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Responses of cotton seed germination characteristics and storage substance metabolism to saline–alkali stress

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Abstract

Background The inhibition of cottonseed germination by soil salinization is a key obstacle affecting cotton production in Xinjiang. The most economical and effective measures to alleviate soil salinization damage are to explore the mechanism of cotton salt tolerance and to cultivate and popularize salt-tolerant varieties. Four cotton varieties were used in this study: the highly saline-alkali-resistant varieties Xinluzhong 82 and Xinluzhong 68 and the saline-alkali-sensitive varieties Xinluzhong 42 and Xinshi H12. These varieties were exposed to three different mixed saline-alkali concentrations: 0 mmol·L^{-1} (control, CK), 219 mmol·L^{-1}, and 365 mmol·L^{-1}.

Results The germination characteristics, root traits, and changes in the storage material contents (crude fat, total protein, and total sugar) during cotton seed germination were analyzed. The results revealed that with increasing mixed saline-alkali concentrations, the relative water absorption, water absorption rate, germination rate, root length, diameter, area, and volume, and total sugar content of cotton decreased by 22.7%–90.1% on average, whereas the crude fat and protein concentrations increased by 7.6% on average. Under saline-alkali stress, cotton seeds undergo metabolic reallocation characterized by a decrease in carbohydrate content and the accumulation of lipids/proteins, potentially mitigating energy deficits and structural damage.

Conclusions Under saline-alkali stress, highly resistant cotton varieties presented increased growth and adaptation during early root emergence. This was achieved by increasing water uptake and adjusting the root diameter. Under 365 mmol·L^{-1} saline-alkali stress, damages in these resistant varieties were correlated with their crude fat, protein, and storage substance contents. Changes in stored nutrients likely support cotton growth under stress. These findings reveal the physiological mechanisms underlying saline-alkali resistance in cotton and provide a scientific basis for the breeding of tolerant varieties. This study identified the key factors affecting cottonseed germination and offered a new direction for breeding programs.

Keywords Saline-alkali, Cotton seeds, Germination period, Root characteristics, Storage substances

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Core ideas

1. Saline-alkali stress reduces cotton seed germination and rooting ability.
2. The storage material content is related to the salt and alkali resistance of cotton.
3. The decreased hydrolysis of sugars and proteins has been shown to improve resistance to saline-alkali stress.

Introduction

Cotton is an economically important crop and a staple agricultural product worldwide (Mao 2016). China's cotton output reached 5.879 million tons in 2021, making China the world's largest cotton producer (Ding et al. 2021). Cotton is more resistant to saline-alkali conditions than other crops, and it is also one of the main crops used to improve saline-alkali land (Ashraf et al. 2018). Xinjiang is an important cotton-producing area in China. The area of saline-alkali soil has reached 847.6 hm^2 (Du et al. 2021), accounting for 37.7% of arable land, and has been increasing annually, resulting in different degrees of soil salinization (Chen 2020). Therefore, the rational development of saline-alkali soils and the achievement of high cotton yields in saline-alkali areas are current challenges and the focus of cotton research.

Seed germination is the most important and fragile stage of crop production (Fang et al. 2017), and it represents the beginning of plant growth and development. The natural conditions that generally cause stress and inhibit seed germination include both salinity and alkalinity. This mixed salt and alkali stress inhibits seed germination, mainly through the inhibition of water absorption by seeds, ion toxicity, high pH values, and ion imbalance (Sperdouli et al. 2012; Degenhardt et al. 2000). With the increases of salt and alkali concentrations, the germination parameters (Caldwell et al. 2008), relative water absorption rates, water absorption rates, seedling morphological indices (Zhang et al. 2018), and root morphological indices (Kuai 2015) of cotton seeds decrease significantly, which inhibits cotton seed germination and the normal development of cotton seedling roots (Zhang et al. 2014). Saline-alkali stress significantly inhibits the growth of cotton, resulting in reduced root length, surface area, and volume, and significantly increasing relative conductivity, malondialdehyde (MDA) content, antioxidant enzyme activity, and proline content (Guo et al. 2020). Moreover, cotton plants also respond to salt stress by maintaining their balance of K and Na ions (Ali et al. 2013). In addition, K, Ca, and Mg also play an important role in improving the salt tolerance of cotton. Li et al. (2019b) reported that with increasing mixed saline-alkali concentrations, the germination rate, germination potential, and average germination time of cotton seeds tended to decrease.

Previous works have shown that physiological and biochemical changes, including carbohydrate metabolism, hydrolysis, and the transformation of storage substances, are involved in seed germination (Liu et al. 2019). Under saline-alkali stress, an increase in protein content is a physiological response of plants that increases plant resistance but reduces damage (Zhang 2018). Many plants increase their contents of reducing sugars (glucose, fructose), sucrose, and fructan under salt and alkali stress (Wang et al. 2014). Moreover, the contents of soluble sugar and soluble protein of cotton seedlings were found to increase under 0.6% salt stress (Khatkar et al. 2000), and cotton plants with high contents of soluble sugar and soluble protein presented greater resistance under the same salt stress conditions. Studies have also shown that at the early and middle stages of cotton growth, the soluble sugar content was significantly increased under salt stress compared with that in the CK group (control) to protect the water and fertilizer absorption ability of cotton (Amangul-Mambetale et al. 2017). To date, most studies on saline-alkali tolerance in cotton have been conducted with plants at the seedling stage, and the physiological mechanism underlying saline-alkali tolerance at the seed germination stage is still unknown. Additionally, there are differences in the resistance levels of different cotton varieties. Continuously examining the resistance of cotton to mixed saline-alkali soil is the key to achieving efficient production in saline-alkali soil.

Although the type of saline-alkali soil used in production is generally mixed, recent research has focused mostly on damage from single salt (Chen et al. 2019) or alkali (Tanou et al. 2019) stress to crops, only few studies (Sun et al. 2025) have focused on mixed salt and alkali stress. This study addresses a critical gap by investigating combined saline-alkali stress, a physiologically distinct and field-representative condition, to advance ecologically relevant stress tolerance strategies. The cotton seed germination period is the most sensitive one to salt and alkali stress. In this study, two cotton varieties with high saline-alkali resistance and two sensitives were selected as research objects and subjected to a simulated mixed saline-alkali ion environment similar to that in Xinjiang. In this study, (1) the effects of mixed saline-alkali stress on the germination and root characteristics of cotton varieties with different levels of resistance were clarified; (2) the storage substance-based mechanism underlying the response to saline-alkali stress during cotton germination was revealed; and (3) a theoretical basis for the breeding of saline-alkali-tolerant cotton varieties to achieve full germination when sown in saline-alkali soil was provided.

Test materials

The test materials used were highly saline-alkali-resistant varieties, Xinluzhong 82 (L6) and Xinluzhong 68 (L7), which were screened and provided by a division of agriculture, and saline-alkali-sensitive varieties, Xinluzhong 42 (L12), which was provided by Yan's Seed Industry in Xinjiang, and Xinshi H12 (L39), which was provided by Xinjiang Academy of Agricultural Sciences (seed permissions were obtained). The experiment was carried out in the artificial climate chamber of Xinjiang Academy of Agricultural Sciences to simulate the environment of cottonseed germination under field conditions. The relative humidity was 60%, the temperature was 25–28 °C, with a 14 h (light)/10 h (dark) photoperiod, and the light intensity was 283 μmol·m^{-2}·$s^{-1.}$

Experimental design

Test methods

A one-year indoor experiment was carried out at the Xinjiang Academy of Agricultural Sciences. The experimental period was 3 months, and the experiment was repeated 4 times. The experiments were carried out in March, June, September, and December 2022. A two-factor split-plot experiment was carried out. The four cotton varieties were selected as the main plot condition, and the two saline-alkali concentrations and a control were set as the split plot condition: 0 mmol·L^{-1} (pH 7.3, CK), 219 mmol·L^{-1} (pH 9.3), and 365 mmol·L^{-1} (pH 9.8). Using a modified version of Xu's method (Xu 2020) (i.e., Composition of Compound Saline-alkali Solutions, a Reference to the Ion Content Composition of Field Saline-alkali Soil in Tumushuke (Xinjiang)), the saline-alkali solution was prepared with $CaCl_2$, $NaHCO_3$, Na_2SO_4, K_2SO_4, and $MgSO_4 \cdot 7H_2O$ in a ratio of 0.094:0.012:0.170:0.034:0.055 (Table 1). For the four tested varieties, seeds with full grains, complete embryos, no mildew, and a consistent size were selected, soaked and disinfected with 1% sodium hypochlorite for 10 min (Xia 2020), rinsed with distilled water four times, and dried on an ultraclean bench to restore the surface of the seed coat without moisture. Vermiculite and mixed saline-alkali solutions were stirred at a ratio of 1:3 and placed into germination boxes. Each germination box contained 40 seeds, and each treatment was repeated 4 times. Each germination box was filled with 500 g of vermiculite. After the seeds were placed in the box, the lid was secured. The box was weighed daily, and water was added to maintain consistent moisture throughout the germination period. The germination box was placed in a random position, and its position was adjusted every day. The cotton used in this study was planted in the artificial climate chamber of the Xinjiang Academy of Agricultural Sciences. This study conforms to relevant legislation and international, national, and institutional guidelines.

Table 1 Mixtures of salt and alkali solutions

Saline and alkali components in the Xiaohaizi reclamation area of the Third Division		Saline-alkali composition	
Ion	Mass/(mg·g^{-1})	Compound	Ratio/(mol·L^{-1})
Ca^{2+}	0.94	$CaCl_2$	0.094
Cl^-	1.48	$NaHCO_3$	0.012
Na^+	1.03	Na_2SO_4	0.170
HCO_3^-	0.18	K_2SO_4	0.034
SO_4^{2-}	3.07	$MgSO_4 \cdot 7H_2O$	0.055
K^+	0.67	PH	9.800
Mg^{2+}	0.33		
pH	7.80		

Parameter determination methods

(1) Relative water and water absorption (Xia 2020).

Ten grains were placed into each germination box, with three repeats, and the seeds were removed every 2 h. The surface liquid was quickly dried with absorbent towels and weighed on a balance (0.001 g); the data were recorded to determine seed germination (n denotes the number of times weighed, and T represents the weight).

Relative water absorption (%) = (T_n-seed dry weight)/seed dry weight × 100;

Water absorption rate (mg·h^{-1}) = (T_n-T_{n-1})/t;

(2) Germination capacity of seeds.

Germination was considered to have occurred when the length of the embryo was greater than 1/2 of the seed length. The number of germinated seeds was counted every day starting on the first day of the experiment.

Germination rate (%) = (number of germinated seeds on day 7/number of seeds tested) × 100%;

Germination potential (%) = (number of germinated seeds on day 3/number of seeds tested) × 100%;

Germination index (GI) = ∑Gt/Dt;

Average germination time (MTG) = ∑(Gt Dt)/∑ Gt;

In the formulas, Gt refers to the number of germinated seeds at time t, and Dt refers to the corresponding seed germination day.

(3) Root characteristics.

On the 14th day of growth, the cotton plants were removed from the germination box. The roots were then rinsed with water until thoroughly clean, and surface moisture was blotted dry using a paper towel prior to measurement. The roots of the cotton plants were scanned with an Epson scanner (Epson Perfection V850 Pro) in color mode, and root photos were obtained. The root angle was measured by ImageJ software, and the total root length, root surface area, average root diameter, and root volume were obtained by WinRHIZO image analysis software.

(4) Storage substances.

The total sugar content (Ts) was determined with the improved sulfuric acid-anthrone method (Liu 2019). The total protein content (Pr) was determined by the BCA method (Fang et al. 2017). The crude fat content (Ad) was determined by GB/T 6433–2006.

Data processing

The test data were processed by MS Excel 2010 software. Cluster analysis (intergroup connection method), variance analysis ($P < 0.05$), principal component analysis, and regression analysis (stepwise regression) were performed using SPSS 19.0 (SPSS, Chicago, IL, USA). Origin 2021 was used for plotting. The test data in the chart are the means ± standard deviations.

Results and analysis

Water absorption characteristics of cotton seeds

Fig. 1 shows that there was no difference between the embryos of the L6 and L39 varieties when the cotton seeds had absorbed water for 6 h under 219 mmol·L^{-1} mixed saline-alkali conditions. However, with the passage of the germination period, after 24 h of water absorption by the cotton seeds, the embryos of L6 and L39 transformed into radicles, but the root length of L6 significantly differed from that of L39.

As shown in Fig. 2, no significant difference was observed in relative water absorption among L6, L7, and L12 in the CK group at 48 h, with values of 138.9%, 140.6%, and 136.9%, respectively. Moreover, L39 presented the highest relative water absorption at 202.4%.

Under the 219 mmol·L^{-1} mixed saline-alkali stress, the relative water absorption of L12 and L39 was 55.6% and 38.9%, respectively, which was 21% and 4.3% lower than that of L6 and L7.

There was no significant difference in the relative water uptake of the cotton varieties under the 365 mmol·L^{-1} mixed saline-alkali stress ($P > 0.05$). When the germination process continued for 66 h, the water absorption of the highly saline-alkali-resistant varieties L6 and L7 was 81.2% and 83.3%, respectively. The relative water absorption of the saline-alkali-sensitive varieties L12 and L39 was 81.5% and 94.2%, respectively, and the water absorption fluctuated between 80.65% and 95.83% over time, so the seeds could not germinate.

As shown in Fig. 3, the water absorption rates of the different varieties peaked at 2 or 4 h under different mixed saline-alkali concentrations and then decreased with time. Under the 219 mmol L^{-1} mixed saline-alkali stress,

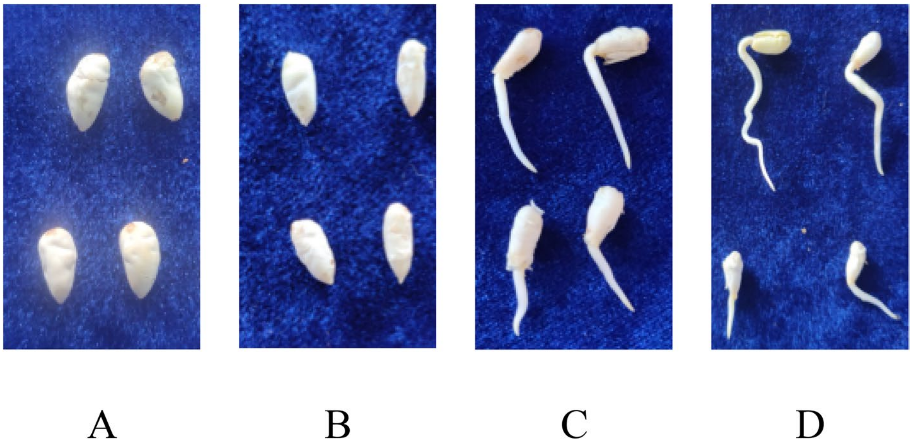

Fig. 1 The germination process of cotton seeds under the 219 mmol·L^{-1} mixed saline-alkali stress. Note: The top row shows L6 seeds; the bottom row shows L39 seeds. A shows the nonabsorbent state of cotton seeds (dry seeds); B shows the state of seeds that had absorbed water for 6 h; C shows the state of seeds that had absorbed water for 24 h (1 mm < germ < 2 cm); D shows the state of seeds that had absorbed water for 24 h (germ > 2 cm)

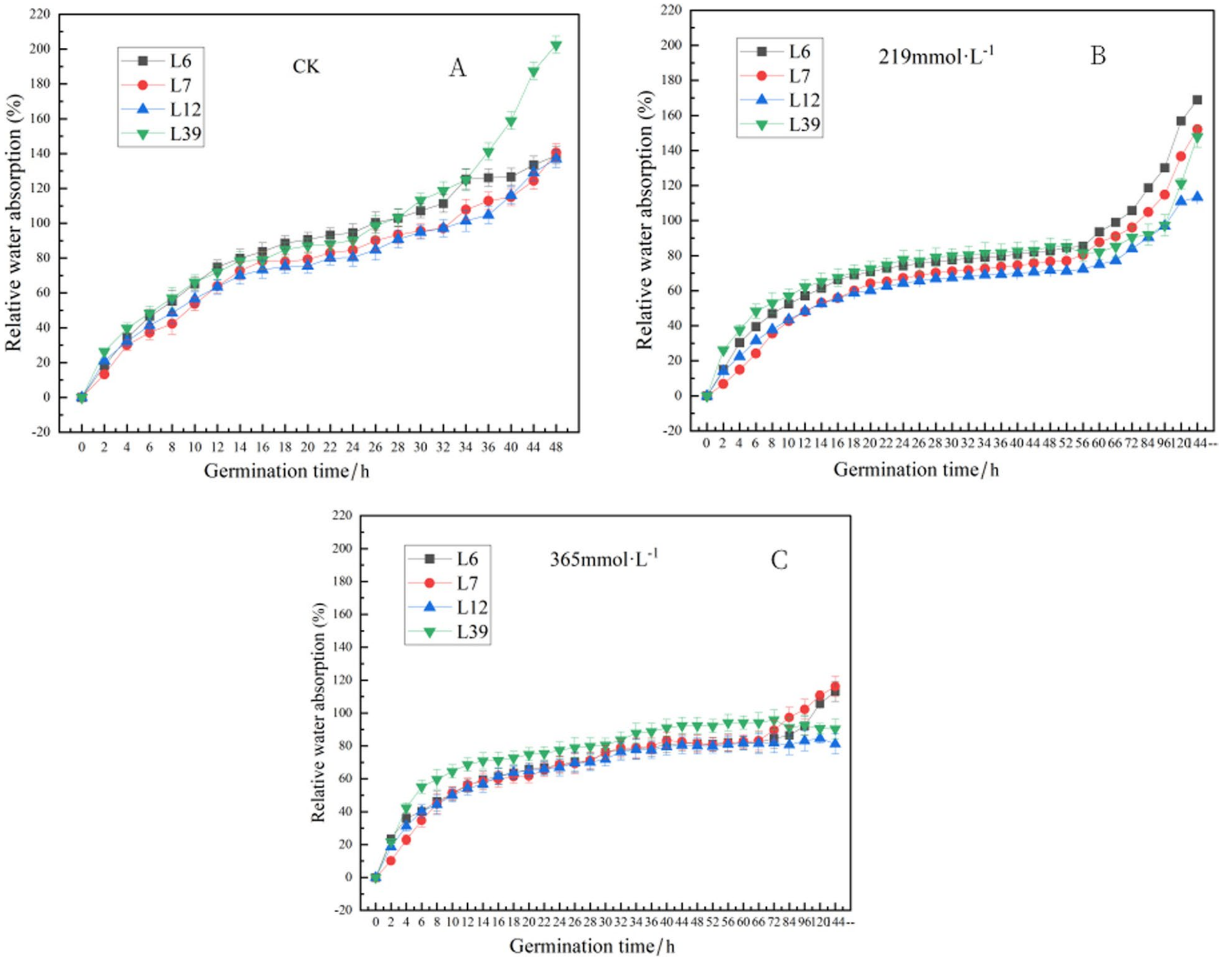

Fig. 2 Changes in the relative water absorption of cotton seeds under different saline-alkali conditions

the water absorption rates of the L12 and L39 seeds were 16.5 mg·h^{-1} and 39 mg·h^{-1}, which were 23 mg·h^{-1} and 45.5 mg·h^{-1} lower than those of the L6 and L7 seeds, respectively. Also under this stress, the water absorption rates of the L12 seeds were lower than that of L6 and L7 seeds by 1.5 mg·h^{-1}, 31.5 mg·h^{-1}, respectively, while the water absorption rate of L39 seeds was 34 mg·h^{-1} and 67.5 mg·h^{-1} lower than that of the L6 and L7 seeds, respectively. This indicated that the seeds of salt-alkali-sensitive cotton varieties could not absorb sufficient water due to the saline-alkali stress, which consequently inhibited the germination process. Under a 365 mmol L^{-1} saline-alkali concentration, the saline-alkali-sensitive varieties did not germinate. The variation trends of the water absorption rates of different cotton varieties at the same concentration were essentially the same. The L39 variety was most affected by mixed saline-alkali stress, and the water absorption rate under 100% mixed saline-alkali stress was 112.1% lower than that under CK, indicating that saline-alkali conditions strongly inhibited the germination and water absorption of the cotton varieties.

Germination characteristics of cotton seeds

The results (Table 2) revealed that with increasing saline-alkali concentration, the germination rate, germination potential, and germination index of the four cotton varieties decreased significantly compared with those of CK, and these values significantly differed from those under saline-alkali stress ($P < 0.01$). Under saline-alkali stress, the average germination time first increased but then decreased, indicating extremely significant differences from CK ($P < 0.01$). When the saline-alkali concentration was 365 mmol·L^{-1}, L12 and L39 could not germinate. Under the 219 mmol·L^{-1} saline-alkali treatment, the average germination rate and germination potential of the L6 and L7 varieties were 93.75% and 91.25%, respectively, showing increases of 6.25% and 16.25%

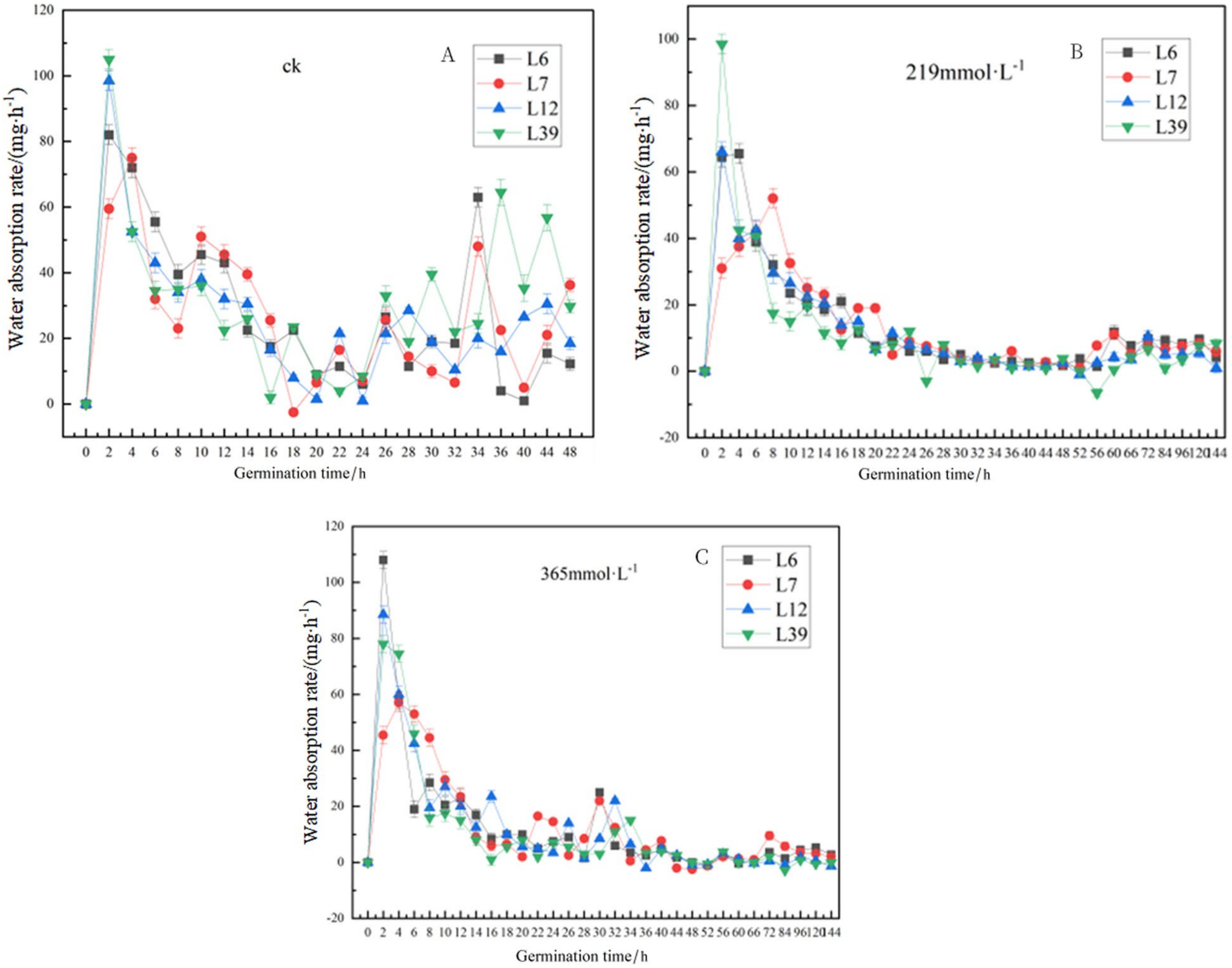

Fig. 3 Changes in the water absorption rate of cotton seeds under different saline-alkaline concentrations

compared withthe L12 and L39 varieties, whereas the mean germination time decreased by 35.8%. No significant difference in the germination rate was found in L6, L7, L12, or L39 under CK; according to the analysis of the cotton seed germination index, there were significant differences between the mixed saline-alkali stress levels and varieties. The interaction effect between the mixed saline-alkali stress level and cotton variety was extremely significant ($P < 0.01$).

Root characteristics of cotton at the germination stage

The root indices of cotton following seed germination were subjected to analysis of variance ($F < 3$, $P < 0.05$). The results (Table 3) revealed that the root length, root diameter, root area, and root volume of the cotton varieties showed very significant differences among the mixed saline-alkali treatments at various concentrations, and the root length, root area, and root volume showed extremely significant differences among the varieties. In terms of the root length, root diameter, root area, and root volume of cotton, the differences in the interaction effects among the varieties and concentration treatments were extremely significant, indicating that saline-alkali stress had an obvious inhibitory effect on the root system of cotton and that, under saline-alkali stress, the root indices of cotton plants with different high saline-alkali tolerances presented significant differences in resistance to saline-alkali stress.

The results (Table 3) revealed that with increasing saline-alkali concentrations, the root length, diameter, surface area, and volume of the same cotton variety significantly differed. With increasing mixed saline-alkali concentration to 365 mmol·L^{-1}, the root length, diameter, surface area, and volume of the four cotton varieties decreased significantly by an average of 90.1%, 58.3%, 86% and 88%, respectively, compared with CK (the clean water treatment). The saline-alkali stress had a more pronounced effect on the radicle length, indicating

Table 2 The effect of saline-alkaline stress on the germination indices of cotton seeds

Variety	Treatment / (mmol·L^{-1})	Germination rate /%	Germination potential/%	Germination index	MTG	Relative salt damage rate
L6	0 (CK)	97.5 ± 5a	95.0 ± 5.8ab	23.7 ± 2.1e	5.8 ± 0.1c	-
	219	95.0 ± 5.8a	95.0 ± 5.8ab	17.0 ± 1.3 cd	6.1 ± 0.1bc	2.5e
	365	45.0 ± 7.1c	1.3 ± 0.5d	5.0 ± 1.0f	2.5 ± 0.4d	54.0c
L7	0 (CK)	97.5 ± 5a	95.0 ± 5.8ab	18.7 ± 2.6bc	6.1 ± 0.2bc	-
	219	92.5 ± 9.7ab	87.5 ± 15abc	15.4 ± 1.3de	6.4 ± 0.2ab	5.0e
	365	36.3 ± 6.3c	1.25 ± 0.5d	4.2 ± 1.5f	2.5 ± 0.4d	62.9b
L12	0 (CK)	100.0a	82.5 ± 9.6bc	19.9 ± 1.7b	6 ± 0.1bc	-
	219	82.5 ± 9.6b	75.0 ± 12.9c	13.5 ± 1.5e	6.2 ± 0.2b	17.5d
	365	-	-	-	-	100.0a
L39	0 (CK)	100.0a	97.5 ± 5a	23.8 ± 1.1a	5.8 ± 0.1c	-
	219	92.5 ± 9.6ab	75 ± 12.9c	13.8 ± 0.6e	6.6 ± 0.2a	7.5e
	365	-	-	-	-	100.0a
Source of variation	C	***	***	***	***	***
	V	***	***	***	***	***
	C×V	***	***	***	***	***

Lowercase letters indicate significant differences within a column ($P < 0.05$), *, **, and *** indicate significant ($P < 0.05$), extremely significant ($P < 0.01$) and very significant ($P < 0.001$), respectively

"-" indicates that no germination was observed

that it inhibits radicle elongation, thereby reducing the root surface area and volume, which enhanced the resistance to the saline-alkali stress. At the concentration of 256 mmol·L^{-1}, the root length, diameter, surface area, and volume of L6 and L7 decreased by an average of 60.4%, 46.1%, 79.8%, and 88%, respectively, compared with CK levels. In contrast, the reductions of L12 and L39 were more substantial, averaging 79.8%, 67.7%, 86.1%, and 89.7%, respectively. These results indicated that the saline-alkali stress mainly reduced the water absorption area of L12 and L39 plants by inhibiting the increase in root length and diameter, resulting in poor saline-alkali tolerance. Under the 365 mmol·L^{-1} mixed saline-alkali stress, the root volume of the L6 and L7 varieties decreased by 93.4% and 96.9%, the root diameter decreased by 43.5% and 60.6%, and the root length decreased by 79.4% and 79.9%, respectively, compared with CK. These findings indicate that under the saline-alkali stress, cotton plants maintain normal physiological activities mainly by altering their root diameter, thereby reducing the damage caused by saline-alkali stress.

Storage substances of cottonseeds during germination

Figure 4 shows that the initial total sugar content of L6 was 13.5% greater than that of L39. Under mixed saline-alkali conditions, the total sugar content of the different cotton varieties first decreased but then increased with germination time. With increasing cotton seed germination time, the total sugar content of L6 was consistently greater than that of L39, and the difference in sugar content between L6 and L39 was obvious at 6 h, reaching 18 mg·g^{-1}. Under the 219 mmol·L^{-1} mixed saline-alkali stress, an increase in the total sugar content occurred at 72 h, which was 48 h later than when the increase occurred in CK, indicating that saline-alkali stress inhibited the normal development of the cotton seeds. Under the 365 mmol·L^{-1} mixed saline-alkali stress, both the L6 and L39 cotton varieties presented a time-dependent decrease in total sugar content. Specifically, in the L6 variety, total sugar consumption followed the pattern CK (0 mmol·L^{-1}) > 219 mmol·L^{-1} > 365 mmol·L^{-1} during the initial 4-h germination period. Notably, no increase in total sugar content was observed throughout the germination process under stress conditions. These results suggest that saline-alkali-tolerant cotton varieties may adapt to stress conditions by modulating sugar metabolism, particularly through reduced hydrolysis rates for total sugars, as a potential stress-coping mechanism.

Figure 5 shows that the initial protein content of L6 was 37.4% higher than that of L39. Under mixed saline-alkali conditions, as the cotton seeds germinated, the protein content first decreased but then increased, and the protein content of the L6 and L39 varieties during germination was lower than the initial value. Under CK conditions, with increasing germination time, the protein content of L6 and L39 decreased sharply. L6 presented the lowest value of 90 mg·g^{-1} at 12 h, which was 64.57% lower than the initial value. L39 showed the lowest

Table 3 The effects of saline-alkali stress on protein content in cotton seeds

Treatment / ($mmol \cdot L^{-1}$)	Variety	Length / mm	Diameter / mm	Area / (mm^2)	Volume / (mm^3)
CK	L6	26.26a	0.14a	12.11a	0.44a
219		18.56b	0.05b	3.39b	0.04b
365		5.40c	0.08c	1.40c	0.02c
CK	L7	19.89a	0.19a	12.21a	0.59a
219		8.56b	0.15b	4.05b	0.15b
365		3.99c	0.07c	0.96c	0.01c
CK	L12	26.15a	0.16a	13.76a	0.57a
219		11.91b	0.14d	5.24b	0.18b
365		-	-	-	-
CK	L39	22.75a	0.16a	11.65a	0.47a
219		7.89b	0.07b	1.81b	0.03b
365		-	-	-	-
Average across varieties					
L6		16.74a	0.09 cd	5.64c	0.17b
L7		10.81c	0.14a	5.74b	0.25a
L12		12.60b	0.10b	6.33a	0.25a
L39		10.21d	0.07d	4.49d	0.16b
Average across saline-alkaline treatments					
CK		23.76a	0.14a	9.74a	0.38a
219		11.73b	0.11b	5.66b	0.21b
365		2.35c	0.05c	1.36c	0.04c
Significance test between factors					
Variety		***	*	***	***
Concentration		***	***	***	***
Variety × Concentration		***	*	*	***

Lowercase letters indicate significant differences within a column ($p < 0.05$), *, ** and *** indicate significant ($p < 0.05$), extremely significant ($p < 0.01$) and very significant ($p < 0.001$), respectively

"-" indicates that no germination was observed

value of 80 $mg \cdot g^{-1}$ at 4 h, which was 47.7% lower than the initial value, and the value then increased slowly to 123 $mg \cdot g^{-1}$. During germination under the 219 $mmol \cdot L^{-1}$ mixed saline-alkali stress, the inflection points of L6 and L39 both moved to 48 h, and the protein content of L6 was 34 $mg \cdot g^{-1}$ higher than that of L39. During germination under the the 365 $mmol \cdot L^{-1}$ mixed saline-alkali conditions, the protein contents of L6 and L39 were significantly different, and the protein content of L39 fluctuated between 108 and 149 $mg \cdot g^{-1}$. At 48 h, the protein content of the L6 variety during germination under the the 365 $mmol \cdot L^{-1}$ mixed saline-alkali conditions was 1.87% and 37.42% greater than that under the CK and the 219 $mmol \cdot L^{-1}$ mixed saline-alkali conditions, respectively, indicating that with the increase in the saline-alkali concentration to a certain extent, the protein content in the cotton plants increased, thereby conferring resistance to damage from adverse conditions.

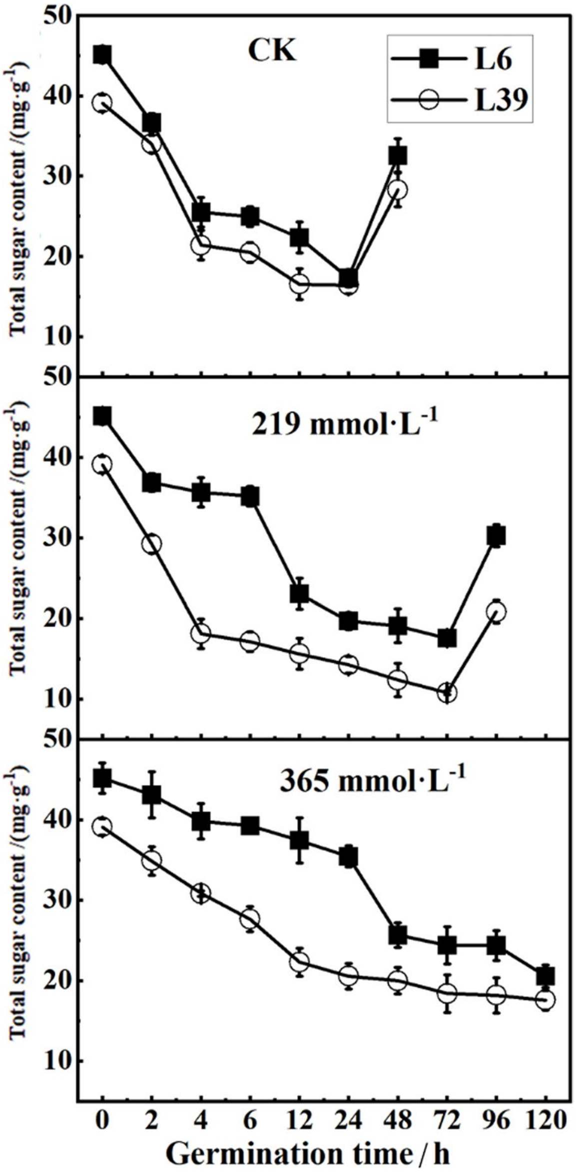

Fig. 4 The effect of saline-alkali stress on the total sugar content in cotton

The results (Fig. 6) revealed that the crude fat concentration in L6 embryos was 5% greater than that in L39 embryos at the beginning of saline-alkali stress. Under germination conditions, the crude fat concentration of L6 was consistently greater than that of L39. Under the 219 $mmol \cdot L^{-1}$ mixed salt and alkali stress, the peak value for L6 was 36.4% at 4 h, and that for L39 was 31.1% at 2 h. Under the 365 $mmol \cdot L^{-1}$ mixed saline-alkali stress, the first peak for L6 was 34.0% at 2 h, and the peak for L39 was 29.8% at 4 h. Within 0–4 h, the crude fat accumulated rapidly, and the concentrations in L6 and L39 were 2.1% and 2.7% higher, respectively, than those in under the 365 $mmol \cdot L^{-1}$ mixed

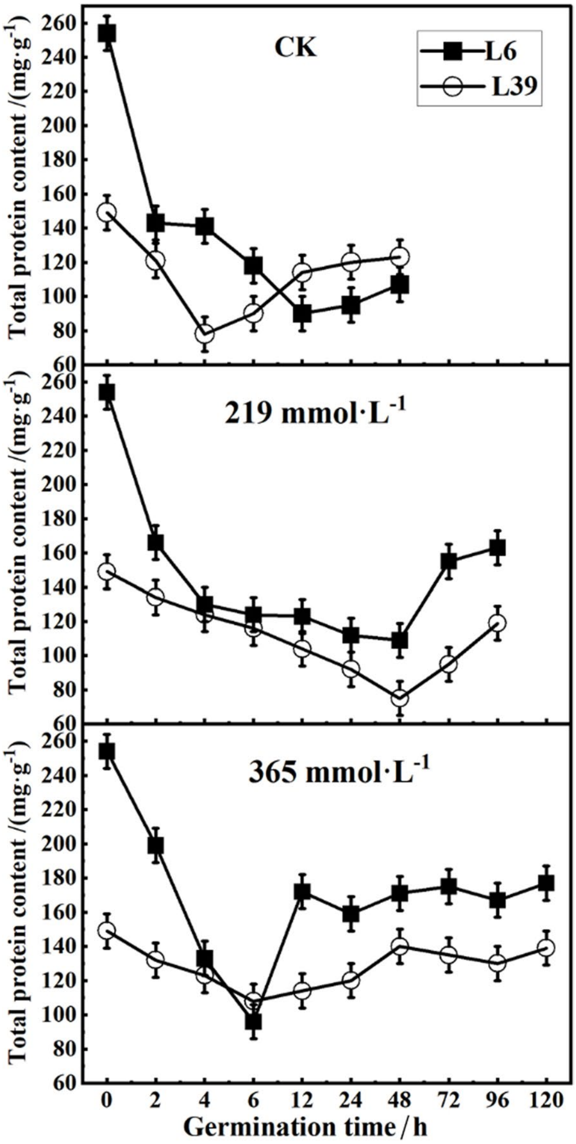

Fig. 5 The effects of saline-alkali stress on protein content in cotton

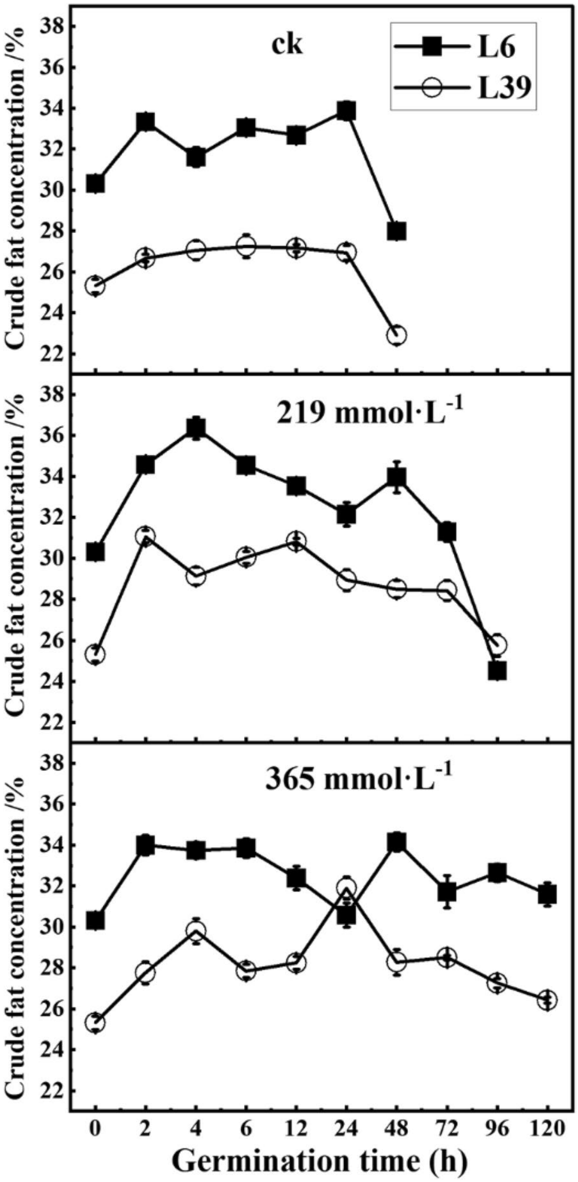

Fig. 6 The effects of saline-alkali stress on the crude fat concentration in cotton seeds

saline-alkali stress. This result indicated that crude fat was made available for subsequent cotton seed germination through early rapid accumulation.

Principal component analysis

Figure 7 shows that under the CK treatment, the germination index of the saline-alkali-resistant varieties was correlated with the root length, root diameter, root volume, root surface area and water absorption rate. Under the 219 mmol·L^{-1} treatment, the germination rate of the salt-tolerant varieties was significantly correlated with the germination potential, relative water absorption, total sugar content and MTG index of cotton. Under 365 mmol·L^{-1} mixed saline-alkali stress, the relative salt damage of the salt-tolerant varieties was correlated with the crude fat, total protein and storage substance contents. These results indicate that, under high saline-alkali stress, highly saline-alkali-tolerant varieties can resist saline-alkali damage to ensure normal germination by producing new storage substances (protein, total sugar, and crude fat) and altering their contents. Highly saline-alkali-tolerant varieties could be suitable for cultivation under saline-alkali stress conditions.

The first sorting axis explained 84.6% of the physiological indices, and the second sorting axis explained 15.4%

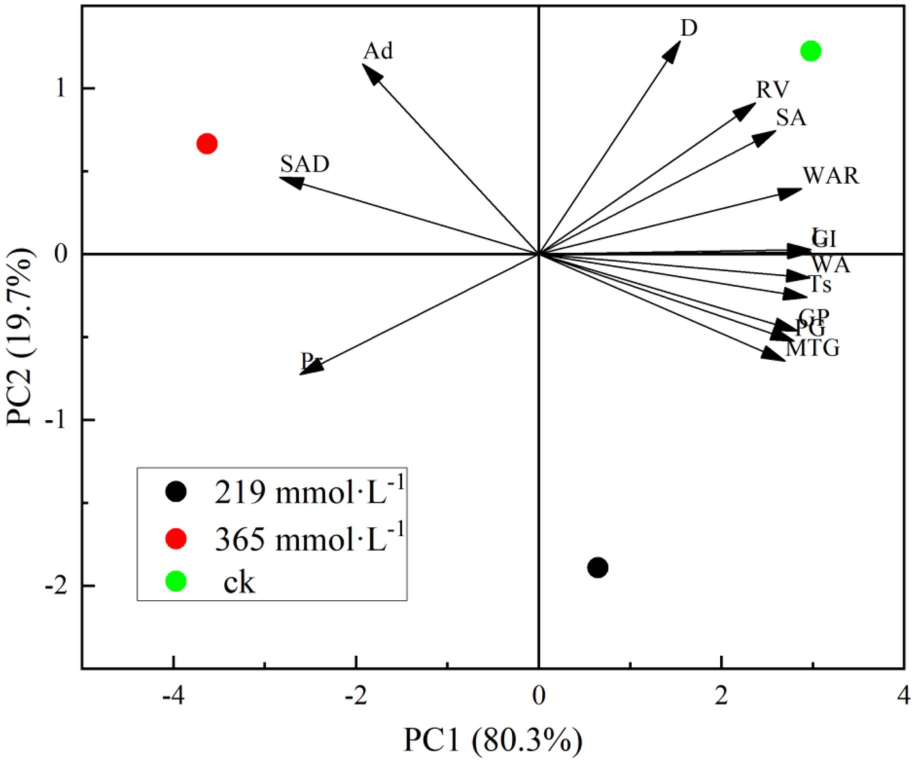

Fig. 7 Principal component analysis of highly saline-alkali-resistant cotton seeds under saline-alkali stress. GP, germination rate; PG, germination potential; GI, germination index; L, root length; D, root diameter; SA, root surface area; RV, root volume; WAR, water absorption rate; WA, relative water absorption; SAD, relative saline-alkali damage; Ad, crude fat; Ts, total sugar; Pr, total protein

of the physiological indices (Fig. 8). These two axes can be considered the main component axes. Under the CK treatment, the saline-alkali-sensitive varieties promoted germination mainly by increasing water absorption, root volume, root surface area, root length, root diameter and total sugar content. Under the 219 mmol·L^{-1} mixed saline-alkali treatment, the saline-alkali-sensitive varieties resisted saline-alkali stress mainly by producing crude fat at relatively high concentrations. Because fat is not an osmoregulatory substance, it cannot guarantee osmotic balance in vivo. Under 365 mmol·L^{-1} mixed saline-alkali stress, saline-alkali damage was reduced by changes in the protein and crude fat concentrations, but the plants of the saline-alkali-sensitive cotton varieties were close to death and could not rely on physiological changes to resist the external environment and maintain their own growth.

Discussion

The active absorption of water is the basis of seed germination under salt and alkali stress (Fang et al. 2017). The water absorption process of seeds enables the necessary material exchange between the seeds and the external environment. In this study, under 365 mmol·L^{-1} mixed saline-alkali stress, the germination rate of the four cotton varieties during the first three days was 0 (Fig. 9), indicating that under 365 mmol·L^{-1} mixed saline-alkali stress, it was difficult for the cotton varieties to absorb water at the early stage of germination, and the water potential of the seeds increased. The water absorption rate slowed or was inhibited entirely, and the seeds temporarily entered a shallow dormancy state to resist damage from mixed saline-alkali stress (Li et al. 2018). Lei et al. (2016) reported that with increasing saline-alkali concentrations, damage to seed water absorption increased, and seed germination was inhibited. This study revealed that with increasing mixed saline-alkali concentration, the relative water absorption, water absorption rate and germination characteristics of cotton decreased, indicating that saline-alkali stress inhibited the active absorption of water by cotton, thereby impairing germination.

The ability of seeds to germinate is the basis of plant survival (Rajjou et al. 2012). The germination rate, germination potential and germination index are generally used to evaluate seed germination ability. These indices not only reflect seed germination ability but also indicate the strength of seed resistance to salt and alkali stress (Zhu and Gao 2002). Many studies have shown

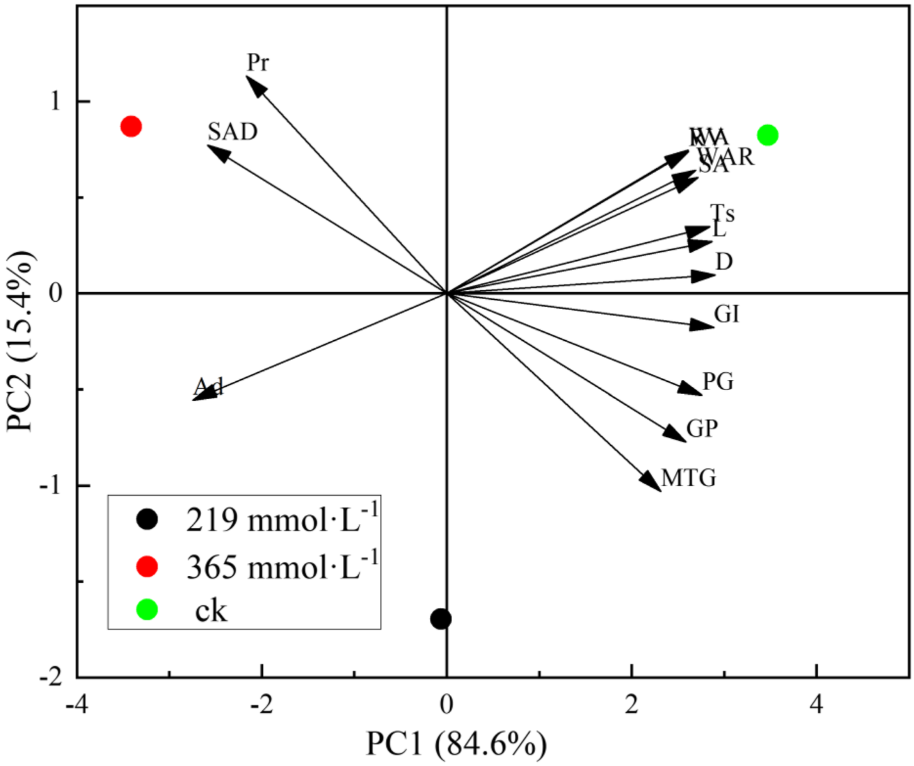

Fig. 8 Principal component analysis of saline-alkali-sensitive cotton seeds under saline-alkali stress. GP, germination rate; PG, germination potential; GI, germination index; L, root length; D, root diameter; SA, root surface area; RV, root volume; WAR, water absorption rate; WA, relative water absorption; SAD, relative saline-alkali damage; Ad, crude fat; Ts, total sugar; Pr, total protein

that high concentrations of saline-alkali stress inhibit the seed germination ability, delay the germination time, and even lead to seed death (Zhang et al. 2018). Some studies have also shown that appropriate saline-alkali stress promotes seed germination (Li et al. 2019a). This study revealed that the germination indices of different cotton varieties were reduced under mixed saline-alkali conditions. When the mixed saline-alkali concentration was increased to 365 mmol·L^{-1}, L12 and L39 could not germinate, while the L6 and L7 varieties could still germinate into seedlings. The salt-sensitive genotypes varieties (L12 and L39) presented markedly low saline-alkali stress tolerance. The damage threshold was quantified at 365 mmol·L^{-1}, which further verified that L6 and L7 are highly saline-alkali-tolerant varieties.

The growth and development of plant roots are closely related to the whole growth process. Roots are the medium linking soils and crops. Nutrients and water in soil are transported to plants through roots. One study revealed that the root length, average root diameter, root volume and surface area of cotton decreased with increasing NaCl concentration (Zhang and Yi 2014). In this study, the root-related indicators decreased with increasing saline-alkali concentration, indicating that mixed saline-alkali stress inhibited the root growth and development of the cotton seedlings. Studies have shown that to prevent excessive accumulation of salt, cotton plants allocate more resources to their roots to alleviate the inhibition of root growth and improve the ability of roots to resist adversity (Egamberdieva et al. 2017; Tian et al. 2025). This study confirmed that the root diameter of the highly saline-alkali-tolerant variety Xinluzhong 82 under 365 mmol·L^{-1} mixed saline-alkali stress increased by 60% compared with that under 219 mmol·L^{-1} mixed saline-alkali stress, indicating that salt-tolerant cotton genotypes exhibit distinct morphological adaptations to saline-alkali stress. Unlike sensitive varieties, tolerant augment root diameter growth, increasing their ability to resist saline-alkali stress. Seed storage materials provide materials and energy for seed germination through respiration and hydrolysis during seed germination (Ming et al. 2018). Soluble sugars and soluble proteins are important osmotic regulators in plants, and their contents directly affect the resistance of crops (Javadipour et al. 2013). The results showed that the total sugar content, protein content and crude fat content of the highly saline-alkali-tolerant varieties were consistently greater than those of the saline-alkali-sensitive varieties under

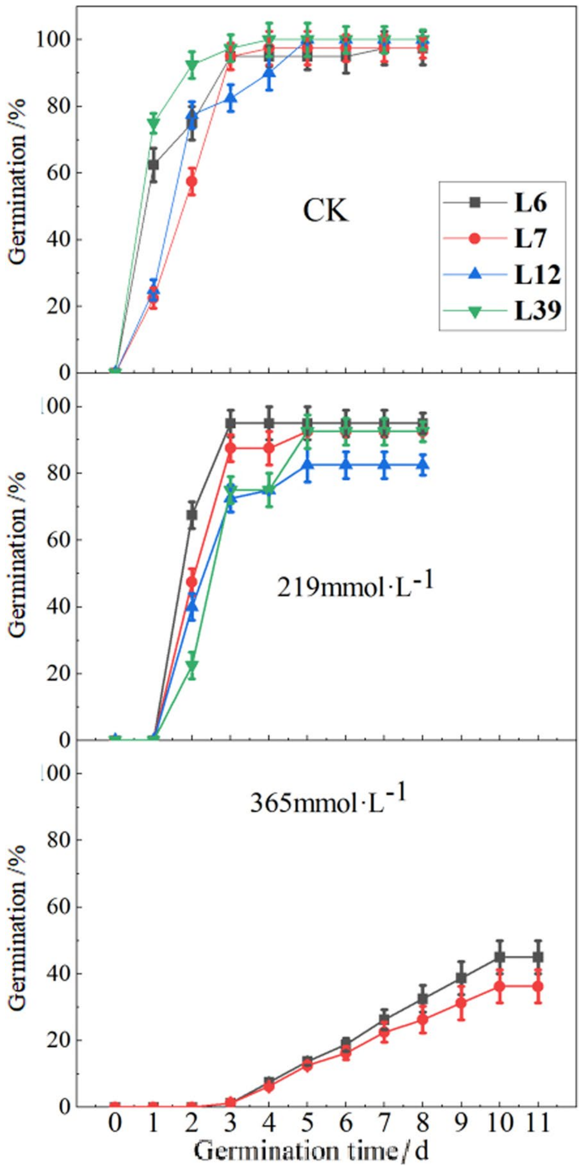

Fig. 9 Changes in the cotton seed germination rate under saline-alkali stress

high saline and alkali stress, which was due to the damaged osmotic adjustment function of soluble protein and soluble sugar in the sensitive saline-alkali cotton varieties under saline-alkali stress (Lin et al 2018), resulting in a decrease in their contents; this finding indicates that the total sugar and protein contents determine the ability of plants to resist saline-alkali stress. It was previously reported (Ashraf et al. 2018; Sun et al. 2025) that under saline-alkali stress, soluble sugars are not only acts as osmotic regulators but also serve as energy sources for the synthesis of other organic solutes. The results revealed that the hydrolysis rate for total sugars decreased in seeds germinated under 219 mmol·L^{-1} mixed saline-alkali stress, which provided the ability for seed germination and maintained normal growth and development, indicating that reducing the hydrolysis rate for total sugars could help seeds resist damage from adverse conditions. The protein content of the saline-alkali-sensitive varieties decreased under 365 mmol·L^{-1} mixed saline-alkali stress, indicating that the cell membrane system of L39 may have been damaged due to saline-alkali stress, resulting in the inhibition of protein hydrolysis and amino acid formation and leading to the synthesis of new substances so that L39 could not germinate. Therefore, highly saline-alkali-tolerant cotton varieties present lower hydrolysis rates for total sugars and proteins during germination than do sensitive varieties, which is correlated with increased internal stability under saline-alkali stress.

Conclusion

The 365 mmol·L^{-1} mixed saline-alkali concentration strongly inhibited the germination of saline-alkali-sensitive cotton seeds, and the germination rate, germination potential and germination index of the saline-alkali-sensitive cotton seeds were all 0; that is, the relative salt damage rate reached 365 mmol·L^{-1}. The increase in mixed saline-alkali stress caused the physiological and morphological indices of the cotton seeds to decrease. Under 365 mmol·L^{-1} saline-alkali stress, the water absorption capacity and root morphological indices of the cotton varieties (L6, L7, L12, and L39) were reduced by an average of 29.6%–43.8% compared with those of the control group, whereas the crude fat and protein contents were increased by an average of 7.6% and 22.7%, respectively. The water absorption characteristics, root morphology indices and storage substance contents of the highly saline-alkali-tolerant varieties were 12.6%–24.8% greater than those of the saline-alkali-sensitive varieties. With increasing amounts of mixed saline-alkali stress, highly saline-alkali-tolerant varieties at the seed germination stage maintained the osmotic balance of their seeds by reducing the amount of water absorbed and the hydrolysis rates for total sugar and total protein during the germination process, which improved their ability to resist saline-alkali stress. When young roots break through the seed coat, highly saline-alkali-tolerant varieties increase the absorption of exogenous water by changing the related indices of the root characteristics, thus exhibiting greater resistance to saline-alkali conditions. For Xinjiang's farmers, prioritizing tolerant varieties (Xinluzhong 82/Xinluzhong 68) could prevent total germination failure in moderate-salinity soils. Theoretically, the biphasic resistance model challenges single-trait breeding paradigms, advocating for synergistic selection of osmotic regulators (sugar/protein conservation) and root architects.

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Authors' contributions
Chen Y: Data curation, formal analysis, writing-original draft preparation, funding acquisition and writing–review and editing; Duan JB: field experiment, data curation, review and editing; Meng FX, Gao N, Yi Q, Jiao TQ, and Zhang HX: field experiment, data curation, formal analysis and investigation; Luo HH and Tian LW: conceptualization and supervision. All the authors have read and approved the final manuscript.

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Data availability
All the data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflicts of interest.

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