

Article

Investigating Salt Tolerance in Melon During Germination and Early Seedling Stages

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Abstract: This study aimed to investigate the correlation between salt tolerance during the germination and seedling stages in melons by analyzing 10 melon varieties under NaCl stress during germination and seedling stages. We found that 200 mM is the appropriate concentration for screening salt-tolerant germplasm in melons. Salt stress led to a decline in germination and seedling growth parameters, while antioxidant enzyme activities and osmotic substance contents significantly increased. Specifically, the activities of CAT and SOD increased by up to 27.22-fold and 6.35-fold, respectively, and soluble protein and proline contents increased by up to 1.03-fold and 1.05-fold, respectively. Varietal differences in salt tolerance traits were observed. Correlation and principal component analyses revealed that 6 germination indicators could be consolidated into 1 comprehensive indicator, accounting for 79.225% of the variance, while 16 seedling-stage indicators were reduced to 3 comprehensive indicators, with a cumulative contribution rate of 75.089%. Membership function and cluster analyses categorized the 10 varieties into 3 groups at both stages, identifying ‘Xindongfangmi’ and ‘Jinyuliuxing’ as salt-tolerant varieties. Additionally, a significant positive correlation ($r = 0.834$) was found between the comprehensive membership function values of germination and seedling stages. These results provide a scientific basis for assessing melon salt tolerance, indicating that germination-stage salt tolerance may predict seedling-stage salt tolerance. By utilizing PCA, comprehensive evaluation, and cluster analysis of relevant indicators under salt stress during the germination period of melon, the salt tolerance of the seedling stage can be quickly identified. The implementation of rapid salt tolerance screening at the germination stage can facilitate the selection of salt-tolerant germplasm and the development of salt-tolerant melon varieties.

Keywords: melon; salt tolerance; germination period; seedling stage; correlation



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1. Introduction

Salt stress is a major abiotic factor that severely impacts plant growth and limits agricultural productivity, particularly in crop cultivation [1]. The escalation of global soil salinization has increasingly constrained agricultural development, especially in the crop production sector. Common strategies to alleviate the effects of salt stress include breeding salt-tolerant varieties and enhancing cultivation techniques [2–6], salt-tolerant varieties are one of the most cost-effective and efficient methods for addressing salinity issues [7–10]. However, breeding depends on a robust, efficient plant salt tolerance evaluation system

and a diverse pool of salt-tolerant germplasm resources [7,11]. Therefore, a rapid and efficient salt tolerance identification system is crucial.

The germination and seedling stages are the most sensitive to salt stress and are pivotal in the growth and development of vegetables [12–14]. Salt stress can notably reduce germination vigor, germination rate, and germination index [14]. It disrupts reactive oxygen species (ROS) balance, heightens membrane lipid peroxidation, decreases chlorophyll content, and reduces root activity, leading to diminished biomass accumulation [15–18]. Studies indicate a potential correlation between salt tolerance resistance to salt stress during the seedling stage, although the precise nature of this relationship remains unclear [16,19,20]. Therefore, most current research focuses on studying salt tolerance separately for two physiological periods [15,18,20].

Melon (*Cucumis melo* L.) is a major crop with great agronomic and economic interest, considered a gourmet food. Several studies have reported the salinity effects on melon plants, and focused on crop yield, fruit quality characteristics, ion concentrations, and water relation [21]. They concluded that melon plants are sensitive, or moderately sensitive, to salinity and demonstrated considerable genotypic variation in the response of melon plants to salinity stress. The screening of salt tolerant germplasm in melons is extremely important for exploring salt-tolerant genes and cultivating salt-tolerant varieties.

Interestingly, not all NaCl solution concentrations will adversely affect melons. For example, 50 and 100 mM NaCl can promote the synthesis of aromatic compounds in sweet melon [22]. Current research has found that 150 and 200 mM are suitable salt concentrations for salt tolerance testing of melons [16,23].

Principal component analysis (PCA) and membership function methods are effective for comprehensive evaluations, as they show the influences among variables, efficiently handle large data sets on agronomic traits, and enhance assessment accuracy [24]. In this study, we used 10 melon varieties to investigate salt stress-related parameters during germination and seedling stages to determine germination-stage parameters that could serve as indicators of salt tolerance at the seedling stage. We employed PCA and membership function methods to evaluate and classify melon varieties across both stages.

Since germination is an especially sensitive stage to salt stress, it is advantageous for large-scale screening of salt tolerance due to its high throughput, ease of operation, and short assessment cycle. However, it is still unclear whether the salt tolerance during the germination stage can represent the salt tolerance during the seedling stage. Our study aims to analyze the relationship between salt tolerance during the germination and seedling stages of melon and to explore the degree of closeness between salt tolerance during these stages. Our goal is to develop a rapid and accurate method for evaluating salt tolerance in melon.

2. Materials and Methods

2.1. Materials

Ten different first-generation hybrid materials of melon were collected from Xinjiang Academy of Agricultural Sciences, Shandong Xuri Agricultural Technology Co., Ltd. (Jinan, China), Shandong Academy of Agricultural Sciences, Zhengzhou Fruit Research Institute, and Wuwei Antaida Seed Industry Co., Ltd. (Antaida, Wuwei, China). The seeds were stored in a seed refrigerator for future use, with specific details on number, variety name, and type provided in Table S1.

2.2. Salt Tolerance Identification at the Germination Stage

The experiment was conducted from 15 April to 21 April 2021 in the Vegetable Research Institute of Shandong Academy of Agricultural Sciences laboratory. Uniform-sized

seeds were selected, soaked in water for two hours, and placed in a high-precision constant temperature and humidity chamber set at 29 °C and 90% humidity. After light drying, 20 seeds were positioned on a double-layer filter paper in a 9 cm culture dish, covered by another layer of filter paper. Measures of 5 milliliters of NaCl solutions with concentrations of 0, 50, 100, 150, 200, and 250 mM were added, respectively, and the culture dishes were returned to the chamber to facilitate germination. To maintain a stable salt concentration, the filter paper was replaced on the third day and 5 milliliters of the corresponding concentrations of NaCl solution were added. A total of 10 melon varieties were used as experimental materials, each variety receiving 6 different concentrations of NaCl treatment, each with 3 repetitions, 20 seeds per repetition, for a total of 3600 seeds.

Germination was recorded daily. The seed is considered to have germinated when the radicle emerges straight from the seed coat, reaches a length of 0.3 cm or more, and appears white [25].

Germination Potential (GP) = (Number of germinated seeds in first 3 days/Total number of seeds) $\times$ 100%. Germination Rate (GR) = (Number of germinated seeds in first 5 days/Total number of seeds) $\times$ 100%. Germination Index (GI) = $\sum (G_t/D_t)$, where G_t = number of seeds germinated on day t , D_t = number of germination days [25].

Radicle Length (RL): Three seeds were randomly selected on the fifth day, and radicle length was measured from the cotyledon junction to the root tail, with 0.1 cm accuracy [25].

Fresh Weight (FW): After 5 days of salt treatment, three seeds were randomly selected, drying surface moisture and weighed by a balance with an accuracy of 0.01 g (Mettler Toledo Co., Ltd., Shanghai, China) [25].

Vitality Index (VI) = GI $\times$ Fresh weight [25].

Relative Salt Tolerance Coefficient: Indicator under treatment/indicator under control $\times$ 100% [25].

2.3. Salt Tolerance Identification at the Seedling Stage

The experiment was conducted from 1 May to 31 May 2021 in the Vegetable Research Institute of Shandong Academy of Agricultural Sciences laboratory. The germinated seeds of 10 varieties melon were transplanted into trays filled with half-strength Hoagland nutrient solution. Half-strength Hoagland nutrient solution was renewed every 5 days (10 mL per plant). The composition of the Hoagland nutrient solution is detailed in Table S2. When the seedlings reached the three-leaf stage, the Hoagland nutrient solution was replaced with nutrient solution containing 0 or 200 mM NaCl. The selection of salt concentration is based on the previous salt concentration screening results, which have demonstrated that 200 mM concentrations can significantly distinguish the salt tolerance of melon varieties and effectively identify salt tolerant germplasm of melons [25]. Seedlings having similar growth vigor were selected and treated for 7 days with 200 mM NaCl. Each treatment included three replicates of 30 plants each, totaling 1800 plants. Thress seedlings were randomly selected from each replicate of each treatment, and a total of 9 melon seedlings were clipped and collected for growth and physiological analyses. All samples were frozen in liquid nitrogen and stored at -80 °C. Three biological replicates were performed for each sample in this study.

Growth Indicators:

Plant height (PH) was measured from the stem base to the highest growth point using a ruler with an accuracy of 0.1 cm (Deli Group Co., Ltd., Shanghai, China).

Maximum root length (MRL) was measured from the root stem connection to the root tip using a ruler with an accuracy of 0.1 cm (Deli Group Co., Ltd., Shanghai, China).

Stem thickness (ST) was measured at 0.5 cm below cotyledon attachment with a vernier caliper with an accuracy of 0.1 mm (Deli Group Co., Ltd., Shanghai, China).

After 7 days of salt treatment, the aboveground and underground parts were separated and washed with clean water; absorbent paper was used to absorb surface moisture. Aboveground fresh weight (AFW) and underground fresh weight (UFW) were measured using a balance with an accuracy of 0.01 g (Mettler Toledo Co., Ltd., Shanghai, China). The separated aboveground and underground parts were dried at 70 °C to a constant weight and weighed with a balance with an accuracy of 0.01 g to obtain the aboveground dry weight (ADW) and underground dry weight (UDW).

Seedling index (SI) = (ST/PH + UDW/ADW) × total plant dry weight [26].

Physiological Indicators:

Root activity (RA) was observed using the triphenyltetrazolium chloride (TTC) reduction method [27]. Extract chlorophyll (chl) using acetone and then determine the chlorophyll content by UV spectrophotometry [27]. The contents of proline (PRO), soluble protein (SP), and MDA were determined using the sulfosalicylic acid-indanone colorimetric method, the coomassie Brilliant Blue (G250) colorimetric method, and the thiobarbituric acid colorimetric method [28]. The activities of SOD, POD, and CAT were determined using the nitroblue tetrazole (NBT) photochemical reduction method, the guaiacol method, and the hydrogen peroxide (H₂O₂) ammonium molybdate catalytic decomposition method [28].

2.4. Statistical Analysis

Data were analyzed using IBM SPSS Statistics 26 and Microsoft Excel 2019 for membership function analysis, PCA, cluster analysis, correlation analysis, and tabulation [24].

Membership function analysis: Use Formula (1) to calculate the membership function values of individual indicators for each material. $X(\mu)$ represents the membership value of the μ -th indicator, X is the evaluation parameter such as the mean relative germination rate, X_{max} is the maximum value of a certain evaluated indicator, and X_{min} is the minimum value of the corresponding evaluated indicator. Calculate the average membership function value using formula (2), where X_{ij} is the j -th evaluation parameter indicator for the i -th material, $\bar{x}_i$ represents the mean of the i -th indicator, and n represents the number of varieties. Calculate the comprehensive membership function value using Formulas (3)–(5), where V_i represents the standard deviation coefficient of the i -th evaluation parameter index, W_i represents the weight coefficient of the i -th evaluation parameter index, m represents the number of evaluation parameter indicators, $\mu(X_i)$ represents the membership value of the i -th evaluation parameter index, and D represents the comprehensive evaluation score (the larger the D value, the stronger the salt tolerance of the variety).

$$X(\mu) = \frac{X - X_{min}}{X_{max} - X_{min}} \quad (1)$$

$$\bar{x}_i = \sum_{j=1}^n x_{ij} \quad (2)$$

$$V_i = \frac{\sqrt{\sum_{j=1}^n (x_{ij} - \bar{x}_i)^2}}{\bar{x}_i} \quad (3)$$

$$W_i = \frac{v_i}{\sum_{i=1}^m v_i} \quad (4)$$

$$D = \sum_{i=1}^m (w_i \times \mu(x_i)) \quad (5)$$

Multiple trait values were converted into one comprehensive index using PCA, and the salt tolerance of 10 melon materials was comprehensively evaluated using the fuzzy mathematical membership function method.

PCA: Multivariate data were condensed into one or more comprehensive indices using PCA, facilitating a comprehensive evaluation of melon salt tolerance.

Cluster analysis: Based on the relative indicator membership function values and the comprehensive membership function values, the Euclidean distance and the average distance between groups connection method are used to classify 10 melon materials. The salt tolerance of each type of melon material is defined according to the size of the comprehensive membership function values.

Correlation analysis: Conduct correlation analysis on the four salt tolerance levels in the germination and seedling stages of melon to determine the correlation of salt tolerance coefficients between the two stages.

3. Results

3.1. Effects of Different NaCl Concentrations on Seed Germination of 10 Melon Varieties

3.1.1. Effects of Different NaCl Concentration on Germination Potential of 10 Melon Varieties

To determine the appropriate NaCl concentration for salt tolerance screening during the germination period of melon, we set NaCl treatments at six concentrations of 0, 50, 100, 150, 200, and 250 mM, and calculated the germination potential (Figure S1). Compared with the control, there was no significant change in the germination potential of all materials when the salt concentration was 50 mM. When the salt concentration was 100 mM, the germination potential of 'Jintiancui' and 'M135' significantly decreased. When the salt concentration was 150 mM, the germination potential of the six materials significantly decreased. When the salt concentration was 200 mM, the germination potential of seven materials significantly decreased. At 250 mM, the germination potential of all materials significantly decreased (Table S3).

3.1.2. Effects of Different NaCl Concentration on Germination Rate of 10 Melon Varieties

We also calculated the germination rates of 10 varieties of melon under different concentrations of NaCl treatment (Figure S2). Compared with the control, there was no significant change in the germination potential of all materials when the salt concentration was 50 mM. When the salt concentrations were 100 and 150 mM, the germination potential of 'Jintiancui' significantly decreased. When the salt concentration was 200 mM, the germination potential of six materials significantly decreased. At 250 mM, the germination potential of all materials significantly decreased (Table S4). Considering that 60% of the varieties showed a decrease in germination rate and potential under 200 mM NaCl treatment, 40% of the varieties still showed no significant changes in germination rate and potential. Given this, it seems that 200 mM NaCl can effectively identify the salt tolerance of melon materials. We comprehensively analyzed the changes in germination rate and germination potential of 10 varieties of melon under different concentrations of NaCl treatment and ultimately selected 200 mM as the appropriate concentration of NaCl for melon with strong salt tolerance.

3.2. Salt Tolerance Identification During the Germination Stage of 10 Melon Varieties

3.2.1. Statistical Analysis of Traits During the Germination Stage

In the control group, trait coefficients of variation (CV) ranged from 0.12 to 0.51, ordered from highest to lowest as follows: vitality index, fresh weight, germination index, radicle length, germination potential, and germination rate. Under 200 mM NaCl treatment, CVs ranged from 0.37 to 0.96, with the same descending order. To control for differences among melon varieties, relative salt tolerance coefficients for each trait were calculated. All indicators decreased under salt stress. CVs for salt tolerance across traits ranged from 0.19 to 0.99, highest for relative germination potential and lowest for the relative

vitality index, indicating variable responses to 200 mM NaCl treatment across varieties and underscoring the limitations of single-trait evaluations for salt tolerance (Table 1).

Table 1. Statistical analysis of melon characters at germination stage under 200 mM NaCl treatment and control conditions.

Treatment	Parameter	Germination Potential	Germination Rate	Germination Index	Radicle Length (cm)	Fresh Weight (g)	Vitality Index
Control	Min.	0.21	0.32	21.88	1.53	0.10	2.37
	Max.	1.00	1.00	169.25	14.06	1.89	171.05
	Average	0.92	0.94	103.6	10.26	0.40	42.66
	SD	0.13	0.11	29.35	2.25	0.16	21.62
	CV	0.14	0.12	0.28	0.22	0.4	0.51
200 mM NaCl treatment	Min.	0.00	0.23	0.00	0.00	0.04	0.00
	Max.	1.00	1.00	96.25	3.68	0.34	31.11
	Average	0.58	0.37	34.12	1.36	0.15	5.79
	SD	0.31	0.32	26.57	0.89	0.06	5.58
	CV	0.53	0.86	0.78	0.65	0.37	0.96
Comparison with control	Min.	0.00	0.00	0.02	0.03	0.30	0.27
	Max.	0.97	0.99	0.71	0.29	0.57	0.51
	Average	0.44	0.63	0.38	0.18	0.43	0.37
	SD	0.44	0.38	0.26	0.10	0.08	0.08
	CV	0.99	0.60	0.70	0.53	0.19	0.21

The varieties with the highest relative germination potential, relative germination rate, relative germination index, relative radical root, relative fresh weight, and relative vitality index are ‘M135’, ‘Jintiancui’, ‘Jinyuliuxing’, ‘Xindongfangmi’, ‘Baicuimi’, and ‘Baicuimi’, respectively. The varieties with the lowest relative germination potential, relative germination rate, relative germination index, relative radical root, relative fresh weight, and relative vitality index are ‘Wanmei 9’ and ‘Huaxiami’, ‘Huaxiami’, ‘Huaxiami’, ‘Huaxiami’, ‘Wanmei9’, and ‘Wanmei9’, respectively (Table 2).

Table 2. Statistical analysis of salt tolerance coefficient of melon at germination stage.

	Germination Potential	Germination Rate	Germination Index	Radicle Length	Fresh Weight	Vitality Index
Huangmengcui	0.14	0.80	0.27	0.18	0.37	0.34
Wanmei 9	0.00	0.85	0.06	0.04	0.30	0.27
Baicuimei	0.09	0.50	0.13	0.14	0.57	0.51
Zhongtian 5	0.07	0.85	0.23	0.20	0.45	0.41
Jinyuliuxing	0.89	0.90	0.71	0.29	0.47	0.42
Jintiancui	0.38	0.23	0.69	0.12	0.40	0.32
Tianhongyu	0.91	0.75	0.54	0.29	0.45	0.40
Huaxiami	0.00	0.25	0.02	0.03	0.39	0.28
Xindongfangmi	0.95	1.00	0.55	0.28	0.51	0.43
M135	0.97	0.59	0.55	0.24	0.37	0.31

From the information of maximum, minimum, and coefficient of variation, there are different ranges of variation in different varieties of melons, indicating that different traits respond differently to 200 mM NaCl treatment. Relying solely on a single indicator of salt tolerance coefficient cannot accurately evaluate the salt tolerance of melons.

3.2.2. Correlation Analysis of Salt Tolerance Coefficients During the Germination Stage

Correlation analysis revealed significant positive correlations among the six salt tolerance traits, with the strongest correlation between relative germination potential and germination index ($r = 0.972$) and the weakest between relative fresh weight and vitality index ($r = 0.684$). This strong inter-trait correlation suggests overlapping information across these indicators (Table 3).

Table 3. Correlation of indexes at germination stage of melon under 200 mM NaCl treatment.

	RGP	RGR	RGI	RRL	RFW	RVI
RGP	1	0.796 **	0.858 **	0.691 **	0.253 **	0.719 **
RGR		1	0.972 **	0.857 **	0.518 **	0.908 **
RGI			1	0.848 **	0.464 **	0.924 **
RRL				1	0.684 **	0.870 **
RFW					1	0.684 **
RVI						1

Note: ** $p < 0.01$ —the difference is extremely significant. RGR—relative germination rate; RGP—relative germination potential; RGI—relative germination index; RRL—relative radicle length; RFW—relative fresh weight; RVI—relative vitality index.

3.2.3. PCA of Salt Tolerance During Germination

PCA results indicated a cumulative contribution rate of 100% across six independent components, with the first principal component (PC1) contributing nearly 80% of the total variance. The PC1 formula, derived from indicator weights, primarily reflects salt stress effects on germination and seed growth. This suggests PC1 as a key factor in evaluating melon seed salt tolerance (Table 4).

Table 4. Principal component eigenvalues and cumulative contribution rate of Salt Tolerance During Germination.

Variable	PC1	PC2	PC3	PC4	PC5	PC6
RGP	0.443	−0.231	−0.271	0.111	0.020	0.800
RGR	0.442	0.106	−0.283	0.345	−0.697	−0.327
RGI	0.440	−0.139	−0.415	0.029	0.653	−0.427
RRL	0.427	0.160	0.109	−0.867	−0.167	−0.027
RFW	0.378	−0.502	0.742	0.188	0.016	−0.127
RVI	0.299	0.798	0.332	0.284	0.255	0.127
Characteristic value	4.753	0.843	0.186	0.142	0.063	0.012
Contribution rate	79.225	14.057	3.094	2.375	1.057	0.193
Cumulative contribution rate	79.225	93.282	96.376	98.751	99.807	100

Note: RGP—relative germination potential; RGR—relative germination rate; RGI—relative germination index; RRL—relative radicle length; RFW—relative fresh weight; RVI—relative vitality index.

3.2.4. Comprehensive Evaluation and Cluster Analysis of Germination-Stage Salt Tolerance

Average and comprehensive membership function values during germination were highest for ‘Xindongfangmi’ (0.951 and 1, respectively) and lowest for ‘Huaxiami’ (0.025 and 0.000, respectively) (Table 5). Based on Euclidean distance and the Wald clustering method, the comprehensive membership values clustered the 10 melon varieties into 2 categories, effectively grouping them by 200 mM NaCl treatment (Figure 1).

3.3. Salt Tolerance Identification During the Seedling Stage of 10 Melon Varieties

3.3.1. Statistical Analysis of Traits During the Seedling Stage

In the control group, CVs for seedling growth traits ranged from 0.08 to 0.36, with plant height showing the highest and stem diameter the lowest variability. In the treatment group, CVs ranged from 0.08 to 0.38, with the highest for plant height and the lowest for stem diameter. Salt stress reduced plant height, fresh weight (both above and below ground), and dry weight. CVs of comparison with control ranged from 0.08 to 0.30, with underground fresh weight showing the highest and stem diameter showing the lowest variation under salt stress (Table 6).

Table 5. The average membership function value, comprehensive membership function value, and ranking of the relative salt tolerance coefficient and various traits during the germination period of different varieties under 200 mM NaCl treatment.

Varieties	Average Membership Function	Comprehensive Membership Function	Ranks
Xindongfangmi	0.951	1.000	1
Tianhongyu	0.912	0.958	2
Jinyuliuxing	0.909	0.955	3
Jintiancui	0.497	0.518	4
Huangmengcui	0.370	0.387	5
Zhongtian 5	0.342	0.359	6
Wanmei 9	0.214	0.216	7
M135	0.186	0.178	8
Baicuimei	0.148	0.135	9
Huaxiami	0.025	0.000	10

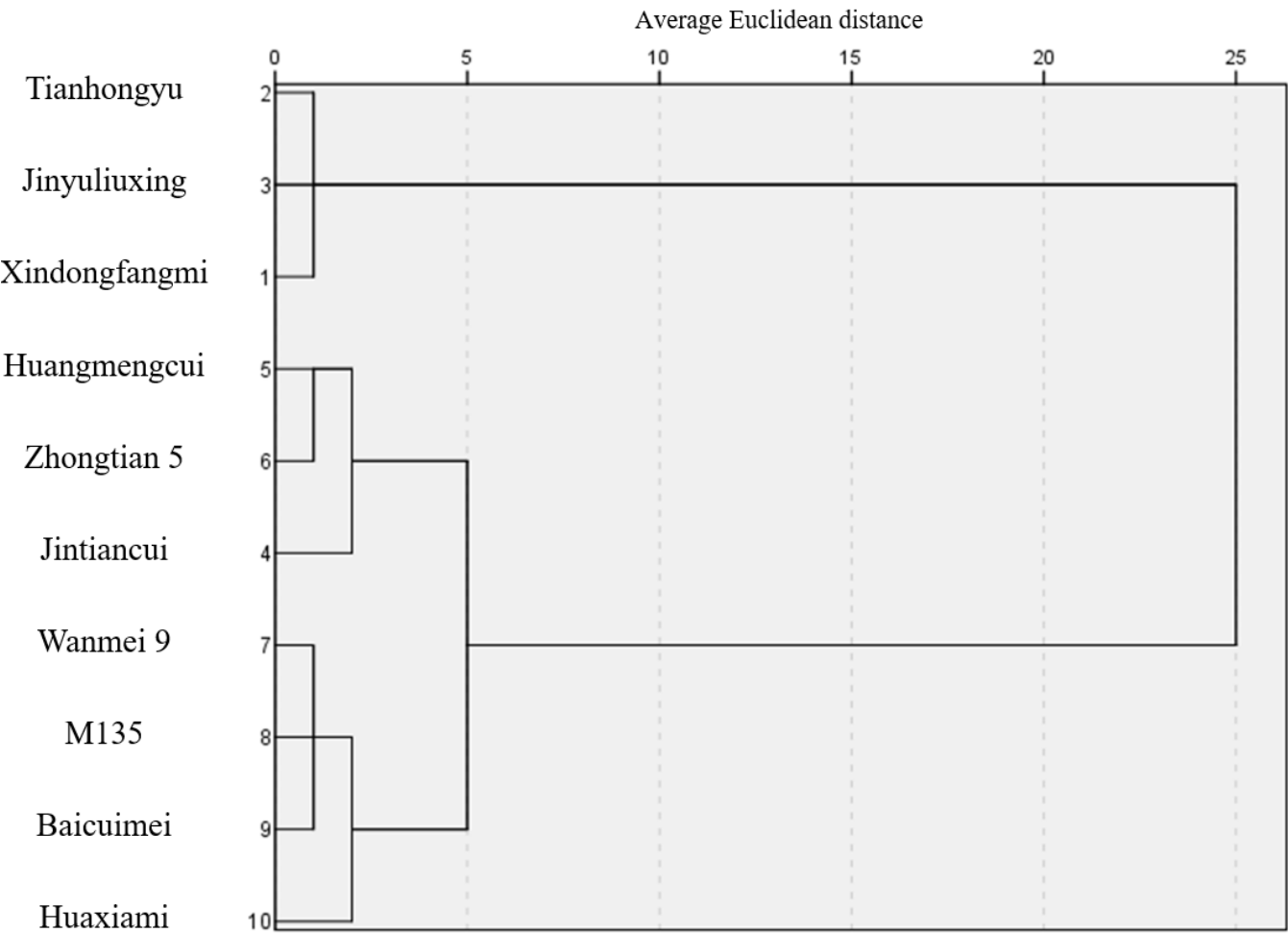


Figure 1. Cluster diagram of tolerance of different varieties under 200 mM NaCl treatment during germination period.

In the control group, CVs for seedling physiological traits ranged from 0.01 to 0.76, with POD activity showing the highest and CAT activity the lowest variability. In the treatment group, CVs ranged from 0.01 to 0.34, with the highest for root activity and the lowest for CAT activity. Salt stress reduced chlorophyll content and POD activity, while SOD activity increased across varieties. CVs of comparison with control ranged from 0.01 to 1.78, with CAT activity showing the highest and proline content the lowest variation under salt stress (Table 7).

Table 6. Statistical analysis of melon growth characters at seedling stage under 200 mM NaCl treatment and control conditions.

Treatment	Parameter	Plant Height	Stem Diameter	Maximum Root Length	Aboveground Fresh Weight	Underground Fresh Weight	Aboveground Dry Weight	Underground Dry Weight	Seedling Index
Control	Average value	11.97	3.71	13.54	11.23	1.4	1.25	0.11	0.47
	Minimum value	5.33	3.27	10.4	7.38	1.13	0.88	0.1	0.239
	Maximum value	17.69	4.24	17.82	15.22	2.33	1.79	0.18	0.702
	Standard deviation	4.35	0.29	2.18	2.64	0.35	0.29	0.03	0.15
	CV	0.36	0.08	0.16	0.23	0.25	0.23	0.23	0.31
200 mM NaCl treatment	Average value	10.1	3.28	11.4	7.14	0.69	0.85	0.08	0.33
	Minimum value	5	2.81	7.72	4.4	0.48	0.57	0.06	0.22
	Maximum value	15.91	3.71	16.81	9.9	0.89	1.15	0.1	0.54
	Standard deviation	3.86	0.27	3.01	1.83	0.16	0.18	0.01	0.09
	CV	0.38	0.08	0.26	0.26	0.23	0.21	0.18	0.28
Comparison with control	Average value	0.85	0.89	0.83	0.64	0.51	0.69	0.72	0.72
	Minimum value	0.68	0.75	0.70	0.48	0.35	0.55	0.47	0.58
	Maximum value	0.94	1.00	1.03	0.81	0.79	0.91	0.95	0.94
	Standard deviation	0.08	0.07	0.10	0.09	0.15	0.11	0.17	0.11
	CV	0.09	0.08	0.12	0.15	0.30	0.16	0.23	0.15

Table 7. Statistical analysis of melon physiological characters at seedling stage under 200 mM NaCl treatment and control conditions.

Treatment	Parameter	Chlorophyll Content	Root Activity	MDA Content	POD Activity	CAT Activity	SOD Activity	Soluble Protein Content	Proline Content
Control	Average value	1.71	996.54	2.07	1340.66	1279.86	873.55	2.55	15.86
	Minimum value	1.42	559.92	1.64	533.33	1266.31	852.7	2.16	8.03
	Maximum value	1.95	2055.08	2.64	3905.21	1295.56	899.01	3.18	24.05
	Standard deviation	0.19	487.49	0.4	1021.63	8.76	14.29	0.28	6.6
	CV	0.11	0.49	0.19	0.76	0.01	0.02	0.11	0.42
200 mM NaCl treatment	Average value	1.37	610.83	2.45	3105.21	1291.92	916.39	1.49	27.24
	Minimum value	1.05	284.6	1.68	1347.92	1261.08	889.97	1.23	17.97
	Maximum value	1.81	980	3.71	4260.42	1313.22	942.9	1.89	39.27
	Standard deviation	0.27	206.21	0.72	812.54	16.32	14.99	0.19	7.13
	CV	0.2	0.34	0.3	0.26	0.01	0.02	0.13	0.26
Comparison with control	Average value	0.79	0.68	1.19	0.59	4.52	3.20	1.01	1.02
	Minimum value	0.59	0.39	0.70	0.44	0.77	1.29	0.98	1.00
	Maximum value	0.93	1.05	1.70	0.72	27.22	6.35	1.03	1.05
	Standard deviation	0.11	0.24	0.28	0.09	8.04	1.70	0.01	0.01
	CV	0.14	0.36	0.23	0.15	1.78	0.53	0.01	0.01

The varieties with the highest relative plant height, relative stem diameter, relative maximum root length, relative aboveground fresh weight, relative underground fresh weight, relative aboveground dry weight, relative underground dry weight, and relative seedling index are ‘Huaxiami’, ‘Baicuimi’, ‘Huaxiami’, ‘Jinyuliuxing’, ‘Huaxiami’, ‘Huangmengcui’, ‘Huaxiami’, and ‘Huangmengcui’. Meanwhile, the varieties with the highest relative plant height, relative stem diameter, relative maximum root length, relative above-

ground fresh weight, relative underground fresh weight, relative aboveground dry weight, relative underground dry weight, and relative seedling index are ‘Xindongfangmi’, ‘M135’, ‘M135’, ‘M135’, ‘Zhongtian 5’, ‘M135’, ‘Jintiancui’, and ‘Tianhongyu’ (Table 8).

Table 8. Statistical analysis of salt tolerance coefficient for growth indicators of melon at seedling stage.

	Plant Height	Stem Diameter	Maximum Root Length	Aboveground Fresh Weight	Underground Fresh Weight	Aboveground Dry Weight	Underground Dry Weight	Seedling Index
Huangmengcui	0.86	0.90	0.83	0.56	0.53	0.91	0.88	0.94
Wanmei 9	0.83	0.84	0.78	0.59	0.44	0.80	0.71	0.80
Baicuimei	0.91	1.00	0.77	0.73	0.49	0.74	0.73	0.83
Zhongtian 5	0.85	0.92	0.74	0.70	0.35	0.64	0.57	0.67
Jinyuliuxing	0.94	0.98	1.03	0.81	0.67	0.70	0.94	0.77
Jintiancui	0.84	0.85	0.85	0.65	0.36	0.65	0.47	0.63
Tianhongyu	0.80	0.84	0.77	0.57	0.40	0.56	0.56	0.58
Huaxiami	0.94	0.93	0.94	0.66	0.79	0.71	0.95	0.73
Xindongfangmi	0.79	0.87	0.91	0.64	0.68	0.60	0.78	0.65
M135	0.68	0.75	0.70	0.48	0.40	0.55	0.60	0.62

The varieties with relative chlorophyll content, relative root activity, relative malondialdehyde content, relative POD activity, relative CAT activity, relative SOD activity, relative soluble protein content, and relative proline content are ‘Huangmengcui’, ‘Wanmei 9’, ‘M135’, ‘M135’, ‘Huaxiami’, ‘Xindongfangmi’, ‘Wanmei 9’, and ‘Baimengcui’. Meanwhile, the varieties with the highest relative chlorophyll content, relative root activity, relative malondialdehyde content, relative POD activity, relative CAT activity, relative SOD activity, relative soluble protein content, and relative proline content are ‘Jintiancui’, ‘Xindongfangmi’, ‘Xindongfangmi’, ‘Xindongfangmi’, ‘Jintiancui’, ‘Huaxiami’, ‘Huaxiami’, and ‘Tianhongyu’ (Table 9).

Table 9. Statistical analysis of salt tolerance coefficients for physiological indicators of melon at seedling stage.

	Chlorophyll Content	Root Activity	MDA Content	POD Activity	CAT Activity	SOD Activity	Soluble Protein Content	Proline Content
Huangmengcui	0.93	0.98	1.04	0.63	1.73	2.76	1.02	1.01
Wanmei 9	0.72	1.05	1.24	0.67	2.53	2.98	1.03	1.03
Baicuimei	0.90	0.42	1.43	0.63	3.41	2.25	1.02	1.05
Zhongtian 5	0.85	0.77	1.17	0.52	1.92	3.69	1.01	1.03
Jinyuliuxing	0.85	0.71	1.31	0.66	1.41	1.96	1.01	1.01
Jintiancui	0.59	0.53	1.07	0.50	0.77	3.62	1.02	1.03
Tianhongyu	0.73	0.48	0.93	0.51	4.02	1.42	1.01	1.00
Huaxiami	0.71	0.51	1.31	0.60	27.22	1.29	0.98	1.02
Xindongfangmi	0.76	0.39	0.70	0.44	0.84	6.35	1.03	1.01
M135	0.90	0.93	1.70	0.72	1.39	5.66	1.00	1.04

From the information of maximum, minimum, and coefficient of variation, there are different ranges of variation in different varieties of melons, indicating that different traits respond differently to salt stress. Relying solely on a single indicator of salt tolerance coefficient cannot accurately evaluate the salt tolerance of melons.

3.3.2. Correlation Analysis of Salt Tolerance Coefficients During the Seedling Stage

Salt tolerance coefficients exhibited significant positive correlations across 11 indicator pairs (e.g., plant height and root dry weight, shoot dry weight and fresh weight, maximum root length, and underground fresh weight). Negative correlations were found among seven indicator pairs, such as between plant height and seedling index or stem diameter and malondialdehyde content. The strongest positive correlation was between stem thickness and aboveground fresh weight ($r = 0.924$), while the weakest was between aboveground fresh weight and proline content ($r = 0$) (Table 10).

Table 10. Correlation of indexes in the seedling stage of melon under 200 mM NaCl treatment.

	RPH	RSD	RMRL	RAFW	RUFW	RADW	RUDW	RSI	RCHI	RRA	RMDA	RPOD	RCAT	RSOD	RSP	RPRO
RPH	1	0.687 *	−0.352	0.765 **	−0.328	0.809 **	−0.194	−0.743 *	0.525	0.569	−0.561	−0.248	0.626	0.252	−0.311	−0.108
RSD		1	−0.131	0.924 **	−0.001	0.790 **	−0.094	−0.171	0.27	0.232	−0.725 *	−0.673 *	0.244	0.256	−0.341	−0.137
RRL			1	−0.249	0.871 **	−0.228	0.858 **	0.616	0.114	−0.395	0.11	−0.038	−0.679 *	−0.153	0.592	0.135
RAFW				1	0.005	0.916 **	−0.058	−0.28	0.211	0.431	−0.770 **	−0.533	0.486	0.309	−0.403	−0.318
RUFW					1	−0.027	0.918 **	0.667 *	−0.084	−0.249	−0.009	−0.113	−0.549	0.033	0.439	−0.115
RADW						1	−0.03	−0.322	0.211	0.564	−0.699 *	−0.429	0.519	0.138	−0.376	−0.271
RUDW							1	0.524	0.236	−0.107	−0.003	0.017	−0.497	0.121	0.641 *	0.000
RSI								1	−0.262	−0.418	0.094	−0.195	−0.697 *	−0.140	0.445	0.107
RCHI									1	0.231	−0.365	−0.087	0.125	0.280	0.459	0.365
RRA										1	−0.044	−0.400	0.493	0.623	0.120	0.142
RMDA											1	0.148	−0.478	0.081	0.304	0.475
RPOD												1	0.129	−0.508	−0.112	−0.398
RCAT													1	0.055	−0.501	−0.344
RSOD														1	0.392	0.181
RSP															1	0.477
RPRO																1

Note: RPH—relative plant height; RSD—relative stem diameter; RMRL—relative maximum root length; RAFW—relative aboveground fresh weight; RUFW—relative underground fresh weight; RADW—relative aboveground dry weight; RUDW—relative underground dry weight; RSI—relative seedling index; RCHI—relative chlorophyll content; RRA—relative root activity; RMDA—relative malondialdehyde content; RPOD—relative POD activity; RCAT—relative CAT activity; RSOD—relative SOD activity; RSP—relative soluble protein content; RPRO—relative proline content. * indicates that the difference is significant ($p < 0.05$). ** indicates that the difference is extremely significant ($p < 0.01$).

3.3.3. PCA of Salt Tolerance During the Seedling Stage

PCA of seedling-stage traits yielded nine independent components with a cumulative contribution rate of 100%. The first three components accounted for 75% of the variance, representing the primary salt tolerance indicators. The PC1, PC2, and PC3 functions were derived based on trait weights, with key indicators identified as follows:

PC1: Plant height, aboveground fresh weight, aboveground dry weight, and CAT activity.

PC2: Maximum root length, underground fresh and dry weights, and POD activity.

PC3: Malondialdehyde, root activity, SOD activity, proline, and soluble protein content.

These principal components highlight the major physiological effects of 200 mM NaCl treatment on melon seedlings (Table 11).

Table 11. Principal component eigenvalues and cumulative contribution rate.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
RPH	0.3617	0.1119	0.0640	0.2294	−0.0763	0.2922	−0.1955	−0.1252	−0.1321
RSD	0.3409	0.2418	−0.1586	−0.1171	0.0362	−0.0286	−0.0676	0.0904	0.2175
RRL	0.3352	0.2132	−0.1388	−0.0238	0.0696	0.3013	0.2702	−0.3014	0.1486
RAFW	0.3226	−0.1713	−0.0064	0.2130	0.1964	−0.0831	0.3522	0.7118	−0.1156
RUFW	0.2944	0.2705	−0.1375	−0.2179	−0.2508	−0.0636	−0.2472	−0.0232	−0.0633
RADW	−0.2802	0.2153	−0.1733	−0.2851	−0.0410	−0.3168	0.4557	−0.1298	0.1817
RUDW	−0.2667	0.3293	−0.1477	0.1483	−0.0772	0.2896	−0.0776	0.1855	−0.5147
RSI	−0.2586	−0.1766	0.3294	−0.1712	0.1955	0.4246	−0.2415	−0.0255	0.0716
RCHL	0.2329	0.1124	0.3594	−0.0131	0.4310	0.1935	0.3924	−0.2435	−0.0853
RRA	−0.2276	0.2800	0.3211	0.2269	0.0620	−0.2480	0.1912	−0.1530	−0.3743
RMDA	−0.1974	0.3908	−0.1062	0.2736	0.2212	0.1480	−0.0259	0.0348	0.3496
RPOD	−0.2072	0.3723	−0.2424	0.0180	0.2422	0.1701	−0.0690	0.3037	0.1569
RCAT	−0.1052	0.0848	0.4739	−0.1229	−0.4577	0.2857	0.2041	0.3176	0.3743
RSOD	0.0979	0.2264	0.3882	−0.1221	0.3966	−0.4129	−0.4298	0.1275	0.1431
RSP	−0.1215	−0.3182	−0.1765	0.5366	0.1506	−0.0182	−0.0532	−0.1600	0.3413
RPRO	0.1015	0.2307	0.2520	0.5128	−0.3928	−0.2143	−0.0273	−0.0719	0.1514
Characteristic value	6.013	3.556	2.445	1.49	1.1	0.593	0.484	0.186	0.132
Contribution rate	37.583	22.227	15.279	9.313	6.877	3.705	3.024	1.163	0.828
Cumulative contribution rate	37.583	59.810	75.089	84.402	91.28	94.985	98.009	99.172	100

3.3.4. Comprehensive Evaluation and Cluster Analysis of Seedling-Stage Salt Tolerance

Average and comprehensive membership function values for salt tolerance were highest for ‘Xindongfangmi’ (0.749 and 0.820) and lowest for ‘Wanmei 9’ (0.233 and 0.208) (Table 12). Cluster analysis, using Euclidean distance and the Wald method, categorized the 10 varieties into 2 groups, effectively distinguishing their salt tolerance levels (Figure 2).

Table 12. The average membership function value, comprehensive membership function value, and ranking of the relative salt tolerance coefficient and various traits at seedling stage of different varieties under 200 mM NaCl treatment.

Varieties	Average Membership Function	Comprehensive Membership Function	Ranks
Xindongfangmi	0.749	0.820	1
Jinyuliuxing	0.665	0.625	2
Huaxiami	0.516	0.598	3
Huangmengcui	0.382	0.573	4
Tianhongyu	0.496	0.568	5
Jintiancui	0.512	0.452	6
M135	0.365	0.452	7
Zhongtian 5	0.286	0.360	8
Baicuimei	0.363	0.321	9
Wanmei 9	0.233	0.208	10

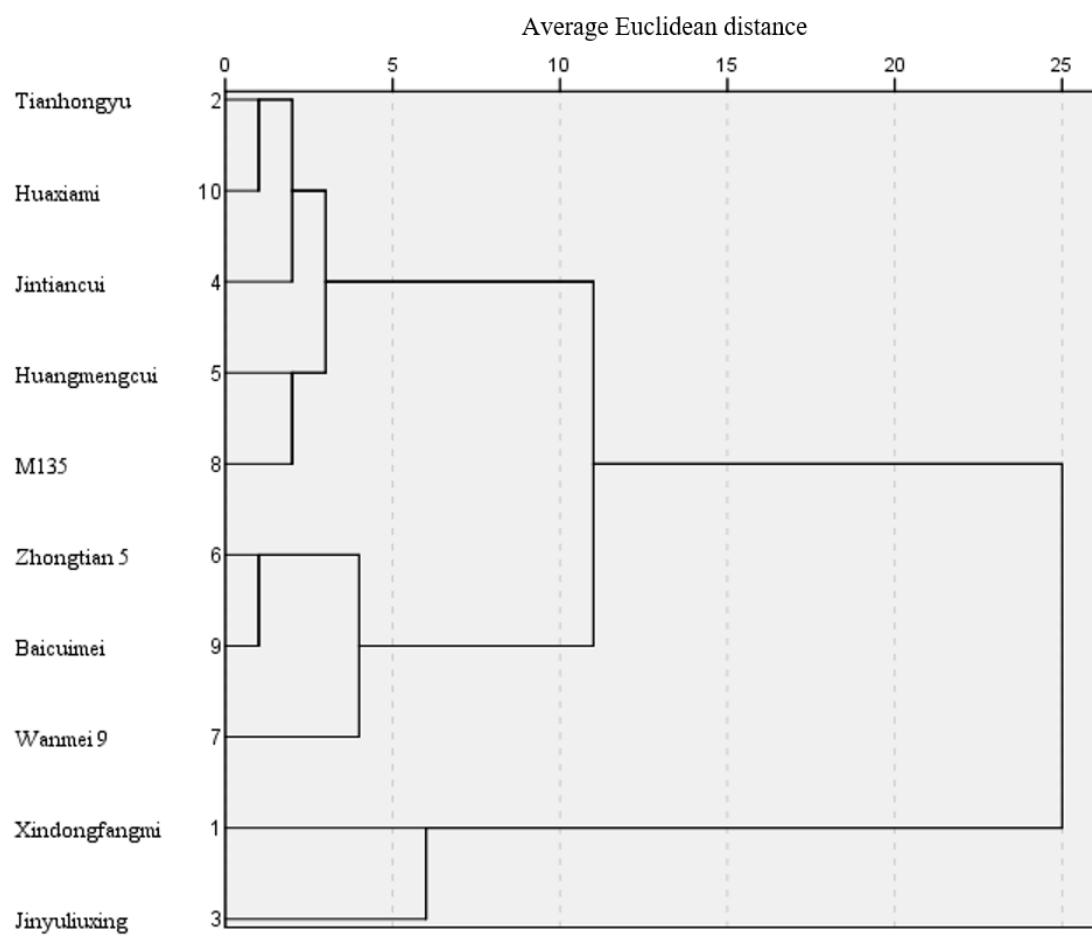


Figure 2. Cluster diagram of salt tolerance of different varieties at seedling stage.

3.4. Correlation Analysis Between Germination and Seedling Stages Under 200 mM NaCl Treatment

A highly significant positive correlation was observed among the four calculated values for germination and seedling stages, with the strongest correlation between average and comprehensive membership function values during germination ($r = 0.892$) and the weakest correlation between average values in germination and seedling stages ($r = 0.711$) (Table 13). This consistency suggests that one membership function value could largely represent the others, confirming a predictive relationship between salt tolerance at germination and seedling stages.

Table 13. Correlation of average membership function value, comprehensive membership function value at germination, and seedling stage of melon under 200 mM NaCl treatment.

	GAMF	GCMF	SAMF	SCMF
GAMF	1	0.892 *	0.719 *	0.711 *
GCMF		1	0.834 *	0.829 *
SAMF			1	0.792 *
SCMF				1

Note: GAMF—average membership function value during germination period; GCMF—comprehensive membership function value during germination period; SAMF—average membership function value at seedling stage; SCMF—comprehensive membership function value at seedling stage. * indicates that the difference is significant ($p < 0.05$).

4. Discussion

This study found no significant change in the germination of all melons at 50 mM NaCl compared to the control. Interestingly, ‘Huangmengcui’, ‘Jintiancui’, and ‘M135’ germinated with 50 mM NaCl slightly better than the control. This shows that melon seeds tolerate low-concentration salt. It can even promote their germination. On one hand, it may be due to the absorption of inorganic salt ions from the salt solution. This increases the cell fluid concentration and reduces the cell water potential. It enhances the seeds’ water absorption and improves the germination rate. It may be due to the activation effect of trace Na⁺ on respiratory enzymes. As NaCl concentration increased, the above indicators fell significantly. This shows that osmotic stress and ion toxicity from NaCl inhibited seed germination. Our study found that, compared to the control, 200 mM NaCl significantly reduced the variation in germination potential, germination rate, germination index, embryonic root length, fresh weight, and vitality index. This result shows that 200 mM NaCl is suitable for screening salt-tolerant melon germplasm. This agrees with some studies [24,28,29]. Also, saline–alkali soil has a salt content above 6‰. Our chosen NaCl concentration is 11.6‰, much higher than that. This can further improve the accuracy of salt-tolerant germplasm screening.

The seed germination stage is the initial phase where plants encounter salt stress, affecting water absorption, seed swelling, and germination rate [29]. Indicators such as radicle length, fresh weight, germination index, vitality index, germination rate, and germination potential are reliable metrics for assessing salt stress effects during this stage [29–34]. *Halostachys caspica* seeds can withstand low-salt stress. However, various indicators significantly decline as salt concentration increases [34,35]. Our study found that all varieties had reduced germination potential, rate, and index under 200 mM NaCl. However, there are differences in the changes of different varieties under salt stress. For example, the relative germination potential of M135 was 0.97, while the relative germination potential of ‘Wanmei 9’ was only 0.00. So, we chose 200 mM NaCl for screening salt-tolerant melon varieties. This concentration is also similar to that in the previous literature [22,23].

The root system is the main plant organ sensitive to salt stress. Its characteristics are key to the plant’s stress response [36]. High salt levels in the root environment cause ion and osmotic stress. This forces roots to reduce their contact with the environment, often by shortening. It is a way to decrease salt toxicity [36,37]. Root vitality shows plant health and stress response. Salt stress can promote the accumulation of ROS in plant cells. Low levels of ROS can activate signaling pathways, while excessive accumulation of ROS can damage cell membrane structure. Lipid oxidation produces various secondary products, exacerbating oxidative damage. MDA is the main product of polyunsaturated fatty acid peroxidation and an important indicator of membrane lipid peroxidation. Some studies have reported a significant upregulation of MDA content in vegetables under salt stress [38–40]. Our research results indicate that MDA content fluctuates in all melon. The plant reduces this by raising antioxidant enzyme activity. These activity levels indicate the plant’s resilience to adverse conditions [26,41,42]. In this study, most melon varieties showed either a continuous increase in POD, CAT, and SOD activity, or an initial increase followed by a decrease. The rise suggests better stress tolerance. The decline implies low enzyme activity to counter free radicals, disrupting plant metabolism. Salt stress causes a rise in protective compounds like proline, soluble sugars, and proteins. They boost osmotic pressure, stabilize cells, and help plants absorb water for growth [43–45]. Proline accumulation is a common physiological response of plants under various abiotic stresses [46,47]. The accumulation of proline can reduce protein hydrolysis, stabilize subcellular structure, eliminate free radicals, and increase redox potential. Higher levels of proline can also prevent cell dehydration, maintain internal cell stability, and reduce salt stress’s harmful effects. Previous studies

have shown that the proline content in watermelon increases with the duration of salt stress, and salt tolerant varieties accumulate more proline than salt sensitive varieties [48–50]. This study found that proline content varied significantly among different melon varieties under salt stress conditions. This variation reflects inherent differences in their physiological mechanisms and genetic predispositions to adapt to such adverse environmental stressors. In contrast, soluble protein content across these varieties tended to decrease uniformly. This decline may be attributed to heightened protease activity at elevated salt concentrations, which can degrade proteins, or to reduced osmotic regulation capabilities, both of which compromise protein stability and functionality within the plant cells. The impact of salt stress is visibly evident in melon seedlings, manifesting as stunted growth characterized by reduced height, diminished root length, thinner stems, and lower overall biomass accumulation. These observable changes underscore the detrimental effects of salt stress on plant physiology, highlighting its capacity to disrupt normal growth patterns and developmental processes.

A high correlation suggests that one feature can explain changes in another. This study reveals significant positive correlations among the six salt tolerance traits at the germination stage, with the strongest correlation between relative germination potential and germination index ($r = 0.972$). The correlation data indicates that relative germination potential can partially reflect melon's salt tolerance during germination, aligning with previous findings [21]. However, considering its correlation coefficient with the relative fresh weight of 0.253, it is still necessary to comprehensively consider the contribution of various traits to salt tolerance. Among the 120 pairs of indicators during the seedling stage, only 18 pairs showed significant correlation. It can be expected that there is a positive correlation between growth indicators during the seedling stage, such as plant height, aboveground dry and fresh weight, and strong seedling index. In addition, there is a positive correlation between stem thickness and aboveground dry and fresh weight, and a positive correlation between maximum root length and underground dry and fresh weight, which has been reported in many related studies [17,23,51]. No significant correlation was observed between physiological indicators during the seedling stage. Notably, there is a negative correlation between stem thickness and MDA content, as well as POD activity. Additionally, CAT activity shows a significant negative correlation with maximum root length and the strong seedling index. Further research is required to fully understand these relationships. Consequently, we conclude that a single indicator during the seedling stage is insufficient to determine melon salt tolerance. Future studies should employ PCA and comprehensive membership analysis.

Salt tolerance in melon varieties is influenced by multiple factors, making it impossible for a single indicator to fully represent a plant's salt tolerance capacity. Therefore, comprehensive indicators are essential for accurate assessments. Previous studies have used membership function values from various indicators to evaluate germplasm resources for different crops, showing high reliability [24]. This study integrates PCA, cluster analysis, and membership function analysis to thoroughly assess melon salt tolerance, offering a more precise reflection of each variety's resilience. PCA results indicate that relative germination potential, relative germination rate, relative germination index, and relative embryonic root length are key indicators for assessing salt tolerance during the germination phase of melons. Additionally, relative plant height, relative aboveground and underground dry and fresh weight, relative CAT activity, relative maximum root length, and relative POD activity are important indicators for evaluating salt tolerance in melon seedlings.

In real-world agricultural settings, the salt tolerance of crops during germination is crucial for maintaining a high emergence rate in saline soils, making it essential for

assessing crop salt tolerance. Additionally, the method used to identify germination periods offers several benefits, including time efficiency, ease of use, high repeatability, and straightforward comparison of results across various studies [14,30]. This period is crucial for the initial assessment of plant salt tolerance. Nonetheless, it is uncertain whether salt tolerance during germination consistently predicts overall salt tolerance in subsequent stages. This study reveals a significant positive correlation between salt tolerance indicators at the germination and seedling stages, indicating that germination-stage evaluations reliably reflect salt tolerance at the seedling stage. Although clustering based on germination potential and rate may be imprecise, it suffices for preliminary classification of salt tolerance. These findings support the use of germination-based screening for selecting salt-tolerant germplasm and breeding salt-tolerant crop varieties.

5. Conclusions

This study evaluated 10 melon varieties exposed to 200 mM NaCl to assess salt tolerance during germination and seedling stages. Salt stress reduced germination and seedling growth but increased antioxidant enzyme activity and osmotic compound levels. Variability in salt tolerance was observed among the varieties. PCA condensed 6 germination-stage indicators into 1 comprehensive indicator with a contribution rate of 79.225%, and 16 seedling-stage indicators into 3 indicators with a cumulative contribution rate of 75.089%. Membership function analysis and cluster analysis grouped the 10 melon varieties into three clusters at both stages. ‘Xindongfangmi’ and ‘Jinyuliuxing’ were identified as salt-tolerant varieties. A significant positive correlation ($r = 0.834$) was found between the comprehensive membership function values for germination and seedling stages. The results indicate that salt tolerance during germination can predict salt tolerance during the seedling stage. Utilizing PCA, comprehensive evaluation, and cluster analysis of indicators under salt stress during germination can efficiently identify salt tolerance in the germination and seedling stage. This study offers a methodological approach for rapid and effective evaluation of crop salt tolerance, aiding in the selection and cultivation of salt-tolerant germplasm for breeding.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae11040397/s1>, Figure S1: Germination potential of 10 melon varieties under different NaCl concentrations; Figure S2: The germination rate of 10 melon varieties under different NaCl concentrations; Table S1: Tested melon materials; Table S2: Composition of improved Hogland nutrients solution; Table S3: Effects of Different NaCl Concentrations on Germination Potential of 10 Melon Varieties; Table S4: Effects of Different NaCl Concentrations on Germination Rate of 10 Melon Varieties.

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