022). The essential problem of plant salt-resistant mechanism is Na^+ and another ion metabolism. In environments with high Na^+ concentration, plants adopt salt-repellent, salt-excretion, and compartments to avoid large amounts of Na^+ entering the metabolically active parts of the body. Controlling the uptake of Na^+ by roots and the transfer of Na^+ to leaves is one of the mechanisms of plant salt tolerance (Wu, 2018). In the experiment, Na^+ concentration was very high in the stress environment, and a large amount of Na^+ was absorbed by the seeds during germination. However, the concentration of Na^+ in the radicle germ did not change with the increase of stress intensity. The concentration of Na^+ in the radicle germ remained at a relatively low stress level (100 mM NaCl, 20 mM Na_2CO_3) (Figure 3 A, B), indicating that the radicle germ could still maintain a suitable concentration of Na^+ under high stress. The results indicated that the absorption degree of Na^+ in the radicle of *A. fruticosa* was low.

The concentration of K^+ in the radicle germ remained unchanged under salt stress, although the concentration of K^+ decreased under alkali stress. Potassium plays an important role in regulating osmotic potential, maintaining normal physiological activities and maintaining Na^+/K^+ balance and to improve salt-alkali tolerance (Sardans and Peñuelas, 2021). Na^+/K^+ is one of the important indicators to measure the salt tolerance of plants (Wu, 2018). In this study, since both Na^+ and K^+ maintained relatively stable concentration. Na^+/K^+ ratio was also maintained at relatively low stress levels (100 mM NaCl, 20 mM Na_2CO_3). Some studies have pointed out that the damage degree of saline-alkali stress on seed vitality is closely related to the Na^+/K^+ ratio, which is consistent with the change of the activity of (Na^+-K^+) –ATPase. Therefore, the Na^+/K^+ ratio can reflect the true level of seed vitality (Geng *et al.*, 2021).

Chlorophyll is a magnesium porphyrin compound, and metal Mg is a component element of chlorophyll, which plays an important role in the absorption, transfer and conversion of light energy in photosynthesis. Mg^{2+} and K^+ together act as the corresponding ions of H^+ to promote photophosphorylation (Sardans and Peñuelas, 2021). The concentration of free Mg^{2+} under salt stress was higher than that under control, and the concentration of free Mg^{2+} under alkali stress was also maintained at the level of control. The suitable concentration of free Mg^{2+} in the radicle and germ under salt-alkali stress is beneficial to the regulation of osmotic potential and chlorophyll biosynthesis.

Under salt and alkali stress, the concentration of Cl- in the radicle and germ of *S. fruticosa* increased significantly. Salinity and alkalinity induced osmotic and ionic stresses inhibit the germination (Panuccio *et al.*, 2014) and induce ion toxicity (Ludwiczak *et al.*, 2021). The results of this experiment are consistent with this

conclusion. It can be seen from the experimental data that when saline-alkali stress reaches a certain level (100 mM NaCl, 20 mM Na_2CO_3), Na^+ concentration and Na^+/K^+ ratio remain relatively stable. This indicates that the effect of ion stress is maintained within a certain limit (Figure 3 A-B), while the growth of radicle and germ is positively correlated with stress intensity. In other words, with the increase of stress intensity, the influence on growth is greater which indicates that the influence of osmotic stress caused by salt is much greater than that of ion toxicity under high-intensity saline-alkali stress. The concentration Na^+ was significantly lower under Na_2CO_3 stress was compared to NaCl stress. Because Na_2CO_3 stress not only absorbed cation and anion during seed germination, but also was affected by high pH value. It can be concluded that the effect of alkali stress on high pH value is stronger than osmotic stress and Na^+ toxicity.

Plants accumulate different osmolyte in response to stress conditions to tolerate the toxic effects of salinity and alkaline stresses (Ozturk *et al.*, 2021). Soluble sugar is not only an effective osmoregulator in plants, but also a carbon frame and energy source for the synthesis of other organic solutes in plants (Keiluweit *et al.*, 2015). In this experiment, soluble sugar concentration of radicle germ was significantly increased under salt stress and alkali stress (P <0.05), indicating that soluble sugar is the osmotic regulator of radicle germ in response to salt and alkali stress.

Organic acids are another organic solute regulated by osmosis in plants. They are not only intermediate products of carbon metabolism, but also major compounds in plant response to heavy metal stress, and (Barra Caracciolo and Terenzi, 2021; Liu *et al.*, 2022). When the concentration of inorganic cations in plants is too high, a large amount of organic acids will accumulate, which has a buffer effect on cations (Hachiya *et al.*, 2012). The results showed that organic acid concentration increased first and then decreased with the enhancement of stress, but was significantly higher than that of the control (P <0.05). This indicates that accumulation of organic acid is also one of the response strategies to salt and alkali stress, and the response degree of alkali stress is greater than that of salt stress, which is conducive to alleviating the effect of high pH under alkali stress. Previous studies have shown that the concentration of organic acid components accumulated by different plants is different. The oxalic acid accumulation in the stems, leaves and skill of *Radix alkalium* (Wang *et al.*, 2023)and the citric acid accumulation in the grass of *Radix acanthica* all exceed 90% of the total organic acid components (Wijeyaratne and Bellanthudawa, 2017; Zhen, 2022). Malic acid and citric acid accumulate most in wheat (Xu *et al.*, 2013), and malic acid accumulate most in *Leymus chinensis* (Lin and Muja, 2016).

The response of organic acid components to salt stress and alkali stress was different. Under salt stress, tartaric acid was the main organic acid accumulated, which was positively correlated with stress degree. The accumulation of formic acid and lactic acid under low stress (<100 mM) was significantly higher than that under control (P <0.05), but significantly decreased under high stress. The increase of proline concentration is generally regarded as a sensitive stress index of plant response to salt stress (Javeed *et al.*, 2021). Other free amino acids accumulate in different plants to varying degrees (Wu *et al.*, 2020) and a total of 16 free amino acids were detected in this experiment (Zhang *et al.*, 2023).

Conclusions

The seed germination of *Amorpha fruticosa* has strong salt-alkali tolerance. Further, proper concentration of salt can promote seed germination and radicle growth, and the planting effect can be improved after soaking the seeds in proper concentration of salt solution.

Authors' Contributions

ZWG, YGM prepared the manuscript. JJD, KJD, JTL, XNL, LJH, ZJW, CSM participated in data collection. AR reviewed the manuscript. SAA and MJA reviewed the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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