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Identification and Evaluation of Celery Germplasm Resources for Salt Tolerance

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Abstract: This study evaluated the salt tolerance in 40 celery germplasm resources to clarify the different salt tolerances of celery germplasm. A gradient treatment with different concentrations of NaCl solutions (100, 200, and 300 mmol·L^{-1}) was used to simulate salt stress. After 15 days of salt treatment, 14 indicators related to plant growth, physiology, and biochemistry were determined. The results showed that different celery varieties responded differently to salt stress. Notably, there were significant variations in below-ground dry weight, root–crown ratio, antioxidant enzyme activity, and soluble protein content among the accessions under salt stress. Principal component analysis was used to identify important indices for evaluating salt tolerance, including plant height, spread, content of soluble protein, and so on. A comprehensive evaluation was conducted utilizing the salt damage index, principal component analysis, affiliation function analysis, and cluster analysis. The 40 celery germplasms were classified into five highly salt-tolerant, seven salt-tolerant, fifteen moderately salt-tolerant, nine salt-sensitive, and four highly salt-sensitive germplasms. SHHXQ, MXKQ, XBQC, XQ, and TGCXBQ were highly salt-tolerant germplasms, and BFMSGQ, HNXQ, ZQ, and MGXQW were highly salt-sensitive germplasms. The results of this study provide a reference for the variety of celery cultivation in saline areas and lay a foundation for the selection and breeding of salt-tolerant varieties of celery.

Keywords: celery; salt tolerance evaluation; salt damage index; affiliation function; principal component analysis; cluster analysis

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Citation: Wu, L.; Du, J.; Zhang, Y.; Xue, Y.; Jiang, C.; Lu, W.; Zheng, Y.; Zhou, C.; Xiong, A.; Li, M. Identification and Evaluation of Celery Germplasm Resources for Salt Tolerance. *Agronomy* **2024**, *14*, 1048. https://doi.org/10.3390/agronomy14051048

Academic Editors: Daniele Antichi, Christian Frasconi and Marco Fontanelli

Received: 8 April 2024
Revised: 9 May 2024
Accepted: 10 May 2024
Published: 15 May 2024

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1. Introduction

Soil salinization is one of the major abiotic stress factors that affect global agricultural production, which has detrimental effects on the normal growth and development of plants, including limiting water absorption and respiration, yellowing, wilting, and even death of plants [1]. The high-salinity environment causes secondary stresses such as osmotic, ionic, and oxidative stress in plants, which causes changes in ionic homeostasis, cellular activity responses, and other aspects of the plant [2]. To resist salt stress, plants typically make adaptive changes in morphology and physiological processes to alleviate secondary stresses induced by stress conditions [3]. Soil salinization affects the growth and development of vegetable crops, resulting in reduced yields, lower quality, and major challenges in sustainable agricultural development [4]. Presently, the effect of salt stress on plants has attracted widespread attention. The selection and breeding of salt-tolerant materials, revealing the mechanism by which plants become salt-tolerant in salt-stressed environments, are significant for developing and utilizing saline soil resources.

Celery (*Apium graveolens* L.), an important leafy vegetable belonging to the Apiaceae family, has high nutritional and medicinal value and a distinct odor, which is favored by the majority of consumers [5]. Celery has been cultivated for over 2000 years, and it boasts a rich collection of germplasm resources as well as a wide variety of cultivars [6]. Celery can be divided into local and western celery, based on the source. Local celery varieties are characterized by short plants, slender petioles, and more hollow structures with a strong aroma, whereas western celery plants are tall, have thick petioles, exhibit vigorous growth, and are less aromatic. The color of the petiole can be used to further divide celery into five distinct accessions: green, white, yellow, purple, and red. Lastly, it can also be divided based on the presence or absence of a medullary cavity in the petiole into hollow and solid celery. The rich germplasm resources and strong adaptability of celery have contributed to its extensive global cultivation [7]. Salt stress inhibits the growth and photosynthesis of celery and reduces the quality of celery [8], and damages the photosynthetic and antioxidant systems of celery and the structure of cell membranes [9]. Moreover, the expression of the *AgSUT1* gene in celery was significantly inhibited under salt stress [10]; the activity of mannitol-6-phosphate reductase was significantly increased under salt stress, which subsequently increased mannitol biosynthesis in celery [11]. As may be seen from above, we know that the practical implications of conducting research on salt stress tolerance in celery, screening salt-tolerant germplasm resources, and selecting and breeding new salt-tolerant varieties are of great significance for salinized land use.

Currently, standards and methods for salt tolerance identification have been established, and several salt-tolerant germplasm resources have been screened in vegetable crops, such as tomato [12], cucumber [13], pepper [14], and eggplant [15]. Li et al. [13] analyzed the phenotypic and physiological indices of 18 cucumber germplasm resources under salt treatment and identified six salt-tolerant and salt-sensitive cucumber germplasms based on a comprehensive evaluation of phenotype, integrated viability, cluster analysis, and principal component analysis. Gyanagoudar et al. [15] evaluated the salinity response of 110 eggplant germplasms at the germination stage using the value of affiliation function (MFV) and classified them into five categories: highly salt-tolerant, salt-tolerant, moderately salt-tolerant, salt-sensitive, and highly salt-sensitive. Currently, there are only a few studies on the evaluation, screening, and identification of salt-tolerant germplasms in celery. Therefore, this study can provide a theoretical basis for the cultivation of celery in saline and alkaline regions and the selection and breeding of salt-tolerant varieties by studying the adaptability of different germplasm resources of celery to salt stress and by screening and evaluating a number of celery varieties with strong salinity tolerance.

2. Materials and Methods

2.1. Plant Material

The plant materials utilized in this study included 40 celery accessions collected by the College of Horticulture of Sichuan Agricultural University and planted at the breeding base located at Sichuan Agricultural University (103°51′29″ E, 30°42′20″ N). The names and sources of the 40 varieties are shown in Table S1.

Celery seeds were sterilized and disinfected by soaking in warm water at 50 °C for 30 min; subsequently, they were removed from warm water and soaked in water at 20 °C for 24 h. The seeds were then sown in seedling hole trays (50 holes) and placed in an incubator to germinate. Once the seedlings grew 2–3 true leaves, the robust seedlings with consistent growth were transferred to nutrient pots (14 cm × 16 cm) (substrate: nutrient soil:perlite:vermiculite = 3:1:1). These pots were then subjected to routine outdoor management, during which 30 mL of nutrient solution ($Ca(NO_3)_2$ 2.0 mol·L^{-1}; K_2SO_4 2.0 mol·L^{-1}; $Ca(H_2PO_4)_2$·$CaHPO_4$ 2.0 mol·L^{-1}; $CaSO_4$ 1.0 mol·L^{-1}; $MgSO_4$ 4.0 mol·L^{-1}) (Sangon Biotech Co., Ltd., Shanghai, China) was applied every two days per pot. After the varieties had reached seedling age in about 30 days, they were subjected to salt stress by irrigation of salt solution on the roots. Ten plants of each variety were treated and compared before and after treatments, setting up three repetitions with a total of 30 plants.

Different concentrations of NaCl (Sangon Biotech Co., Ltd., Shanghai, China) solution (100, 200, and 300 mmol·L^{-1}) were used for gradient treatment to simulate salt stress, and the salt concentration was increased once every three days. When the salt concentration reached 300 mmol·L^{-1} on the ninth day, the seedlings were investigated for salt damage every two days, and the salt damage symptom level of each variety was recorded three times. After 15 days of salt stress treatment, growth indices were measured, and the growth status of seedlings was recorded to calculate the salt damage index and determine the salt tolerance level of each species according to the salt tolerance grading standard [16]. Fresh samples were then frozen with liquid nitrogen, transferred to 50 mL test tubes, and stored at −80 °C for determining physiological and biochemical indices with three replicates.

2.2. Salt Damage Symptom Classification and Salt Damage Index

The grading standard of salt damage symptoms was established as described by Guan et al. [16], and the salt tolerance grading criteria were improved, as shown in Table 1.

Table 1. Classification criteria of salt injury symptoms of celery seedlings.

Grade	Symptoms of Salt Injury	Salt Damage
0	Plants with normal growth, erect petioles, no yellowish leaves	none
1	Plants mildly chlorotic, petioles bent downward, leaves crumpled and yellowed	slight
2	Plant chlorosis, petiole water loss and collapse, 1–2 true leaves water loss and crumpling	moderate
3	Plants moderately chlorotic, most petioles collapsed, 2–3 true leaves crumpled and scorched	severe
4	Plant severe chlorosis, collapse or even death, 3–4 true leaves withered and scorched	very severe

The salt damage index reflects the degree of salt damage to plants. In the course of this investigation, the salt damage symptoms of celery seedlings were graded, statistics were compiled, and the salt damage index was calculated.

Salt damage index = Σ (number of salt damage levels × number of plants with corresponding salt damage levels)/(total number of plants × number of highest salt damage levels) × 100%.

The salt tolerance of each celery germplasm was determined on the basis of the salt damage index. Those with a salt damage index of 0~20% were classified as highly salt-tolerant varieties, 20.1~40% as salt-tolerant varieties, 40.1~60% as moderately salt-tolerant varieties, 60.1~80% as salt-sensitive varieties, and 80.1~100% as highly salt-sensitive varieties.

2.3. Measurement of Growth Indicators

A tape ruler was used to measure the height of the plant from the base of the stem to the growth point of the seedling, and a soft tape ruler was used to measure the size of the widest part of the leaf cluster as the spread. After rinsing the plants with deionized water, the above-ground and below-ground portions were separated. The absorbent paper was utilized to draw out the water, and the fresh weight of the plants was weighed with a precision balance. The newspaper-wrapped fresh samples of the experimental varieties were dried in a clean oven at 60 °C to a constant weight. The dry mass of the above-ground and below-ground parts of the experimental varieties was weighed with a precision balance (Sartorius Trading Co., Ltd., Shanghai, China) to calculate the moisture content of the plants and the root–crown ratio.

$$\text{Moisture content (\%)} = [(\text{fresh weight} - \text{dry weight})/\text{fresh weight}] \times 100\%.$$

$$\text{Root-crown ratio} = \text{below-ground fresh weight}/\text{above-ground fresh weight}.$$

2.4. Measurement of Physiological and Biochemical Indicators

Chlorophyll was extracted and measured using the acetone–ethanol mixture method. Fresh leaf samples were thoroughly ground in the dark using 80% acetone (Sangon Biotech Co., Ltd., Shanghai, China) (5 mL) and 95% alcohol (Sangon Biotech Co., Ltd., Shanghai, China) (5 mL) as solvents and centrifuged at 9000× *g* for 10 min at 4 °C. Absorbance readings of the collected supernatant at 645 nm and 663 nm were used for the estimation of chlorophyll a and chlorophyll b contents, respectively [17].

The superoxide dismutase (SOD) activity was calculated by recording the decrease in the absorbance of the superoxide nitro blue tetrazole complex. The peroxidase (POD) and catalase (CAT) activities were determined by the guaiacol method [18] and the hydrogen peroxide extraction method [19], respectively. For SOD activity determination, three milliliters of reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 14.5 mM methionine, 2.25 mM nitrotetrazolium blue, 60 µM riboflavin, and 30 µM EDTA-Na_2 (Sangon Biotech Co., Ltd., Shanghai, China). For POD activity assessment, 30 µL of crude enzyme solution was added to 3 mL of reaction solution and 40 µL of H_2O_2 (0.25%) (Sangon Biotech Co., Ltd., Shanghai, China), mixed quickly to start the reaction, and then the absorbance was measured at 470 nm. For CAT activity, 0.5 g of fresh leaf sample was ground with 3 mL of phosphate buffer (pH 7.0) (Sangon Biotech Co., Ltd., Shanghai, China), the enzyme was extracted in a total volume of 10 mL, and then the solution absorbances were measured at 240 nm for 3 min.

The proline content of leaves was measured using the sulfosalicylic acid method [14]. Initially, 0.2 g of fresh leaf tissue was subjected to extraction and subsequently mixed with 10 mL of 3% sulfosalicylic acid (Sangon Biotech Co., Ltd., Shanghai, China). The resulting filtrate was then analyzed for absorbance at 520 nm to determine malondialdehyde content utilizing the thiobarbituric acid method. Absorbance readings at 532 and 600 nm were further taken to estimate MDA content, while absorbance at 600 nm was measured to estimate soluble protein content using the Caulmers Brilliant Blue G-250 Staining method.

2.5. Data Analysis

Salt tolerance coefficients were used to evaluate the salt tolerance of celery by converting the indicators of each trait during analysis and calculating the affiliation function for a comprehensive evaluation. The value of the affiliation function and its average for each index were calculated using the affiliation function method, and the average value of the affiliation function was used to represent the comprehensive evaluation of salt tolerance of different celery varieties. The formula for calculating the relevant indices is as follows:

$$\text{Salt tolerance coefficient (X)} = \text{salt stress assay}/\text{control assay} \times 100\%$$

The formula for calculating the affiliation function is as previously described [20]:

When the measured value is positively correlated with salt tolerance, $F_{ij} = (X_{ij} - X_{min})/(X_{max} - X_{min})$;

When the measured value is negatively correlated with salt tolerance, $F_{ij} = 1 - (X_{ij} - X_{min})/(X_{max} - X_{min})$.

F_{ij} represents the value of the affiliation function of the trait indicators of different varieties; X_{ij} represents the salt tolerance coefficient of indicator j of germplasm i; X_{min} represents the minimum value of the salt tolerance coefficient of each indicator among 40 germplasms; and X_{max} represents the maximum value of the salt tolerance coefficient of 40 germplasms.

Microsoft Excel 2021 and Origin Pro 2021 software were used for data summarization, computational analysis, and post-graphing. IBM SPSS Statistics 24.0 was used to analyze the growth and physiological and biochemical indicators of celery by principal component analysis, classify the individual indicators into composite indices, and determine the score value of each composite index for comprehensive evaluation. The score of each comprehensive index was obtained using the formula: $D_i = w_{i1}Z_1 + w_{i2}Z_2 + \ldots w_{in}Z_n$,

where $w_{ij} = \theta_j / \sqrt{\lambda i}$, which denotes the weight of each variable in the principal components, θ_j is the coefficient corresponding to each variable in the component matrix, and $\sqrt{\lambda i}$ is the open root value of the corresponding eigenvalue of the ith principal component. The integrated salinity tolerance evaluation value (D) of each celery germplasm was calculated using the formula: $D = \alpha_1 D_1 + \alpha_2 D_2 + \ldots \alpha_n D_n$, where α_i is the percentage of the variance of the ith principal component [21].

The data were analyzed using the salt damage index, principal component analysis, and affiliation function analysis. The scored data obtained from the three classification methods were analyzed using cluster analysis for a comprehensive evaluation of the salt tolerance level of different varieties.

3. Results

3.1. Effect of Salt Damage Symptoms and Salt Damage Index of Seedlings of Different Celery Materials under NaCl Stress

Different celery seedlings grew well without any adverse symptoms under normal growing conditions. Salt stress induced discernible changes in the phenotypes of celery seedlings, mainly characterized by plant chlorosis, petiole bending, loss of green coloration and gradual yellowing, and finally, wilting and death. The salt damage index of each celery germplasm was calculated according to its classification standard. The salt damage index was combined with the salt tolerance grading standard to determine the salt tolerance grade of each germplasm (Table 2). The results showed that treatment with salt solution yielded eight highly salt-tolerant germplasms with salt tolerance grade 1, accounting for 20.0% of the total. Among these, MXKQ, JYBaoQ, and SHHXQ were extremely salt-tolerant, as evidenced by their delayed onset of salt damage symptoms, minor salt damage symptoms, and better growth conditions. The remaining varieties included six salt-tolerant, seven moderately salt-tolerant, ten salt-sensitive, and nine highly salt-sensitive germplasms, accounting for 22.5%, among which SHWTLXQ and HNXQ were the most intolerant, manifesting severe salt damage symptoms earlier on and the worst growth conditions.

Table 2. Salt damage index and salt tolerance ranking of celery seedlings of different materials under NaCl stress.

Materials	Salt Injury Index/%	Ranking	Materials	Salt Injury Index/%	Ranking
MXKQ	2.5	1	JYZQ	60.0	21
SHHXQ	7.5	2	OZHDXQ	62.5	22
JYBaoQ	7.5	3	WTX	65.0	23
JYBQ	10.0	4	HCBQ	67.5	24
XQ	12.5	5	HFXQ	70.0	25
BQ	12.5	6	TGBGHXQ	72.5	26
XXQ	15.0	7	HYSQ	72.5	27
LLHXQ	17.5	8	OSTZYXQ	75.0	28
TGCXBQ	22.5	9	FLL	75.0	29
HCHQ	27.5	10	SJSSHNXQC	77.5	30
HYNCXQ	27.5	11	JHQC	80.0	31
MWHH	30.0	12	BLCQ	82.5	32
YLBQ	37.5	13	JNSQ-3	82.5	33
NCW	40.0	14	California emperor	85.0	34
JLLXQ	42.5	15	MGXQW	87.5	35
SJSSQ	45.0	16	HHXQ	90.0	36
KXDYQ	47.5	17	BFMSGQ	92.5	37
BG-1	52.5	18	ZQ	95.0	38
XBQC	55.0	19	SHWTLXQ	97.5	39
YDLXQ	60.0	20	HNXQ	10.0	40

3.2. Effect of NaCl Stress on the Growth Indices of Seedlings of Different Celery Materials

The plant phenotypes of all 40 celery germplasm resources were significantly different (Figure S1). Significant variations were observed in the growth traits (above-ground fresh and dry weights, below-ground fresh and dry weights, plant height, and spread) of the 40 celery varieties under salt stress (Figure 1 and Table S2). The above-ground fresh weight ranged from 10.86 to 22.21 g, whereas the below-ground fresh weight ranged from 1.36 to 3.21 g. Additionally, the spread ranged from 11.27 to 22.30 cm, with an average of 16.18 cm, a maximum value of 18.29 cm, and a minimum value of 7.41 cm. The height of the subject plants varied from 13.33 to 24.80 cm, with an average height of 18.89 cm. Among them, the plant height and fresh weight of different celery varieties decreased significantly under salt stress treatment compared with those before treatment, which indicated that there were significant differences in the growth traits of celery.

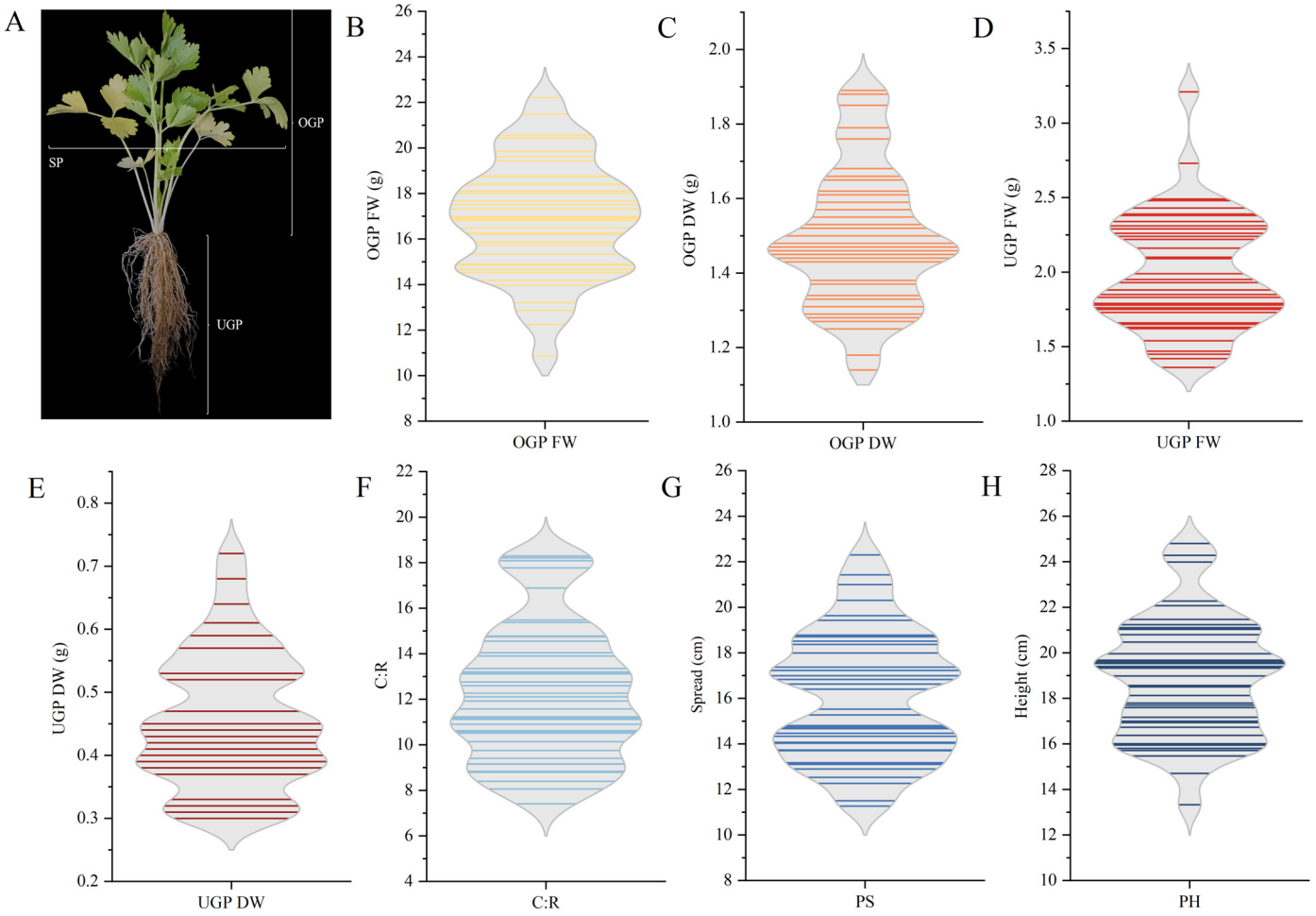

Figure 1. Effects of NaCl stress on morphological indices of celery seedlings with different materials. Notes: plant growth phenotype under NaCl stress (**A**); OGP FW: above-ground fresh weight (**B**); OGP DW: above-ground dry weight (**C**); UGP FW: below-ground fresh weight (**D**); UGP DW: below-ground dry weight (**E**); C: R: root–crown ratio (**F**); PS: plant spread (**G**); PH: plant height (**H**).

3.3. Effect of NaCl Stress on Physiological and Biochemical Indices of Seedlings of Different Celery Materials

The physiological and biochemical traits (Chl, SP, PRO, MDA, SOD, POD, and CAT) of the 40 celery varieties were significantly different before and after treatments, as well as among various germplasms, as shown in Figure 2 and Table S3. The Chl content ranged from 1.61 to 2.17 mg/g, with an average of 1.83 mg/g; the SP content ranged from 22.97 to 59.67 μg/g, with a mean of 45.88 μg/g; the PRO content had a maximum value of

834.45 μg/g and a minimum of 256.55 μg/g, with an average of 544.47 μg/g; the MDA content had a maximum value of 49.72 nmol/g and a minimum value of 15.48 nmol/g; and the average SOD, POD, and CAT contents of each celery germplasm were 246.11, 19.49, and 3.31 U/g, respectively.

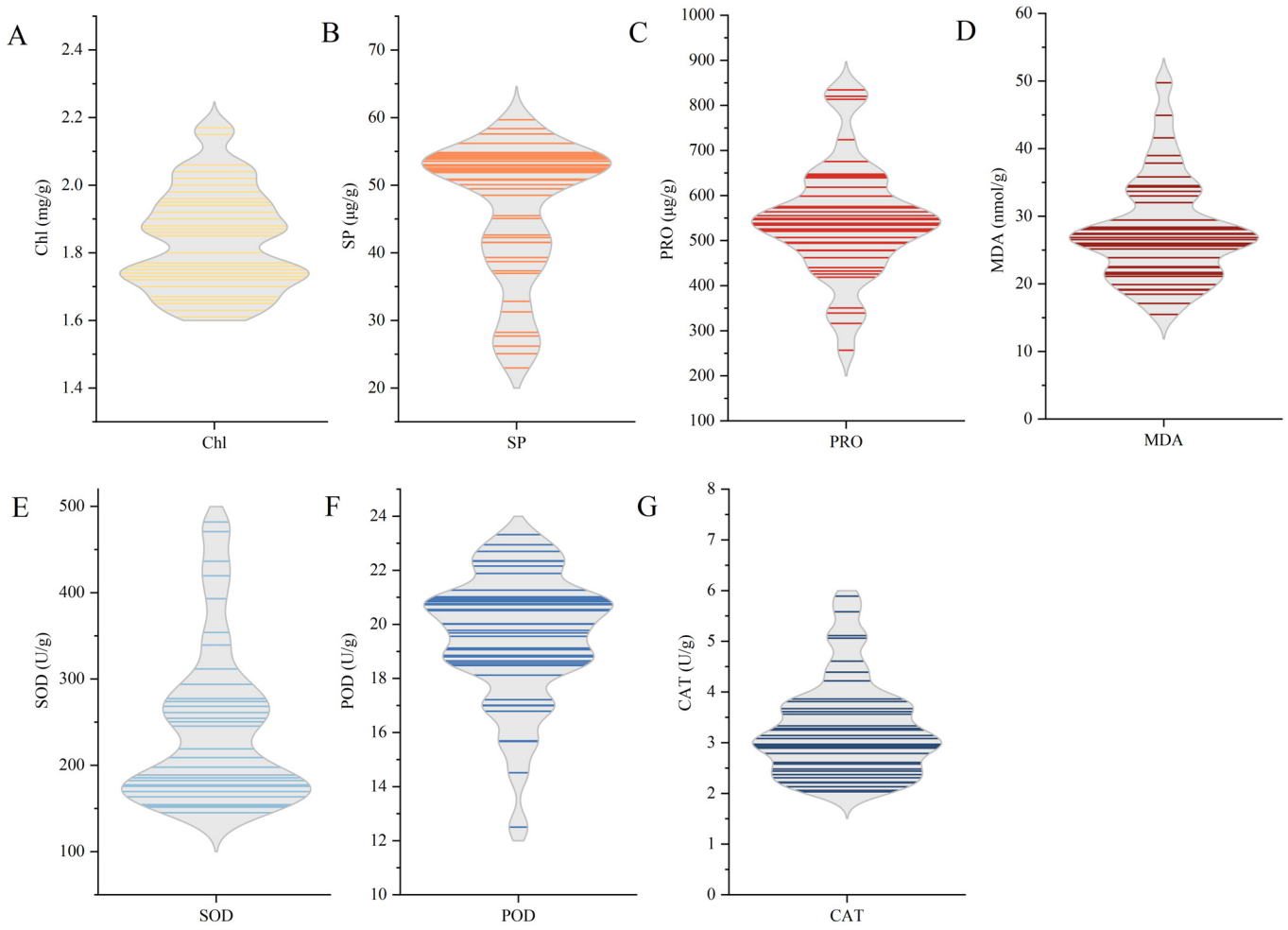

Figure 2. Effects of NaCl stress on physiological and biochemical indices of celery seedlings with different materials. Notes: Chl: chlorophyll (**A**); SP: soluble protein (**B**); PRO: proline (**C**); MDA: malondialdehyde (**D**); SOD: superoxide dismutase (**E**); POD: peroxidase (**F**); CAT: catalase (**G**).

3.4. Correlation Analysis of Various Indices and the Salt Damage Index of Seedlings of Different Celery Varieties under NaCl Stress

Figure 3 and Table S4 indicate that the salt tolerance coefficient provides a more accurate representation of the results derived by comparing each indicator before and after treatment. The correlation analysis between the salt tolerance coefficient of celery growth and physiological indices and the salt damage index revealed that there were significant or highly significant correlations between them. For instance, the salt damage index exhibited a positive correlation with the malondialdehyde content, a significant negative correlation with the below-ground dry weight, SOD, POD, and Chl content, and a highly significant negative correlation with the plant height and CAT content. This suggests that the salt tolerance of celery is easily affected by antioxidant enzyme activities and plant growth traits. It was concluded that plant height, dry and fresh weight in growth indices, antioxidant enzyme activities, and Chl content in physiological indices can serve as important reference indices for the evaluation of plant salt tolerance.

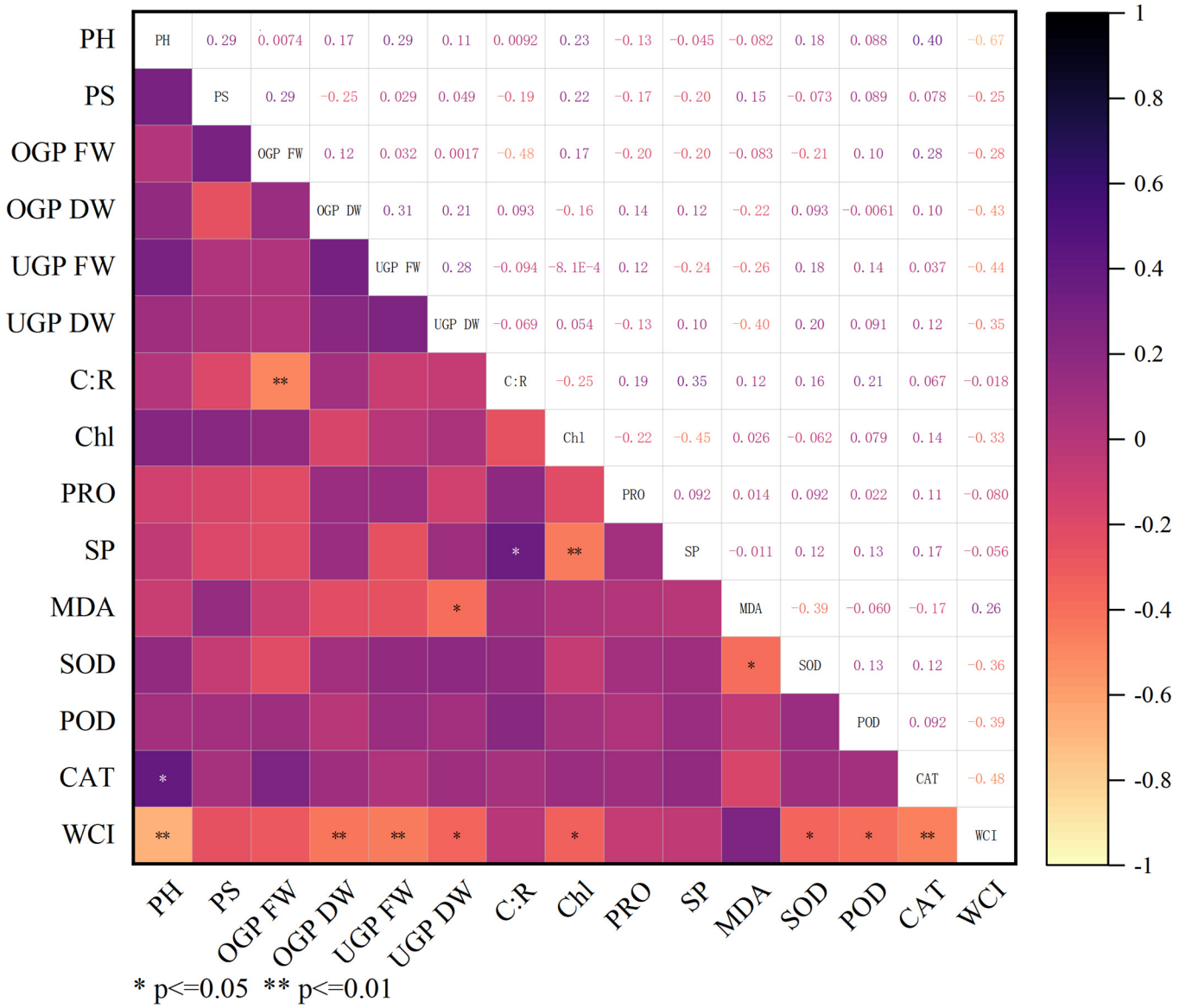

Figure 3. Correlation of seedlings of different celery cultivars under NaCl stress. Note: WCI: salt damage index; * represents significant correlations at the 0.05 level ($p \leq 0.05$); ** represents significant correlations at the 0.01 level ($p \leq 0.01$).

3.5. Principal Component Analysis of Each Index in Seedlings of Different Celery Varieties under NaCl Stress

A certain correlation was observed between the salt damage index and the growth and physiological indices of different celery varieties. Therefore, principal component analysis was used to visualize the changes in various traits among celery germplasm resources. As shown in Table 3, ten key traits out of fourteen selected were simplified into six independent composite indicators, and the cumulative contribution rate of their eigenvalues reached 86.1%, indicating that these six principal components could summarize most of the trait-related information. The first principal component accounted for 25.1% of the total morphological traits. The factor loadings of above-ground fresh weight, root–crown ratio, and SOD were the highest, and the second principal component accounted for 17.0%, with the highest factor loadings being for PH and below-ground fresh weight, indicating that the growth traits of the first and second principal components were the main morphological traits. The factor loading for the third principal component was highest for Chl and SP, accounting for 14.1% of the total morphological characteristics.

The fourth principal component exhibited the highest MDA, accounting for 12.5%. The fifth principal component had the highest factor loading of PRO with 10.2%. The sixth principal component had the highest SP loading, accounting for 7.2%. These six principal components revealed important indices related to the salt tolerance of celery. However, these were insufficient to classify salt-tolerant varieties accurately, and further validation is needed to determine them.

Table 3. Principal component feature vectors and contribution rates of celery seedlings of different materials under NaCl stress.

Trait	Factor Loading					
	C1	C2	C3	C4	C5	C6
PH	−0.004	0.658	−0.226	−0.237	0.490	0.258
PS	−0.463	0.719	0.028	0.008	0.073	0.190
OGP FW	−0.816	0.207	0.262	−0.159	−0.188	−0.134
UGP FW	0.343	0.717	0.233	0.299	−0.032	−0.392
C: R	0.895	0.156	0.058	0.276	0.088	−0.140
Chl	0.143	0.014	−0.868	0.154	−0.118	0.209
PRO	−0.192	−0.404	0.220	0.057	0.807	−0.068
SP	0.353	−0.007	0.644	0.190	−0.157	0.608
MDA	−0.272	−0.034	−0.118	0.839	0.161	0.059
SOD	0.670	0.048	0.001	−0.488	0.128	−0.002
eigenvalue	2.505	1.697	1.409	1.253	1.022	0.715
contribution/%	25.1	17.0	14.1	12.5	10.2	7.2
cumulative/%	25.1	42.1	56.2	68.7	78.9	86.1

Celery varieties could be categorized into the first two components (PC1 and PC2) based on the PCA results of the traits (Figure 4). Certain varieties, such as SHHXQ, XBQC, and SJSSQ, were clustered in the first quadrant. Most varieties, including HFXQ, JLLXQ, and HYNCXQ, were clustered in the second quadrant; varieties such as HNXQ, BFMSGQ, and MGXQW were scattered in the third quadrant; and varieties such as BG-1, JYZQ, and WTX were clustered in the fourth quadrant. Most traits, such as UGP FW, C: R, and SOD, were clustered in the first quadrant, whereas traits such as PS and OGP DW were clustered in the second quadrant. PH was clustered at the junction of quadrants I and II, whereas SP was clustered at the junction of quadrants I and IV.

Additionally, the factor score coefficients were calculated based on the principal factor eigenvalues and the original component matrix. The data were standardized to obtain values from Z1 to Z10. The factor score coefficients in Table S5 were used to calculate the scores of the six factors (D1 to D6) using the following formula: D1 = −0.003 × Z1 − 0.293 × Z2 − 0.516 × Z3 + 0.217 × Z4 + 0.566 × Z5 + 0.090 × Z6 + . . . + 0.423 × Z10 . . .. . .; The composite value D was calculated based on the percentage of factor variance and factor scores using the following formula: D = 0.25052 × D1 + 0.16975 × D2 + 0.14094 × D3 + 0.12532 × D4 + 0.10223 × D5 + 0.07154 × D6. Table 4 illustrates that SHHXQ, TGCXBQ, XBQC, OSTZYXQ, XQ, and MWHH were highly salt-tolerant varieties, accounting for 15.0%; salt-tolerant varieties with a composite D-value of 0.175~0.465 accounted for 27.5%; moderately salt-tolerant varieties with a composite D-value of −0.078~0.085 accounted for 20.0%; salt-sensitive varieties with a composite D-value of −0.396~−0.159 accounted for 20.0%; and six varieties were regarded as highly salt-sensitive with a composite D-value of −1.368~−0.470, including SJSSHNXQC, ZQ, SHWTLXQ, HNXQ, MGXQW, BQ, and BFMSGQ. These results highly overlapped with the highly salt-sensitive varieties in the salt tolerance classification of celery germplasms based on the salt damage index. For instance, SHHXQ belonged to the highly salt-tolerant varieties in both classifications; however, some differences persisted in the salt tolerance classification of other varieties, which were further analyzed and demonstrated in depth by combining them with the affiliation function method.

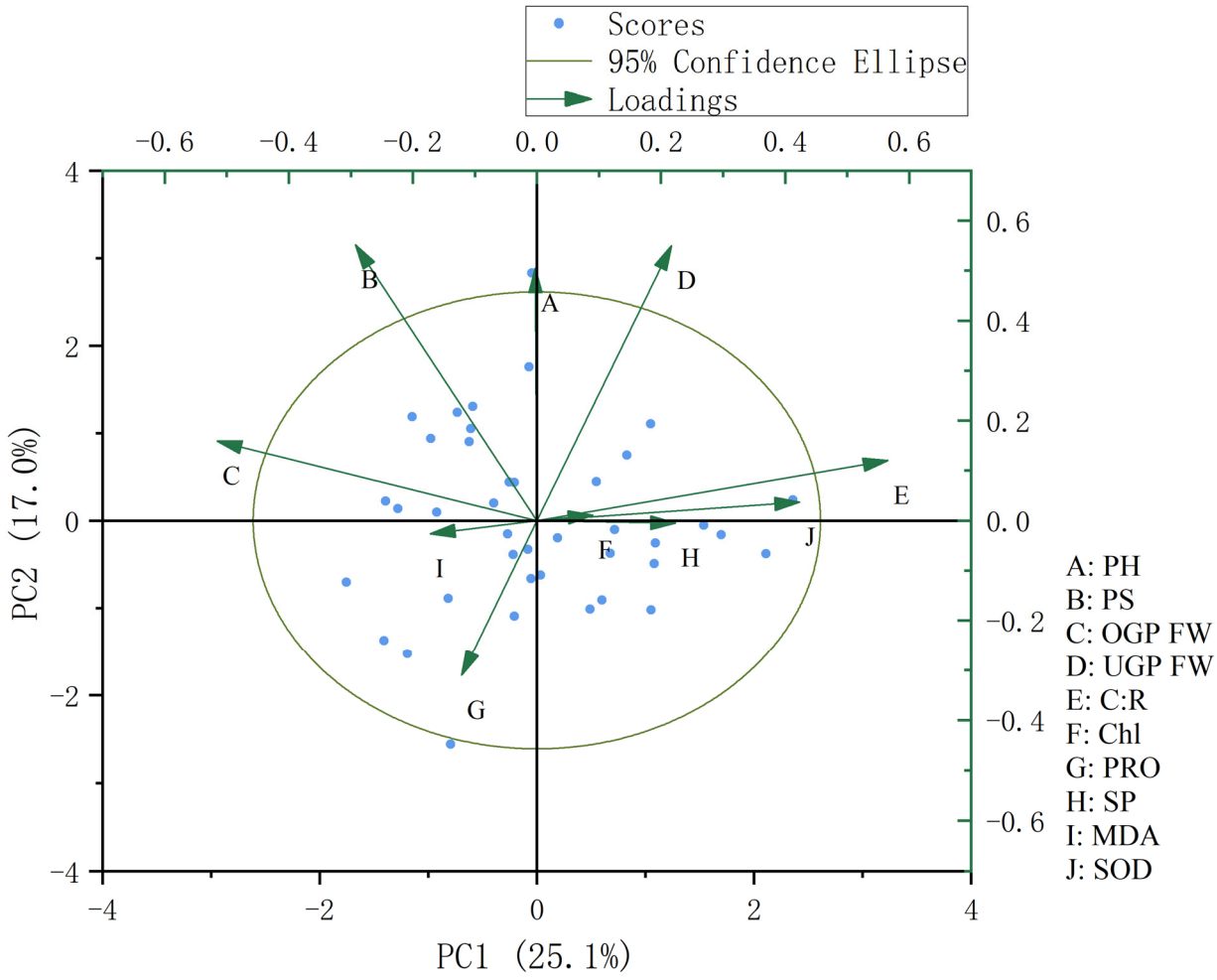

Figure 4. Principal component analysis of seedlings of different celery cultivars under NaCl stress.

Table 4. Principal component scores and comprehensive scores of different varieties of celery for each quality.

Materials	Principal Component Score						Overall Score D-Value	Rank
	D1	D2	D3	D4	D5	D6		
SHHXQ	3.731	0.311	−1.126	−0.598	1.926	1.163	1.034	1
TGCXBQ	2.688	−0.210	1.024	1.574	0.444	−1.041	0.950	2
XBQC	1.661	1.442	−0.372	1.124	0.402	0.851	0.851	3
OSTZYXQ	3.339	−0.492	−0.122	−0.077	0.205	−0.413	0.718	4
XQ	−0.076	3.685	−0.644	1.572	−1.190	−0.692	0.542	5
MWHH	−0.115	2.287	1.650	−1.462	1.148	0.211	0.541	6
BLCQ	−0.086	−0.866	1.136	2.868	0.697	0.599	0.465	7
SJSSQ	1.315	0.973	−0.657	0.323	−1.173	0.909	0.388	8
MXKQ	2.433	−0.068	−0.374	−2.021	0.988	−0.158	0.382	9
TGBGHXQ	1.731	−0.334	−0.261	0.273	0.511	−1.187	0.342	10
JLLXQ	−0.965	1.371	1.166	1.088	0.229	−0.256	0.297	11
HYSQ	0.054	−0.814	2.158	0.617	0.533	−0.196	0.297	12
WTX	1.712	−0.644	1.135	0.106	−1.401	−0.834	0.290	13
NCW	0.867	0.579	1.392	−1.326	−0.911	0.002	0.253	14
JYZQ	1.133	−0.137	−0.164	0.744	−1.060	−0.415	0.193	15
JHQC	1.668	−1.330	0.927	−0.831	−0.755	0.581	0.183	16
HFXQ	−1.162	1.610	1.586	−0.097	−0.126	−0.085	0.175	17

Table 4. *Cont.*

Materials	Principal Component Score						Overall Score D-Value	Rank
	D1	D2	D3	D4	D5	D6		
YDLXQ	−1.550	1.224	0.681	1.556	−0.358	0.156	0.085	18
BG-1	0.301	−0.258	1.317	−0.912	−0.359	0.037	0.069	19
HCHQ	1.069	−0.491	−0.189	0.370	−0.516	−1.202	0.065	20
HCBQ	−0.937	1.700	−0.293	0.639	0.490	−1.516	0.034	21
JYBaoQ	−1.823	1.548	−1.367	0.609	2.711	0.407	−0.004	22
HYNCXQ	−0.985	1.174	0.831	−1.007	−0.566	0.847	−0.054	23
LLHXQ	−0.631	0.264	−0.894	−0.333	1.160	1.267	−0.072	24
JNSQ-3	−0.426	−0.201	−0.339	−0.234	1.267	0.142	−0.078	25
FLL	0.952	−1.187	−0.382	−0.223	−0.374	−1.061	−0.159	26
KXDYQ	0.774	−1.318	−1.676	−0.238	−1.029	1.831	−0.270	27
Californa emperor	−2.030	0.176	1.665	−0.601	−0.561	1.339	−0.281	28
OZHDXQ	−0.344	−0.505	−0.603	−0.577	0.219	−0.313	−0.329	29
YLBQ	−0.330	−1.423	0.660	−1.123	−0.258	0.474	−0.364	30
XXQ	−1.459	0.127	−0.428	0.540	−1.357	1.243	−0.386	31
JYBQ	−0.401	0.575	−1.751	−1.490	0.526	−0.134	−0.392	32
HHXQ	−1.293	−1.162	1.744	−0.715	−0.575	0.386	−0.396	33
SJSSHNXQC	−0.330	0.566	−1.996	−0.834	−1.035	0.118	−0.470	34
ZQ	−1.256	−3.336	0.675	1.156	0.864	−0.225	−0.569	35
SHWTLXQ	−0.132	−0.428	−2.988	1.154	−1.578	−0.460	−0.576	36
HNXQ	−1.886	−1.991	−1.024	1.860	−0.134	1.093	−0.657	37
MGXQW	−2.228	−1.791	−0.568	−0.119	2.101	−0.992	−0.813	38
BQ	−2.208	0.292	−0.383	−1.997	−0.182	−1.218	−0.914	39
BFMSGQ	−2.778	−0.916	−1.144	−1.359	−0.922	−1.260	−1.368	40

3.6. Analysis of the Affiliation Function Values of Each Index for Seedlings of Different Celery Cultivars under NaCl Stress

To improve the scientific and rational perspective of celery salt tolerance evaluation, the affiliation function value of each index of celery germplasm was calculated and averaged as the degree of affiliation, using the salt tolerance coefficient of each index as the basis. A comparison of germplasm salt tolerance was conducted (Table S6). The results, as shown in Table 5, revealed that the average affiliation function values of the 40 celery varieties ranged from 0.228 to 0.718; the varieties were categorized into five classes based on these values. A total of five (12.5%) germplasms from the total germplasm resources tested exhibited high salt tolerance, with average affiliation function values of 0.614~0.718. MXKQ, with an average affiliation value of 0.718, was the most salt-tolerant germplasm. Among the remaining resources, 12 were regarded as salt-tolerant with an average affiliation function value of 0.512~0.595; 10 were moderately salt-tolerant with an average affiliation function value of 0.461–0.508; seven were salt-sensitive with an average affiliation function value of 0.372~0.454; and six (15%) were highly salt-sensitive germplasm resources, with HNXQ showing the highest degree of salt sensitivity, with an average affiliation function value of 0.228.

Table 5. Subjection function values of different materials of celery seedlings under NaCl stress.

Materials	Subjection Function	Ranking	Materials	Subjection Function	Ranking
MXKQ	0.718	1	XBQC	0.493	21
SHHXQ	0.704	2	SJSSQ	0.482	22
BQ	0.655	3	YDLXQ	0.476	23
JYBQ	0.642	4	OSTZYXQ	0.471	24
LLHXQ	0.614	5	HYSQ	0.463	25
JYBaoQ	0.595	6	TGBGHXQ	0.462	26

Table 5. *Cont.*

Materials	Subjection Function	Ranking	Materials	Subjection Function	Ranking
HCHQ	0.571	7	HFXQ	0.461	27
XXQ	0.554	8	JHQC	0.454	28
MWHH	0.543	9	HCBQ	0.440	29
YLBQ	0.541	10	FLL	0.429	30
XQ	0.534	11	JNSQ-3	0.424	31
WTX	0.533	12	SJSSHNXQC	0.401	32
HYNCXQ	0.531	13	MGXQW	0.384	33
JYZQ	0.526	14	BLCQ	0.372	34
TGCXBQ	0.525	15	HHXQ	0.369	35
KXDYQ	0.519	16	BFMSGQ	0.362	36
JLLXQ	0.512	17	California emperor	0.358	37
OZHDXQ	0.508	18	SHWTLXQ	0.308	38
NCW	0.498	19	ZQ	0.266	39
BG-1	0.498	20	HNXQ	0.228	40

3.7. Comparative Validation of the Salt Tolerance Identification of Different Celery Varieties

A correlation analysis was conducted between the salt damage index, the composite D-value of the principal component analysis, and the average affiliation function value of 40 celery varieties to verify the consistency and reliability of the results of the salt tolerance identification based on the direct evaluation method of the salt damage index and the comprehensive evaluation method of the principal component analysis and affiliation function. As displayed in Figure 5, the salt damage index was highly negatively correlated with the composite D-value and the average affiliation function value, while the composite D-value of the principal component analysis exhibited a significant positive correlation with the average affiliation function value, indicating that the salt tolerance of celery germplasms can be comprehensively evaluated and validated using the salt damage index, composite D-value of the principal component analysis, and average affiliation function value.

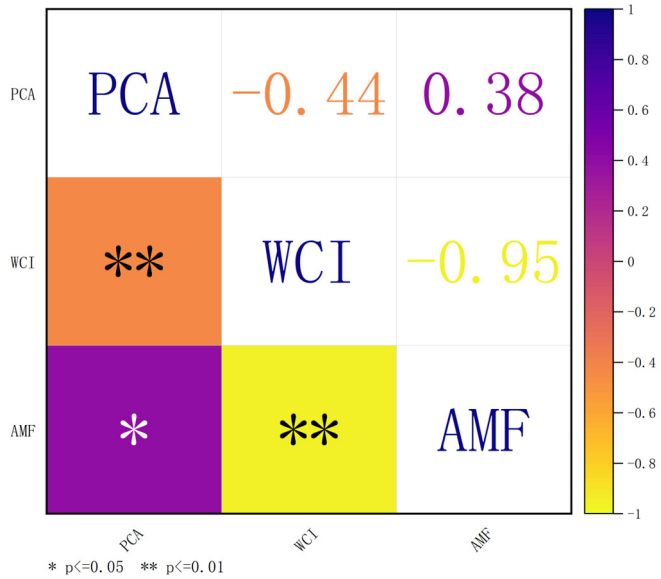

Figure 5. Correlation analysis of salt damage index, comprehensive D-value, and membership function value of different celery materials. Notes: PCA: principal component analysis; WCI: salt damage index; AMF: average membership function; * represents significant correlations at the 0.05 level ($p \leq 0.05$); ** represents significant correlations at the 0.01 level ($p \leq 0.01$).

3.8. Cluster Analysis of Salt Tolerance of Seedlings of Different Celery Varieties and the Mechanism of Celery Response to Salt Stress

The results of salt-tolerant germplasm classification using different methods were determined by individual classification evaluations of the salt damage index, principal component analysis, and affiliation function. However, some differences were observed among the different results. Therefore, to validate the salt tolerance of different celery germplasms and to ensure the accuracy of salt-tolerant germplasm classification, an in-depth cluster analysis was conducted based on the salt damage index, principal component analysis composite D-value, and the mean value of the affiliation function of 40 celery germplasms, on the basis that all three analyses showed significant or highly significant correlations. As depicted in Figure 6, 40 celery germplasms were classified into five categories according to their salt tolerance ability. Among these, five copies (12.5%) were classified as highly salt-tolerant germplasm, with TGCXBQ being the most representative; seven copies (17.5%) of germplasms were salt-tolerant; 15 copies (37.5%) of germplasms were moderately salt-tolerant; nine copies (22.5%) of germplasms were salt-sensitive; and four copies (10.0%) of germplasms were highly salt-sensitive, including BFMSGQ, HNXQ, ZQ, and MGXQW, with ZQ being the most representative.

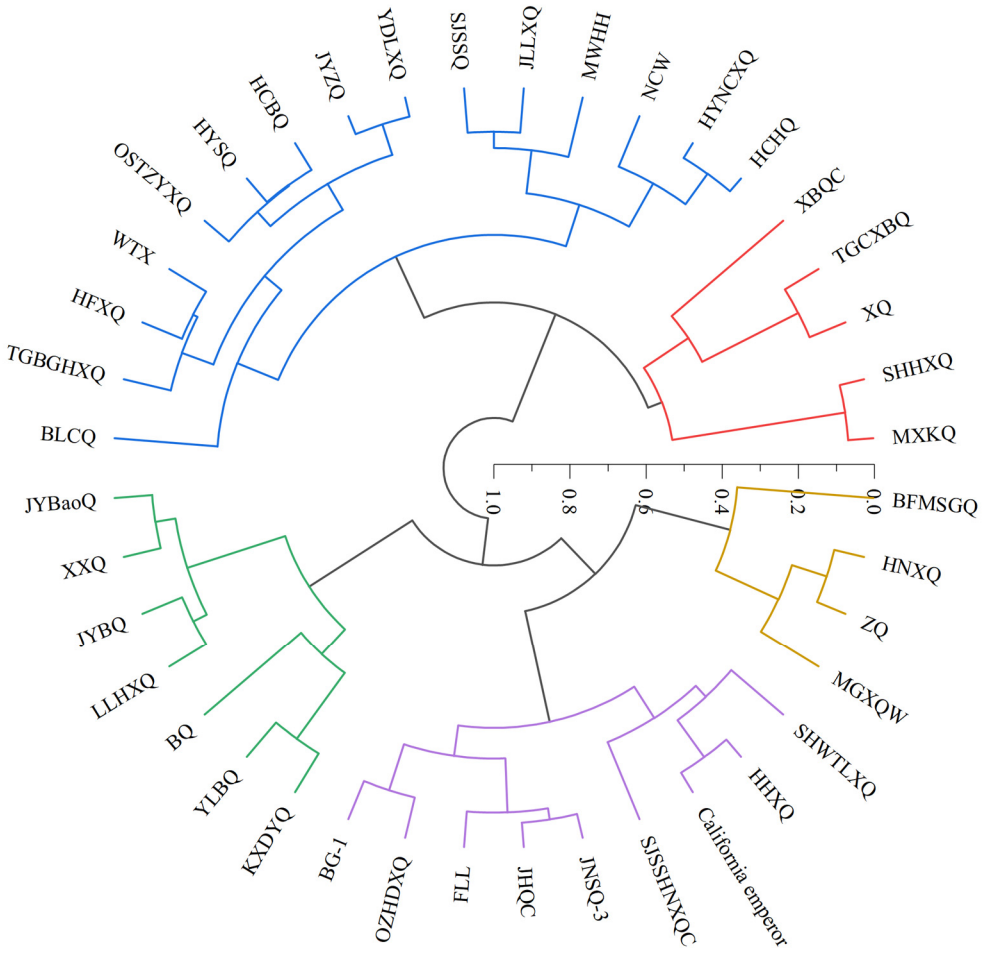

Figure 6. Cluster analysis of salt tolerance of 40 celery germplasm. Notes: Those with similar distances are divided into one category; different colors represent a category.

3.9. Mechanisms of Salt-Tolerant and Salt-Sensitive Celery Varieties' Responses to Salt Stress

According to the results mentioned above, the growth and physiological indices of salt-tolerant and salt-sensitive varieties varied significantly before and after treatment, which elucidated that the responses of celery varieties with different tolerances to salt stress and its physiological mechanisms were significantly different. The results showed that

salt-tolerant celery varieties exhibited enhanced growth adaptability and tolerance under salt stress. Under salt stress, celery seedlings reduced the degree of plasma membrane damage by increasing antioxidant enzyme activities (SOD, POD, and CAT). The salt-tolerant celery varieties increased their enzyme activities more, resulting in less accumulated MDA compared with sensitive varieties. Additionally, the salt-tolerant celery reduced the degree of plasma membrane damage by decreasing the decline in soluble protein content and increasing proline content under salt stress. Moreover, the salt-tolerant celery varieties retained more chlorophyll content to maintain normal photosynthesis.

4. Discussion

Since salt tolerance is a multifaceted and complex quantitative trait that is affected by numerous genetic and non-genetic factors, it is difficult for a single trait to reflect the salt tolerance of plants [22]. In this experiment, seven growth and seven physiological and biochemical indices were determined under salt stress. The germplasm resources were comprehensively evaluated using the salt damage index, principal component analysis, affiliation function analysis, and cluster analysis. The established celery salt-tolerance evaluation system and screening method can provide a basis for improving the efficiency of celery salt-tolerance screening and cultivating new varieties of salt-tolerant celery.

Under salt stress, plants adjust their biomass allocation by reducing plant height, root length, and fresh and dry mass to cope with a salt-stressed environment [23]. In this study, under salt solution watering, the indicators of celery plant height, spread, fresh mass, and dry mass were significantly different from those before treatment, and the magnitude of the difference was also significantly different among the different varieties, which indicated that there were differences in the strength of salt tolerance among the different materials, which was consistent with the results of previous studies on peppers [24] and cabbage [25]. Previous studies have shown that the efficiency of photosynthesis tends to decrease significantly when plants are under salt stress [26,27]. In this study, the chlorophyll content in celery leaves was significantly decreased after watering with a salt solution, and this result is consistent with those of previous studies.

Salinity generally impairs the structure and function of cell membranes, alters membrane lipids, and increases membrane permeability [28]. In addition to osmotic pressure and ionic stress, salinity induces reactive oxygen species (ROS) overproduction, leading to membrane lipid peroxidation and membrane damage [29]. In contrast, the antioxidant enzyme system, comprising SOD, CAT, and POD, plays an important role in maintaining cellular expansion pressure and scavenging ROS, thus maintaining metabolic homeostasis in plants [30,31]. The results of this study demonstrated an increasing trend of SOD, POD, and CAT contents in celery leaves compared with the pre-treatment values, which is consistent with the previous findings. Particularly, the salt-tolerant varieties were significantly stronger in the above activities than the sensitive varieties. Additionally, Bu et al. [32] found that NaCl stress damages the structure of cell membranes, resulting in a significant increase in malondialdehyde (MDA) content in leaves, and this increase is proportional to the concentration of NaCl. In this experiment, the MDA content in celery leaves after salt stress treatment was significantly higher than that before treatment. However, the MDA content of salt-tolerant varieties was significantly lower than that of sensitive varieties.

Osmoregulation is important for the growth, development, and adaptation of plants to environmental changes [33]. Soluble proteins are key components of osmoregulation [34]. Proline is a major organic osmoregulator in plants with various functions, such as osmoregulation, protein structure stabilization, and ROS scavenging under salt stress [35]. Previous studies on wheat and rice showed that proline accumulation was positively correlated with salt tolerance in plants [36]. In this experiment, the proline content of celery germplasm was significantly higher after salt stress treatment compared with that before treatment, while the soluble protein content decreased significantly. In contrast, the salt-tolerant varieties showed a greater increase in proline content while experiencing a relatively small decrease in soluble protein compared with the sensitive varieties. These results suggest that

salt-tolerant varieties can maintain osmoregulatory capacity under salt stress by enhancing the activities of ROS-related enzymes and accumulating osmoregulatory substances, thus maintaining the integrity of leaf cell membranes to ensure relatively stable plant growth.

By analyzing each index, it was found that the salt tolerance evaluation of each celery germplasm was highly variable, and it was not possible to accurately screen the salt-tolerant germplasms. Therefore, a comprehensive evaluation was needed. It has been proven that the comprehensive evaluation method considers the correlation between indicators and the difference in significance among different indicators. This provides a more scientific and accurate reflection of the resistance exhibited by different varieties [37]. Zhou et al. [38] graded the salt tolerance of 20 soybean varieties by determining the emergence potential and other indices and evaluated the theoretical discriminating concentration by using cluster analysis and the subordinate function method, then classified 20 soybean varieties into three salt tolerance classes by applying cluster analysis. It was evident that the use of the salt damage index for grading salt-tolerant germplasms has a certain accuracy. Similarly, principal component analysis and the subordinate function method are relatively reliable evaluation methods for adversity tolerance evaluation. Yu et al. [39] analyzed the salt tolerance coefficients of 14 traits using salt tolerance coefficients, principal component analysis, and subordinate function analysis to screen 20 alfalfa varieties into one highly salt-tolerant, two salt-tolerant, four moderately salt-tolerant, and thirteen salt-sensitive varieties. Kanawapee et al. [40] performed a multivariate cluster analysis based on the salt tolerance scores of rice survival and the Na^+/K^+ ratio, which laid the foundation for screening different crop varieties. In this study, the salt tolerance of celery germplasms was analyzed using the salt damage index, principal component analysis, and affiliation function method, respectively. To comprehensively identify and evaluate the salt tolerance of celery germplasms, this study used the salt damage index, the integrated D-value of the principal component analysis, and the average affiliation function value for cluster analysis. As a result, forty celery germplasms were classified into five categories, which established a framework for the screening and classification of salt-tolerant celery germplasms.

5. Conclusions

In this study, we identified the salt tolerance of 40 celery germplasms. The salt damage index, principal component analysis, and affiliation function analysis were used for simple classification, whereas cluster analysis was employed for a more comprehensive evaluation. Finally, forty celery germplasm resources were classified into five highly salt-tolerant, seven salt-tolerant, fifteen moderately salt-tolerant, nine salt-sensitive, and four highly salt-sensitive germplasms. Among them, SHHXQ, MXKQ, XBQC, XQ, and TGCXBQ were highly salt-tolerant, while BFMSGQ, HNXQ, ZQ, and MGXQW were highly salt-sensitive germplasms. This study established a comprehensive and accurate set of methods for the identification of salt tolerance in celery, which provides a reference for the screening, evaluation, and utilization of salt-tolerant germplasms in celery.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14051048/s1, Figure S1: Phenotype of the plant growth of different celery varieties under conditions of NaCl stress. Table S1: Names and types of 40 celery germplasm materials; Table S2: Effects of NaCl stress on morphological indices of celery seedlings with different materials; Table S3: Effects of NaCl stress on physiological and biochemical indices of celery seedlings with different materials; Table S4: Salt tolerance index of different materials of celery seedlings under NaCl stress; Table S5: Factor score coefficients for the main extraction factors in principal component analysis; Table S6: Subjection function values of different materials of celery seedlings under NaCl stress.

Author Contributions: Conceptualization, M.L., C.Z. and A.X.; Methodology, L.W., J.D., Y.Z. (Yidan Zhang) and Y.X.; Resources, C.J., W.L., Y.Z. (Yangxia Zheng) and C.Z.; Data curation and visualization, L.W. and J.D.; Writing-original draft preparation, L.W. and J.D.; Funding acquisition, M.L. All authors have read and agreed to the published version of the manuscript.

Funding: The research was supported by the National Natural Science Foundation of China (32002027), and the National Natural Science Foundation of Sichuan Province (2022NSFSC1674), Jiangsu seed industry revitalization project [JBGS(2021)068], Key Research and Development Program of Jiangsu (BE2022386), Key R&D Program Project of Xinjiang Province (2023B02020).

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

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