Effects of salt stress on seed germination and respiratory metabolism in different *Flueggea suffruticosa* genotypes

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ABSTRACT

The selection and utilization of ornamental plants that are highly tolerant to salt are helpful for landscape construction and the ecological protection of coastal and arid areas. To evaluate salt tolerance, one of the most used methods is the observation of seed germination under salt stress. Therefore, this work aimed to evaluate the influence of different concentrations of NaCl in water absorption, germination, and respiratory metabolism in seeds of different *Flueggea suffruticosa* genotypes. P2 and P27, salt-sensitive and salt-tolerant line s of *F. suffruticosa*, were chosen for treatment with 0, 40, 80, 120, 160, 200, and 240 mM NaCl. *F. suffruticosa* under salt stress exhibited inhibition of seed germination. The seeds of *F. suffruticosa* have different times for the physiological phases of water absorption with different NaCl concentrations. Salt stress retarded the seed water absorption process, and it depended on seed genotypes for *F. suffruticosa*. Soluble sugars accumulated in both P2 and P27 under salt stress. Meanwhile, the activities of hexokinase, 6-phosphofructokinase, pyruvate kinase, pyruvate dehydrogenase, citrate synthase, and glucose-6-phosphate dehydrogenase were overall increased in P27 after salt treatment, which caused increases in pyruvic acid and citric acid. The citrate synthase and glucose-6-phosphate dehydrogenase activities decreased in P2. These results suggest that the respiratory metabolism of salt-tolerant *F. suffruticosa* was enhanced, compared with the salt-sensitive line, to ameliorate the repression of seed germination under salt stress. The different changes in respiratory metabolism could influence the degree of salt tolerance.

Subjects Biochemistry, Plant Science

Keywords *Flueggea suffruticosa*, Salt stress, Germination, Water absorption, Respiratory metabolism

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INTRODUCTION

Salt stress is a primary and complex abiotic stress that has become a critical obstacle to the growth, development, and landscaping use of ornamental plants (*Liang et al., 2017*). Climate change, increased CO_2 emissions, and alterations in precipitation regimes have led to the widespread degradation of ecosystems (*Pelage et al., 2019*). More than 950 million

hectares of land worldwide are affected by salinity, which accounts for more than 6% of the world's land (*Bui et al., 1994*; *Chen et al., 2020*; *Jiang et al., 2021*). Approximately 90 million hectares of land in China are affected by salt, and the trend could become even worse with changes in the ecological environment (*Bui et al., 1994*; *Munns & Tester, 2008*). Salt stress significantly affects the morphology and physiological and biochemical activity of ornamental and crop plants (*Liang et al., 2014*; *Muchate et al., 2016*; *Wu et al., 2019*). To manage a saline environment, plants have evolved respiratory metabolism to respond at the biochemical level, including glycolysis, the tricarboxylic acid (TCA) cycle and pentose phosphate pathway (PPP) (*Jacoby, Taylor & Millar, 2011*; *Yang & Guo, 2018*; *Jiang et al., 2020*).

Glycolysis is one of the major provide carbon sources for energy metabolism and is common in virtually all tissues of plants (*Bhagavan, 2001*). In the glycolytic pathway, hexokinase (HK) converts glucose into glucose-6-phosphate. Phosphofructokinase (PFK) catalyzes the conversion of fructose 6-phosphate (F6P) to fructose-1, 6-bisphosphate (F1-6BP), or the transfer of phosphate to F6P. Pyruvate kinase (PK) catalyzes the conversion of phosphoenolpyruvate and ADP into pyruvic acid (PA) and ATP (*Plaxton & Podestá, 2006*). They are associated not only with regulatory points but are also critical for signaling under environmental stress. The TCA cycle and PPP are also important for protection under abiotic stress conditions (*Fernie, Carrari & Sweetlove, 2004*). Pyruvate dehydrogenase (PDH), citrate synthase (CS), and glucose-6-phosphate dehydrogenase (G6PDH) are thought to be the key rate-limiting enzymes in the TCA cycle and PPP, respectively (*Tovar-Méndez, Miernyk & Randall, 2003*). These respiratory metabolism pathways are ubiquitous in plants. The intermediate products of these pathways can be used to synthesize other substances. *Zhang et al. (2011)* found that glycolysis and TCA cycle were altered in widespread metabolic networks, which were the responses of tobacco (*Nicotiana tabacum*) to salinity. *Huang et al. (2003)* showed that salt stress could lead to an up-regulation of *Os6PGDH* in rice shoots (*Oryza sativa*), indicating that 6PGDH in plants may play an important role in cell division and salt response.

Flueggea suffruticosa (Pall.) Baill. is a deciduous shrub in the Euphorbiaceae family that is widely distributed in northeast China, Mongolia, Russia, Japan, and North Korea (*Park et al., 2019*). *F. suffruticosa* can grow 1–2 m in height and is highly adaptable to cold, drought and barren conditions (*Du et al., 2020*). *F. suffruticosa* is recognized as a potential landscaping plant and is highly valued for ornamental, ecological and medicinal purposes (*Raj & Luczkiewicz, 2008*; *Qin et al., 2008*). Research on *F. suffruticosa* has primarily focused on the chemical and medicinal components because it is rich in securinega-type alkaloids (*Ohsaki et al., 2003*; *Yuan et al., 2005*; *Qin, Liang & Guo, 2009*; *Raj, Kokotkiewicz & Luczkiewicz, 2015*). However, to our knowledge, there have been no reports on salt tolerance in these species. Seed germination is the first and critical stage that can be very sensitive to salt stress depending on the plant's tolerance mechanisms (*Hajihashemi et al., 2020*). It is highly significant for landscaping to select salt-tolerant genotypes of *F. suffruticosa* during seed germination under controlled conditions (*Rasel et al., 2020*).

Therefore, in this study, salt tolerance of 30 *F. suffruticosa* germplasm resources collected from the northeastern Hebei Province of China was comprehensively evaluated at the germination stage. The seeds of two *F. suffruticosa* genotypes (highly salt-sensitive seeds in Jiaoshan, Shanhaiguan and highly salt-tolerant seeds in Fengshan, Fengning) were used (1) to examine the effects of salinity on respiratory metabolism, (2) to explore the salt tolerance mechanism, and (3) to select salt-tolerant ecotypes of *F. suffruticosa* which can be used for preventing water loss and soil erosion on saline land. These findings may facilitate the selection and utilization of *F. suffruticosa* in coastal and arid areas for landscape construction and ecological protection.

MATERIALS AND METHODS

Plant materials

The mature fruits of *F. suffruticosa* from diverse genotypes were harvested from 30 provenances in the northeastern Hebei Province of China (Fig. 1). Detailed information on the provenance data from the original seed collection sites is shown in Table 1. Within several days of harvest, the seeds were abscised from the naturally dried fruit and collected in order of the location data for the salt tolerance screening test. The seeds of two *F. suffruticosa* genotypes (highly salt-sensitive in Jiaoshan and Shanhaiguan (P2) and highly salt-tolerant in Fengshan and Fengning (P27)) were chosen for further tests according to the results of salt tolerance of *F. suffruticosa*. All the chemicals used in this study were of analytical grade.

Experimental design

A salt tolerance screening test was established to ascertain the salt-tolerant ability of *F. suffruticosa* seeds according to the method described by *Wu et al. (2019)* and *Fu et al. (2020)*, with some modifications. Seeds of different *F. suffruticosa* genotypes were sterilized with 1% sodium hypochlorite for 15 min, rinsed in distilled water five times, and placed in a Petri dish (nine cm diameter, two layers of filter paper on the bottom) containing 12 mL of NaCl solution with 0 (CK) and 200 mM, and germinated under dark conditions in a constant temperature incubator (Dongnan, Ningbo, China) at 25 ± 0.5 °C for 12 days. Each treatment contained three independent biological replicates. Seeds were considered to germinate when the seed coat was broken and the radicle was visible (*Chen et al., 2020*).

To evaluate the salt tolerance of *F. suffruticosa* at the germination stage, the germination rate, relative germination rate, germination potential, relative germination potential, germination index, relative germination index, relative salt harm rate, fresh weight, relative fresh weight, root length and relative root length were determined at 12 days after salt stress treatment (DAT). There were calculated using the formulae below (*Deng et al., 2017*; *Sikder et al., 2020*; *Xu et al., 2021*).

Germination rate = total germinated seeds/total seeds × 100%.

Relative germination rate = germination rate of salt treatment/germination rate of control × 100%.

Figure 1 **Sample plots of *Flueggea suffruticosa* in the northeastern Hebei Province of China.**
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Germination potential = number of germinated seeds in specified dates/ the tested seed number × 100%, germination potential was confirmed at the peak of sprouting.

Relative germination potential = germination potential of salt treatment/ germination potential of control × 100%.

Germination index = ΣGt/Dt, where Gt is expressed as the germination seed number in t days, and Dt refers to the day number of germination.

Relative germination index = germination index of salt treatment/germination index of control × 100%

Relative salt harm rate = (germination rate of control − germination rate of salt treatment)/ germination rate of control × 100%

Relative fresh weight = Fresh weight of salt treatment/Fresh weight of control × 100%

Relative root length = Root length of salt treatment/Root length of control × 100%

Hierarchical cluster analysis was also used to evaluate salt tolerance based on the relative germination rate, relative germination potential, relative germination index, relative salt

Table 1 Information on the provenance data from the original seed collection sites of *Flueggea suffruticosa*.

Provenance code	Site of seed collection	Latitude (N)	Longitude (E)	Altitude (m)	Orientation	Slope (°)	Agrotype
P1	Geziwo, Beidaihe	39°50′29″	119°32′06″	10	Northeast	5	Gravel
P2	Jiaoshan, Shanhaiguan	40°02′28″	119°05′11″	50	South	15	Loam
P3	Tianmashan, Funing	39°58′33″	119°14′59″	133	East	20	Loam
P4	Jieshishan, Changli	39°43′57″	119°09′52″	150	South	12	Gravel
P5	Fengjiashan, Lulong	39°47′56″	119°05′56″	25	West	10	Loam
P6	Xiaxinzhuang, Lulong	40°03′37″	118°58′55″	127	North	15	Loam
P7	Dushan, Qinglong	40°27′55″	118°58′12″	550	Southeast	7	Loam
P8	Huaguoshan, Qinglong	40°08′04″	119°23′04″	350	Southeast	5	Gravel
P9	Gangou, Qinglong	40°29′41″	119°12′45″	422	North	18	Gravel
P10	Baiyangyu, Qian'an	40°11′26″	118°43′20″	156	North	40	Gravel
P11	Hetaoyuan, Qianxi	40°11′37″	118°33′04″	120	West	35	Loam
P12	Tiemenguan, Qianxi	40°24′07″	118°24′32″	146	North	45	Loam
P13	Huashanfeng, Luanzhou	39°57′14″	118°21′27″	120	North	18	Loam
P14	Panjiayu, Fengrun	39°58′48″	118°18′47″	175	North	15	Gravel
P15	Shangdiancun, Kuancheng	40°40′16″	118°34′08″	379	North	17	Gravel
P16	Erquandi, Pingquan	40°47′08″	118°37′38″	413	Northeast	20	Gravel
P17	Shihucun, Pingquan	41°16′07″	118°35′14″	796	Northeast	20	Gravel
P18	Shuangluanqu, Chengde	40°57′34″	117°52′53″	456	Northeast	15	Loam
P19	Liuzhangzi, Chengde	40°43′20″	117°43′23″	497	East	16	Gravel
P20	Sanjiazhen, Chengde	41°15′09″	118°13′36″	566	East	45	Gravel
P21	Xiaomiaozi, Chengde	41°19′33″	118°33′18″	530	West	30	Gravel
P22	Yixunhegou, Longhua	41°19′55″	117°46′49″	639	South	18	Gravel
P23	Dalianggou, Longhua	41°28′14″	117°44′42″	607	West	25	Gravel
P24	Miaogong Reservoir, Weichang	41°44′00″	117°51′05″	750	West	15	Gravel
P25	Sanchakou, Weichang	41°41′35″	117°03′49″	902	West	20	Gravel
P26	Yushudixia, Fengning	41°28′01″	117°71′04″	939	West	35	Gravel
P27	Fengshan, Fengning	41°12′20″	117°11′38″	597	Northeast	20	Loam
P28	Xingzhoucun, Luanping	41°00′47″	117°22′04″	457	East	25	Gravel
P29	Xinglong town	40°24′01″	117°29′57″	589	South	25	Loam
P30	Heyanzi, Xinglong	40°21′53″	117°53′21″	287	Northwest	20	Loam

harm rate, relative fresh weight, and relative root length of the samples (Fig. 2). The salt tolerance was divided into four levels with a Euclidean distance of 6 between each level: highly salt tolerant (HST), salt tolerant (ST), moderately salt tolerant (MST), salt sensitive (SS). In addition, the *F. suffruticosa* inbred lines with a relative germination rate of less than 2% were classified as highly salt sensitive (HSS).

The seeds of two *F. suffruticosa* genotypes (HSS in Jiaoshan and Shanhaiguan (P2) and HST in Fengshan and Fengning (P27)) were chosen for the salt stress test as shown in Table 2. All the seeds were randomly divided into seven groups, each containing 30 uniform seeds. According to the results of a preliminary germination test, *F. suffruticosa* seeds would not germinate at NaCl concentrations greater than 240 mM. The experimental treatments were as follows: (1) no salt treatment (control, CK); (2) 40 mM NaCl treatment

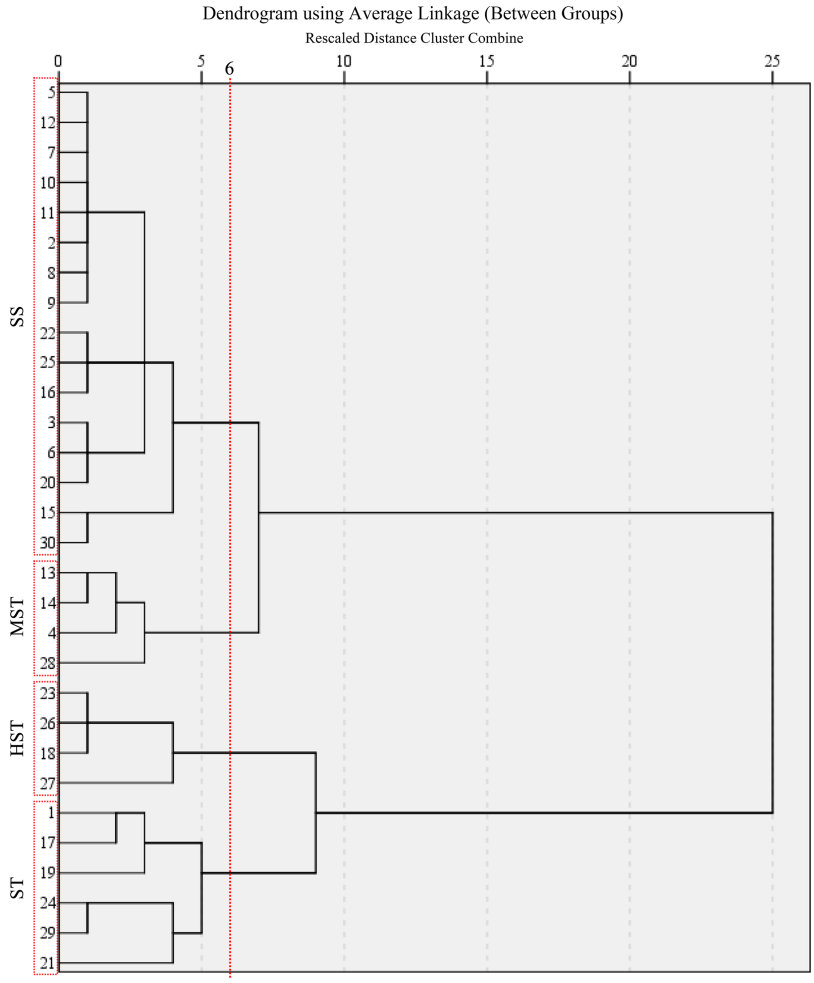

Figure 2 **Cluster analysis plots of salt tolerance in 30 *F. suffruticosa* germplasms.**
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(S40); (3) 80 mM NaCl treatment (S80); (4) 120 mM NaCl treatment (S120); (5) 160 mM NaCl treatment (S160); (6) 200 mM NaCl treatment (S200); and (7) 240 mM NaCl treatment (S240). The *F. suffruticosa* seeds were sterilized with 1% sodium hypochlorite for 15 min. After being rinsed with distilled water five times, 30 seeds were sown in a Petri dish (nine cm diameter) with two layers of filter paper and cultured at 25 ± 0.5 °C for 12 days in an incubator in the dark. Three independent biological replicates were used for each treatment. The germination rate was measured at 12 DAT.

Determination of water absorption

For water absorption, seeds were immersed in petri dishes containing 12.5 ml of the solution for each treatment, and successive weighing was carried out to measure the fresh weight gain every four hours until germination. The water on the seed epidermis should be blotted with absorbent paper before measurement. Then the seeds were placed in the treatment solution again after measurement. As the water content absorbed in each time

Table 2 Results of salt tolerance screening test of *F. suffruticosa* seeds.

Provenance code	Grades of saline resistant	Provenance code	Grades of saline resistant	Provenance code	Grades of saline resistant
P1	ST	P11	SS	P21	ST
P2	HSS	P12	HSS	P22	SS
P3	SS	P13	MST	P23	HST
P4	MST	P14	MST	P24	ST
P5	HSS	P15	SS	P25	SS
P6	SS	P16	SS	P26	HST
P7	HSS	P17	ST	P27	HST
P8	SS	P18	HST	P28	MST
P9	SS	P19	ST	P29	ST
P10	HSS	P20	SS	P30	SS

Notes.
HST, highly salt tolerant; ST, salt tolerant; MST, moderately salt tolerant; SS, salt sensitive; HSS, highly salt sensitive.

is calculated as follows, wherein W_i and W_f are the initial and final weight of seeds at each time point, respectively (*Sousa, Maia & Morais, 2016*).

$$\% \text{ of absorbed water} = (W_f – W_i)/ W_i \times 100\%$$

Determination of soluble sugar content

The soluble sugar content was determined at different time points (3, 5, 7, 9, 11 DAT) using the method described by *Chen et al. (2020)* with slight modifications and included three independent biological replicates. Glucose (0.1 g) was weighed and diluted into 100 mL volumetric flasks to obtain a standard solution of 1 mg mL^{-1}. Then the standard solution was diluted to 5 concentration gradients, including 0 μg mL^{-1}, 10 μg mL^{-1}, 20 μg mL^{-1}, 30 μg mL^{-1}, 40 μg mL^{-1} and 50 μg mL^{-1}. A total of 2 ml standard solutions and 4 ml acid-anthrone reagent were added to test tubes for 10 min. The reaction mixtures were determined by measuring the absorbance at 620 nm using a UV2600 spectrophotometer (Shimadzu, Kyoto, Japan) for making a standard curve. All germinated seeds were ground into powder after oven-drying at 85 °C for 4 h. A total of 0.1 g dried samples and 15 mL of 80% ethanol were added to test tubes and placed in boiling water for 30 min. The reaction mixture was transferred into a centrifuge tube and centrifuged at 800 g for 30 min, and the supernatant was transferred into test tubes. These steps were repeated twice, and the supernatants were combined. The supernatant (two mL) and sulfuric acid-anthrone reagent (four mL) were mixed. The soluble sugar contents were determined by measuring the absorbance of each sample at 620 nm using a UV2600 spectrophotometer (Shimadzu) and calculated using a standard curve.

Determination of glycolysis metabolizing enzyme activity

The activities of HK, PFK and PK were determined at different time points (3, 5, 7, 9, 11 DAT) using assay kits (HK-2-Y, PFK-2-Y and PK-2-Y, respectively; Suzhou Comin Biotechnology Co., Ltd., Suzhou, China). Three independent biological replicates were

assessed. All germinated seeds were rapidly frozen in liquid nitrogen and stored at −80 °C until analysis.

NADPH is the product of HK-related reactions, which has characteristic absorption peak at 340 nm. While PFK and PK activities can be reflected by the determination of NADH decline rate at 340 nm. 0.1 g samples were ground in a mortar with one mL of corresponding extraction solution and transferred into EP tubes. The homogenate was centrifuged at 8,000 g 4 °C for 10 min, and the supernatant was collected. Related reagents were added to the above filtrate according to the manufacturer's instructions. The reaction mixtures were measured at 340 nm with a UV2600 spectrophotometer (Shimadzu). The enzyme activity was calculated and expressed in nmol min^{-1} g^{-1} (fresh weight (FW)).

Determination of TCA cycle-related enzyme activity

The activities of PDH and CS were determined at different time points (3, 5, 7, 9, 11 DAT) using assay kits (BC0380, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China; BC1060, Beijing Solarbio Science & Technology Co., Ltd.). Three independent biological replicates were assessed. All germinated seeds were rapidly frozen in liquid nitrogen and stored at −80 °C until analysis.

PDH catalyzes the dehydrogenation of PA, resulting in a reduction in absorbance at 605nm. 0.1 g samples were ground into a homogenate with one mL and 10 μL of corresponding reagents in a mortar. The homogenate was then collected and centrifuged at 11,000 g 4 °C for 10 min. Related reagents were added to the supernatant according to the manufacturer's instructions. The reaction mixtures were measured at 605 nm with a UV2600 spectrophotometer (Shimadzu). The enzyme activity was calculated and expressed in nmol min^{-1} g^{-1} (FW).

CS-related reactions cause the transfer of DTNB into TNB, which has characteristic absorbance at 412 nm. 0.1 g samples were ground into a homogenate with one mL and 10 μL of corresponding reagents in a mortar. The homogenate was centrifuged at 600 g 4 °C for 10min. The supernatant was then collected and centrifuged at 11,000 g 4 °C for 10min. Related reagents were added to the supernatant according to the manufacturer's instructions. The reaction mixtures were measured at 412 nm with a UV2600 spectrophotometer (Shimadzu). The enzyme activity was calculated and expressed in nmol min^{-1} g^{-1} (FW).

Determination of G6PDH activity

The activity of G6PDH was determined at different time points (3, 5, 7, 9, 11 DAT) using a glucose-6-phosphate dehydrogenase activity assay kit (QYS-231047, Qiyi Biological Technology (Shanghai) Co., Ltd., Shanghai, China). Three independent biological replicates were assessed. All germinated seeds were rapidly frozen in liquid nitrogen and stored at −80 °C until analysis.

G6PDH activity can be reflected by the determination of NADPH increase rate at 340 nm because it catalyzes the conversion of $NADP^+$ to NADPH. 0.05 g samples were ground in a mortar with one mL of the corresponding extraction solution and transferred into EP tubes. The homogenate was centrifuged at 8,000 g 4 °C for 10 min, and the

supernatant was collected. Related reagents were added to the above filtrate according to the manufacturer's instructions. The reaction mixtures were measured at 340 nm with a UV2600 spectrophotometer (Shimadzu). The enzyme activity was calculated and expressed in nmol min^{-1} g^{-1} (FW).

Determination of the contents of PA and CA

The contents of PA and CA were measured at different time points (3, 5, 7, 9, 11 DAT) using pyruvic acid and citric acid assay kits (BC2200 and BC2150, respectively; Beijing Solarbio Science & Technology Co., Ltd.) according to the manufacturer's instructions. Three independent biological replicates were assessed. All germinated seeds were rapidly frozen in liquid nitrogen and stored at −80 °C until analysis. The reaction mixtures were measured at 520 nm for PA and 545 nm for CA with a UV2600 spectrophotometer (Shimadzu). The PA content was calculated using a standard curve and expressed in μg g^{-1} (FW), while the CA content was calculated and expressed in μmol g^{-1} (FW).

Statistical analysis

Microsoft Excel 2013 (Redmond, WA, USA) was used for data processing. SPSS 21.0 (IBM, Inc., Armonk, NY, USA) was used to analyze variance (ANOVA). Differences among the treatment means were assessed using a least significant difference (LSD) test at a $p < 0.05$ threshold.

RESULTS

Effect of salt stress on the germination rate

As shown in Fig. 3, the germination rate in P2 and P27 decreased as the NaCl concentration increased, reaching its minimum at 240 mM NaCl. Under normal conditions (CK), the seed germination rate reached 84.44% and 85.56% in P2 and P27, respectively. In comparison, the germination rate was only about 1.11% and 5.56% under 240 mM NaCl treatment, indicating that NaCl indeed inhibited seed germination. Compared with the CK treatment, the germination rate in P2 was reduced by approximately 9.21%, 28.95%, 56.58%, 64.47%, 96.05% and 98.68%, respectively, at the 40, 80, 120, 160, 200, and 240 mM NaCl treatments. While the germination rate in P27 was reduced by approximately 0.00%, 7.79%, 25.97%, 35.06%, 88.31% and 93.51%, respectively, at the 40, 80, 120, 160, 200, and 240 mM NaCl treatments.

Effect of salt stress on the water absorption

There are three phases in germination: phase I, phase II, phase III, which represent imbibition and imbibitional damage, the lag phase and the completion of germination, respectively (*Bewley et al., 2013*). For the water absorption of the seeds P2 and P27, the physiological phase I imbibition of the CK treatment occurred in about 24 h with a noticeable increase to stabilize water absorption, and phase II was extended to about 60 h. The phase III of the CK treatment occurred in about 108 and 96 h in P2 and P27, respectively, with increases in absorption to germination. Seed germination was delayed by salt stress. For seeds under salt stress, is that phase I went up to approximately 36 and

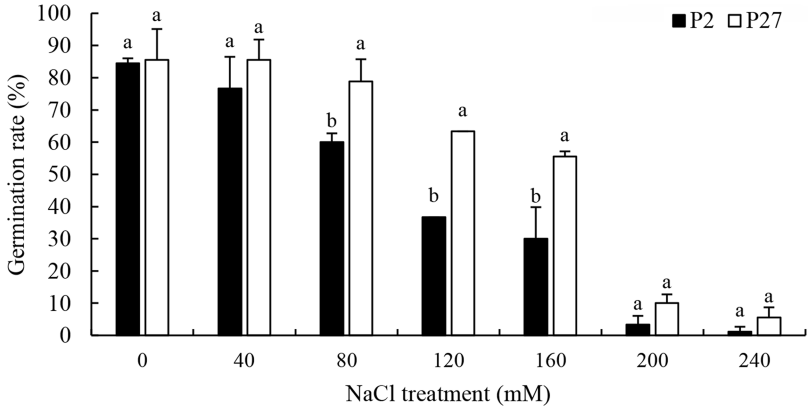

Figure 3 Effects of salt stress on germination rate of *F. suffruticosa* in P2 and P27. The data are the means of three replicates (±SE), and treatments with different letters are significantly different at a $p <$ 0.05 threshold.

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28 h in P2 and P27 at 240 mM NaCl, respectively, when there was a more significant increase in water absorption since phase II was extended up to 120 and 108 h. The phase III occurred in about 264 and 228 h in P2 and P27 at the highest salinity level, respectively, until germination (Table 3).

Different changes in relative absorption value were detected in P2 and P27 under salt stress. By comparing the relative absorption of distilled water (CK) with the other treatments in P2, the relative absorption values of phase II under salt stress were lower than CK except for the seeds that were exposed to 80 mM NaCl. The relative absorption values of phase II in P2 was decreased by approximately 1.05%, 2.12%, 0.46%, 1.98%, and 8.36%, respectively, at the 40, 120, 160, 200, and 240 mM NaCl treatments. In contrast, the relative absorption values of phase II with salt treatments were higher in P27, which increased by 4.90%, 10.51%, 9.04%, 29.47%, 10.62%, and 3.61%, respectively, compared with the CK treatment. However, the relative absorption values of phase III in P2 and P27 under salt stress were higher than those with CK treatments.

Effect of salt stress on the soluble sugar content during seed germination

As shown in Fig. 4A, as the salt concentrations increased, salt stress overall increased the soluble sugar content in P2. A significant increase was observed at 5, 7, and 9 DAT. Compared with the CK treatment, the soluble sugar content in P2 increased by approximately 35.37%, 44.85%, 76.56%, 38.65%, and 17.65% at the highest level (240 mM) at 3, 5, 7, 9, and 11 DAT, respectively. In P27, the content of soluble sugar increased first and then decreased at 5, 7, and 11 DAT, and continued to increase at 3 and 9 DAT as the salt concentrations increased (Fig. 4B). Compared with the CK treatment, the soluble sugar content in the NaCl seeds of P27 was significantly higher. As the time of salt stress continued, the soluble sugar content in P27 increased by approximately 56.70%, 77.33%, 56.35%, 42.93%, and 75.14% at the highest level (240 mM) at 3, 5, 7, 9, and 11 DAT, respectively, compared with the CK treatment.

Table 3 Percentage of water absorption of *F. suffruticosa* seeds in P2 and P27 before germination.

Time (h)	Percentage of water absorption (%)													
	CK		S40		S80		S120		S160		S200		S240	
	P2	P27	P2	P27	P2	P27	P2	P27	P2	P27	P2	P27	P2	P27
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4	15.95 ± 1.99	14.21 ± 1.58	15.34 ± 1.77	16.27 ± 1.66	16.13 ± 1.59	16.6 ± 1.87	14.86 ± 1.96	18.04 ± 1.67	15.95 ± 1.35	22.84 ± 1.94	14.69 ± 1.51	17.8 ± 1.68	14.84 ± 1.86	11.77 ± 1.50
8	21.07 ± 2.42	18.22 ± 1.88	19.86 ± 1.97	20.93 ± 1.83	20.61 ± 1.49	20.54 ± 1.67	17.72 ± 2.07	20.80 ± 1.76	19.41 ± 1.84	25.57 ± 2.02	18.85 ± 2.08	21.99 ± 1.98	18.76 ± 1.99	20.16 ± 1.86
12	21.89 ± 2.59	19.72 ± 1.83	20.93 ± 1.86	21.87 ± 1.78	21.77 ± 1.70	22.82 ± 1.83	18.67 ± 1.98	22.63 ± 1.79	21.26 ± 1.81	26.67 ± 2.00	19.83 ± 2.00	23.35 ± 1.92	19.79 ± 1.96	21.39 ± 2.14
24	22.35 ± 2.57	22.42 ± 1.85	21.64 ± 1.96	23.73 ± 1.86	22.92 ± 1.92	24.38 ± 1.99	20.93 ± 1.99	24.36 ± 1.70	21.51 ± 1.86	28.85 ± 1.97	21.05 ± 2.00	24.71 ± 1.97	20.14 ± 1.89	22.72 ± 2.10
28	22.35 ± 2.57	22.62 ± 1.94	22.12 ± 1.88	23.73 ± 1.86	23.56 ± 1.69	25.00 ± 1.90	21.52 ± 2.02	24.67 ± 1.68	21.88 ± 1.80	29.29 ± 1.98	21.54 ± 1.78	25.03 ± 1.86	20.25 ± 1.88	23.34 ± 2.07
32	22.35 ± 2.57	22.62 ± 1.94	22.12 ± 1.88	23.73 ± 1.86	23.82 ± 1.62	25.00 ± 1.90	21.88 ± 2.08	24.67 ± 1.68	22.13 ± 1.76	29.29 ± 1.98	21.66 ± 1.70	25.03 ± 1.86	20.37 ± 1.87	23.44 ± 2.14
36	22.35 ± 2.57	22.62 ± 1.94	22.12 ± 1.88	23.73 ± 1.86	23.82 ± 1.62	25.00 ± 1.90	21.88 ± 2.08	24.67 ± 1.68	22.25 ± 1.74	29.29 ± 1.98	21.91 ± 1.76	25.03 ± 1.86	20.48 ± 1.89	23.44 ± 2.14
48	22.47 ± 2.61	22.62 ± 1.94	22.24 ± 1.91	23.83 ± 1.79	23.82 ± 1.62	25.00 ± 1.90	21.88 ± 2.08	24.77 ± 1.65	22.25 ± 1.74	29.4 ± 1.99	21.91 ± 1.76	25.13 ± 1.89	20.48 ± 1.89	23.44 ± 2.14
60	22.58 ± 2.57	23.12 ± 2.01	22.35 ± 1.84	23.94 ± 1.77	23.94 ± 1.59	25.00 ± 1.90	21.88 ± 2.08	24.87 ± 1.62	22.37 ± 1.73	29.4 ± 1.99	22.15 ± 1.72	25.13 ± 1.89	20.48 ± 1.89	23.44 ± 2.14
72	23.17 ± 2.63	26.23 ± 1.98	22.95 ± 1.81	25.28 ± 1.80	24.07 ± 1.82	26.35 ± 1.89	21.88 ± 2.08	25.28 ± 1.57	22.5 ± 1.71	30.05 ± 1.94	22.15 ± 1.72	25.13 ± 1.89	20.48 ± 1.89	23.54 ± 2.11
84	26.08 ± 2.69	32.73 ± 2.33	24.73 ± 1.86	30.67 ± 2.20	24.07 ± 1.82	30.60 ± 2.50	22.35 ± 2.14	26.81 ± 1.69	22.62 ± 1.70	30.71 ± 1.97	22.15 ± 1.72	25.97 ± 1.92	20.48 ± 1.89	23.54 ± 2.11
96	31.66 ± 3.10	41.64 ± 2.28	31.63 ± 1.80	37.93 ± 1.95	26.25 ± 1.92	37.45 ± 2.25	23.54 ± 2.30	29.97 ± 1.79	23.11 ± 1.64	31.91 ± 2.08	22.15 ± 1.72	27.54 ± 2.14	20.48 ± 1.89	23.54 ± 2.11
108	32.71 ± 2.95		38.29 ± 2.15	44.25 ± 2.22	29.45 ± 2.29	45.33 ± 2.11	25.45 ± 2.12	34.96 ± 2.89	23.86 ± 1.58	35.63 ± 2.29	22.15 ± 1.72	29.11 ± 2.07	20.71 ± 2.04	25.69 ± 2.14
120			44.59 ± 2.34		34.19 ± 2.14	52.39 ± 2.08	28.3 ± 2.33	40.98 ± 3.04	25.96 ± 1.99	40.22 ± 2.18	22.89 ± 1.68	32.15 ± 2.14	21.75 ± 2.22	28.25 ± 2.15
132					41.74 ± 2.00		31.39 ± 2.55	48.11 ± 2.63	29.05 ± 2.17	44.37 ± 2.21	23.99 ± 1.70	37.8 ± 2.84	23.25 ± 2.05	30.5 ± 2.21
144							36.03 ± 2.27		32.39 ± 1.86	46.89 ± 2.09	26.19 ± 1.69	43.14 ± 3.67	24.97 ± 2.06	32.96 ± 2.15
156									35.48 ± 2.00		28.15 ± 1.99	45.65 ± 3.55	26.7 ± 1.99	35.41 ± 2.14
168									38.94 ± 2.10		30.84 ± 2.20		28.54 ± 2.26	38.59 ± 2.17
180											32.93 ± 1.98		29.92 ± 2.09	41.15 ± 2.19
192											35.25 ± 1.91		31.76 ± 2.09	43.81 ± 2.44
204											38.07 ± 1.92		33.72 ± 2.12	46.57 ± 2.46
216											40.27 ± 1.71		35.56 ± 2.12	49.03 ± 2.23
228											42.59 ± 1.77		37.4 ± 1.99	50.56 ± 2.22
240											44.55 ± 1.78		39.13 ± 1.76	
252											46.14 ± 1.71		40.74 ± 2.13	
264													42.35 ± 1.97	

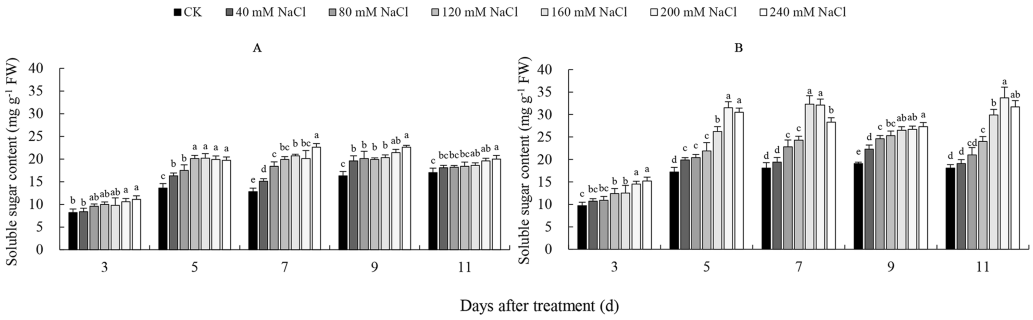

Figure 4 Effects of salt stress on the soluble sugar content during seed germination. (A) Soluble sugar content in P2. (B) Soluble sugar content in P27. The data are the means of three replicates (±SE), and treatments with different letters are significantly different at a $p < 0.05$ threshold.
Full-size DOI: 10.7717/peerj.15668/fig-4

Effect of salt stress on the glycolysis metabolizing enzyme activity during seed germination

With increasing salt concentrations, the HK activity in P2 reached its maximum value at 160 mM NaCl, then reduced at 3 and 5 DAT. An increase in the concentration of salt treatment was accompanied by a significant increase in the HK activity in P2 at 7, 9, and 11 DAT (Fig. 5A). As stress time went on, a decrease in HK activity of P2 was observed in CK treatment. Compared with the CK treatment, the HK activity of P2 increased by approximately 81.57%, 181.66%, and 133.30% at the 240 mM NaCl concentration at 7, 9, and 11 DAT, respectively. With the increase in salt concentrations, a significant increase in the HK activity of P27 was observed at 3, 5, 7, and 9 DAT, which was observed at higher levels (80, 120, 160, 200, and 240 mM) of salinity at 11 DAT (Fig. 5B). At 3, 5, 7, and 9 DAT, the HK activity of P27 reached its maximum at 200 mM NaCl, with increases of 108.33%, 222.54%, 167.06%, and 290.47%, respectively, compared with their respective control.

PFK activity increased first and then decreased in the seeds P2 and P27 after salt treatment (Figs. 5C, 5D). With the increase in salt concentrations, the PFK activity of P2 increased the most significantly following treatment with 120 mM NaCl at 3, 7, 9, and 11 DAT, which increased by 36.95%, 40.07%, 20.35%, and 11.25%, respectively, compared with the CK treatment. In P27, the activity of PFK at all the levels of NaCl was higher than that with the CK treatment in general. A more significant increase occurred following exposure to 40 and 80 mM NaCl at 3 and 5 DAT. Compared with the CK treatment, PFK activity increased significantly at 7, 9, and 11 DAT under salt stress.

A similar response was observed in the activity of PK in P2 and P27 seeds under salt stress (Figs. 5E, 5F). Simultaneously, the PK activity increased first and then decreased under different concentrations of salt stress. The PK activity increased over time and reached its maximum on 11 DAT. Compared with the CK treatment, the PK activity with the salt treatment was simultaneously higher, and it increased to higher levels in P27 than in P2. At 11 d, the PK activity of P2 under the salt stress treatment increased by only 12.01%, 13.28%, 16.94%, 10.64%, 2.71%, and 0.92%, respectively, compared with the CK treatment. However, compared with the CK treatment, the PK activity of P27 increased by

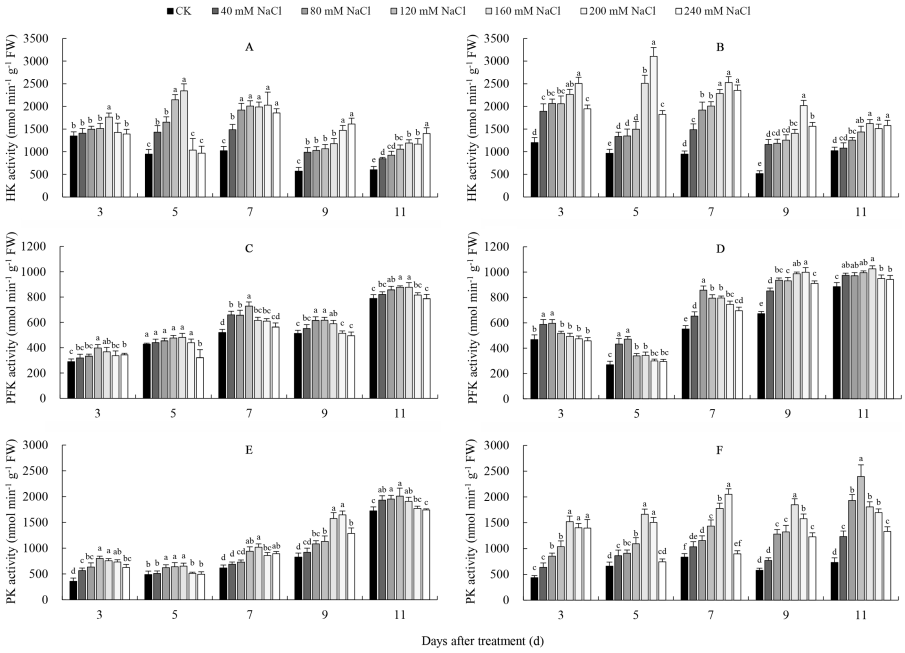

Figure 5 Effects of salt stress on the glycolysis metabolizing enzyme activity during seed germination. (A) HK activity in P2. (B) HK activity in P27. (C) PFK activity in P2. (D) PFK activity in P27. (E) PK activity in P2. (F) PK activity in P27. The data are the means of three replicates (±SE), and treatments with different letters are significantly different at a $p < 0.05$ threshold.

Full-size DOI: 10.7717/peerj.15668/fig-5

approximately 68.61%, 164.29%, 227.61%, 147.12%, 132.14%, and 82.01%, respectively, which was much higher than that of P2.

Effect of salt stress on the TCA cycle-related enzyme activity during seed germination

The PDH activity in the seeds of P2 increased first and then decreased after salt treatment. At 9 DAT, the activity of PDH in P2 was slightly lower at 200 and 240 mM NaCl concentrations than that with the CK treatment. Compared with the CK treatment, the PDH activity of P2 significantly decreased by approximately 25.84%, 33.59%, and 30.43% at the 160, 200, and 240 mM NaCl concentrations, respectively, at 11 DAT (Fig. 6A). In P27, the PDH activity was significantly increased at 5, 7, 9, and 11 DAT after salt treatment except for the seeds that were exposed to 40 mM NaCl at 11 DAT (Fig. 6B).

With the increase in salt concentrations, different changes in CS activity in P2 and P27 were apparent over time. At 3, 5, and 7 DAT, the activity of CS in P2 increased first and then decreased under salt stress. At the same time, a decrease was observed at 9, and 11 DAT (Fig. 6C). At 7 and 9 DAT, the CS activity under the CK treatment was significantly higher than those under the 120, 160, 200, and 240 mM NaCl treatments. As the salt stress continued, the CS activity of P27 increased first and then decreased at 3, 5, and 7 DAT. Salt stress treatment significantly increased CS activity at 9 and 11 DAT. Compared with each CK treatment, the activity of CS in P27 increased by approximately 27.00%, 52.56%, 57.04%, 48.57%, 61.69%, and 62.51% at 9 DAT and increased by approximately 15.40%,

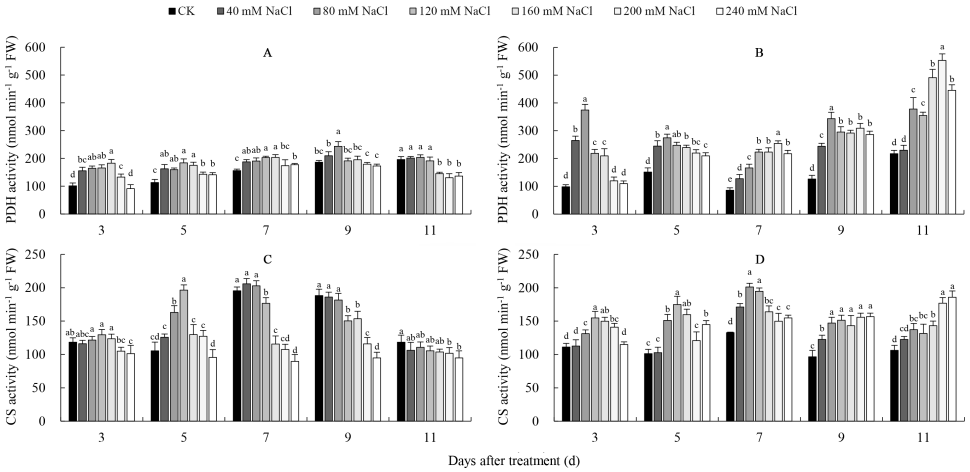

Figure 6 Effect of salt stress on the TCA cycle-related enzyme activity during seed germination. (A) PDH activity in P2. (B) PDH activity in P27. (C) CS activity in P2. (D) CS activity in P27. The data are the means of three replicates (±SE), and treatments with different letters are significantly different at a $p < 0.05$ threshold.

Full-size DOI: 10.7717/peerj.15668/fig-6

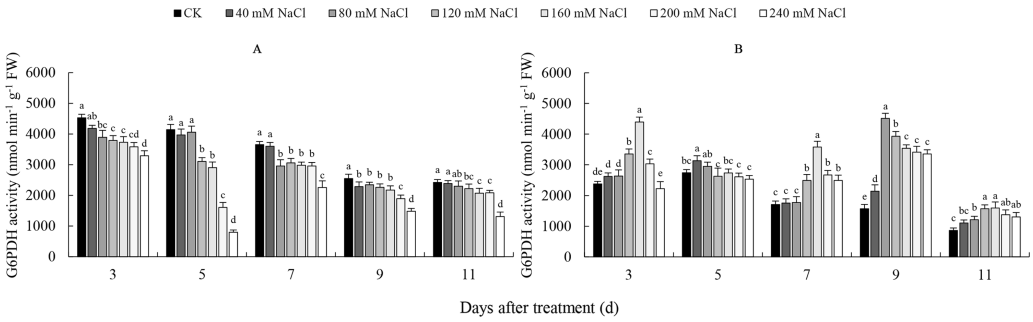

Figure 7 Effect of salt stress on the G6PDH activity during seed germination. (A) G6PDH activity in P2. (B) G6PDH activity in P27. The data are the means of three replicates (±SE), and treatments with different letters are significantly different at a $p < 0.05$ threshold.

Full-size DOI: 10.7717/peerj.15668/fig-7

29.20%, 23.85%, 35.00%, 66.71% and 75.00% at 11 DAT as the salt stress time continued (Fig. 6D).

Effect of salt stress on the G6PDH activity during seed germination

Different changes of G6PDH activity were detected in P2 and P27 under salinity stress. With increasing salt concentrations, the NaCl treatment in P2 caused a general decrease in the activity of G6PDH (Fig. 7A). The G6PDH activity in seeds with the CK treatment was significantly higher compared with those treated with higher levels (120, 160, 200, and 240 mM) of NaCl (Fig. 7A). The activity of G6PDH in P27 increased first and then decreased with the increase in the salt concentrations (Fig. 7B). The G6PDH activity of P27 under 120, 160, and 200 mM of NaCl stress was significantly higher than that with the corresponding CK treatment at 3, 7, 9, and 11 DAT.

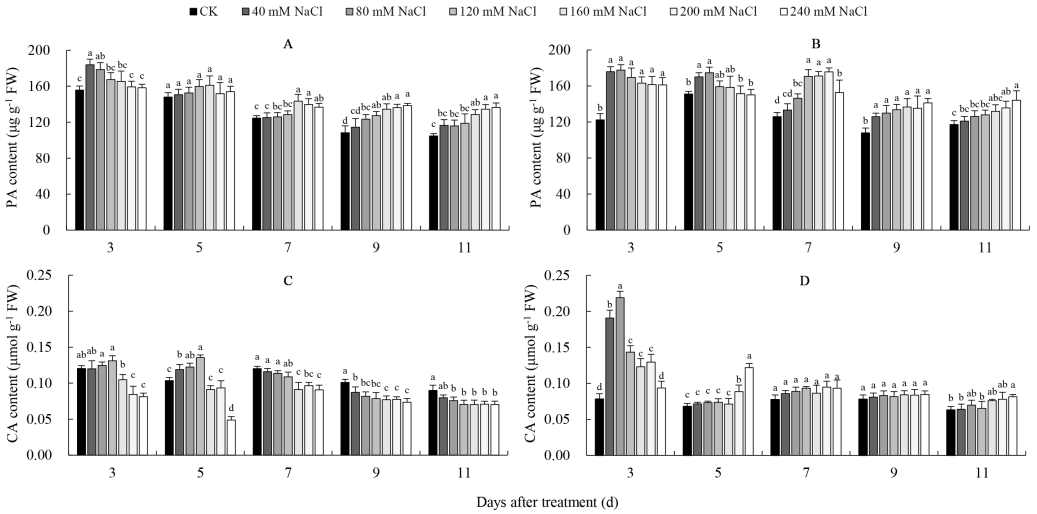

Figure 8 Effect of salt stress on the PA and CA contents during seed germination. (A) PA content in P2. (B) PA content in P27. (C) CA content in P2. (D) CA content in P27. The data are the means of three replicates (±SE), and treatments with different letters are significantly different at a $p < 0.05$ threshold.
Full-size DOI: 10.7717/peerj.15668/fig-8

Effect of salt stress on the PA and CA contents during seed germination

The PA content in P2 and P27 showed similar trends under salt stress. The PA content was increased first, then decreased at 3, 5, and 7 DAT, and continued to increase at 9 and 11 DAT. In P2, no significant change in PA content was detected under the NaCl treatment at 5 DAT. At 7, 9, and 11 DAT, a noticeable change appeared at higher levels (160, 200, and 240 mM) of NaCl (Fig. 8A). With the increase in salt concentrations, the PA content in seeds of P27 increased significantly first and then decreased at 3 and 5 DAT, and an evident increase was observed under the 80, 120, 160, 200, and 240 mM NaCl treatments at 7 and 9 DAT (Fig. 8B). The PA content under each salt stress concentration was higher than the corresponding CK treatment in general.

The effect of salinity on the CA content in P2 and P27 is shown in Figs. 8C, 8D. The CA content of P2 increased first and then decreased with the increase in salt concentration at 3 and 5 DAT. At 7, 9, and 11 DAT, a more significant decrease was observed with the increase in salt concentration. Overall, the content of CA was lower in the seeds exposed to 160, 200, and 240 mM NaCl concentrations compared with the CK treatment. In P27, the content of CA increased first and then decreased under salt stress at 3 DAT, which was significantly higher at the 40, 80, 120, 160, and 200 mM NaCl treatments on the day before germination compared with the CK treatment. The CA content increased at 5, 7, 9, and 11 DAT with the increased salt concentrations. No significant change in the CA content of P27 was apparent at 7, 9, and 11 DAT, while the CA content under salt stress was slightly higher than that with the CK treatment.

DISCUSSION

Large areas of abandoned saline land are distributed in coastal and arid zones worldwide (*Yan et al., 2013*), which has become one of the main factors affecting the plant landscape construction and ecological protection. Seed germination is a complex process regulated by internal metabolic activities and external conditions (*Nonogaki, Bassel & Bewley, 2010*; *Marler, 2019*; *Li et al., 2019*). Among the external conditions, salinity and water are one of the main environmental factors that may influence the germination of seeds. According to *McDonald, Vertucci & Roos (1988)*, water absorption or imbibition is essential for metabolic activity in seed germination. In the present study, seed germination was to varying degrees delayed with the increase of salt concentrations. For the water absorption, the relative absorption values of phase II in P2 were decreased by NaCl treatment, which may cause adverse effects on the metabolic activity of cells.

Meanwhile, the germination rates of salt-stress seeds in P2 and P27 were reduced, indicating that salt stress affects the germination of *F. suffruticosa* seeds. The decrease of water intake and energy supply due to the high salt concentration could be an explanation for the reduced germination attributes (*Bai et al., 2020*). Similar results were observed in *Vicia faba*, wheat, and barley, with a decrease in the germination rate of salt-affected seeds (*Anaya et al., 2015*; *Colmer, Munns & Flowers, 2005*; *Ali et al., 2012*).

A series of physiological and metabolic reactions will be triggered in plants to prevent, reduce, or repair damages under abiotic stress, including salt stress (*Yang et al., 2017*). Sucrose is the major substrate for respiratory metabolism and the primary energy source during seed germination (*Klotke et al., 2004*; *Ren et al., 2020*). It is involved in the osmotic adjustment to increase cytoplasmic solute concentrations and cell osmotic pressure, and it also can be used as a protective agent to adjust the response of plants to salt stress (*Zhong et al., 2015*). *Mbarki et al. (2020)* reported that increasing salt stress increased the accumulation of sugars in the leaves of *Medicago ciliaris*, *M. intertexta*, and *M. scutellate*, but the effect depended on the population. This difference was associated not only with the plant species but was also influenced by different salt concentrations. In this study, the soluble sugar content overall increased in P2 and P27 with increasing salt concentrations. The salt-tolerant lines had generally higher amounts of soluble sugars than the salt-sensitive ones, indicating that the salt-tolerant plant cells could accumulate more sugars to alleviate salt stress. Soluble sugar plays a dual role in stressed plants as a tolerance characteristic and is deemed to be a determining factor in the osmotic adjustment for resistance to salt stress (*Poór, Czékus & Ördög, 2019*; *Jawahar et al., 2019*). In addition, the increase in soluble sugar content could provide sufficient substrate for respiratory metabolism, ensuring energy supply for seed germination. Most enrichment of soluble sugar may be a defensive response that prevents high salinity stress (*Tiwari et al., 2021*). The same results were found for cotton (*Gossypium hirsutum*) seeds, salt-tolerant wheat (*Triticum aestivum*), and safflower (*Carthamus tinctorius*), in which soluble sugar accumulation increased under salt stress (*Chen et al., 2020*; *Kerepesi & Galiba, 2000*; *Ashraf & Fatima, 1995*).

The glycolysis pathway is an essential process of respiratory metabolism, which is related to the response of salt stress (*Zhang et al., 2011*). The main role of the glycolysis pathway is

to produce ATP and pyruvate by oxidizing sucrose (*Millar et al., 2011*). HK, PFK, and PK are the most critical irreversible enzymes in the glycolytic pathway that play an irreplaceable role in catalyzing rate-limiting reactions. *Minhas & Grover (1999)* found that glycolytic enzymes had sufficient flexibility to adjust to increased energy demand and the supply of intermediates to acclimatize to stress conditions. Many studies have suggested that increases in the activities of HK, PFK, and PK would enhance the glycolysis pathway and glycolysis associated with intermediate metabolism and then significantly increase plant resistance, including salt tolerance (*Singla et al., 2003*; *Kang et al., 2011*; *Guo et al., 2017*). Our results showed that although the glycolysis metabolizing enzyme activity in P2 and P27 increased overall after salt treatment, the salt-tolerant line was considerably more significant than that in the salt-sensitive line. Increases in HK, PFK, and PK activities can accelerate the conversion of respective intermediates, suggesting that more ATP can be released for seed germination, thereby enhancing the glycolysis pathway in the salt-tolerant line. In addition, the active glycolysis of P27 provided more PA for the TCA cycle, which had no significant change in P2 at 5 DAT. These results indicate that the representative enzymes involved in glycolysis are active throughout the germination process, probably providing the energy required to support the growing embryo in a salt-tolerant line. Similar results were observed in mangrove trees (*Bruguiera sexangula*) under salt stress with an increase in the activities of PFK and PK (*Suzuki et al., 2005*).

The TCA cycle dominates the carbon metabolizing machinery and provides energy for plant growth (*Millar et al., 2011*). PA links the glycolysis pathway and the TCA cycle (*Jardine et al., 2010*). It plays a vital role in the primary plant metabolism. According to *Wu et al. (2013)*, PA is crucial for barley (*Hordeum vulgare*) to withstand salt stress. *Yang et al. (2017)* found that the TCA cycle was enhanced in salt-tolerant wild soybean (*Glycine max* L.) under neutral salt stress due to the higher CA content than the control. PHD and CS are the key enzymes in the TCA cycle. A previous study showed that PHD is critical to enhancing stress tolerance in wheat (*Liu et al., 2013*). In this study, the *F. suffruticosa* seeds of P27 had more PA content under salt stress, which provided more material for the TCA cycle. The activities of PDH and CS in P27 increased at 5, 7, 9, and 11 DAT, and the CA content of P27 with salt treatment was higher than that of the control after salt treatment. Therefore, the amount of substrate for mitochondrial citric dehydrogenase was increased by the increase of CA content, with the reduction of oxidative damage under salt stress (*Zhong et al., 2015*). However, the activity of CS in P2 decreased at 7, 9, and 11 DAT, resulting in decreases in the content of CA. This disrupted the TCA cycle of the salt-sensitive line and provided fewer precursors for other metabolic pathways. These results indicate that the increases in activities of the enzymes involved in the TCA cycle modulate the suppression induced by salt stress, thereby supporting the salt resistance of P27.

Recently, G6PDH in PPP was reported to be involved in some metal-induced and other stress responses in plants, as the specific activity of G6PDH was found to be modulated by these abiotic stresses (*Van Assche, Cardinaels & Clijsters, 1988*). In the case of aluminum stress, a rapid increase in G6PDH activity was observed in an Al-resistant wheat cultivar (*Slaski et al., 1996*). *Lin et al. (2005)* found that G6PDH may be involved in the activation

of superoxide dismutase (SOD) and peroxidase (POD) activities and the induction of freezing resistance at low temperatures. According to *Valderrama et al. (2006)*, salt stress increases the activity of G6PDH, and thus decreases the level of reactive oxygen species (ROS), indicating that G6PDH is a resistant enzyme in plants under salt stress. Our results showed that a general decrease in G6PDH activity in P2 was apparent with increasing salt concentrations. The activity of G6PDH in P27 at the highest salinity level decreased at 3 and 5 DAT, and was distinctly increased at 7, 9, and 11 DAT. NADPH catalyzed by G6PDH and other antioxidant enzymes are crucial in ROS scavenging and could enable plants to survive under salt stress. The change of G6PDH activity at the highest level may be involved in the different content of ROS in P27, which further experiments should verify.

Various studies have confirmed that plants have adapted different mechanisms to cope with damage under salt stress (*Rizk et al., 2020*). These include strategies that help alleviate ion toxicity such as sodium exclusion and sequestration, and osmotic stress, such as accumulation of sugars (*Bohnert & Gensen, 1996*; *Das, Seal & Biswas, 2016*; *Xu et al., 2022*). The content of soluble sugar in the seeds of *F. suffruticosa* increased, which could provide significant energy for germination and maintain the plant's energy status under salt-stress conditions (*Cornic, 2000*). Distinctive differences in salinity tolerance mechanisms were observed between different *F. suffruticosa* genotypes. The enzyme activities involved in respiratory metabolism highlighted that P27 could modulate the metabolic activity more efficiently than P2 by increasing the amount of ATP synthesis in salt-tolerant lines for seed germination. Thus, the tolerance of P27 against salinity may be enhanced by modulating osmotic adjustment and active transport of ions driven by ATP consumption.

CONCLUSIONS

The influence of different concentrations of NaCl in water absorption, seed germination and the respiratory metabolism of different *F. suffruticosa* genotypes in northeastern Hebei Province were assessed comprehensively. As indicated by the phenotypic data, salt-tolerant *F. suffruticosa* had a higher germination rate than salt-sensitive ones at the same salt concentration. *F. suffruticosa* under salt stress exhibited inhibition of seed germination. The seeds of *F. suffruticosa* have different times for the physiological phases of water absorption with different salt concentrations. Salt stress retarded the seed water absorption process, and it depended on seed genotypes for *F. suffruticosa*. Soluble sugars accumulated in both P2 and P27 under salt stress, which is important for the seeds to adapt to saline environments. The respiratory metabolism of P27 was enhanced, compared with P2, to ameliorate the repression of seed germination under salt stress. To our knowledge, our study is the first to explore the effects of salt stress on *F. suffruticosa* germination. It has implications for selecting excellent salt-tolerant germplasm and landscape application of *F. suffruticosa*. Transcriptome profile analyses of tissue metabolic responses to salt stress in salt-tolerant and salt-sensitive lines of *F. suffruticosa* need to be done to explore the molecular mechanism in further studies.

Abbreviations

HK hexokinase

PFK	6-phosphofructokinase
PK	pyruvate kinase
PDH	pyruvate dehydrogenase
CS	citrate synthase
G6PDH	glucose-6-phosphate dehydrogenase
PA	pyruvic acid
CA	citric acid
TCA	tricarboxylic acid
PPP	phosphate pathway
SOD	superoxide dismutase
POD	peroxidase
ROS	reactive oxygen species
HST	highly salt-tolerant
ST	salt-tolerant
MST	moderately salt-tolerant
SS	salt-sensitive
HSS	highly salt-sensitive
DAT	days after salt stress treatment

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Ningwei Xu conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Bin Lu conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

- Yang Wang conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Xiaoyue Yu performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Nan Yao performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Qijuan Lin performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Xingyou Xu conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Bingshe Lu conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.15668#supplemental-information.

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