

Article



Assessing Germplasm Variation and Tolerance Thresholds of Bermudagrass (*Cynodon dactylon*) to Neutral and Alkaline Salt Stress in Ecological Restoration

Lisi Tang ¹, Wen Li ¹, Qikun Yu ¹, Zongjiu Sun ^{1,2,3} and Peiying Li ^{1,2,3,*}

- ¹ College of Grassland Science, Xinjiang Agricultural University, Urumqi 830052, China; tls1376821784@163.com (L.T.); lwxndcx@163.com (W.L.); yqk13852585307@163.com (Q.Y.); szj@xjau.edu.cn (Z.S.)
- ² Key Laboratory of Grassland Resources and Ecology of Xinjiang, Urumqi 830052, China
- ³ Key Laboratory of Grassland Resources and Ecology of Western Arid Region, Urumqi 830052, China
- * Correspondence: lpy@xjau.edu.cn; Tel.: +86-138-9996-9428

Abstract: Bermudagrass (Cynodon dactylon), a salt-tolerant species surviving in environments with pH up to 9.3, and it exhibits variable germination responses under salt and alkaline stress. This study evaluates the impact of neutral and alkali salts with varying pH levels on bermudagrass seed germination. Six bermudagrass germplasm accessions were analyzed using neutral (NaCl: Na₂SO₄ = 1:1, pH 6.12–7.14) and alkali (NaHCO₃:Na₂CO₃ = 1:1, pH 9.62–9.90) salt treatments. Salt concentrations ranged from 0 to 250 mmol/L, with increments of 25 mmol/L. The assessed parameters included seed germination rate, germination potential, germination index, radicle length, plumule length, seedling weight, and radicle and plumule length ratio. The salt tolerance threshold of each germplasm was calculated using a linear regression fitting model. Critical indicators of salt tolerance were selected through stepwise regression, and the salt-alkali tolerance ranking was determined using a combined membership function and discriminant analysis. The results indicated that the total score decreased with increasing salt concentration under neutral salt stress. Alkali salt stress was more damaging to bermudagrass seedlings than neutral salt stress, inhibiting germination at 50 mmol/L. Neutral salt tolerance thresholds ranged from 31.7 to 207.7 mmol/L, while alkaline salt tolerance thresholds ranged from 16.9 to 53.3 mmol/L. The six germplasm accessions exhibited different responses to salt and alkali stress. Key indicators for neutral salt tolerance included plumule length, radicle and plumule length ratio, and seedling weight. For alkali salt tolerance, key indicators were germination potential, radicle length, and seedling weight, which can be used to screen for resistant germplasms. Our study demonstrates that alkaline salts inhibit seed germination and seedling growth more than neutral salts, and pH affects root growth and the radicle-to-plumule length ratio in seedlings. This research has significant ecological implications, providing insights into the adaptation strategies of bermudagrass in salt-affected and alkaline environments, which could aid in the restoration and management of degraded ecosystems.

Keywords: bermudagrass (*Cynodon dactylon*); neutral and alkaline salt stress; salt tolerance thresholds; germplasm evaluation; sustainable cultivation in saline-alkali areas

1. Introduction

Soil salinity, affecting over 800 million hectares of land, poses a severe threat to global agriculture [1]. Salinity factors not only constrain plant growth but can also lead to plant death [2,3]. Planting salt-tolerant grasses has proven to be an effective strategy for enhancing ecology in combating these challenges [4]. Documented successes in cultivating salt-tolerant plants on saline-alkali soils highlight their constructive role in ecology through mechanisms such as secreting organic acids from roots, regulating soil pH and infiltration patterns, and absorbing inorganic salts from the soil [5,6]. Notably, neutral salts (including



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). NaCl and Na₂SO₄) and alkaline salts (primarily NaHCO₃ and Na₂CO₃) represent two distinct forms of salt stress [7,8]. Alarmingly, due to their high conductivity and pH levels, NaHCO₃ and Na₂CO₃ are more detrimental to vegetation than neutral salts [9]. Given the prevalence of high pH in saline areas, cultivating salt-alkali tolerant plants is crucial for restoring saline-alkali soils and ensuring sustainable development [10].

Seed germination, the most critical stage in the plant life cycle, is particularly sensitive to stress and is often used as an indicator to assess plant adaptability to saline-alkali stress [6]. Understanding how seeds respond to both neutral and alkaline salts is fundamental for successful plant establishment. In coping with saline-alkali stress, plant seeds have evolved multiple mechanisms, including osmotic adjustment to maintain water balance, ion exclusion and chelation to reduce ion toxicity, antioxidant defense systems to resist oxidative damage, gene expression regulation to enhance stress resistance, and seed dormancy and germination strategies to adapt to environmental changes. These mechanisms act synergistically to ensure successful seed germination and growth in salinealkali environments. However, different germplasms exhibit significant variation in their responses to saline-alkali stress [11]. Consequently, screening forage grass varieties for resistance to saline and alkaline stress holds significant importance [12]. While numerous studies on salt-alkali tolerance in grasses have been conducted, most have focused on responses to neutral salt stress, with limited attention given to alkaline salt stress, but the interplay between pH and salt ions, rather than pH alone, is the most crucial factor affecting seed germination and seedling growth [13]. Therefore, the impact of pH should not be examined in isolation; a comprehensive exploration of the variation in salt concentration is also imperative.

Bermudagrass, a valuable warm-type grass germplasm resource, is widely used in various lawn establishments. It maintains its growth vigor under various stress conditions, such as drought and salinization, through adaptive strategies like enhancing water-use efficiency, increasing stress resistance, promoting root development, adjusting growth morphology, and regulating gene expression and metabolic pathways [14]. Previous research has identified neutral salt-tolerant bermudagrass germplasm, which has demonstrated robust growth in soil with a pH of 8.7 and is recognized as highly salt tolerant [15,16]. Moreover, bermudagrass has shown less damage from alkaline salts compared to other turfgrasses, making it a suitable candidate for ameliorating saline-alkali land [17]. However, high-quality bermudagrass varieties are often not amenable to widespread planting in saline-alkali soils. Therefore, exploring additional saline-alkali-tolerant germplasm from wild resources and investigating the extent of saline-alkali resistance and its underlying mechanism is essential for developing new bermudagrass varieties tolerant to saline-alkali conditions.

Xinjiang, with its diverse soil salt compositions and heavy soil salt accumulation, presents a unique opportunity for such research [18]. The main components of saline-alkali soil in southern Xinjiang are carbonates, whereas those in northern Xinjiang are composed of sulfates and chlorides [19]. Bermudagrass possesses widely distributed ecotypes in both the north and south of Xinjiang. It has been reported that Xinjiang bermudagrass can grow well on heavy saline and alkali soil with a pH of 9.3 [20]. While previous studies have reported seed germination responses of bermudagrass to NaCl [21], there is limited information on the germination responses of different bermudagrass accessions to varying salt and alkali stresses. Considering Chai et al.'s [15] previous study of the neutral salt effect on 50 bermudagrass accessions in Xinjiang, it is imperative to investigate the adaptability of bermudagrass to survive in alkaline saline-alkali land in Xinjiang. This study primarily examines the effect of different salt concentrations and salt types (neutral or alkaline salt) on six accessions with varying degrees of salt resistance. We hypothesize that (1) despite similarities, salt and alkali stresses elicit distinct germination responses, especially under different salt concentration regimes in salt-alkali environments; (2) neutral and alkaline salt stresses possess differing germination thresholds; and (3) there are discernible differences among different bermudagrass accessions in the comprehensive evaluation of resistance to neutral and alkaline salts.

2. Materials and Methods

2.1. Experimental Materials and Growth Conditions

Experimental samples were collected from different ecological areas in Xinjiang to ensure representativeness, including three samples from southern Xinjiang and three from northern Xinjiang (Table 1).

Materia	l Code	C1	C7	C12	C20	C21	C33
Material col	lection site	NXJ(I)	NXJ(II)	SXJ(III)	SXJ(IV)	SXJ(V)	NXJ(VI)
Terretter	Ν	43°88.459′	$43^{\circ}86.434'$	39°05.370′	39°25.899′	39°48.623′	43°52.964′
Location	Е	81°36.130′	81°23.547′	$77^{\circ}07.688'$	75°56.520′	$76^{\circ}13.418'$	81°17.551′
Elevatio	on (m)	639	594	1180	1280	1260	596
Mean Rain	fall (mm)	319.8	314.7	74.7	67.4	88.5	314.7
Mean temperature (°C)		8.9	9	11.7	12	13.1	9

Table 1. Bermudagrass seed collection sites and the seed characteristics.

NXJ—Northern Xinjiang; SXJ—Southern Xinjiang; I—Yining City; II—Cha County; III—Yuepuhu County; IV—Kashghar City; V—Aux County.

2.2. Salt-Alkali Stress Treatment

The experiment was conducted in an incubator (model GTOP-380B, Zhejiang Top Instrument Co., Ltd., Hangzhou, China) at Xinjiang Agricultural University (Xinjiang, China) in April 2021 (Figure 1). Bermudagrass seeds were sterilized in 75% alcohol for 30-60 s and washed with distilled water three times. Fifty healthy seeds from each treatment were evenly placed on double filter paper (pH 7.0) in Petri dishes (90 mm diameter), with 10 mL of sterilized saline solution added. The salt treatments consisted of neutral salt (N) (NaCl and Na₂SO₄ at 1:1, pH 6.12–7.14) and alkaline salt (A) (NaHCO₃ and Na₂CO₃ at 1:1, pH 9.62–9.90). Salt concentrations were set at nine levels (0, 25, 50, 75, 100, 125, 150, 200, and 250 mmol/L) (Figure 1). pH and conductivity values for each treatment are shown in Table 2. In total, the experiment comprised 15 treatments with 3 replicates per treatment (270 Petri dishes in total). The germination experiment was conducted for 21 days, with seedlings grown in an incubator under a photoperiod of 16 h light and 8 h dark, 65% relative humidity, and a light intensity of 11,000 Lx. The distilled water in the Petri dishes was weighed daily to maintain a constant weight using a precision balance (Model B-320C, Explorer OHAUS, Santa Clara, CA, USA). The number of germinated seeds (embryo length > $0.5 \times$ seed length) was recorded daily. The radicle and plumule lengths of 10 randomly selected seedlings from each replication were measured using a ruler with 1 mm accuracy. After drying the seedlings in an oven (model 100–800, Memmert, Schwabach, Germany) at 80 °C for 48 h, they were weighed using an analytical balance (Model B-320C, Explorer OHAUS, Santa Clara, CA, USA) with an accuracy of 0.0001 g. The chemicals NaCl, Na₂SO₄, NaHCO₃, and Na₂CO₃ used in this experiment were procured from Tianjin Kemiou Chemical Reagent Co., Ltd. in Tianjin, China. They were of guaranteed reagent (GR) grade with a purity level greater than 99.8%.

Table 2. The total salt mass, pH, and electrical conductivity (EC) at different neutral and alkaline salt concentrations.

Treatment	Concentration (mmol/L)	Total Mass of Salt (g)	pН	EC (mS/cm)
Control	0	0.00 ± 0.00	7.14 ± 0.03	0.01 ± 0.00
	25	3.30 ± 0.16	6.92 ± 0.08	0.87 ± 0.00
	50	6.58 ± 0.06	6.37 ± 0.02	1.69 ± 0.00
	75	9.29 ± 0.07	6.13 ± 0.03	2.50 ± 0.00
NaCl: Na ₂ SO ₄	100	13.36 ± 0.25	5.90 ± 0.07	3.27 ± 0.01
	125	16.43 ± 0.30	5.96 ± 0.08	3.89 ± 0.01
	150	19.46 ± 0.15	5.94 ± 0.01	4.66 ± 0.00
	200	25.10 ± 0.62	5.96 ± 0.02	6.14 ± 0.01



Table 2. Cont.

Figure 1. Picture of representative plants at the time of harvesting, along with the experimental setup and the primary instruments used.

2.3. Data Processing and Evaluation Methods of Indicators

Our evaluation of these six accessions included parameters such as seed germination rate, germination potential, germination index, radicle length, embryo length, seedling weight, and the ratio of radicle to embryo length. We used a linear regression model to calculate the salt tolerance threshold for each germplasm. Key indicators of salt tolerance were identified through stepwise regression, and a combination of membership functions and discriminant analysis was used to determine the levels of salt and alkaline tolerance.

The seed germination indicators, including the germination rate (*GR*), the germination index (*GI*), the germination potential (*GP*), the ratio of radicle and the plumule length (*RRP*), and the salt tolerance coefficient of each index (*SC*) [22], were calculated as follows:

$$GR(\%) = \frac{The normal number of germinated seeds}{The total number of seeds tested} \times 100\%$$
(1)

$$GP(\%) = \frac{The number of normal germinated seeds that reached the peak}{The total number of seeds tested} \times 100\%$$
(2)

$$GI = \sum \frac{Gt}{Dt}$$
(3)

where G_t is the number of germinated seeds, and Dt is the corresponding time to Gt in days.

$$RRP = \frac{radicle \ length}{plumule \ length} \tag{4}$$

$$SC = \frac{The \ value \ of \ the \ index \ measured \ under \ salt \ treatment}{The \ value \ of \ the \ index \ measured \ under \ the \ control}$$
(5)

SC values of each indicator were used to carry out correlation and perform the principal component analysis (PCA). Formulas (6)–(10) are used to calculate the $R(X_t)$, the weight coefficient (W_j) [23], and the Membership composite score (*D*: D_N for neutral salts, and D_A for alkaline salts). The calculation of the evaluation index includes the following steps: The membership function of positive correlation between variables and neutral or alkaline salts according to (6), the membership function of negative correlation according to (7), and the coefficient of standard deviation *S* according to (8). W_j is expressed by formula (9) based on the factor weight, and when combined with the membership function R(Xt), the comprehensive evaluation index *D* according to (10) is as follows:

$$R(Xt) = \frac{(Xt - Xmin)}{(Xmax - Xmin)}, t = 1, 2, 3, \dots, n.$$
 (6)

$$R(Xt) = \frac{(Xmax - Xt)}{(Xmax - Xmin)}, t = 1, 2, 3, \dots, n.$$
(7)

R(Xt) represents the average membership function value for the target trait's resistance to neutral and alkaline salts. A total of t indicators is considered. *Xmin* and *Xmax* represent the minimum or maximum value, respectively, of the *Xt* value for each variable.

$$S = \frac{\sqrt{\sum_{t=1}^{n} (X_{it} - \overline{X}_t)^2}}{\overline{X}_t},$$
(8)

$$Wj = \frac{S_t}{\sum_{t=1}^m S_t},\tag{9}$$

$$D = \sum_{t=1}^{n} [R(X_t) \times W_j], t = 1, 2, 3, \dots, n.$$
(10)

2.4. Statistical Analysis and Graphing

Data processing and graph drawing were conducted using Microsoft Excel 2021 (Microsoft Co., Ltd., Redmond, WA, USA) and Origin 2021 (Origin Lab, Northampton, MA, USA). Statistical analysis was performed using a one-way or multivariate analysis, as well as a cluster analysis, via the IBM SPSS Statistics 26 program (IBM SPSS Statistics, IBM corp., Chicago, IL, USA), with a significance level set at p < 0.05.

3. Results

3.1. Effect of Salinity Interactive in Different Bermudagrass Accessions of Neutral and Alkali Salt Tolerance

There were significant differences among different salinity concentrations (p < 0.01), accessions (p < 0.01 or 0.05), and salt types (p < 0.01) for GR, GP, GI, PL, RL, and RRP (Table 3), but SW was only affected by the salt concentration (p < 0.01). Significant interaction effects existed between salinity concentration and accessions for GP, PL, and RL (p < 0.05), between salinity concentration and salt types for GR, GI, PL, RL, and RRP (p < 0.05), and between accessions and salt types for GR, GP, GI, PL, and RL (p < 0.05). There was a significant three-way interaction effect (salinity concentration × accessions × salt types) for GR and RL (p < 0.05).

Item	GP	GR	GI	PL	RL	RRP	SW
Salinity concentration (SC)	30.27 **	43.82 **	34.69 **	37.59 **	39.82 **	21.05 **	31.62 **
Accessions (G)	23.73 **	64.25 **	40.51 **	7.96 **	3.73 **	2.41 *	0.92 ^{NS}
Salt types (ST)	7.56 **	23.94 **	12.75 **	66.13 **	120.33 **	85.24 **	1.56 ^{NS}
$SC \times G$	1.83 *	1.65 ^{NS}	1.3 ^{NS}	2.69 **	2.59 **	1.49 ^{NS}	1.17 ^{NS}
SC imes ST	1.97 ^{NS}	9.35 **	3.62 *	5.01 **	12.44 **	3.80 *	0.27 ^{NS}
G imes ST	2.74 *	4.84 **	2.89 *	3.02 *	6.80 **	1.81 ^{NS}	0.23 ^{NS}
$SC \times G \times ST$	1.33 ^{NS}	2.87 *	2.27 ^{NS}	0.8 ^{NS}	2.78 *	1.5 ^{NS}	0.39 ^{NS}

Table 3. Variance analysis of three-way ANOVA on seed germination of bermudagrass by salinity concentration, accessions, and salt types.

GP—germination potential; GR—germination rate; GI—germination index; PL—plumule length; RL—radicle length; RRP—Ratio of radicle and plumule length; SW—seedling weight. * p < 0.05; ** p < 0.01; NS—no significant difference.

3.2. Seed Germination and Seedling Growth Response of Bermudagrass Accessions under Neutral and Alkali Salt Stress

In this study, to explore the differences in seed germination response to various salt concentrations under neutral and alkaline salt stress in six bermudagrass accessions, the data were fitted with a Coefficient of determination of $R^2 \ge 0.85$, and a linear graph was drawn for comparison (Figure 2 and Table 4). The study found that, for the majority of indicators in the six bermudagrass accessions, both neutral and alkaline salt stress decreased gradually as salinity concentration increased and ultimately reached zero.

We observed a minimal promotional effect in neutral salt stress but not in alkaline salt stress. At the same time, there were significant differences among the six bermudagrass accessions. The germination potential of C7 and C21, the radicle length of C12 and C21, the ratio of radicle and plumule length of C20 and C21, and the seedling weight of C1 and C21 were significantly higher under 25 mmol/L neutral salts than CK (p < 0.05). The radicle length of C20, the ratio of radicle and plumule length of C20, the ratio of radicle and plumule length of C20, mmol/L neutral salts than CK (p < 0.05). The radicle length and the seedling weight of C20, C33, and C7 were significantly higher under 50 mmol/L neutral salts than CK (p < 0.05).

Table 4. Regression analysis of the relationship between salinity concentration and germination parameters of bermudagrass seeds under salt-alkali stress.

	Indicator		21	C	27	C	12	C	20	C	21	C	33
marcator		Ν	Α	Ν	Α	Ν	А	Ν	Α	Ν	Α	Ν	Α
	Model number	2 **	2 **	2 *	3 **	2 *	4 **	1 *	2 **	2 *	2 **	2 **	2 **
GP R ²	R ²	0.935	0.915	0.915	0.939	0.94	0.887	0.882	0.85	0.866	0.863	0.87	0.872
CD	Model number	3 **	3 **	5 **	3 **	2 **	2 **	2 **	2 **	4 **	3 **	5 **	2 **
GR R ²	R ²	0.928	0.961	0.898	0.91	0.961	0.905	0.904	0.876	0.886	0.861	0.883	0.886
01	Model number	2 **	2 **	2 **	3 **	2 **	2 **	2 **	2 **	2 **	2 **	3 **	2 **
GI	R ²	0.879	0.876	0.876	0.94	0.982	0.906	0.975	0.917	0.892	0.92	0.885	0.862
БТ	Model number	2 **	4 **	2 **	4 **	4 **	3 **	3 **	3 **	4 **	3 **	2 **	3 **
KL	R ²	0.97	0.912	0.878	0.892	0.897	0.855	0.864	0.906	0.859	0.912	0.952	0.859
DI	Model number	2 **	2 **	2 **	2 **	2 **	2 **	1 **	2 **	1 **	3 **	2 **	2 **
PL	\mathbb{R}^2	0.947	0.873	0.877	0.879	0.863	0.888	0.936	0.859	0.904	0.852	0.887	0.88
DDD	Model number	2 **	3 **	2 **	4 **	2 **	3 **	3 **	2 **	4 **	3 **	2 **	3 **
KRP	R ²	0.97	0.863	0.899	0.885	0.902	0.874	0.891	0.851	0.882	0.927	0.855	0.878
CIAT	Model number	4 **	2 **	3 **	2 **	4 **	2 **	2 **	2 **	5 **	2 **	4 **	4 **
SW	R ²	0.962	0.877	0.945	0.854	0.861	0.874	0.856	0.888	0.855	0.886	0.869	0.869

Model number 5quintic model; Model number 4quartic model; Model number 3cubic model; Model number 2quadratic model; Model number 1linear model. N.A. GR.GP.GI.PL.RL.RRP.SW represents Nneutral salt treatments; Aalkaline salt treatments; GRgermination rate; GPgermination potential; GIgermination index; RLradicle length; PLplumule length; RRPRatio of radicle and plumule length; SWseedling weight. The R² values in the table correspond to those of the fitted curves shown in the figures. * $p \le 0.05$, ** $p \le 0.01$.

However, neutral and alkaline salts exerted different effects on seed germination of the bermudagrass accessions. Specifically, C33, C1, and C20 could not germinate at neutral

salt concentrations of 100, 125, and 150 mmol/L, respectively. Only C7, C12, and C21 could germinate at a 150 mmol/L concentration of neutral salt. In contrast, when the alkaline salt concentration reached 50 mmol/L, five accessions could not germinate; only C21 could germinate under 75 mmol/L alkaline salt, but its germination rate was only 0.67%. At a salt concentration of 75 mmol/L, the germination potential of C33 was reduced to zero under neutral salt stress, while that of C20, C12, C21, C7, and C1 was 30.67, 17.33, 8.67, 8.00, and 4.00%, respectively. Furthermore, only C21 had a 0.67% germination potential under alkaline salt stress, while the rest of the accessions reached zero at 75 mmol/L. These results indicate that alkaline salt stress has a more pronounced inhibitory effect on germination than neutral salt treatment in bermudagrass accessions.



Treatment concentration (mmol/L)

Figure 2. Germination potential (GP), germination rate (GR), germination index (GI), radicle length (RL), plumule length (PL), the ratio of radicle and plumule length (RRP), and seedling weight (SW) of bermudagrass seeds under different salt-alkali stresses of different concentrations of salinity. Blue and red dots indicate respective values under neutral and alkaline salt stresses for each parameter, while dashed lines of the same colors represent the fitted curves as salinity concentrations increase. refer to Table 4 in the supporting information section.

The difference in seedling growth indicators between neutral and alkaline salts was more pronounced than that observed in germination indicators (Figure 2). Since alkaline salt stress showed minimal germination at 75 mmol/L, we compared the growth indicators of the two salt stresses at 50 mmol/L. Under neutral salt stress, the ranges of radicle length, the plumule length, the ratio of radicle to plumule length, and the seedling weight were 8.21–24.62 mm, 12.38–18.15 mm, 0.66–1.36, and 0.32–0.58 mg, respectively. Under alkaline salt stress, these ranges were 3.17–6.25 mm, 9.78–14.42 mm, 0.28–0.53, and 0.23–0.36 mg, respectively. These findings indicate that alkaline salt stress has a more significant impact on the growth of bermudagrass seedlings than neutral salt stress, particularly in inhibiting root length.

The standard for calculating thresholds involves reducing each index to 50% under control conditions (CK) to evaluate the tolerance of different germplasms under neutral and alkaline salt stress (Figure 3). The thresholds for SW, RRP, RL, PL, GI, GP, and GE under neutral salt stress ranged from 60.1–207.7, 54.1–144.3, 47.1–106.5, 63.7–129.5, 32.8–63.2, 31.7–62.2, and 38.9–91.4 mmol/L, respectively. Under alkaline salt stress, the thresholds for SW, RRP, RL, PL, GI, GP, and GE ranged from 44.5–53.3, 20.3–29.6, 16.9–23.8, 41.8–50.4, 24.4–45.8, 19.4–47.2, and 27.1–52.3 mmol/L, respectively (Figure 2).



Figure 3. Tolerance thresholds of neutral salts and alkaline salts for each germination parameter. Germination potential (GP), germination rate (GR), germination index (GI), radicle length (RL), plumule

length (PL), the ratio of radicle and plumule length (RRP), and seedling weight (SW). They were used as the salt or alkali tolerance threshold when the germination parameters of bermudagrass were reduced to 50% at CK.

3.3. Comprehensive Evaluation of Salt and Alkali Tolerance in Bermudagrass

The salt tolerance coefficient or alkali tolerance coefficient can reflect salt tolerance, further elucidating the correlation between indicators, and comparing the saline-alkali resistance of six bermudagrass accessions. Therefore, we replaced the original values with the neutral salt tolerance coefficient (SC_N) and the alkaline salt tolerance coefficient (SC_A). Given the significant correlation observed among the indicators (Figure 4), this study carried out PCA to avoid information redundancy among the indicators under neutral or alkaline salt stress. PCA is a functional multivariate method that evaluates significant distinguishing features of variability, identifies relationships among growth indicators, and groups them based on similar properties.



Figure 4. Correlation analysis of neutral salt and alkaline salt stress in bermudagrass seedlings.

The PCA extracted a common factor with a cumulative variance contribution rate of 85%, which was conducted on germination and growth indicators under salt and alkali stress. We calculated the coefficients for each index in the comprehensive scoring model by determining the typical factor score, and these extracted scores were then weighted to form the neutral or alkaline salt tolerance indices of the bermudagrass accessions. In the present investigation, performing PCA converted seven indicators into two new comprehensive indexes that explained 90.9% and 94.7% of the original index information described under neutral and alkaline salt stress treatment, respectively (Figure 5). Under neutral salt stress, PC1 showed the highest percentage of variation (79.7%), followed by 11.2% for PC2. Germination rate, index, potential, and radicle length were positively influential traits in PC1, while the ratio of radicle and plumule length, plumule length, and seedling weight were significant characteristics in PC2. Under alkaline salt stress, PC1 showed the highest percentage of variation (84.6%), followed by 10.1% for PC2. Seedling weight, radicle length, the ratio of radicle and plumule length, and plumule length were positively influential traits in PC1, while germination potential and germination rate were significant characteristics in PC2.

The trend of different bermudagrass accessions in response to increasing salt concentration differs under neutral and alkaline salt stresses (Table 5). The tolerance coefficients of these accessions to neutral and alkaline salts were ranked using the membership function fuzzy comprehensive score value. The results showed that the response trends of different accessions under neutral salt treatment varied. Specifically, C1, C20, and C33 exhibited an

increase followed by a decrease with increasing salt concentration, peaking at 50 mmol/L, indicating a promotion effect at low salt concentrations. In contrast, the remaining three accessions decreased with increasing salt concentration, whereas no promotion effect at low concentrations was observed.



Figure 5. PCA of neutral salt and alkaline salt stress in bermudagrass seedlings. PC1—the first principal component; PC2—the second principal component.

	Material	D	N	D _A			D _N		D _A	
Concentration		SC _N Value	Rank	SC _A Value	Rank	Material	SC _N Value	Rank	SC _A Value	Rank
25		0.438	2	0.359	2		0.632	2	0.523	2
50		0.488	1	0.601	1		0.748	1	0.663	1
75		0.342	3	0.070	3		0.483	4	0.070	3
100		0.193	4	0.070	3	C2 0	0.464	5	0.070	3
125	CI	0.095	5	0.070	3	C20	0.508	3	0.070	3
150		0.095	5	0.070	3		0.328	6	0.070	3
200		0.095	5	0.070	3		0.095	7	0.070	3
250		0.095	5	0.070	3		0.095	7	0.070	3
25		0.650	1	0.307	2		0.765	1	0.479	2
50		0.519	2	0.609	1		0.560	2	0.621	1
75		0.335	4	0.070	3	C2 1	0.463	3	0.070	3
100		0.495	3	0.070	3		0.451	4	0.070	3
125	C/	0.095	6	0.070	3	C21	0.265	6	0.070	3
150		0.313	5	0.070	3		0.422	5	0.070	3
200		0.095	6	0.070	3		0.095	7	0.070	3
250		0.095	6	0.070	3		0.095	7	0.070	3
25		0.719	1	0.380	2		0.558	2	0.477	2
50		0.618	2	0.625	1		0.582	1	0.731	1
75		0.420	5	0.070	3		0.238	3	0.070	3
100	C12	0.474	3	0.070	3	C 22	0.095	4	0.070	3
125		0.176	6	0.070	3	C33	0.095	4	0.070	3
150		0.438	4	0.070	3		0.095	4	0.070	3
200		0.095	7	0.070	3		0.095	4	0.070	3
250		0.095	7	0.070	3		0.095	4	0.070	3

Table 5. A comprehensive evaluation of salt concentration and accessions under neutral and alkaline salt stress.

Alkaline salt treatment showed no significant differences in bermudagrass accessions compared with neutral salt treatment. All accessions increased and then decreased with increasing salt concentration, similar to the neutral salt stress treatment, indicating a peak at 50 mmol/L. All alkaline salt stress treatments showed a particularly low salt concentrations promotion effect.

A comprehensive evaluation of the different accessions revealed differences in tolerance coefficients under neutral and alkaline salt stresses. The neutral and alkaline salts tolerance coefficients of the different bermudagrass accessions were ranked by calculating the mean of the membership function fuzzy comprehensive score value (Table 6). Their ranks (1–6) were significantly higher than those of the alkaline salt stress treatments (7–12) under neutral salt stress. Bermudagrass seedlings suffered more harm under alkaline salt stress with high pH conditions than under neutral salt stress. All performed less well under alkaline salt stress than under neutral salt stress. In the different bermudagrass accessions, based on the size of the membership function fuzzy comprehensive score value, the rank order (highest to lowest) of the scores was C20, C21, C12, C7, C33, and C1 under neutral salt stress. At the same time, it was C33, C20, C21, C12, C1, and C7 under alkaline salt stress. Combining bermudagrass accessions into three levels of tolerance-high salt or alkaline, medium salt or alkaline, and sensitive salt or alkaline is necessary. Cluster analysis is also required to find accessions with both neutral salt and alkali salt tolerance, providing technical support for subsequent suggestions on cultivating accessions in saline-alkali land.

Material	SC _N Value Average	Internal Ranking of Neutral Salt Treatment	Total Ranking of Each Treatment	SC _A Value Average	Internal Ranking of Alkaline Salt Treatment	Total Ranking of Each Treatment
C1	0.230	6	6	0.173	5	11
C7	0.325	4	4	0.167	6	12
C12	0.379	3	3	0.178	4	10
C20	0.419	1	1	0.201	2	8
C21	0.390	2	2	0.190	3	9
C33	0.232	5	5	0.204	1	7

Table 6. Comprehensive evaluation and ranking of various accessions.

3.4. Clustering Based on Morphological Traits and Tolerance of Bermudagrass Accessions

Cluster analysis was performed to group the bermudagrass accessions based on similar properties. The dendrogram was generated using the Euclidean squared distance method, considering all morphological traits and systematically clustering the D value. Cluster analysis grouped the six accessions into three distinct groups (Group 1—Higher, Group 2—Medium, and Group 3—Sensitive). Under neutral salt stress, Group 1 consisted of 1 accession, Group 2 of 3 accessions, and Group 3 of 2 accessions. Furthermore, under alkaline salt stress, Group 1 consisted of 3 accession, Group 2 of 2 accessions, and Group 3 of 1 accession (Figure 6).

Fisher discriminant analysis (Bayes Coefficient) was used to validate the accuracy of the neutral and alkaline salt resistance assessments and clustering results (Figure 7). Under neutral salt stress, C20 showed the highest projected score, while C21, C12, and C7 exhibited moderate scores, and C33 and C1 had the lowest. Under alkaline salt stress, C33, C21, and C20 demonstrated the highest projected score, C12 and C1 showed moderate scores, and C7 had the lowest. The result of the discriminant function was 100% consistent with that of the Euclidean squared distance method.



Figure 6. Cluster analysis of accessions materials under neutral and alkaline salt stress in bermudagrass.



Figure 7. Discriminant analysis verification of bermudagrass accessions materials cluster results in neutral salt and alkaline salt stress.

3.5. Screening Critical Indicators of Salt Tolerance of Bermudagrass

A predictive model was created using Pearson's correlation and multiple regression (Table 7). The D value of the membership function was taken as the independent variable, and the salt tolerance coefficient value SC of each index was considered the dependent variable. Using correlation analysis, a stepwise regression equation was established to screen the indicators and quantify the relationship between dependent and independent variables in bermudagrass. An optimal regression equation was derived to identify three morphological and yield indicators (plumule length, ratio of radicle and plumule length, and seedling weight) and was used as the screening indicators for bermudagrass varieties with neutral salt tolerance. Simultaneously, a combined stepwise regression equation and correlation analysis method were used to identify three indicators (germination potential, radicle length, and seedling weight) for evaluating alkaline salt tolerance. The fitting curve R^2 of neutral salt and alkaline salt stress reached 0.999.

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Salt Type	Stepwise Regression	R ²	F Value	<i>p</i> -Value
Neutral salt resistance	$D_N = 0.31PL + 0.35RRP + 0.38SW$	0.999	14,214.04	0.000
Alkaline salt resistance	$D_A = -0.1GR + 0.61RL + 0.51SW$	0.999	10,221.56	0.000

Table 7. Model prediction of neutral salt and alkaline salt resistance in bermudagrass germplasm materials.

4. Discussion

4.1. Adaptation and Variability of Bermudagrass Germplasm to Two Types of Salt

It is worth noting that different germplasms of bermudagrass exhibit disparate adaptive mechanisms when faced with neutral and alkaline salts. In this study, two neutral salts, NaCl and Na₂SO₄, were selected for mixing at a ratio of 1:1. The findings of Chai Yan [15] on neutral salt (NaCl) treatment are similar to those in this paper. Our study found that high pH may be one of the crucial environmental limiting factors for the germination and establishment of bermudagrass in saline-alkali soil. The experimental results showed that the germplasms of bermudagrass exhibited significant differences in seed germination patterns when subjected to neutral salt and alkaline salt stress. Under the same Na⁺ concentration, the growth inhibitory effect of alkaline salt stress was greater than that of neutral salt stress. It has been reported that alkaline salts are more stressful than neutral salts to many plants, such as Reaumuria soongorica [24], Japonica Rice [25,26], Kentucky Bluegrass (Poa pratensis) [27], Alfalfa (Medicago sativa) [28], and Brassica napus [29]. It is well known that alkaline salt stress and neutral salt stress share common stress factors, but alkaline salt stress exhibits a higher level of involvement of high pH and CO_3^{2-}/HCO_3^{-} . Therefore, owing to the high pH and CO_3^{2-}/HCO_3^{-} involvement, alkaline salt stress has a greater adverse effect on plants than neutral salt stress.

However, at lower salinity levels (0–50 mmol/L), the seed germination rate was notably higher even under alkaline salt stress treatment at elevated pH. This phenomenon suggested that high pH had no inhibitory effect on seed germination under low salinity conditions, rather it exhibited a specific regulatory impact on seed germination under common salinity conditions. The pH range of alkaline salts in this study was 9.62–9.90. The interaction between high salt concentrations and high pH values significantly inhibits seed germination and seedling growth, potentially leading to seed structural breakdown, seedling malformation, and death. This phenomenon is observed in multiple plant species, including Brassica rapa [30], Halostachys capsica [31], and Reaumuria soongorica [24]. After 21 days, the stressed seeds were transferred to distilled water in this experimental group. It was found that most of the seeds recovered and germinated again (data not listed), indicating that bermudagrass seeds adopt a dormant adaptation strategy in response to high salinity and pH, enabling them to survive the harsh external environment by remaining in a non-germinated state. Under alkaline salt stress, the seedlings' radicle length, plumule length, and seedling weight were reduced. This may be attributed to the high pH environment in the Petri dish, which caused ion imbalance and metabolic imbalance in the plant, ultimately disrupting the plant cell membrane structure. High pH under alkaline salt stress is an essential factor that cannot be ignored when considering the planting and application of bermudagrass in saline-alkali land in Xinjiang in the future.

The early seedling stage is another critical period for plants to survive under salinealkali conditions [32]. Our results indicate that the radicle length, plumule length, and other indicators of seedling growth were all 1.94–2.70 times greater under the same concentration of neutral salt stress compared to alkaline salt stress. Seedling damage is significant, and these findings are consistent with previous studies on linseed [33] and alfalfa (*Medicago sativa*) [34]. Bermudagrass grew well under lower salinity stress but experienced hindered radicle growth under higher pH conditions, with a decreased ratio of radicle to plumule length, indicating that bermudagrass radicles are more sensitive to salinity and pH stress. These results are consistent with those of a study on *Arabidopsis* [35]. This phenomenon may be attributed to high pH and its interaction with ionic stress. The high pH environment, especially in combination with high salt stress, can cause ion imbalance, metabolic disturbance, disrupt the structure and function of root cells, and reduce seedling elongation [36].

4.2. Differences in Salt Tolerance Thresholds and Indicator Screening

There were significant differences in the salt tolerance thresholds between neutral and alkaline salt stresses in bermudagrass. The germplasm of Xinjiang bermudagrass exhibited different seed germination patterns when subjected to neutral salt and alkaline salt stress. The threshold range of each index under neutral salt stress was determined to be 31.7–207.7 mmol/L, while the threshold range for alkaline salt stress was 16.9–53.3 mmol/L. The primary reason for this difference may be that alkaline salt stress shares the same salt concentration stress characteristics as neutral salt stress but additionally increases high pH stress, resulting in more harmful effects.

The results of stepwise regression analysis among various stress factors indicate that saline-alkali stress has differing effects on the germination and early seedling stages of bermudagrass. Under the influence of high pH, alkali-resistant bermudagrass germplasm may need to germinate rapidly to avoid competition at the seedling stage. Compared with seed germination, the seedling stage is much more complex. The radicle needs to absorb numerous nutrient elements while maintaining osmotic and ion balance. However, a high pH value will strongly inhibit the ion absorption of plant cells, disrupting radicle growth and usually affecting the growth of the plant's radicles. The critical indicators for selection of germplasm under neutral salt stress were plumule length, the ratio of radicle to plumule length, and seedling weight; the key indicators for selection of germplasm under alkaline salt stress were germination potential, radicle length, and seedling weight. Whether under neutral salt stress or alkaline salt stress, seedling growth indicators are the primary indicators used to screen germplasm. It is shown that the germination rate and germination index of seeds have little effect on selecting salt-tolerant bermudagrass seeds. In the future germplasm screening process, more attention should be paid to indicators such as seedling growth.

4.3. Similarities and Differences in Salt Tolerance Evaluation Methods and the Variability of Their Results, Differences, and Similarities in the Final Screening Materials

This text is based on a threshold evaluation method involving the construction of a multivariate linear model to initially compare the response patterns of various bermudagrass germplasms to neutral and alkaline salt stresses as the stress salt concentration increases. The results were obtained by calculating thresholds: Under neutral salt stress, the average threshold ranking for each index was C20 > C21 > C12 > C7 > C33 > C1. Under alkaline salt stress, the ranking was C20 > C12 > C21 > C33 > C1 > C7. Despite obtaining these rankings, the overlap between the indexes was not considered, making the results less intuitive. Correlation analysis results verified significant overlap between the indicators, suggesting a dimensionality reduction process, such as PCA, is required. The results were obtained by combining two different cluster analysis methods: the Euclidean distance method and discriminant analysis. Through the integrated analysis of affiliation functions, the cluster analysis results under neutral salt treatment indicated that C20 was a highly salt-tolerant material, C21, C12, and C7 were moderately salt-tolerant materials, and C33 and C1 were salt-sensitive materials. The alkaline cluster analysis results showed that C20, C21, and C33 were highly alkali-resistant materials, C12 and C1 were moderately alkali-resistant materials, and C7 was a sensitive alkaline material. C20, which exhibited the highest tolerance to neutral salt, also demonstrated stable stress resistance under saline-alkali stress and can be recommended as the preferred germplasm for reseeding and establishment of bermudagrass in saline-alkali areas for practical applications. The results of neutral salt stress and threshold evaluation were consistent. The ranking of C12 was significantly lower under alkaline salt stress, possibly due to the removal of overlap between the indicators. However, regardless of the method used, the salinity tolerance of C20 remained stable, making it a recommended germplasm for bermudagrass in saline areas of Xinjiang. This study compared the effects of Xinjiang bermudagrass germplasms on seed germination under neutral salt stress and alkaline salt stress. The initial exploration of differences in the adaptation of these germplasms to neutral and alkaline salt stress lays the foundation for future physiological and molecular research on salinity and alkali stress tolerance.

5. Conclusions

This preliminary study investigates the responses of Xinjiang bermudagrass under neutral salt stress and high pH alkaline salt stress. A linear decrease in each index was observed as salt concentration increased, with seeds and seedlings experiencing greater damage under alkaline salt stress. Specifically, the threshold ranges for the indicators were 31.7–207.7 under neutral salt stress and 16.9–53.3 under alkaline salt stress. PCA and membership function were employed to comprehensively evaluate the salinity and alkali resistance of six Xinjiang bermudagrass samples. Key indicators for identifying salinity and alkali resistance were as follows: for neutral salt, plumule length, radicle length, and seedling weight; for alkaline salts, germination potential (defined as the percentage of seeds germinating within a specified time), radicle length, and seedling weight. Our findings underscore the importance of considering seedling growth indicators in further screening for salinity-tolerant bermudagrass. The comprehensive evaluation revealed that C20 exhibits high salt and alkali resistance; C21 shows high alkali resistance and moderate salt resistance; C33 is salt-sensitive but highly alkali-resistant; C12 has average alkali and salt resistance; C7 has average salt resistance but is alkali-sensitive; and C1 is generally alkali-resistant but salt-sensitive. These results indicate that bermudagrass seed germination ability decreases with increasing salt concentration, with alkaline salt causing more significant damage than neutral salt. Consequently, for establishing and reseeding bermudagrass in saline-alkali areas, considering the local soil's saline-alkali composition is crucial. Based on our findings, we recommend selecting appropriate germplasm based on pH levels to enhance bermudagrass cultivation sustainability in these areas, thereby contributing to sustainable development goals.

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