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Growth and physiological characteristics of forage bermudagrass in response to salt stress

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Abstract

Background Bermudagrass (*Cynodon dactylon*) has a long history as an excellent forage grass, and salt stress will inhibit its growth and development. In order to minimize the damage, it is necessary to continuously develop innovative technologies and management strategies.

Results This study evaluated the salt tolerance of new Bermudagrass strains 'FB2019R101' and 'FB2019R105' compared to commercial varieties 'Wrangler' and 'A12359' under simulated soil salinity conditions through seawater irrigation. Through correlation analysis of growth, physiological, and nutritional indicators, and principal component analysis, core indicators and weights for salt tolerance evaluation were identified. The salt-tolerant varieties were 'FB2019R101' and 'FB2019R105'. Under salinity stress, the plants of Bermudagrass varieties with salt tolerance suffered less damage as a whole, which could better regulate the osmotic balance inside and outside cells, accumulate more nutrients and have stronger ability to resist salt damage. The expression level of salt-tolerant variety *CdCINV1*, *CdSPS1*, *CdSUS5*, and *CdSWEET6* was up-regulated under salt stress. *CdCINV1*, *CdSPS1*, *CdSUS5* can promote the transformation of sucrose into glucose and fructose in Bermudagrass under salt stress, and *CdSWEET6* can promote the accumulation of fructose.

Conclusions 'FB2019R101' and 'FB2019R105' exhibited higher salt tolerance, with minimal impact on their biomass, physiological, and nutritional indicators under salt stress. The comprehensive evaluation revealed a salt tolerance ranking of 'FB2019R105' > 'FB2019R101' > 'Wrangler' > 'A12359'. This study provides significant reference for the bioremediation of coastal saline soils and promotes research on the application of Bermudagrass under salt stress conditions. *CdCINV1*, *CdSPS1*, *CdSUS5*, and *CdSWEET6* can improve the salt tolerance of plants by regulating the changes of carbohydrates.

Keywords *Cynodon dactylo*n, Salinity stress, Comprehensive evaluation, Forage quality, Physiological characteristics

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Introduction

Soil salinization is becoming increasingly serious. In China, the area of salinized land is approximately 76 million hectares [1], with coastal saline-alkali land playing a significant role. With economic growth and the intensification of industrial pollution, coupled with the diversification of agricultural production methods, the area of salinized land in China is continuously expanding. These salinized lands almost do not support vegetation growth, severely hindering the sustainable development of agriculture [2]. Currently, the Shan Dong province has more than 600,000 hectares of saline-alkali land, including over 200,000 hectares of undeveloped saline wasteland, mainly distributed in the cities of Dongying, Binzhou, Weifang, and Dezhou, especially in Dongying and Binzhou [3]. Through the development and utilization of saline-alkali land for the planting of Bermudagrass and implementing strategies for the management of saline-alkali land aimed at supplementing arable land, a large area of saline-alkali land has been used and transformed into arable land. This has formed an industrial chain from planting and transformation to sales, providing necessary support for provincial economic and social development.

High salt concentrations cause ion toxicity (particularly sodium ions), osmotic stress, and oxidative damage, which are the main stresses that plants face [4]. This high concentration of salt leads to a decrease in the external environment's osmotic potential, hindering plant water absorption, causing cell dehydration, disrupting normal physiological and biochemical reactions within the cell, disturbing ion balance, and ultimately resulting in plant damage. To adapt to the salt stress environment, plants ensure survival through adjustments in morphology, physiological biochemistry, and genes [5, 6]. Intracellular ion homeostasis is the basic mechanism of plant survival under salt stress [7], the absorption and transport of potassium ions by plants will be inhibited by sodium ions under high salt conditions [8]. To counteract ion imbalance, plants adjust the ratio of sodium to potassium ions in their cells, by transporting sodium ions to vacuoles or expelling them to the apoplast, and reduce the loss of potassium ions to maintain cell ion balance [9]. A previous study demonstrated that the maintenance of a higher K^+/Na^+ ratio was positively correlated with salt tolerance in different plant species [10].

Faced with osmotic stress caused by high salt, plants increase the synthesis of osmotic regulation substances such as carbohydrate [11] and proline [12]. Carbohydrate plays an important role in life, especially in plants. It is found that plant carbon metabolism pathway is closely related to salt tolerance [13, 14]. Sucrose, soluble sugar, glucose, fructose, etc. are abundant carbohydrate in plants, which play various roles in mediating stress tolerance, such as carbon storage, osmotic protection, osmotic stability and free radical scavenging, and can also be used as signal molecules to participate in regulating gene expression [15]. Improvement in salt tolerance via enhanced carbohydrate biosynthesis and metabolism has been reported in many plant species such as wheat [16] and quinoa *(Chenopodium quinoa Willd.)* [17], and rice [18–20] and alfalfa *(Medicago sativa L.)* [21] and Arabidopsis thaliana [22].

Bermudagrass (*Cynodon dactylon*), belonging to the Poaceae family, is both a high-quality turfgrass and forage, renowned for its wide adaptability, strong invasive capacity, and trampling resistance, and is particularly noted for its significant salt tolerance, making it a key plant in improving saline-alkali soil and accelerating the soil desalination process [23]. There is a wide variety of genetic resources in Bermudagrass, with significant differences in salt tolerance among different genotypes. Research by Li et al. found that the salt-tolerant varieties 'Cd026' and 'Cd032', compared to the salt-sensitive variety 'Cd013', could accumulate more chlorophyll and biomass [24]. Studies by Hu et al. and others also indicated that the sodium-potassium ratio significantly increased in the salt-sensitive variety 'C198' compared to the salt-tolerant variety 'C43' [25]. Therefore, evaluating the salt tolerance of Bermudagrass varieties and selecting high salt-tolerance germplasm is of great importance for the effective use and improvement of salinized soils [26]. Identifying or developing salt-tolerant germplasm or cultivated varieties is one of the most effective strategies for mitigating salinity damage to crop productivity [27].

Salt tolerance is a complex trait and possesses species-specific regulatory effects and mechanisms in the plant kingdom [28]. At present, the research on salt tolerance of Bermudagrass mostly uses single salt to simulate salt stress, while the research on direct use of seawater for stress is relatively rare. Considering that the salt composition of coastal saline-alkali soil in China is similar to that of seawater [29], the study of plant salt tolerance needs to consider multi-level factors such as genotype, growth stage, physiological and biochemical reactions and environmental factors, and it is difficult to comprehensively evaluate salt tolerance with a single index. In recent years, the screening of salt-tolerant germplasm resources increasingly relies on multi-index comprehensive evaluation methods, such as principal component analysis, membership function method and regression analysis [30]. In this study, seawater irrigation was used to simulate salt stress, and the changes of physiological indexes such as biomass, chlorophyll, proline, soluble sugar and sucrose, and quality indexes such as crude fiber, crude protein, crude fat and crude ash of different bermudagrass varieties under salt stress were comprehensively investigated. Based on these core evaluation indexes, the salt tolerance of four Bermudagrass varieties

was comprehensively evaluated, and a preliminary evaluation system was established to screen salt-tolerant Bermuda grass varieties suitable for improving coastal saline soil, which provided a theoretical basis for further exploring the response differences of different varieties to salt stress.

Materials and methods

Material

The experimental materials are the new salt-tolerant lines 'FB2019R101' and 'FB2019R105' (hereinafter referred to as 'R101' and 'R105') of Bermudagrass bred by Professor Fu Jinmin, the commercial variety 'Wrangler' and the line 'A12359' which was sequenced by our research group. The research group has cultivated seven new feed Bermudagrass varieties, 'FB2019R101'~'FB2019R107'. Through previous variety comparison experiments, it was found that among these seven new varieties, 'FB2019R101' and 'FB2019R105' are the best varieties with the best growth, the highest yield and the best overwintering rate, while Wrangler is a widely used commercial feed bermudagrass variety developed from the United States [31]. 'A12359' is a variety that has been analyzed by GWAS and sequenced by the whole genome.

Bermudagrass varieties (lines) were collected in Coastal Grass Planting Resource Technology Center Base of Ludong University in Yantai City, China (121°21′E, 37°31′N) in May 2022. After evenly separating the stems of 1/2 lawn of Bermudagrass with a hoe, evenly burying them in the shallow soil layer in the salt pond with a shovel, filling the salt pond with soil in advance and loosening the soil, watering it with tap water every week after planting, and applying compound fertilizer once a month according to the application rate of 20 kg/ mu (about 30 g/m^2).When all varieties (lines) grow evenly and completely cover the soil surface, all the grass in the salt pond will be trimmed to a uniform height of 6 cm, ready to start treatment. The site belongs to temperate continental monsoon climate, and the soil type is loessial soil. During the whole experiment, the average temperature is 25.9 °C and the average precipitation is 180.9 mm.

Experimental design

The experiment aims to simulate the irrigation management strategies of artificial pastures to the greatest extent, and lasts for 40 days from July 10 to August 18, 2023. Adoptting a completely random block design, as shown in Fig. 1, and set up two treatments: a control

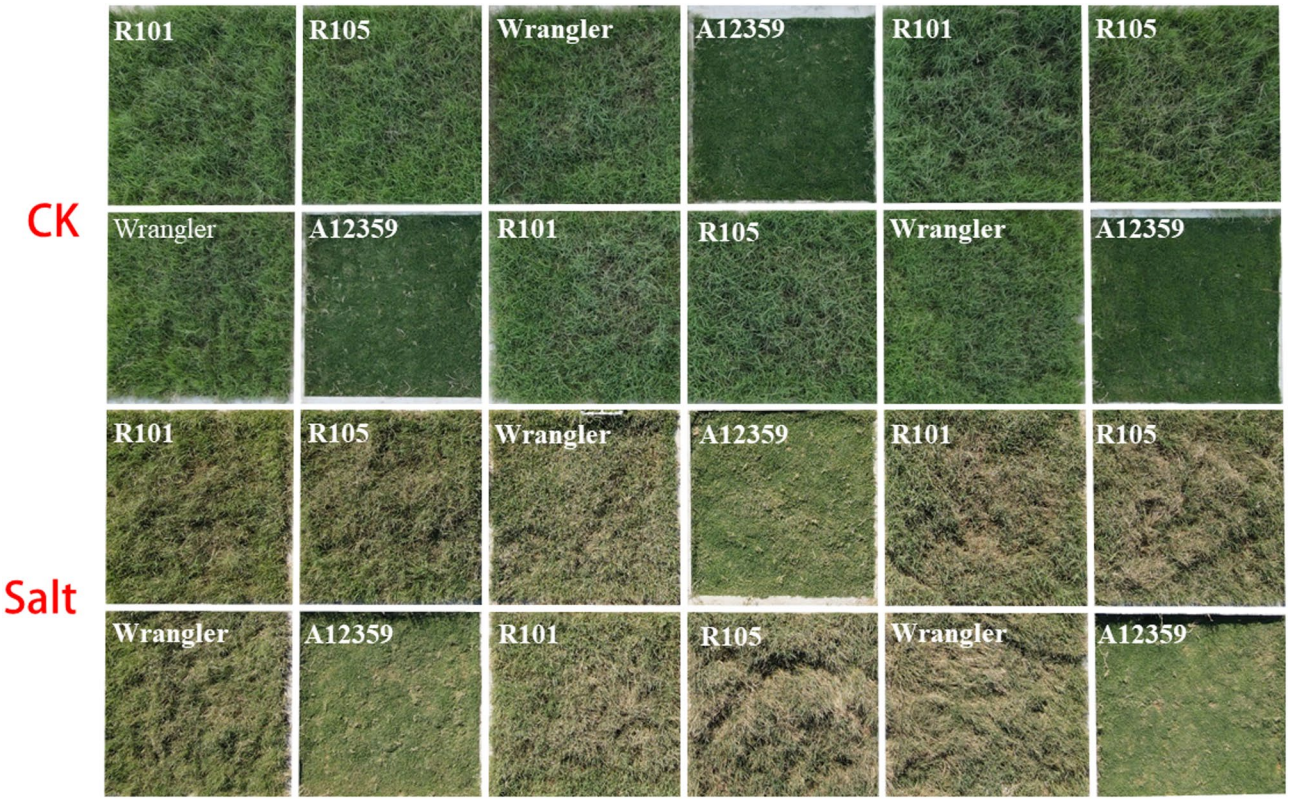


Fig. 1 Phenotypes of four varieties of bermudagrass under salinity stress. 'R101' and 'R105' indicate the varieties 'FB2019R101' and 'FB2019R105' respectively, similarly for the following tables. The photo was taken at the end of salinity treatment on August 18th, 2023, which was the 40th day of salt treatment. The picture shows the distribution map of bermudagrass varieties (lines) designed according to random blocks. The first two lines are the control group, and the third and fourth lines are the salt group. The pipes of the control group and the salt treatment group are not connected with each other

group (CK/ tap water irrigation, soil salinity 0%, the EC is about 3 dS/m) and a salt treatment group (salt/seawater irrigation, soil salinity 1.5%, the EC is about 245 dS/m). By irrigating seawater into the salt ponds of the salt treatment group, the salt content in the soil can reach the corresponding concentration, and at the same time, the same amount of tap water is irrigated to the control group. Each treatment used three salt ponds as three repetitions, making a total of 24 salt ponds. Each salt pond was a cement pond with an area of 4 m^2 (2 m ×2 m) and a depth of 2 m. The bottom of each salt pond was connected with a sewer pipe, so that excess water in the soil in the salt ponds can flow out. The pipes between the salt ponds of the control group and the salt treatment group were not connected with each other, and the salt in the salt treatment group can not be lost to the control group. Salt stress treatment started from 0.5% salt concentration, increased to 1.0% every week, and then increased to about 1.5%. When it was necessary to supply seawater to maintain the salt content, the control group was also irrigated with an equivalent amount of tap water. Once the percentage of withered leaves of Bermudagrass in the salt treatment group was 50%, all plots were harvested uniformly. The salt in the salt treatment needed in the experiment came from seawater, which was purchased from Chengzhi seawater monopoly in Yantai City. To reduce experimental errors, the grass in the residential isolation belts of the plots was pruned weekly during the experiment period.

Measurements

Soil salinity in the salt stress group was monitored and recorded every three days using a salinity meter (Delta-T HH2). Forty days after the treatment ended, measurements including plant height, the percentage of withered leaves, chlorophyll content, and above-ground fresh weight were taken. Plant height was measured with a ruler, chlorophyll content was calculated using a UV spectrophotometer and dimethyl sulfoxide extraction method [32], and above-ground fresh weight was measured with an electronic scale. For each treatment, a quantitative sample of the whole plant was taken, immobilized at 105 °C for 30 min, and then dried at 70 °C to constant weight, and then measured the dry weight, and repeated three times.

Soil salt content

Soil salinity in the salt stress group was monitored and recorded every three days using a salinity meter (Delta-T HH2).

Estimation of shoot height, fresh and dry weight of shoot

Shoot height was recorded from 40-day-old bermudagrass using a meter scale. Bermudagrass in all plots was harvested by using a lawn mower, and the fresh weight was measured by using an ordinary electronic balance, which was calculated as W1, which was the fresh weight of the above-ground part of the sample in the plot. Then ten plants were randomly selected to measure their fresh weight, and recorded as W2; The fresh sample of W2 was killed out in an oven at 105°C for half an hour, then dry it at 75°C to constant weight, and measure its dry weight with a one thousandth balance after cooling, and record it as W3; According to the formula of tool dry weight (kg)=(W3/W2)×W1, the fresh weight and dry weight yield per hectare were calculated.

Photosynthetic pigments analysis (chlorophyll A, B, total chlorophyll and carotenoid)

Photosynthetic pigments were analyzed following the modified method of Arnon [33] and Lichtenthaler and Wellburn [34]. Immediately after the experiment, 0.1 g of fresh leaves were cut and put into a centrifuge tube, and 10 mL of dimethyl sulfoxide was added, which was left in the dark for 72 h. Then, 1 ml of chlorophyll extract was mixed with 2 ml of dimethyl sulfoxide as the solution to be measured, and dimethyl sulfoxide was used to zero, and the absorbance at the wavelengths of 663 nm, 645 nm and 440 nm was measured by ultraviolet spectrophotometer (UV-1700). The calculation formula was:

Chlorophyll A content (mg/g) = [12.72×A663-2.59×A645]×0.3;

Chlorophyll B content (mg/g) = [22.88×A645-4.67×A663]×0.3;

Total chlorophyll (mg/g) = chlorophyll a + chlorophyll b;

Carotenoid content (mg/g) = [4.7×A440-0.27× (CHL A + CHL B)]×0.3.

Relative water content (RWC) determination

We used the method of Weatherley [35] with some modifications to determine the relative water content (RWC). Briefly, about 0.15 g of leaves was mixed evenly and the fresh weight (FW) of each sample was measured immediately after harvest. Then, the samples were incubated for 12 h on a shaker in tubes containing 10 mL water at 30 °C. Following the determination of turgid weight (TW), samples were dried at 80 ∘C for 72 h to determine the dry weight (DW).

$$\begin{aligned} &\mathrm{RWC}\,(\%)\ \text{was calculated as} \\ &((\mathrm{FW}-\mathrm{DW})/(\mathrm{TW}-\mathrm{DW}))\times 100\% \end{aligned} \tag{1}$$

where fresh weight = FW, turgid weight = TW and dry weight = DW.

Proline quantification

We estimated the proline content according to Bates [36] with some modifications. Free proline was extracted

from 0.5 g fresh leaf samples using 3% sulfosalicylic acid (10 mL). The 2 mL extraction volume was mixed with 2 mL of a mixture of glacial acetic acid and acid ninhydrin. After being incubated for 1 h at 100 ∘C, the tubes were placed into ice bath to cool down, 4-mL toluene was added and the upper phase absorbance was measured spectrophotometrically at 520 nm. The free proline was quantified using a standard curve.

Determination of electrolyte leakage (EL)

Electrical leakage (EL) was measured according to Lutts [37] with some modifications. Briefly, after salt stress, 100 mg of leaf tissue were collected from the second youngest leaf of the plants of each treatment. Subsequently, the samples were washed three times with deionized water, cut into (10 mm) pieces, placed in 20 mL distilled deionized water, and incubated on a shaker at 30 ∘C for 24 h. After incubation, The initial conductivity Ci was measured with a conductivity meter, and the maximum conductivity Cmax was measured after sterilization at 120°C for 15 min. Then, the EL (%) was calculated as

$$(\mathrm{Ci} \,/\, \mathrm{Cmax}) \times 100\% \tag{2}$$

Determination of MDA in leaves

According to the methods of Heath and Packer [38] and Ali et al. [39], the status of malondialdehyde (MDA) as an index of lipid peroxidation in Bermuda grass leaf cells was determined. 0.2 g fresh leaf sample was mixed in 2 ml mixed reaction solution of trichloroacetic acid and thiobarbituric acid. After homogenization, the solution was centrifuged at 12,000 rpm at 4 °C for 15 min, and the supernatant was reserved for later use. In addition, 0.5 ml of the supernatant was added to 1 ml of the reaction mixture as a sample to be tested, and the reaction solution was set to zero. The mixed solution was bathed in a 95°C water bath pot for 30 min. Then quickly cooled to room temperature, and centrifuged at 12,000 rpm at 20 degrees Celsius for 30 min. The absorbance of the collected supernatant was measured at 532 and 600 nm(3):

$$\begin{aligned}&\text{Calculation of MDA content } (\mu\text{mol}\cdot\text{g}-1\cdot\text{FW})\\&=\frac{(\text{OD532}-\text{OD600})\times \text{ volume of total mixer}}{\text{Extinction co}-\text{effcient }\left(155\,\text{mM}^{-1}\cdot\text{cm}^{-1}\right)\times \text{ sample weight }(0.2\text{g})}\\&\qquad\frac{(0.5\,\text{mL supernatant}+1\,\text{mL RM})\times 1000}{}\end{aligned} \tag{3}$$

where D532 and D600 denote absorbance reading, and 1000 is used to convert μM from mM of extinction co-efficient.

Carbohydrate content (soluble sugar, sucrose, glucose and fructose)

The contents of soluble sugar, sucrose, glucose and fructose were determined by HPLC, and the glucose content in the sample was determined by HPLC.

Activities of key enzymes in sucrose metabolism

Activities of sucrose phosphate synthase, sucrose synthase and invertase were determined by HPLC, and the glucose content in the sample was determined by HPLC.

Sodium/potassium contents

After the test, take about 0.1 g of dried leaf powder which has been dried to constant weight, weigh and record it, put it in a digester tube, add 10 mL of concentrated sulfuric acid into the digester tube, and then run it in a graphite digester at 420°C for 120 min. After digestion, the digestion tube was cooled to room temperature, and the solution was diluted 10 times. Then, the content of sodium ion and potassium ion in feed Bermuda grass was determined by flame spectrophotometer (Meta-analysis F500, Shanghai), and finally the sodium-potassium ratio was calculated.

Nutritional quality content (crude protein, crude fat, crude fiber, crude ash)

Dry samples were used to determine crude protein, crude fat, crude fiber, and crude ash content. Crude protein content was determined using an automatic Kjeldahl nitrogen analyzer (Hanon K9860, Jinan) with the Kjeldahl method, crude fat content by a fat determination instrument (Hanon SOX406, Jinan) using the hot immersion-gravimetric method, crude fiber content by a crude fiber determination instrument (Hanon F800, Jinan) and muffle furnace (KSL-1200X, Hefei) using the acid-base washing method, and crude ash content by incineration at 580 °C for 3 h. Each treatment was replicated three times to ensure the accuracy and reliability of the data.

Evaluation of Bermudagrass salt tolerance

The salt tolerance coefficient: calculated as the average measured value under different salt concentration treatments divided by the control group's measured value, multiplied by 100%.

Data normalization: The fuzzy mathematical membership function method is applied to normalize the salt tolerance coefficient, converting the measured data using the fuzzy mathematical membership degree formula. The membership function formula is as following:

$$\mathrm{U}\left(\mathrm{X}_{ijk}\right) = \left(\mathrm{X}_{ijk} - \mathrm{X}_{\min}\right)/\left(\mathrm{X}_{\max} - \mathrm{X}_{\min}\right) \tag{4}$$

In formula (4), $U(X_{ijk})$ represents the membership degree of the kth indicator under the jth stress concentration for

the ith variety, with X_{max} and X_{min} being the maximum and minimum values of the kth indicator, respectively.

The *Weighting Coefficient D* value: The coefficient of variation method is used to calculate the standard deviation coefficient V_j with formula (5), and the weights of each salt tolerance indicator W_j are obtained by normalizing with formula (6).

$$Vj = \left[(X_{ij} - \bar{X}_{ij})^2 \right]^{1/2} / \bar{X}_{ij} \quad (5)$$

$$Wj = V_j / V_j \quad (6)$$

The calculation formula for *Weighting Coefficient* D value of germplasm salt tolerance is as following:

$$D = (X_{ij} \times W_j) \quad (7)$$

The bigger the *D* value, the higher salt tolerance [40].

Gene expression analysis

RNA extraction

Bermudagrass leaf samples (100–200 mg) were ground with liquid nitrogen. RNA was isolated using FastPure Universal Plant Total RNA Isolation Kit (Vazyme, Nanjing). Gel electrophoresis was used to measure the purity of RNA concentrations.

cDNA synthesis

cDNA synthesis was done by using Hifair® AdvanceFast 1st Strand cDNA Synthesis SuperMix (Yeasen, Shanghai) for qPCR. The reaction mixture consisted of Total RNA, DNA Digester Mix, Hifair® AdvanceFast SuperMix and RNase-free H_2O. Incubation was done on PCR instrument according to the instructions attached to the kit.

Real-time quantitative PCR (qRT-PCR)

Primers for qRT-PCR used in this study were designed by Primer Premier 5, which are given in Table 1. The reaction mixture for qRT-PCR included Hieff® qPCR SYBR Green Master Mix (1×)(Yeasen, Shanghai), (iQ™ SYBR® Green (BioRad) master mix (2×), 1 µL of both forward as well as reverse primers (50 pM each), Sterile ultrapure water and diluted cDNA (1:10) template 2 µg. Carry out amplification according to the program setting in the instruction manual. Actin gene primers forward.

Table 1 List of primers used for qRT-PCR

Gene name	Primer-F	Primer-*R*
CdCINV1	5′-TGATGATGTGGAGGCGCG-3′	5′-TAACTGCGCCTGTGCTCC-3′
CdSWEET6	5′-CACTGAAGGAGGGGCAGC-3	5′-CGGCCTCCTTCTCCTCCT-3′
CdSUS5	5′-CCAGCACGGTGGTAGCAA-3	5′-TTTGGCATCTCCTGGGCG-3′
CdSPS1	5′-TAACGGATGCGCTGCACA-3	5′-CTCTGAGACGGCCTCCCT-3′

5′- TCTGAAGGGTAAGTAGAGTAG-3′ and reverse

5′- ACTCAGCACATTCCAGCAGAT- 3′ were normalized. QuantStudioTM Real-Time PCR Software (Thermofisher) measured the Ct values of samples for selected target gene expression. The selected genes' expression levels were calculated using the $2^{-\Delta\Delta Ct}$ calculation method provided by Vinje et al. [41].

Data statistical analysis

Data were recorded as means standard deviation (SD) from three replications. Statistical analysis of the data was carried out using SPSS 19.0 software, with results presented as mean values and their standard errors. To assess the significance of differences between treatments, two-way analysis of variance (ANOVA) was used, followed by further comparison of data groups using Duncan's multiple range test. Principal component analysis (PCA), pearson correlation coefficient and graphical presentation were done using Origin 2022 (OriginPro, Version 2022. OriginLab Corporation, Northampton, MA, USA).

Results and analysis

Soil salt concentration

From the beginning of the experiment, the soil salinity of the salt stress group was measured every 3 to 4 days (Table 2). In the initial phase of the treatment, from day 1 to 7, the soil salinity was maintained at about 0.5%. In the subsequent period from day 7 to 20, the salinity increased to 1.0%, and from day 21 until the end of the experiment, the salinity level was stable at about 1.5%.

The effects of salinity stress on Bermudagrass growth

Salinity stress inhibited the growth of Bermudagrass plants, with significant growth differences observed among different varieties. The varieties 'R101', 'R105' and 'A12359' showed significantly better growth potential than 'Wrangler' (Fig. 1). The picture shows that 'R101', 'R105' and 'Wrangler' have the smallest growth difference, which is an upright growth form, and 'A12359' is shorter with creeping stems. After salt treatment, the leaves of four bermudagrass in salinity treatment group all showed wilting and yellowing in different degrees, among which 'R101', 'R105' and 'Wrangler' showed lodging, while 'Wrangler' showed the earliest signs of wilting, and 'Wrangler' was the most affected by salt stress.

Salinity stress limited the growth of Bermudagrass plants (Fig. 2), the plant heights of 'R101', 'R105', 'Wrangler', and 'A12359' in salt treatment groups decreased by

Table 2 Changes in soil salinity during experimental treatments in the salt stress group (%)

Variety	Treatment	Treatment days/d												
		1	2	3	4	5	6	7	8	9	10	11	12	13
FB2019	1	0.3 ± 0.05e	0.4 ± 0.05de	0.5 ± 0.05d	1.3 ± 0.15b	1.3 ± 0.13b	1.2 ± 0.1b	1.0 ± 0.13c	1.4 ± 0.16b	1.3 ± 0.14b	1.2 ± 0.05b	1.2 ± 0.13b	1.6 ± 0.19a	1.6 ± 0.04a
R101	2	0.4 ± 0.04f	0.4 ± 0.04f	0.5 ± 0.08f	1.0 ± 0.19e	1.1 ± 0.04de	1.2 ± 0.12 cd	1.1 ± 0.08de	1.6 ± 0.12a	1.4 ± 0.11bc	1.1 ± 0.15de	1.3 ± 0.09c	1.5 ± 0.11ab	1.6 ± 0.23a
	3	0.3 ± 0.04f	0.3 ± 0.08f	0.4 ± 0.08f	1.0 ± 0.09e	1.0 ± 0.01e	1.4 ± 0.10abc	1.2 ± 0.21d	1.3 ± 0.08bcd	1.4 ± 0.23abcd	1.2 ± 0.09 cd	1.2 ± 0.21 cd	1.5 ± 0.14ab	1.5 ± 0.06a
FB2019	1	0.4 ± 0.08ef	0.3 ± 0.05f	0.4 ± 0.07e	1.1 ± 0.08 cd	1.1 ± 0.06 cd	1.2 ± 0.04c	1.0 ± 0.11d	1.4 ± 0.11b	1.4 ± 0.07b	1.1 ± 0.09d	1.5 ± 0.09b	1.7 ± 0.12a	1.5 ± 0.08b
R105	2	0.5 ± 0.08f	0.3 ± 0.07 g	0.5 ± 0.07f	1.0 ± 0.04e	1.3 ± 0.10 cd	1.4 ± 0.09bc	1.2 ± 0.19d	1.6 ± 0.06b	1.3 ± 0.10 cd	1.2 ± 0.09d	1.3 ± 0.20 cd	1.7 ± 0.10a	1.5 ± 0.11b
	3	0.5 ± 0.04e	0.4 ± 0.06f	0.5 ± 0.07e	1.1 ± 0.09d	1.0 ± 0.06d	1.3 ± 0.07c	1.0 ± 0.06d	1.2 ± 0.11c	1.6 ± 0.07a	1.2 ± 0.06c	1.4 ± 0.06b	1.4 ± 0.08b	1.5 ± 0.05a
Wran-	1	0.4 ± 0.08 h	0.3 ± 0.05 h	0.6 ± 0.07 g	1.0 ± 0.07f	1.0 ± 0.10f	1.2 ± 0.07e	1.0 ± 0.02f	1.5 ± 0.07c	1.4 ± 0.10d	1.2 ± 0.13e	1.3 ± 0.11de	1.8 ± 0.13a	1.7 ± 0.06b
gler	2	0.4 ± 0.05f	0.4 ± 0.08f	0.5 ± 0.08f	1.2 ± 0.05de	1.2 ± 0.08e	1.5 ± 0.07a	1.2 ± 0.11e	1.4 ± 0.08ab	1.4 ± 0.11bc	1.2 ± 0.08de	1.2 ± 0.05de	1.4 ± 0.11bc	1.3 ± 0.07 cd
	3	0.5 ± 0.08e	0.5 ± 0.04e	0.5 ± 0.07e	1.1 ± 0.05d	1.0 ± 0.08d	1.1 ± 0.05c	1.2 ± 0.05c	1.2 ± 0.04c	1.5 ± 0.09b	1.2 ± 0.12c	1.2 ± 0.08d	1.6 ± 0.07a	1.6 ± 0.08a
A12359	1	0.4 ± 0.08 g	0.5 ± 0.05 g	0.4 ± 0.07 g	1.0 ± 0.10f	1.0 ± 0.08f	1.2 ± 0.04e	1.0 ± 0.04f	1.3 ± 0.08d	1.5 ± 0.07b	1.3 ± 0.05 cd	1.2 ± 0.07e	1.6 ± 0.07a	1.4 ± 0.04bc
	2	0.3 ± 0.05 fg	0.3 ± 0.04 g	0.4 ± 0.04f	1.0 ± 0.07e	1.0 ± 0.11e	1.3 ± 0.04c	1.2 ± 0.08 cd	1.4 ± 0.08b	1.4 ± 0.04b	1.2 ± 0.08d	1.5 ± 0.05b	1.8 ± 0.05a	1.5 ± 0.09b
	3	0.5 ± 0.08f	0.4 ± 0.05f	0.4 ± 0.05f	1.2 ± 0.05d	1.0 ± 0.04e	1.2 ± 0.05d	1.2 ± 0.05 cd	1.6 ± 0.08a	1.4 ± 0.07b	1.2 ± 0.07 cd	1.3 ± 0.08c	1.5 ± 0.15b	1.4 ± 0.08b

Different lowercase letters within the same row indicate significant differences between the different days in the same treatments at the 0.05 level, similarly for the following tables. Data are means ± standard error with five replicates each

11.59%, 8.56%, 16.45%, and 30.04% respectively, compared to the control group (Fig. 2A). The percentage of withered leaves showed an increasing trend under salinity stress (Fig. 2B), with 'Wrangler' having the highest rate at 42.24%, and 'R101' the lowest at 31.73%. Compared to the control, the percentage of withered leaves of the four varieties increased by 214.09%, 201.74%, 221.08%, and 206.18%, respectively.

Based on the performance of plant height and the percentage of withered leaves, the salt tolerance of varieties from high to low is 'R105', 'R101', 'Wrangler', and 'A12359'. These results indicate that salt stress inhibited the growth of different Bermudagrass varieties to varying degrees, and the inhibition was closely related to their salt tolerance.

After salinity treatment, the biomass of the four Bermudagrass varieties varied, but all were lower than the biomass under control conditions (Fig. 3). Compared to the control group, the fresh weight of Bermudagrass significantly decreased (Fig. 3A). The fresh weight of 'R101', 'R105', 'Wrangler', and 'A12359' after salt stress decreased by 48.77%, 49.63%, 51.59%, and 52.07%, respectively, with 'R101' having the highest fresh weight in the treatment group at 13,375 kg/ha, and 'A12359' the lowest at 5,212.5 kg/ha. The differences in fresh weight between the control and treatment groups for 'R101', 'R105', 'Wrangler', and 'A12359' were significant ($P < 0.05$), but the differences among 'R101', 'R105', and 'Wrangler' were not significant. The aboveground biomass of' R101' and' R105' varieties accumulated the most under salt stress, showing good growth conditions.

The dry weight performance also varied among the four varieties (Fig. 3B), with all varieties showing a significant decrease in dry weight after salt stress compared to the control. The dry weights of 'R101', 'R105', 'Wrangler', and 'A12359' decreased by 30.20%, 42.18%, 45.80%, and 45.48%, respectively, with 'R101' having the highest dry weight at 5,955 kg/ha, and 'A12359' the lowest at 2,056.6 kg/ha. The dry weight differences between 'R101' and 'A12359' compared to the other three varieties were significant ($P < 0.05$), while the difference between 'R105' and 'Wrangler' was not significant. According to the dry weight of Bermudagrass varieties under salinity stress, the four varieties were ranked as 'R101' > 'R105' > 'Wrangler' > 'A12359', especially 'R101' and 'R105', which showed that salt-tolerant varieties could accumulate more dry matter in the shoot.

The correlation between fresh and dry weight, the fresh-to-dry weight ratio was used to replace the dry weight indicator and incorporated into the principal component analysis. Based on the salt tolerance coefficients for fresh weight and fresh-to-dry weight ratio, the salt tolerance of the four varieties from highest to lowest was 'R105', 'Wrangler', 'R101', 'A12359'. The results

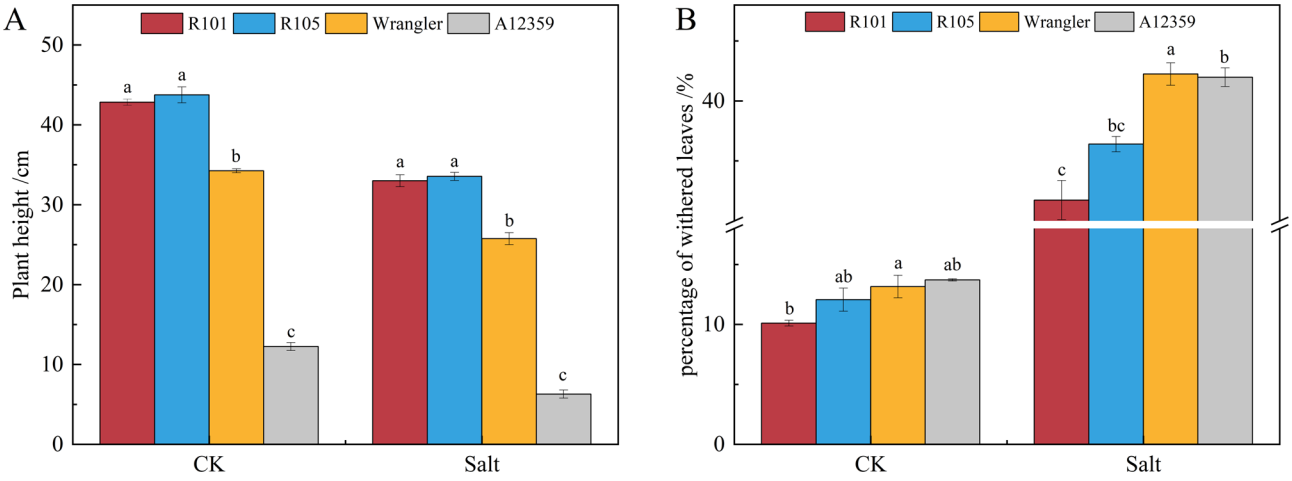


Fig. 2 Plant height and percentage of withered leaves of four varieties of bermudagrass under salinity stress. **A** Plant height of four varieties of bermudagrass under salinity stress; **B** Percentage of withered leaves of four varieties of bermudagrass under salinity stress. Different lowercase letters signify statistically significant differences among the varieties of this material at the $P < 0.05$ level. Data represent means of three replicates. Bars are means ± SD

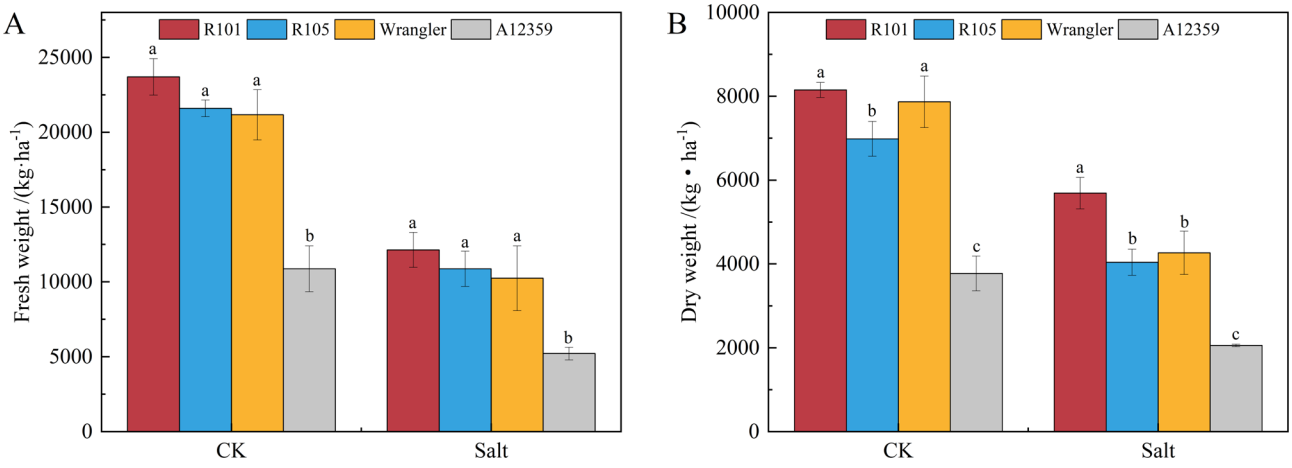


Fig. 3 Biomass of four varieties of bermudagrass under salinity stress. **A** Changes in fresh weight of four varieties of bermudagrass under salinity stress; **B** Changes in dry weight of four varieties of bermudagrass under salinity stress. Different lowercase letters indicate significant differences among different varieties of this material at the $P < 0.05$ level. Data represent means of three replicates. Bars are means ± SD

show that the biomass of all four Bermudagrass varieties was inhibited to varying degrees by salt stress, with the degree of inhibition closely related to their respective salt tolerance.

Effects of salinity on physiological characteristics of bermudagrass

Relative water content, MDA and electrical conductivity

After salt stress, the relative water content of bermudagrass decreased, with obvious differences among varieties (Fig. 4A). Compared with the control, the relative water content of four varieties decreased by 17.28%, 21.77%, 43.17 and 49.83% respectively. After salt stress, the highest relative water content was 'R101', which reached 75.47%, followed by 'R105', 'Wrangler' and 'A12359'. After salt stress, the MDA content of bermudagrass increased significantly (Fig. 4B), which increased by 253.80%, 206.75%, 249.70% and 289.38% respectively compared with the control. After salt stress, the lowest MDA content was 'R105', which was 16.67 mmol/g FW, and the highest was 'A12359'. The electrical conductivity showed an increasing trend under salt stress (Fig. 4C). Compared with the control, it increased by 12.20%, 11.72%, 48.71% and 60.15% respectively. After salt stress, the highest conductivity was 'A12359', reaching 2.79%, and the lowest was 'R105', reaching 2.03%.

Osmotic substance accumulation

Salinity stress significantly increased the soluble sugar in bermudagrass. In the treatment group, the soluble sugar content in the leaves of bermudagrass was notably higher than that in the control group (Fig. 5A), with 'R101', 'R105', 'Wrangler', and 'A12359' showing increases of 50.22%, 47.06%, 24.61%, and 28.27% in soluble sugar

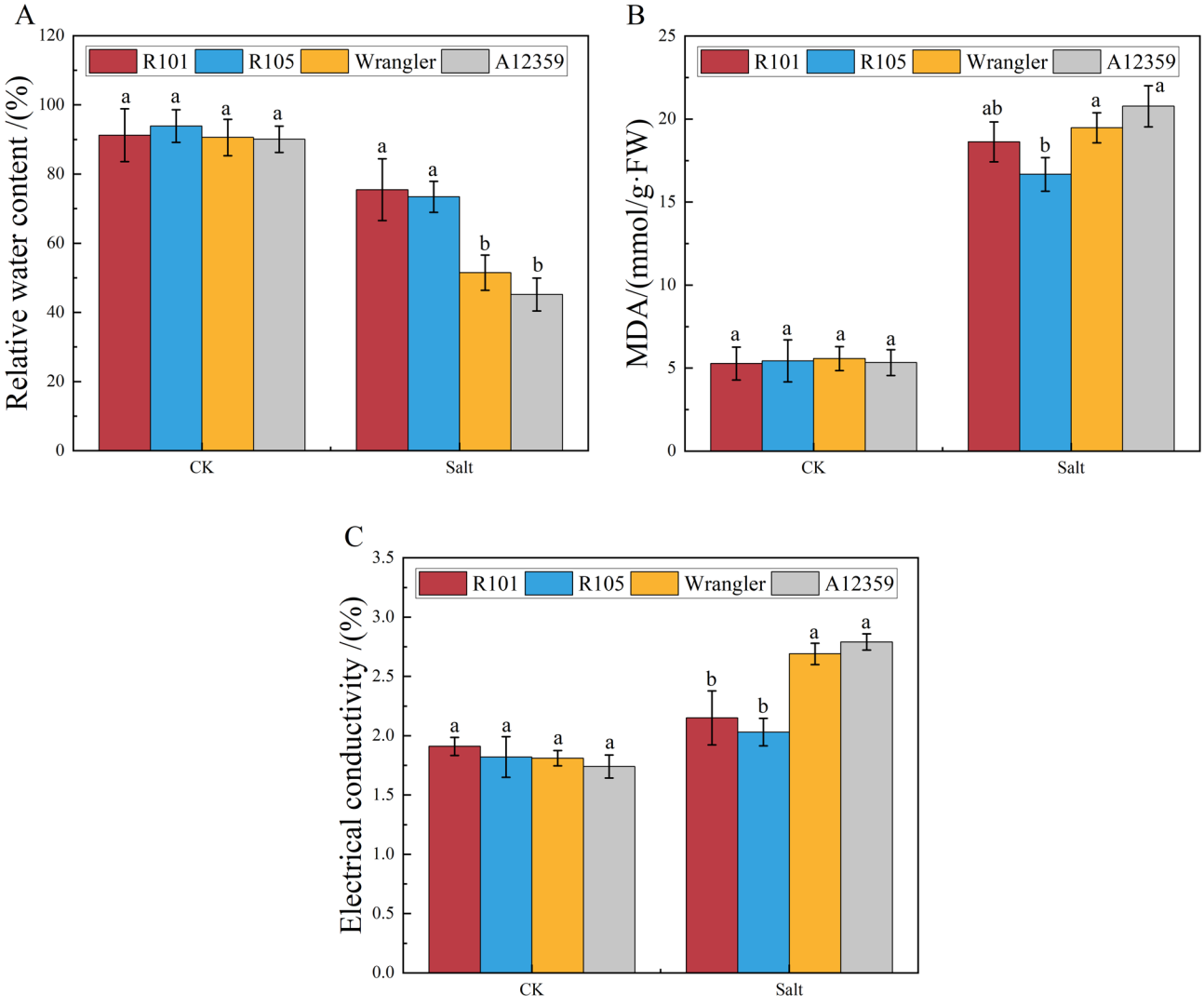


Fig. 4 Physiological changes in four bermudagrass varieties under salinity stress. **A** Variations in relative water content among four bermudagrass varieties under salinity stress; **B** Variations in MDA content among four bermudagrass varieties under salinity stress; **C** Variations in electrical conductivity among four bermudagrass varieties under salinity stress. Different lowercase letters indicate statistically significant differences among the varieties of this material at the $P < 0.05$ level. Data represent means of three replicates. Bars are means ± SD

content, respectively. Under salt stress, 'R101' and 'R105' exhibited a stronger capability to accumulate soluble sugars.

Meanwhile, the sucrose content in bermudagrass leaves showed an opposite trend to that of soluble sugars and proline under salt stress conditions, with the sucrose content in the control group significantly higher than in the treatment group (Fig. 5B), with 'R105' having the highest sucrose content at 21.88 mg/g. Compared to the control group, the reductions in sucrose content for 'R101', 'R105', 'Wrangler', and 'A12359' were 28.41%, 24.02%, 29.12%, and 36.30% respectively, indicating that 'Wrangler' and 'A12359' had a reduced ability to accumulate sucrose following salt treatment.

The contents of fructose and glucose increased in different degrees after salt stress (Fig. 5C, D). Compared with the control group, the fructose contents of bermudagrass 'R101', 'R105', 'Wrangler' and 'A12359' in salt treatment group increased by 34.57%, 52.11%, 30.63% and 26.05, respectively. Compared with the control group, the increase of glucose in the salt treatment group was larger, and the four varieties increased by 970.34%, 855.75%, 540.65% and 834.00% respectively. The variety with the highest glucose content after salt treatment was 'R105', which reached 5.54 mg/g, followed by 'R101', 'Wrangler' and 'A12359'.

The trend in proline content in bermudagrass leaves was similar to that of soluble sugars (Fig. 6A), with the treatment group significantly higher than the control group, and proline content ranging from 214.50 to 334.32 mg/g. Compared to the control group, the increases in proline content for 'R101', 'R105', 'Wrangler',

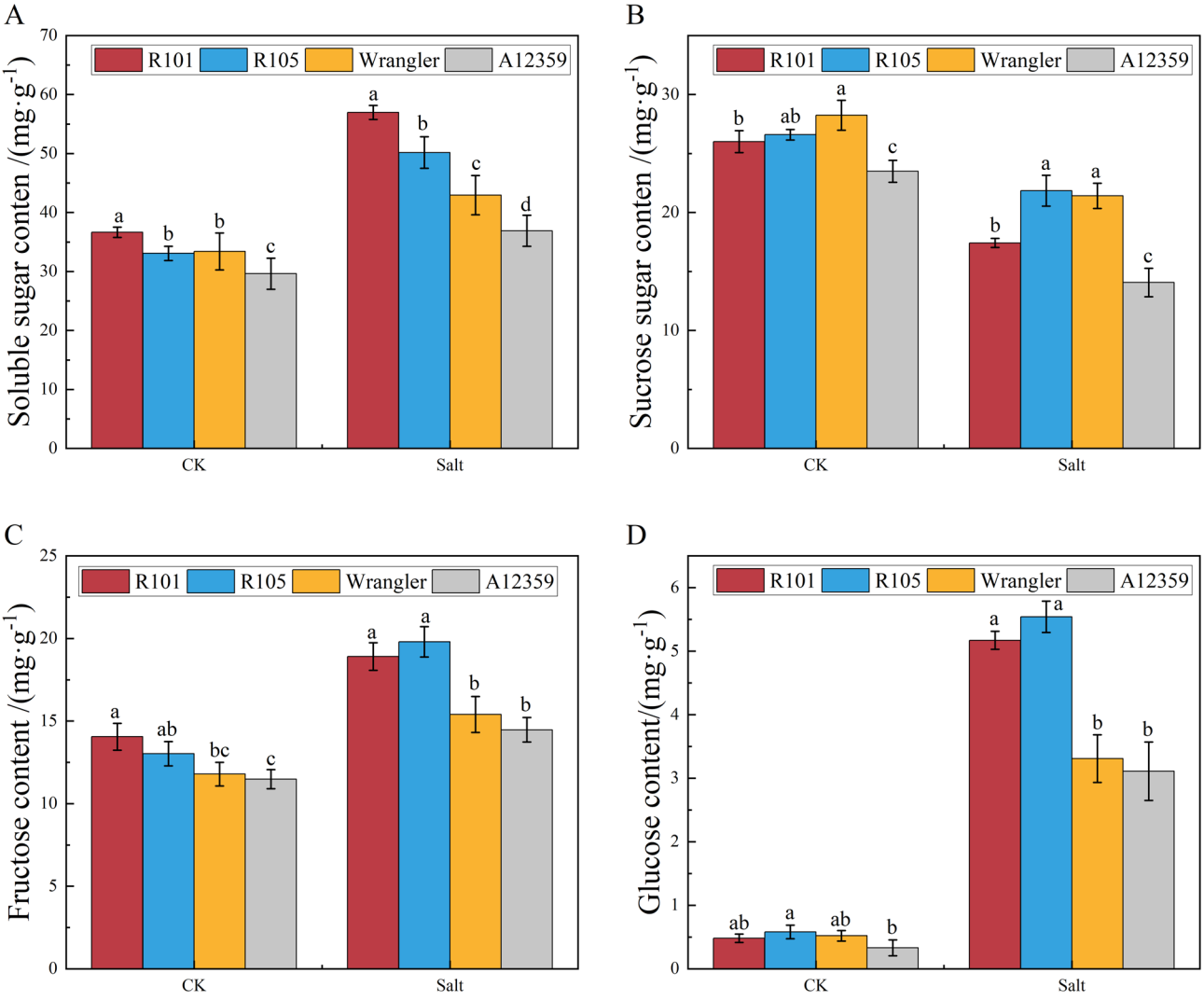


Fig. 5 Changes of carbohydrate content in four bermudagrass varieties under salinity stress. **A** Variations in soluble sugar content among four bermudagrass varieties under salinity stress; **B** Variations in sucrose content among four bermudagrass varieties under salinity stress; **C** Variations in fructose content among four bermudagrass varieties under salinity stress; **D** Variations in glucose content among four bermudagrass varieties under salinity stress. Different lowercase letters indicate statistically significant differences among the varieties of this material at the $P < 0.05$ level. Data represent means of three replicates. Bars are means ± SD

and 'A12359' were 658.15%, 520.51%, 485.96%, and 468.73% respectively, especially 'R105' exhibited the most prominent ability to accumulate proline under salt stress. Recent investigations have shown that SoS and Pro can serve as signaling molecules and their actions are not isolated; instead, they coordinate their actions to improve cell functions to stressors [42].

Ion content

Salt stress can cause the change of plant ion content. In this experiment, the sodium ion content changed significantly after salt stress (Fig. 6B), and the rising trend was obvious. Compared with the control group, the four Bermuda grass varieties increased by 100.10%, 166.40%, 361.68% and 555.58% respectively. After salt treatment, the content of potassium ion did not change as obviously as that of sodium ion, but it also increased (Fig. 6C). Compared with the control group, the content of potassium ion in salt treatment group increased by 27.86%, 29.74%, 30.65% and 32.06% respectively. Salinity stress also led to a significant increase in the Na^+/K^+ ratio in Bermudagrass leaves (Fig. 6D). Among the four varieties, the Na^+/K^+ ratios in the treatment groups were significantly higher than those in the control group, indicating that 'R105' and 'Wrangler' accumulated relatively less sodium under salt stress, with a smaller increase in the Na^+/K^+ ratio, more effectively maintaining the balance of Na^+/K^+ in the above-ground parts.

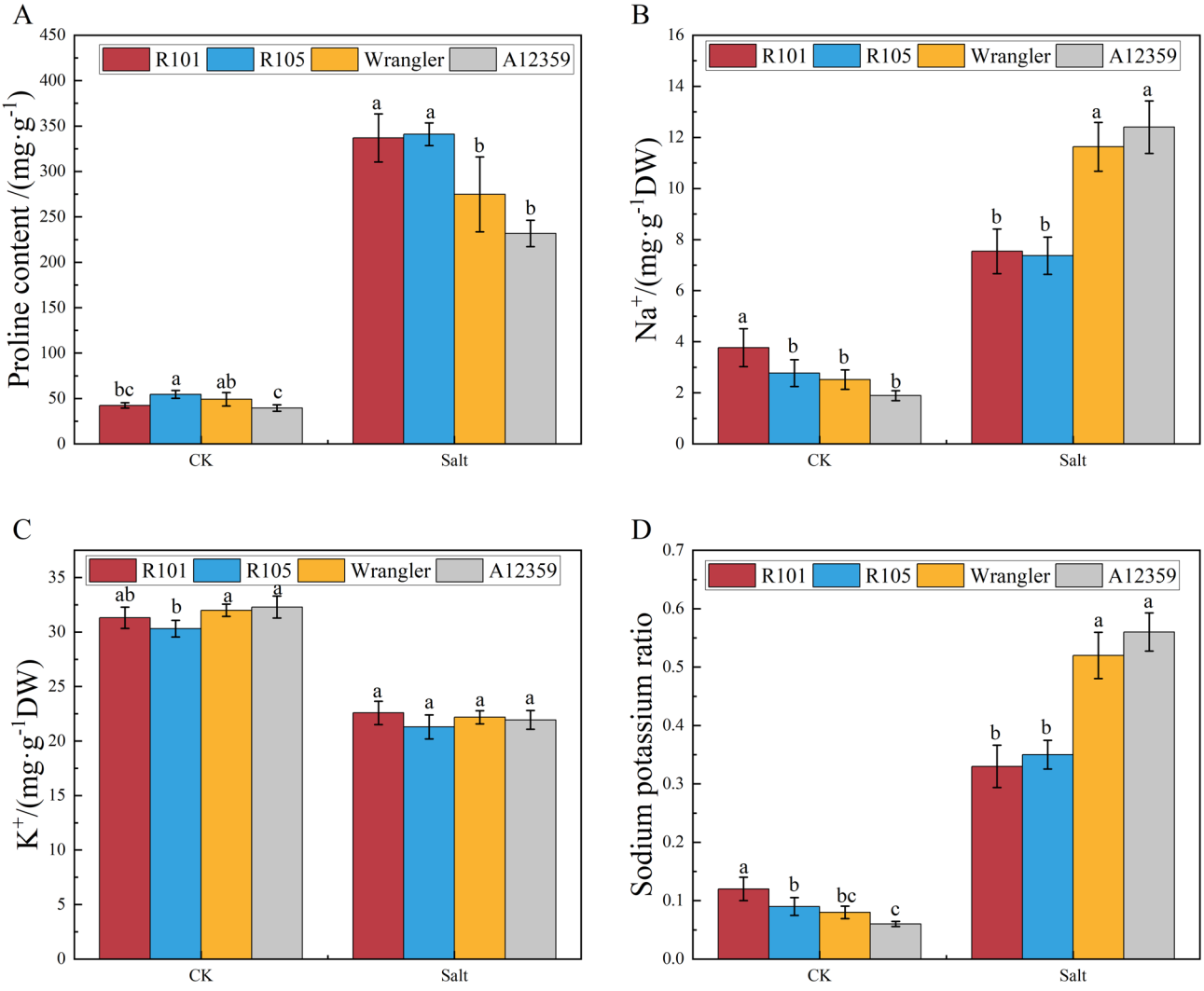


Fig. 6 Changes of proline, sodium and potassium ions content in four bermudagrass varieties under salinity stress. **A** Variations in proline content among four bermudagrass varieties under salinity stress; **B** Variations in sodium ion content among four bermudagrass varieties under salinity stress; **C** Variations in potassium ion content among four bermudagrass varieties under salinity stress; **D** Variations in sodium potassium ratio among four bermudagrass varieties under salinity stress. Different lowercase letters indicate statistically significant differences among the varieties of this material at the $P < 0.05$ level. Data represent means of three replicates. Bars are means ± SD

Activities of key enzymes in sucrose metabolism

Sucrose phosphate synthase activity and sucrose synthase activity of Bermuda grass decreased under salt stress (Fig. 7A, B), and different varieties responded differently to SPS activity under salt stress. Under salt stress, the SPS activity of' 'A12359' decreased the most, to 178.80 U/gFW, while that of 'R101' decreased the least, to 481.73 U/gFW, and the SPS activities of the four varieties decreased by 5.31%, 8.31%, 27.55% and 44.17% respectively. The SS activity of salt treatment group was lower than that of control group, and the SS activity of' Wrangler' decreased the most to 105.44 U/g FW, while that of' R105' decreased the least to 155.08 U/g FW, and the four varieties decreased by 31.49%, 29.89%, 42.26% and 38.86% respectively.

Different from SPS and SS, the activity of INV increased after salt stress (Fig. 7C). The activity of 'A12359' increased the most, reaching 110.82 U/g FW, while that of 'Wrangler' increased the least, reaching 100.37 U/g FW, and the four varieties increased by 34.97%, 56.21%, 30.15%, 73.21% respectively.

Photosynthetic pigment content

After salinity stress, the chlorophyll content in Bermudagrass generally showed a slow decreasing trend, with chlorophyll a, chlorophyll b, and carotenoids all reducing under salt stress. After the salinity stress, the chlorophyll a content in all four varieties displayed a downward trend (Fig. 8A), with 'R105' having the highest content at 6.53 mg/g, and 'A12359' the lowest at 3.54 mg/g. By the

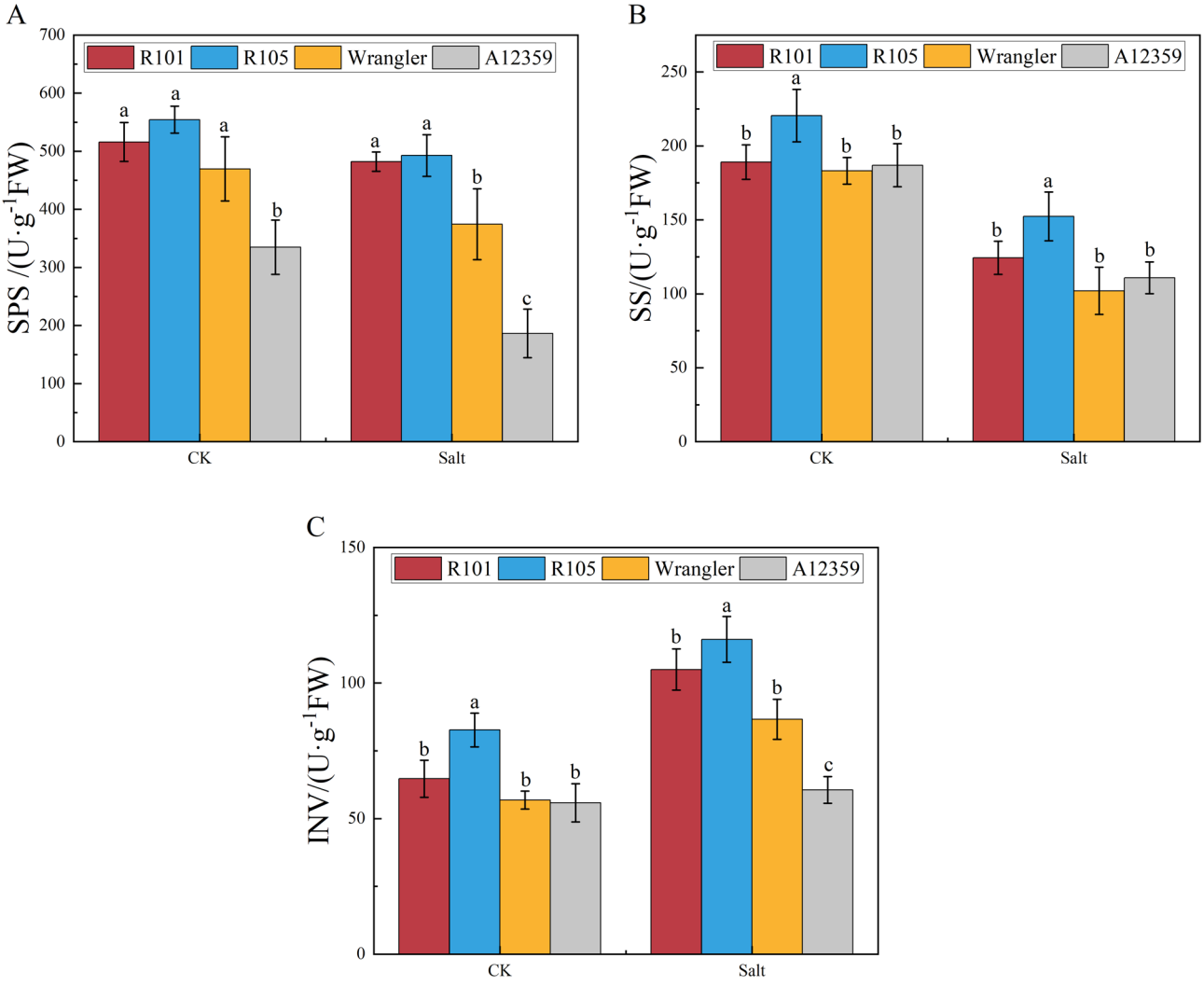


Fig. 7 Changes of key enzyme activities in sucrose metabolism in four bermudagrass varieties under salinity stress. **A** Variations in SPS activity among four bermudagrass varieties under salinity stress; **B** Variations in SS activity among four bermudagrass varieties under salinity stress; **C** Variations in INV activity among four bermudagrass varieties under salinity stress. Different lowercase letters indicate statistically significant differences among the varieties of this material at the $P < 0.05$ level. Data represent means of three replicates. Bars are means ± SD

end of the salinity stress, the chlorophyll a content in the four varieties decreased by 21.71%, 21.61%, 41.21%, and 57.86% compared to the control group (CK), respectively. The chlorophyll b content decreased by 27.29%, 27.72%, 67.38%, and 76.08% compared to the control group, with the highest content being 'R101' at 1.53 mg/g (Fig. 8B). Compared with the control group (CK), the total chlorophyll content decreased by 22.80%, 22.74%, 48.72% and 62.83% respectively. After salt stress, the highest chlorophyll content was 'R105', reaching 7.85 mg/g, and the lowest chlorophyll content was 'A12359', reaching 4.07 mg/g (Fig. 8C). The carotenoid content decreased by 56.51%, 56.70%, 60.00%, and 66.92%, with 'R101' and 'R105' having similar contents at 2.32 mg/g and 2.31 mg/g, respectively (Fig. 8D).

The effects of salinity stress on the forage quality of Bermudagrass

Under salinity stress conditions, the crude protein content in Bermudagrass leaves was generally higher than that in the control group (Fig. 9A). Compared to the control group, the increases in crude protein content in the leaves of 'R101', 'R105', 'Wrangler', and 'A12359' varieties in the treatment group were 13.26%, 8.17%, 44.20%, and 6.91%, respectively. Taking into account the salt tolerance coefficient, 'R101' and 'Wrangler' exhibited the most notable ability to accumulate crude protein under salt stress.

In contrast, the crude fat content in Bermudagrass leaves was higher in the control group than in the treatment group (Fig. 9B). Compared to the control group, the decreases in crude fat content in the leaves of 'R101',

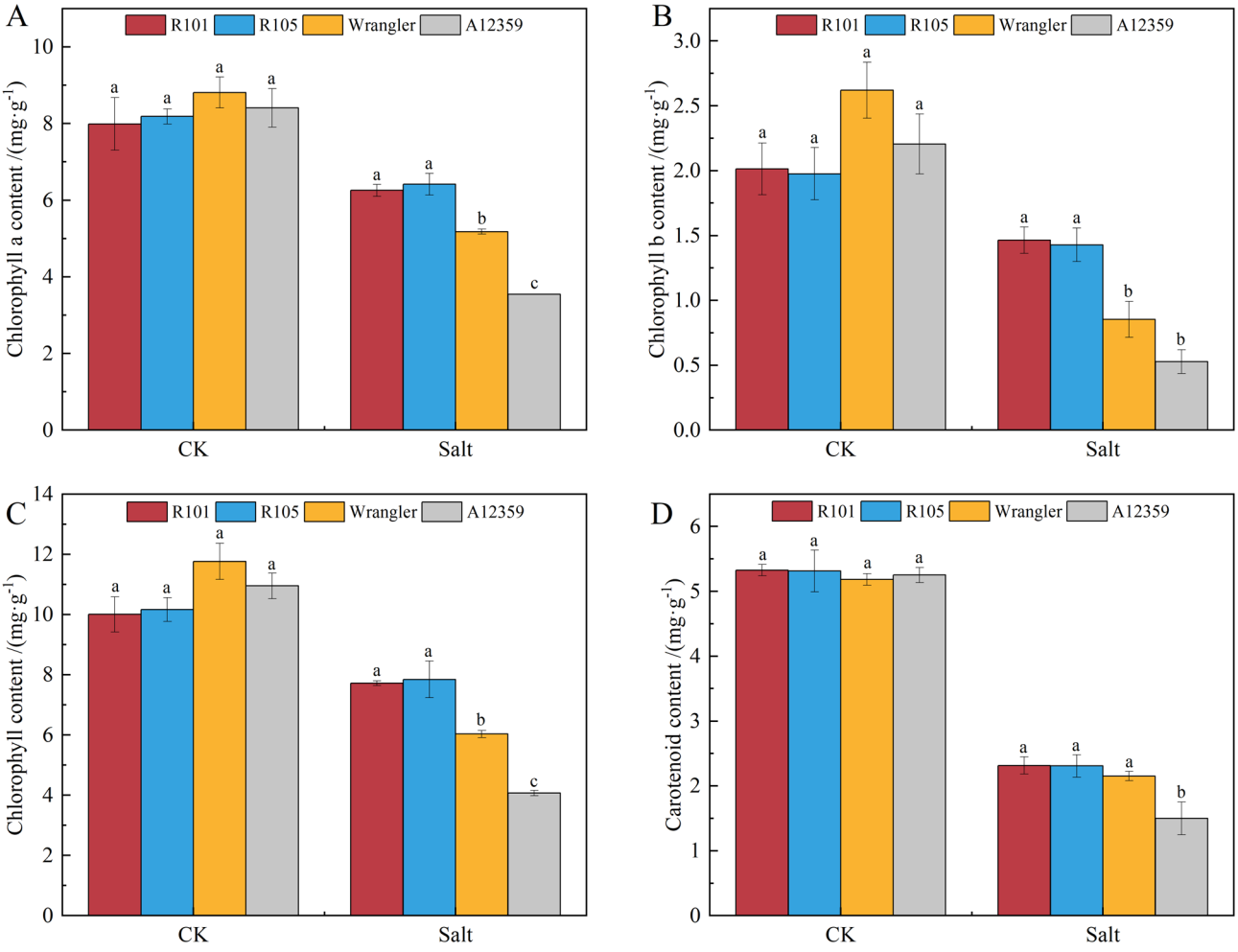


Fig. 8 Photosynthetic pigment content of four varieties of bermudagrass under salinity stress. **A** Variations in Chlorophyll a content among four bermudagrass varieties under salinity stress; **B** Variations in s Chlorophyll b content among four bermudagrass varieties under salinity stress; **C** Variations in total chlorophyll content among four bermudagrass varieties under salinity stress; **D** Variations in Carotenoid content among four bermudagrass varieties under salinity stress. Different lowercase letters indicate statistically significant differences among the varieties of this material at the $P < 0.05$ level. Data represent means of three replicates. Bars are means ± SD

'R105', 'Wrangler', and 'A12359' varieties in the treatment group were 43.48%, 41.00%, 44.32%, and 47.16%, respectively. The impact of salinity treatment on the crude fiber content in leaves of different Bermudagrass varieties varied (Fig. 9C). Compared to the control group, the crude fiber content in 'R101' treatment group leaves decreased by 1.21%, while for 'R105', 'Wrangler', and 'A12359', the crude fiber content in treatment group leaves increased by 2.89%, 7.48%, and 9.82%, respectively. Regarding the crude ash content in Bermudagrass leaves, the control group was also higher than the treatment group (Fig. 9D). Compared to the control group, the increases in crude ash content in the leaves of 'R101', 'R105', 'Wrangler', and 'A12359' varieties in the treatment group were 10.14%, 5.52%, 8.31%, and 23.47%, respectively.

Principal component and correlation analysis for Bermudagrass salt tolerance index

Principal component analysis

The salt tolerance coefficients (STCs) for various physiological indicators of different Bermudagrass varieties are shown in Table 3. It can be observed that the STCs for Bermudagrass varieties vary. The STCs for plant height, fresh weight, fresh-to-dry weight ratio, RWC, chlorophyll a, chlorophyll b, carotenoids, and soluble sugars, SPS, SS decreased (STC less than 1), indicating a reduction in these contents after salinity stress treatment. Conversely, the STCs for the percentage of withered leaves, MDA, sucrose, fructose, glucose, proline, sodium/potassium ratio, INV, crude fat, crude ash, and crude fiber increased (STC greater than 1), meaning that these contents increased after salinity stress treatment. The STC for crude protein showed significant variation after salt stress treatment but generally remained around 1.0.

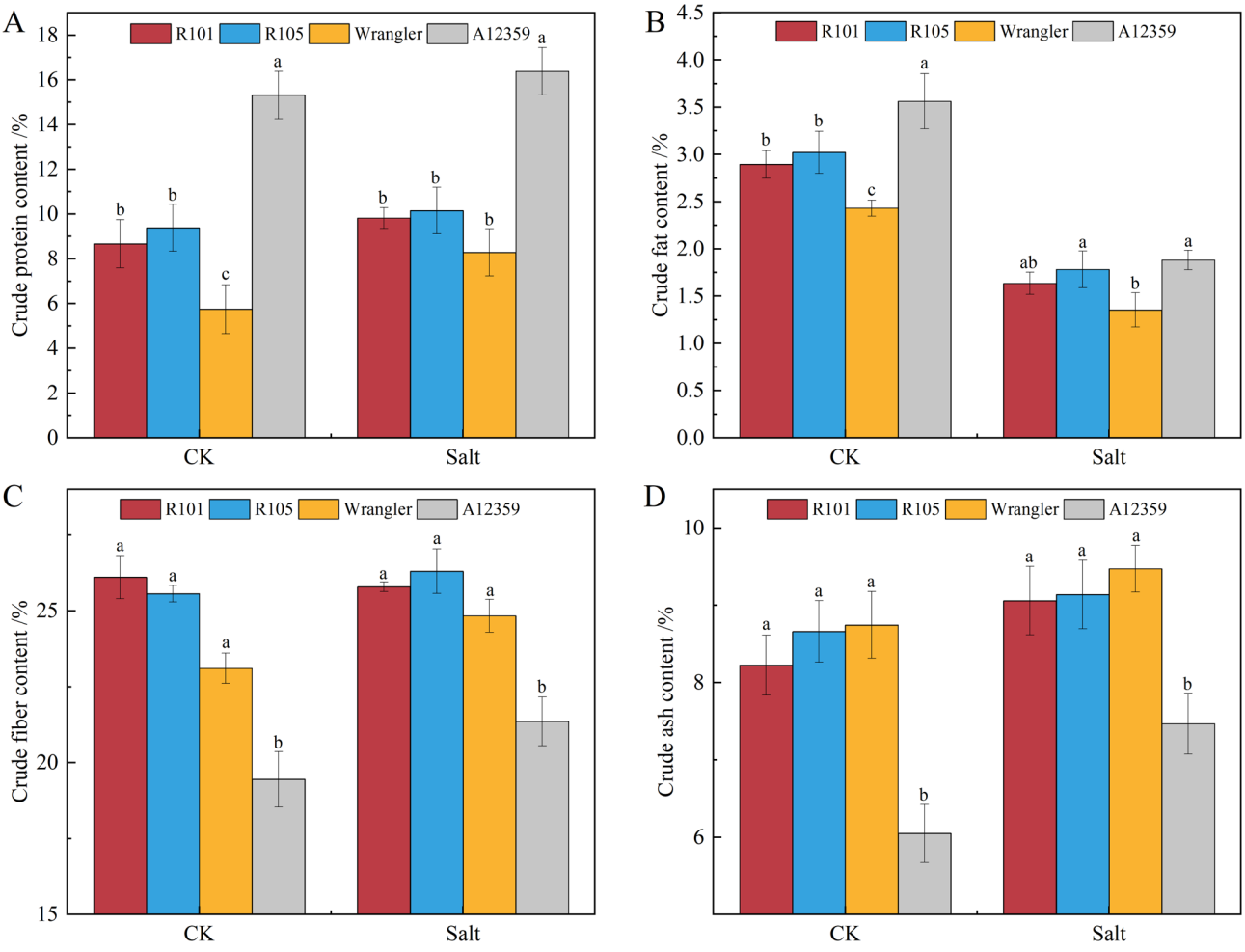


Fig. 9 Forage quality of four varieties of bermudagrass under salinity stress. **A** Crude protein content of four varieties of bermudagrass under salinity stress; **B** Crude fat content of four varieties of bermudagrass under salinity stress; **C** Crude fiber content of four varieties of bermudagrass under salinity stress; **D** Crude ash content of four varieties of bermudagrass under salinity stress. Different lowercase letters indicate statistically significant differences among the varieties of this material at the $P < 0.05$ level. Data represent means of three replicates. Bars are means ± SD

To screen for traits indicative of salt tolerance, a principal component analysis (PCA) was conducted on the salt tolerance coefficients of various indicators. In the dimension reduction process, principal components accounting for more than 80% of the cumulative variance contribution were considered representative. The results in Table 4 show that the cumulative contribution rate of three principal components derived from 23 individual indicators reached 100%, Three principal components (PCs) with characteristic values > 1.0 were extracted from 23 indicators, which explained 100.00% phenotypic variation (Table 4; Fig. 10). The first principal component having the largest eigenvalue and a contribution rate of 64.847%. The absolute values of the eigenvectors for fresh weight, plant height, the percentage of withered leaves, RWC, INV, soluble sugar and sucrose, chlorophyll a, chlorophyll b, carotenoids, and sodium/potassium ratio all exceeded 0.294 (Table 5), indicating these indicators play a dominant role in the first principal component; the second principal component contributed 22.921% (Table 4), with EL, SPS, MDA, proline, CF, EE, SS, FD, crude protein and crude ash's eigenvector absolute values at 0.273 and 1.264 respectively; the third principal component contributed 2.813%, with fructose (0.525) and glucose (0.424) having larger eigenvector absolute values. Among the three components, the first principal component had the highest contribution rate, suggesting these indicators could represent most of the information for the traits under investigation.

Correlation analysis

We performed a correlation analysis to identify how different growth and physiological characteristics were interrelated in the for contrasting bermudagrass under salinity (Fig. 11). The correlation between multiple indicators is extremely significant.

Table 3 Salt tolerance coefficients of various indicators for *Cynodon dactylon* under salt stress

Index	Salt resistance coefficients			
	FB2019R101	FB2019R105	Wrangler	A12359
PH	77.04%	76.69%	75.18%	51.40%
PWL	303.74%	315.75%	345.95%	380.07%
FW	51.23%	50.37%	48.41%	47.93%
FD	73.38%	87.11%	89.32%	87.92%
RWC	82.72%	78.23%	56.83%	50.17%
MDA	353.80%	306.75%	349.70%	389.35%
EL	112.20%	111.72%	148.71%	160.15%
SoS	71.59%	75.98%	70.88%	63.70%
Suc	150.22%	147.06%	124.61%	128.27%
Fru	134.57%	152.11%	130.63%	126.05%
Glu	1070.34%	955.75%	640.65%	934.00%
Pro	314.09%	301.74%	321.08%	306.18%
SPR	278.38%	379.93%	666.54%	965.70%
SPS	93.47%	88.88%	79.74%	55.68%
SS	65.77%	69.08%	55.69%	59.29%
INV	162.30%	140.37%	152.51%	108.47%
Chl a	72.71%	72.28%	32.62%	23.92%
Chl b	43.46%	43.41%	41.51%	28.54%
CA	56.52%	59.00%	55.68%	52.84%
EE	110.14%	105.52%	108.31%	123.47%
Ash	113.26%	108.17%	144.20%	106.91%
CP	98.79%	102.89%	107.48%	109.82%
CF	1145.45%	1228.08%	1347.27%	2650.70%

PH represent plant height, PWL: percentage of withered leaves, Chl a: chlorophyll a, RWC: Relative water content, FW: fresh weight, Chl b: chlorophyll b, INV: invertase, SoS: soluble sugar, Suc: sucrose, CA: carotenoid, SPR: sodium potassium ratio, EL: electrical conductivity, SPS: Sucrose phosphate synthase, MDA: malondialdehyde, Fru: fructose, Glu: glucose, Pro: proline, CF: Crude fiber, EE: crude fat, Ash: crude ash, CP: crude protein, SS: Sucrose synthase, FD: fresh-dry ratio

Table 4 Characteristeristic value and variance contribution rate of principa component analysis of each index

Principa component	Characteristeristic value	Contribution rate/%	Cumulative contribution rate/%
1	14.915	64.847	64.847
2	5.272	22.921	87.767
3	2.813	12.233	100.000

Membership function values and comprehensive evaluation of salt tolerance

Plant salt tolerance mechanisms are diverse, and a single indicator cannot accurately reflect the impact of salt stress on Bermudagrass. Therefore, to scientifically assess plant salt tolerance, it is essential to select indicators that truly reflect plant characteristics [43]. Comprehensive analysis of the membership function values and *D* values for each Bermudagrass variety reveals that indicators such as plant height, fresh weight, chlorophyll a, chlorophyll b, carotenoids, sucrose, sodium-potassium ratio, and crude fiber effectively reflect the tolerance

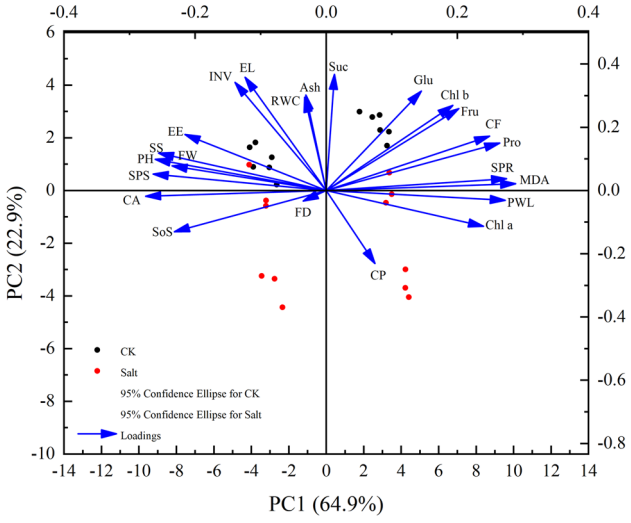


Fig. 10 Principa component analysis (PCA) figure of indicators in bermudagrass. FW, FD, PWL, Chl a, Chl b, CA, SS, Suc, Pro, SPR, EE, Ash, CP, CF represent plant height, fresh weight, fresh dry, percentage of withered leaves, chlorophyll a, chlorophyll b, carotenoid, soluble sugar, sucrose, proline, sodium potassium ratio, crude fat, crude ash, crude protein, Crude fiber, similarly for the following tables

of each variety to NaCl. Based on the comprehensive evaluation *D* values of the salt tolerance coefficients for each indicator (Table 6), under salinity stress, the *D* values for the four Bermudagrass varieties are ranked as: 'R105' > 'R101' > 'Wrangler' > 'A12359', with 'R105' and 'R101' demonstrating stronger salt tolerance capabilities compared to 'Wrangler' and 'A12359', especially 'R105' showing the most significant salt tolerance. By using membership function and index weight method, the comprehensive evaluation value *D* of various quality resources is obtained and ranked. The distribution range of *D* value is 0.458 ~ 0.787. The greater the D value, the stronger the salt tolerance, and vice versa. According to the D value, R105 and R101 were identified as typical salt-tolerant varieties, with the highest D value, followed by Wrangler and A12359, with the lowest *D* value and poor salt tolerance.

Gene expression

Four key genes related to sucrose metabolism, *CdCINV1*, *CdSPS1*, *CdSUS5*, and *CdSWEET6*, were analyzed under salt stress. In the results, the transcription levels of *CdCINV1*, *CdSPS1*, *CdSUS5*, and *CdSWEET6* in salt-tolerant varieties 'R105' and 'R101' were higher than those of 'Wrangler' and 'A12359' (Fig. 12). The expression of *CdCINV1*, *CdSPS1*, *CdSUS5*, and *CdSWEET6* in salt-tolerant varieties increased under salt stress, and the expression levels were significantly different.

Table 5 Principa component analysis matrix and eigenvectior of indicators

Index	Principa component 1	Eigenvector Y1	Principa component 2	Eigenvector Y2	Principa component 3	Eigenvector Y3
PH	0.993	0.362	0.004	0.021	-0.115	0.018
PWL	-0.989	0.359	0	0.000	-0.145	0.028
Chl a	-0.986	0.357	0.15	0.036	0.069	0.006
RWC	0.977	0.351	-0.21	0.070	0.039	0.004
FW	0.974	0.349	-0.219	0.076	-0.057	0.041
Chl b	0.964	0.341	-0.264	0.110	-0.003	0.007
INV	0.957	0.337	0.205	0.067	0.205	0.057
SoS	0.955	0.335	0.233	0.086	-0.186	0.047
Suc	-0.912	0.306	-0.222	0.078	-0.345	0.161
CA	0.909	0.304	0.334	0.177	0.249	0.084
SPR	-0.895	0.294	-0.32	0.162	0.311	0.131
EL	0.881	0.285	0.46	0.335	-0.107	0.015
SPS	0.802	0.236	-0.429	0.292	-0.416	0.234
MDA	-0.795	0.232	-0.415	0.273	0.443	0.266
Fru	0.77	0.218	0.137	0.030	-0.623	0.525
Glu	0.748	0.206	0.356	0.201	0.56	0.424
Pro	0.665	0.162	-0.582	0.537	0.468	0.296
CF	0.44	0.071	-0.893	1.264	-0.09	0.011
EE	-0.157	0.009	0.873	1.208	0.463	0.290
Ash	-0.492	0.089	-0.839	1.116	0.233	0.073
CP	-0.212	0.017	0.787	0.982	0.579	0.454
SS	0.604	0.134	0.689	0.752	-0.4	0.217
FD	-0.584	0.125	0.563	0.502	-0.586	0.465

PH represent plant height, PWL: percentage of withered leaves, Chl a: chlorophyll a, RWC: Relative water content, FW: fresh weight, Chl b: chlorophyll b, INV: invertase, SoS: soluble sugar, Suc: sucrose, CA: carotenoid, SPR: sodium potassium ratio, EL: electrical conductivity, SPS: Sucrose phosphate synthase, MDA: malondialdehyde, Fru: fructose, Glu: glucose, Pro: proline, CF: Crude fiber, EE: crude fat, Ash: crude ash, CP: crude protein, SS: Sucrose synthase, FD: fresh-dry ratio

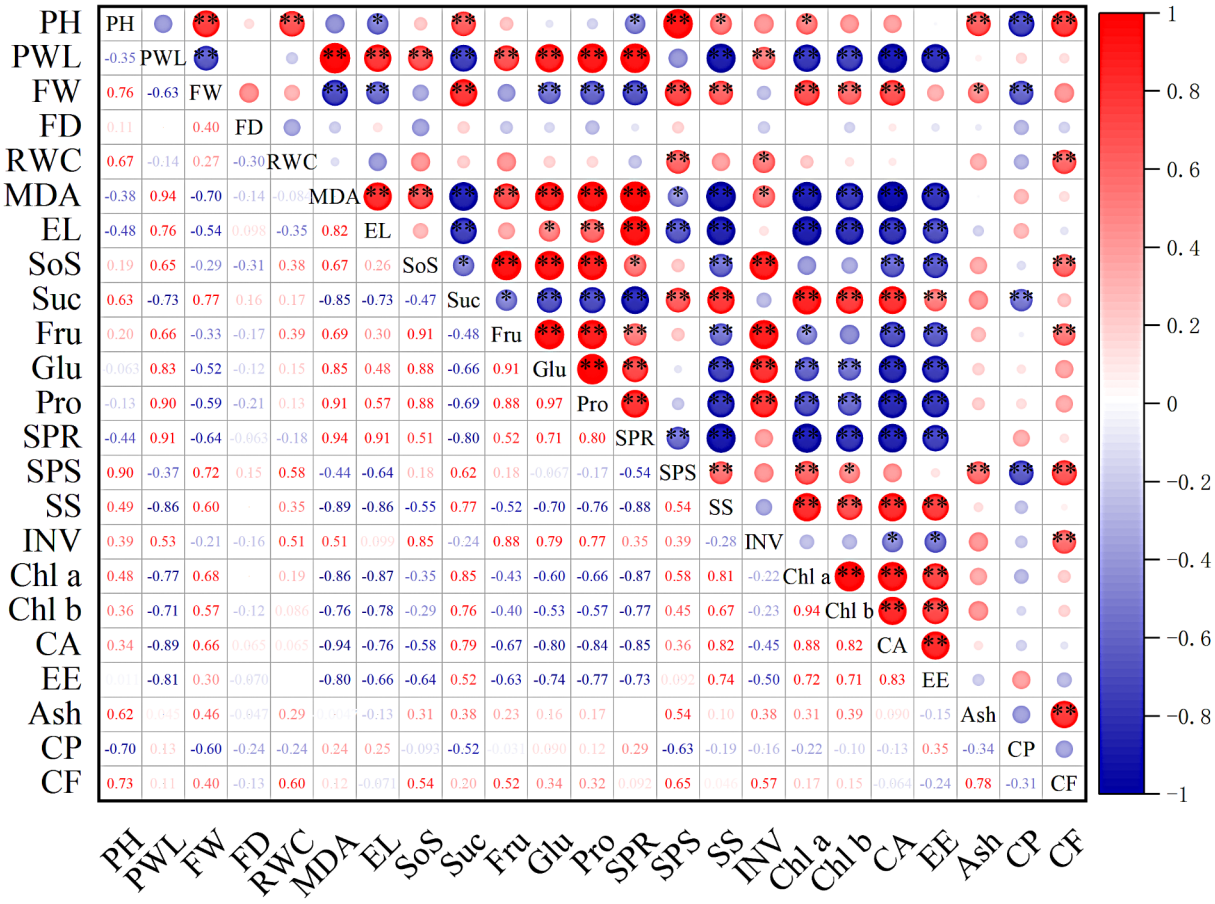
Discussion

The adaptation of plants to salt stress is the result of genetic variation interacting with the environment, manifesting at both the physiological phenotype and biochemical levels. Long-term research in plant stress physiology has identified some key indicators that may reflect a plant's salt tolerance. This study found significant differences in the trends of plant height, fresh weight, chlorophyll content, soluble sugars, proline, sodium-potassium ratio, and crude fat et al. Among different Bermudagrass varieties. Therefore, when evaluating the salt tolerance of Bermudagrass varieties, it is essential to consider multiple indicators. By calculating the correlation indices of these indicators, we identified 23 core indicators for studying the salt tolerance of Bermudagrass varieties. Combining the membership function method and weight calculation, we obtained a dimensionless number, the *D* value, based on which the salt tolerance of Bermudagrass varieties was ranked. This method lays the foundation for establishing an evaluation system for the salt tolerance capacity of Bermudagrass varieties, achieving a comprehensive evaluation of their salt tolerance.

The primary symptoms of salt damage include tip burning, leaf yellowing and rolling, plant wilting, and stunted plant growth [44]. EL, Chl, have been widely used to identify tolerance to environmental stress in various plant species, such as heat stress in creeping bentgrass [45], Salt tolerance of okra germplasm (*Abelmoschus esculentus L.)* [46], drought tolerance [47] and heat tolerance [48] of wheat, and cold tolerance of cool-season grasses [49], and salt tolerance in blue panicgrass *(Panicum antidotale)* [50], drought tolerance of perennial ryegrass [51]. Our current study demonstrated physiological variations in 4 bermudagrass materials under salt stress. R105 and R101 exhibited superior salt tolerance with high levels of biomass, quality and physiological performance than other bermudagrass materials. On the contrary, Wrangler and A12359 were the most susceptible to salt stress. These salt-tolerant and salt sensitive w bermudagrass provide potential materials for breeding, cultivation, and utilization and further studies on the mechanism of salt tolerance in leguminous plants.

The impact of salinity stress on the growth and physiological characteristics of Bermudagrass

Plants are significantly affected by salinized soil throughout their growth and development process, mainly manifested by leaf yellowing, slow growth, reduced vitality, and weakened absorption capacity, which in severe cases may even lead to plant death [52]. In this experiment, the plant height of the salinity treatment group decreased compared to the control group, with significant



* $p \leq 0.05$ ** $p \leq 0.01$

Fig. 11 Heat map for correlation analysis of salt tolerance indexes in bermudagrass. PH, FW, FD, PWL, Chl a, Chl b, CA, SS, Suc, Pro, SPR, EE, Ash, CP, and CF represent plant height, fresh weight, fresh-to-dry weight ratio, percentage of withered leaves, chlorophyll a, chlorophyll b, carotenoids, soluble sugar, sucrose, proline, sodium-potassium ratio, crude fat, crude ash, crude protein, and crude fiber, respectively. This notation applies similarly in subsequent tables. * indicates a significant correlation ($P < 0.05$); ** indicates an extremely significant correlation ($P < 0.01$). The circle size indicates the correlation size

differences in plant height among different varieties. 'R101' and 'R105' experienced a smaller decrease in plant height than 'Wrangler' and 'A12359', indicating that 'R101' and 'R105' have stronger salt tolerance, while 'Wrangler' and 'A12359' have weaker salt tolerance. Zhou et al. [12] also found in rice studies that the morphological growth parameters of plants could be used to evaluate their salt tolerance.

Biomass is one of the key indicators of plant growth and development and is also one of the most reliable indicators for assessing plant salt tolerance [53]. In this study, the biomass of Bermudagrass varieties 'R101' and 'R105' significantly decreased after salt stress, but the decrease was relatively small. This suggests that water can dilute the salt to some extent, and the smallest reduction in dry weight for 'R101' indicates that 'R101' accumulated more dry matter. Its good growth condition enabled the plant to absorb more water and nutrients. Cai Fan and others [54] also found in their study on rice under salinity stress that rice yield was significantly positively correlated with physiological indicators such as the number of green leaves on the main stem and the quality of individual leaves, indicating that salt stress inhibited the plant's physiological characteristics, thereby affecting yield.

Plant growth inhibition caused by salt stress is closely related to the reduction in photosynthetic pigments [55]. In this study, the contents of chlorophyll a, chlorophyll b, and carotenoids in the varieties of Bermudagrass were all lower in the treatment group compared to the control group, indicating that salt stress significantly inhibited the photosynthesis of Bermudagrass and reduced its photosynthetic pigment content. Among them, 'R101' and 'R105' showed a smaller decrease in pigment content, suggesting that these two varieties could maintain higher levels of photosynthetic pigments under salt stress, exhibiting better salt tolerance. Conversely, 'Wrangler'

Table 6 Membership value, weight coefficient, comprehensive evaluation *D* value of each variety under seawater stress

Index	R101	R105	Wrangler	A12359	Weight index
PH	0.714	1.000	0.694	0.000	0.065
PWL	1.000	0.553	0.157	0.000	0.055
Chl a	0.158	0.046	1.000	0.000	0.044
RWC	0.000	0.861	1.000	0.912	0.025
FW	1.000	0.862	0.205	0.000	0.060
Chl b	0.431	1.000	0.480	0.000	0.000
INV	0.990	1.000	0.236	0.000	0.005
SoS	1.000	0.879	0.131	0.000	0.058
Suc	0.320	1.000	0.716	0.000	0.065
CA	0.327	1.000	0.176	0.000	0.060
SPR	1.000	0.733	0.000	0.683	0.030
EL	1.000	0.733	0.000	0.683	0.043
SPS	1.000	0.283	0.000	0.118	0.002
MDA	1.000	0.852	0.435	0.000	0.067
Fru	1.000	0.879	0.637	0.000	0.050
Glu	0.753	1.000	0.000	0.269	0.063
Pro	1.000	0.593	0.818	0.000	0.064
CF	0.877	1.000	0.351	0.000	0.060
EE	1.000	0.963	0.164	0.000	0.068
Ash	1.000	0.886	0.769	0.000	0.068
CP	0.729	1.000	0.487	0.000	0.002
SS	0.642	0.000	0.020	1.000	0.041
FD	0.264	0.000	1.000	0.093	0.004
evaluation *D* value	0.765	0.787	0.458	0.068	
Rank	2	1	3	4	

PH represent plant height, PWL: percentage of withered leaves, Chl a: chlorophyll a, RWC: Relative water content, FW: fresh weight, Chl b: chlorophyll b, INV: invertase, SoS: soluble sugar, Suc: sucrose, CA: carotenoid, SPR: sodium potassium ratio, EL: electrical conductivity, SPS: Sucrose phosphate synthase, MDA: malondialdehyde, Fru: fructose, Glu: glucose, Pro: proline, CF: Crude fiber, EE: crude fat, Ash: crude ash, CP: crude protein, SS: Sucrose synthase, FD: fresh-dry ratio

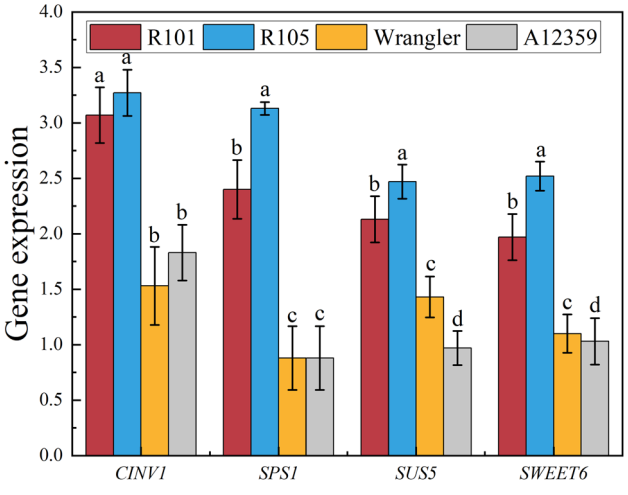


Fig. 12 Analysis of transcript levels of *CdCINV1*, *CdSPS1*, *CdSUS5*, *and CdSWEET6* in bermudagrass. Means of three replicates and standard errors are presented; the same letter above the column indicates no significant difference at $P < 0.05$

and 'A12359' showed a greater reduction in photosynthetic pigment content, indicating weaker salt tolerance. Tian Tian et al. [56] suggested that salt stress causes the production of reactive oxygen species in plants, damaging chloroplasts and thus reducing chlorophyll content.

Sodium ions, the main component of salt damage, increase under salt stress, inhibiting the absorption and transport of potassium ions, thereby affecting plant growth and development [57]. The Na^+/K^+ ratio reflects the ionic balance in the plant and is an important indicator for assessing plant salt tolerance [58]. Salt-tolerant Bermudagrass can maintain a lower Na^+ content in the above-ground parts while keeping a relatively higher K^+ content, according to Satish et al.'s research, it is speculated that the accumulation of sodium ions in roots may maintain normal cell metabolism and limit the transport of Na^+ to leaves, thus limiting the accumulation of Na^+ in leaves [59] to resist salt stress. The higher ions in leaves lead to oxidative stress which affects normal cell functions [60]. In this study, the Na^+/K^+ ratios of the four Bermudagrass varieties significantly increased after salt stress, likely due to the plant's extensive absorption of sodium ions and their transport to vacuoles in the above-ground parts for storage [61], where the SOS1 in the xylem parenchyma cells is directly responsible for sodium ion transport, and NHX transports it to leaf vacuoles [62]. Similar to our research results, Yuichi Tada et al. studied the salt tolerance of 20 species of wild turfgrass, and found that salt stress inhibited excessive Na accumulation in the shoot and roots, and maintained a high Na/K ratio [63]. Hu et al. [25] found that growth inhibition of Bermudagrass under salt stress might be related to osmotic and ionic effects, noting that the reduction in canopy height was correlated with an increase in the Na^+/K^+ ratio. In this experiment, the salt tolerance coefficient for plant height was negatively correlated with the sodium-potassium ratio, further corroborating this view.

Increases in endogenous sucrose, fructose, glucose, soluble sugar contents could be key adaptive responses to salt stress in the 'R105' and the 'R101' through upregulating genes encoding sucrose and fructose, glucose biosynthesis. Plants develop various adaptive strategies against salt stress, such as alterations in defense-associated compounds and carbohydrate [64]. It has been well documented that regulation of sucrose accumulation and metabolism improved tolerance against various types of environmental stresses, including salt stress, by triggering many biochemical reactions in bermudagrass and other plant species [65]. In response to salt stress, the sucrose reduction of salt-tolerant varieties 'R105' and 'R101' is significantly less than that of salt-sensitive varieties 'Wrangler' and 'A12359', and the fructose and soluble sugar contents of 'R105' and 'R101' are also significantly higher than that of 'Wrangler' and 'A12359'.

Bermudagrass can cope with salt stress by increasing soluble sugar content and converting sucrose into fructose and glucose. And these processes are regulated by three key enzymes of sucrose metabolism.

Studies have shown that proline, together with arginine, cysteine and other amino acids, can reduce potassium ion efflux under salt stress, thus maintaining ion homeostasis. Therefore, monitoring proline content under salt stress can be used as an important index to evaluate the salt tolerance mechanism of plants [66]. RWC is a reliable and simple way to assess the water status of a leaf without any need for special equipment. Under salt stress, plants with high RWC content have high salt tolerance. Leaf water potential and osmotic potential can also evaluate leaf water potential, Xue et al. [67] found that the water potential of tomato leaves will decrease, which will lead to the decrease of stomatal regulation of tomato leaves, and then affect the water use efficiency.

The effects of salinity stress on nutritional quality of bermudagrass

Nutritional quality is one of the key reference indicators for selecting superior Bermudagrass varieties. Crude protein, crude fat, crude fiber, and crude ash are the main indicators for assessing forage quality. For Bermudagrass, higher contents of crude protein and crude fat and a lower content of crude ash indicate better quality; conversely, lower quality is indicated.

Crude protein reflects the nitrogen content in the plant and is an important indicator for evaluating forage quality [68]. Increasing the crude protein content of forage can effectively improve its feed quality. In this study, the crude protein content in the leaves of Bermudagrass in the treatment group was generally higher than that in the control group, with 'R101' and 'Wrangler' showing greater increases, indicating that these two varieties have higher quality under salinity treatment. The research results of Ali et al. [69] support this viewpoint, as they found that salt stress is conducive to the synthesis of crude protein in plants, which may be related to the plants' own salt tolerance capacity.

Crude fat contains lipid substances, such as fats, vitamins, fatty acids, etc., which are essential nutrients for animal life activities. This study found that the crude fat content in Bermudagrass leaves decreased after salt stress compared to the control group, a phenomenon also observed by Chen et al. [70] in their study on alfalfa. The decrease was similar across the four varieties, indicating that salinity stress had a minimal impact on the crude fat content of the four Bermudagrass varieties.

Crude fiber, as a major component of plant cell walls [71], limits the intake of forage by animals and affects the digestion speed of food. Crude fiber also influences the creeping ability and support function of Bermudagrass [72]. In this study, salt treatment had different effects on the crude fiber content in the leaves of different Bermudagrass varieties, with a decrease in 'R101' and increases in 'R105', 'Wrangler', and 'A12359'. Research by Uddin et al. [73] also confirmed that salt stress increases the crude fiber content in plants. The larger increases in crude fiber content in 'Wrangler' and 'A12359' suggest that these two varieties have a stronger ability to accumulate crude fiber.

Crude ash is the residue left after plant material is burned at high temperatures [74], and its content reflects the total amount of minerals in the plant [75]. In this study, the crude ash content in plants treated with salt was significantly higher than in the control plants, mainly due to the accumulation of minerals such as Na and Cl in the treated plants [76]. After salt treatment, 'R101' and 'A12359' accumulated more crude ash than 'R105' and 'Wrangler', indicating the latter two have stronger resistance to salt stress.

Comprehensive evaluation of bermudagrass salt tolerance

Plant salt tolerance is a complex quantitative trait controlled by multiple genes, with significant differences in salt tolerance observed between different plants and even among different genotypes of the same species. Evaluating salt tolerance based on only one or two indicators often fails to comprehensively reflect a plant's salt tolerance [77]. The Salt Tolerance Coefficient (STC) reveals the comparative results of control and salt-treated varieties across various indicators and is commonly used as a standard for evaluating crop salt tolerance. This study evaluated the salt tolerance of Bermudagrass material using 23 indicators, finding that although these individual indicators reflect the strength of germplasm material's salt tolerance from different perspectives, the results are inconsistent. For example, 'R105' had the highest STC, but its STC was not the highest for indicators such as plant height, fresh weight, fresh-to-dry weight ratio, chlorophyll b, carotenoids, soluble sugars, proline, sodium-potassium ratio, crude fiber, and crude protein, etc. The salt tolerance evaluation results based on single indicators differ from those based on the comprehensive evaluation D value, indicating that considering multiple indicators is essential in screening for salt tolerance in Bermudagrass germplasm resources [78, 79].

This study, based on 23 growth and physiological indicators of four Bermudagrass varieties, calculated the Salt Tolerance Coefficient (STC) through correlation analysis. It then used principal component analysis to reduce dimensions and identify three non-interfering principal components. Weights were calculated based on the contribution rate of the principal components, and the comprehensive evaluation D value for salt tolerance was calculated using the membership function method. The analysis results show that indicators such as plant

height, fresh weight, chlorophyll a, chlorophyll b, carotenoids, sucrose, and sodium-potassium ratio can reflect the resistance of each Bermudagrass variety to sodium chloride well. Under salt stress, 'R105' ranked first in the comprehensive *D* value, while 'A12359' had the lowest comprehensive *D* value. The construction of this comprehensive evaluation system solves the problem of inability to uniformly compare multiple indicators and provides a basis for the selection and breeding of salt-tolerant quality forages, similar to findings in previous evaluations of salt tolerance in plants such as alfalfa [80], green amaranth [81], and quinoa [82].

The results of correlation analysis show that there are significant differences in salt tolerance among Bermudagrass varieties, which indicates that there are genetic differences among the selected Bermudagrass varieties, and the interaction of most traits is significant (Fig. 11). Our results show that fresh weight is significantly correlated with sucrose and proline content, glucose content and sodium-potassium ratio, indicating that osmotic adjustment and antioxidation play a very important role in salt tolerance. Similarly, RWC, PH, PWL, Chl a, Chl b, FW, INV, SoS, CA and Suc contributed more than 60% to genetic diversity, indicating that considering these traits when selecting differentiated parents will contribute to the genetic improvement plan of Bermudagrass [83]. In addition, fresh weight was negatively correlated with MDA and electrical conductivity, which indicated that it was more beneficial for plants to grow in harsh environment to maintain low MDA and electrical conductivity in vivo under salt stress. To sum up, salt-tolerant varieties can maintain higher growth and quality, lower carbohydrate accumulation, lower conductivity and MDA, higher RWC and photosynthetic pigment content than salt-sensitive varieties. The research of Li et al. also found that plant height, stem diameter, chlorophyll, proline, survival rate and malondialdehyde can be used as important indexes to evaluate the salt tolerance of cucumber [84].

Gene expression analysis

For the sucrose metabolic process, invertase (Inv, EC 3.2.1.26) can catalyze the irreversible hydrolysis of sucrose to fructose and glucose [85]. Sucrose phosphate synthase (SPS, EC 2.4.1.14) converts UDP glucose and fructose-6-phosphate into UDP and sucrose phosphate in cytoplasm [86]. This reaction is an important step, and the generated sucrose phosphate will be rapidly and irreversibly degraded into sucrose. Sucrose synthase (SS, EC 2.4.1.13) can reversibly convert fructose and UDP glucose into sucrose and UDP [87]. Cytosol translocatase (CINV1) can convert sucrose into glucose and fructose, which is the key entry point of carbon into cell metabolism. Studies have shown that glucose is received by its signal receptor hxk1, which in turn activates the expression of transcription factors SPL9 and pap1, thus activating the expression of CINV1/CINV2, enhancing its activity and promoting it to decompose more sucrose into glucose [88]. In this study, qRT-PCR showed that *CdCINV1* of salt-tolerant varieties would increase under salt stress, which indicated that bermudagrass would change sucrose accumulated in the body into glucose and fructose, thus increasing osmotic adjustment substances and maintaining the normal physiological function of cells. After salt treatment, the expression levels of salt-tolerant varieties *CdSUS5* and *CdSWEET6* also increased significantly. SUS5 can control the decomposition of sucrose into glucose and fructose, while *SWEET6* can promote the accumulation of fructose. In this study, after salt treatment, the sucrose content decreased, while the glucose and fructose content increased, which also confirmed the up-regulation of the expression levels of *CdSUS5* and *CdSWEET6*. Recent studies have shown that the transcription factor *ZAT5* can activate the expression of *SWEET6* [89]. The expression of salt-tolerant variety *CdSPS1* was also up-regulated, which indicated that *SPS1* gene also played a role in promoting sucrose decomposition. The expression of these four genes is up-regulated, which shows that bermudagrass can regulate the changes of carbohydrates in plants through these four genes, and then regulate the downstream protein to regulate the excretion and absorption of various substances, so as to make a way to deal with salt damage.

Conclusions

In this study, we employed seawater irrigation to simulate salt stress and evaluated multiple indicators including plant height, the percentage of withered leaves, fresh weight, dry weight, photosynthetic pigments, proline, soluble sugars, sucrose, sodium-potassium ratio, crude fat, crude protein, crude fiber, and crude ash, to assess the salt tolerance of four Bermudagrass varieties. Through comprehensive analysis, we identified eight key indicators - plant height, fresh weight, chlorophyll a, chlorophyll b, carotenoids, RWC, sucrose, sodium-potassium ratio, and Soluble sugar - as the core basis for evaluating the salt tolerance capacity of Bermudagrass varieties. Using the membership function method and comprehensive *D* value analysis, we determined the comprehensive ranking of salt tolerance among these four Bermudagrass varieties, from highest to lowest as: 'FB2019R105' > 'FB2019R101' > 'Wrangler' > 'A12359', *CdCINV1*, *CdSPS1*, *CdSUS5*, and *CdSWEET6* can improve the salt tolerance of plants by regulating the changes of carbohydrates. This research provides a scientific basis for the application and selection of Bermudagrass varieties in salt-stressed environments.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06281-8.

Supplementary Material 1

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Author contributions
Xinyu Cui performed most of the experiments and wrote the article with contributions of all the authors; An Shao modified the manuscript. Jianmin Chen and Shuang Li provided technical assistance; J.F. supervised the experiments.

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Data availability
The data that support this study are available in the article and accompanying online supplementary material.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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