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Article

Identification of salt tolerance of different Chinese kale cultivars under NaCl stress at seedling stage

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Abstract: In order to understand the salt tolerance of different Chinese kale varieties at seedling stage and screen out the varieties with strong salt tolerance at seedling stage, the seeds of 26 Chinese kale varieties were used as materials. The growth indexes, physiological indexes, seedling rate and salt damage index of different Chinese kale varieties were compared and cluster analyzed by means of tissue culture. The results showed that: (1) under 160 mmol·L^{-1} NaCl stress, the different Chinese kale varieties at growth indexes, physiological indexes, seedling rate and salt damage index were significantly different, the relative seedling rate of R4, R14 and R32 reached 100.00%, and the salt damage index was the lowest, which was 20.00%; The correlation analysis showed that the relative seedling rate, the relative content of soluble protein, the relative content of soluble sugar and the relative value of Leaf SPAD were all negatively correlated with the salt damage index, and the relative conductivity and the relative content of malondialdehyde were all positively correlated with the salt damage index. (2) The average value of 14 salt tolerance indexes was used to cluster analyze the salt tolerance of 26 Chinese kale varieties. The tested materials were divided into four categories: high salt tolerance, moderate salt tolerance, low salt tolerance and salt sensitivity. R14, R25, R30 and R32 were highly salt tolerant varieties and R24 was salt sensitive varieties, which laid a foundation for further breeding and genetic research of salt tolerant varieties.

Keywords: Chinese kale; salt stress; seedling stage; salt resistance

1. Introduction

Chinese cabbage (Brassica oleracea var. alboglabra bailey), a variety of cabbage, is a specialty vegetable in southern my country, rich in nutrients, and its introduction and cultivation area is gradually expanding. Salt stress has become one of the main factors affecting the growth and development, yield and quality of vegetable crops [1,2]. According to statistics, the total area of saline soil in my country is about 36 million hm^2, of which the salinized area of cultivated land is 9.209 million hm^2 [3–5], the area of saline soil is still gradually expanding.

Relevant studies have shown that the most economical and effective way to overcome soil salinization is to maximize the salt tolerance of the variety itself [6,7], so it is imminent to breed varieties with strong salt tolerance. At present, the research on the salt tolerance of vegetable crops mainly focuses on the research on the seed germination stage and the seedling stage. The seed germination stage is mainly through the Petri dish germination method [8–10], and the seedling stage is when the plant grows to a certain size. The treatment of salt stress [11–13], but there is a lack of continuous observation process between the two methods, and culture on MS medium can well show the continuous changes under salt stress, Literature [14] and Chinese cabbage seeds were used as test materials, and the method of plant tissue culture was used to study the salt tolerance of seeds at germination and seedling stages. The purpose of this experiment was to identify the salt tolerance of different kale cultivars at the seedling stage. Using the seeds of 26 kale varieties as test materials, tissue culture was used to analyze the differences in salt tolerance of different kale varieties under salt stress. The kale varieties with strong salt tolerance were screened out, which provided theoretical basis for expanding the planting area of kale and salt-tolerant breeding in the future.

2. Materials and methods

2.1. Materials to be tested

The seeds of 26 different kale varieties were used as test materials, all provided by the breeding group of the Department of Horticulture, Hebei North University; the experiment was carried out in the tissue culture laboratory of the Department of Horticulture, College of Agriculture and Forestry Science and Technology, Hebei North University from November 2021 to January 2022.

2.2. Test method

The salt tolerance was identified by tissue culture, with "MS + 30 $g·L^{-1}$ sucrose + 6 $g·L^{-1}$ agar + 0 $mmol·L^{-1}$ NaCl (pH5.84)" as the control medium and "MS + 30 $g·L^{-1}$ sucrose + 6 $g·L^{-1}$ agar + 160 $mmol·L^{-1}$ NaCl (pH 5.84)" as the treatment medium, and sterilized at high temperature and autoclave at 121 °C for 21 min. Select plump and unbroken seeds for disinfection, the disinfection treatment combination is "75% alcohol (30s) + 2% NaClO (16min)", inoculate on the ultra-clean workbench after disinfection, put 5 seeds in each bottle, and inoculate 5 bottles for the control. Inoculate 5 bottles, seal them, and place them in a culture room for incubation at room temperature. The growth status of the plants was observed on the 10th, 20th and 40th days of inoculation; the seedling rate and salt damage level were recorded after the 20th day (refer to the method of Wang et al. [15] with a slight modification). Three bottles were selected for control and treatment, and the number of lateral roots, plant height, root length, fresh weight of shoots and roots, leaf SPAD value (SPAD-502Plus portable chlorophyll meter), cell membrane permeability (conductivity method), Soluble protein content (Coomassie brilliant blue G-250 staining method), soluble sugar content (anthrone colorimetric method) and malondialdehyde content (thiobarbituric acid method); after 40 d of culture, the seedling rate and Salt damage level.

$$\text{Salt damage index (\%)} = \sum \text{(number of affected plants} \times \text{salt damage level)/(highest level of salt damage} \times \text{total number of plants)} \times 100$$

$$\text{Relative value (\%)} = \text{(measured value of salt stress treatment/measured value of control treatment)} \times 100$$

2.3. Data processing

The experimental data were organized and tabulated by Excel 2010, and processed by SPSS 17.0 and DPS 7.05 software.

3. Results and Analysis

3.1. The effect of NaCl stress on the growth indexes

The growth indexes of 26 kale varieties under NaCl stress for 20 d are shown in **Table 1**. It can be seen from **Table 1** that the relative lateral root number, relative plant height, relative root length, relative shoot fresh weight and relative root fresh weight of different kale varieties under NaCl stress all showed significant differences.

The seedlings of R24 all died under NaCl stress for 20 d. The varieties with larger relative lateral roots are R1, R4, R11, R12, R14 and R25, which are 18.18%, 18.75%, 33.56%, 24.67%, 33.96% and 41.43%, respectively, and the relative lateral root number of R25 is significantly higher than the remaining ones 21 cultivars of R3, R9, R18, R20, R23 and R28 had no lateral roots.

The variation range of relative plant height was between 7.54% and 29.53%. The relative plant heights of R6, R10 and R12 were larger, which were 26.43%, 26.53% and 29.53%, respectively. The relative plant height of R12 was significantly higher than that of R6 and R12. Varieties other than R10. The variation range of the relative root length was between 3.11% and 23.72%. The relative root length of R1, R6, R12, R14, R25 and R32 was larger, and the relative root length of R25 was the largest, and its value was significantly higher than the remaining 21 accessions. Variety. The relative aboveground fresh weight of R9 was the largest, reaching 86.95%, which was significantly higher than other varieties. The relative root fresh weight of R25 was the largest, which was 28.57%, which was significantly higher than that of other varieties.

Table 1. Effects of NaCl stress on growth indexes of different Chinese kale cultivars (%).

Variety	Relative lateral roots	Relative plant height	Relative root length	Relative shoot fresh weight	Relative root fresh weight
R1	18.18 ± 6.06abc	12.93 ± 5.18efgh	15.91 ± 2.54abcd	34.60 ± 4.20efghij	11.43 ± 2.86cde
R3	0.00 ± 0.00c	13.23 ± 0.00efgh	3.38 ± 0.00e	24.72 ± 0.00ghij	0.07 ± 0.00h
R4	18.75 ± 18.75adc	17.50 ± 2.49cdef	10.20 ± 4.90cde	23.12 ± 3.16hij	8.51 ± 2.13cdef
R6	15.10 ± 7.26bc	26.43 ± 0.46ab	14.03 ± 5.66abcde	51.48 ± 13.52bcd	14.13 ± 4.04cd
R7	3.15 ± 0.45c	20.93 ± 0.41bcd	5.07 ± 0.01de	52.54 ± 6.39bcd	5.14 ± 0.47efgh
R9	0.00 ± 0.00c	18.88 ± 2.45cde	6.71 ± 2.59de	86.95 ± 1.04a	10.90 ± 2.39cdef
R10	5.36 ± 5.36c	26.53 ± 0.27ab	9.69 ± 5.73cde	40.10 ± 1.99defg	9.09 ± 0.00cdef
R11	33.56 ± 17.36ab	11.74 ± 1.00fgh	11.05 ± 3.63bcde	43.18 ± 5.54cdef	14.63 ± 6.36c
R12	24.67 ± 0.00abc	29.53 ± 0.00a	15.70 ± 0.00abcd	60.05 ± 0.00b	20.95 ± 0.00b
R13	3.56 ± 0.00c	9.06 ± 0.00gh	4.15 ± 0.00e	38.60 ± 0.00defgh	22.03 ± 0.00b
R14	33.96 ± 0.00ab	13.91 ± 0.03efgh	20.90 ± 0.11ab	24.06 ± 6.24ghij	0.51 ± 0.10gh
R18	0.00 ± 0.00c	11.34 ± 1.78fgh	4.58 ± 1.48e	47.03 ± 4.75bcde	0.01 ± 0.01h
R19	1.44 ± 1.44c	22.94 ± 4.28bc	10.84 ± 2.20bcde	60.44 ± 8.94b	7.59 ± 2.23cdefg
R20	0.00 ± 0.00c	15.77 ± 0.00defg	3.11 ± 0.00e	37.40 ± 0.00defghi	3.83 ± 0.00fgh
R21	1.25 ± 1.25c	12.75 ± 2.47efgh	10.08 ± 2.32cde	38.27 ± 2.13defgh	7.18 ± 4.08defgh
R22	2.24 ± 2.24c	8.42 ± 1.69h	3.69 ± 0.22e	29.80 ± 4.45fghij	5.38 ± 0.54efgh
R23	0.00 ± 0.00c	16.97 ± 0.61cdef	5.52 ± 2.00de	32.69 ± 1.32efghij	4.93 ± 1.87efgh
R24	—	—	—	—	—
R25	41.43 ± 20.00a	22.36 ± 0.00bcd	23.72 ± 0.00a	56.74 ± 0.00bc	28.57 ± 0.00a
R28	0.00 ± 0.00c	7.54 ± 0.24h	3.84 ± 0.03e	26.95 ± 6.60fghij	12.83 ± 2.84cd
R29	2.54 ± 2.54c	20.78 ± 2.83bcd	6.39 ± 1.31de	33.85 ± 3.54efghij	4.90 ± 0.00efgh

Table 1. (*Continued*).

Variety	Relative lateral roots	Relative plant height	Relative root length	Relative shoot fresh weight	Relative root fresh weight
R30	14.15 ± 4.72bc	22.26 ± 2.77bcd	8.97 ± 4.30cde	33.47 ± 0.99efghij	0.05 ± 0.02h
R32	5.07 ± 1.80c	22.28 ± 0.85bcd	19.51 ± 7.83abc	19.73 ± 0.46j	8.75 ± 1.47cdef
R33	2.21 ± 2.21c	17.36 ± 2.75cdef	10.42 ± 1.90bcde	43.15 ± 6.97cdef	13.85 ± 1.54cd
R34	0.38 ± 0.00c	17.24 ± 2.32cdef	8.45 ± 3.05de	24.76 ± 4.43ghij	0.03 ± 0.01h
R35	8.18 ± 8.18c	12.83 ± 2.13efgh	11.86 ± 4.85bcde	21.39 ± 1.08ij	5.34 ± 1.29efgh

Note: The date are mean values ± SD, Different lowercase letters indicate significant differences in the same column ($p < 0.05$). The same below. Indicates that seedlings of R24 were dead on 160 $mmol \cdot L^{-1}$ NaCl medium.

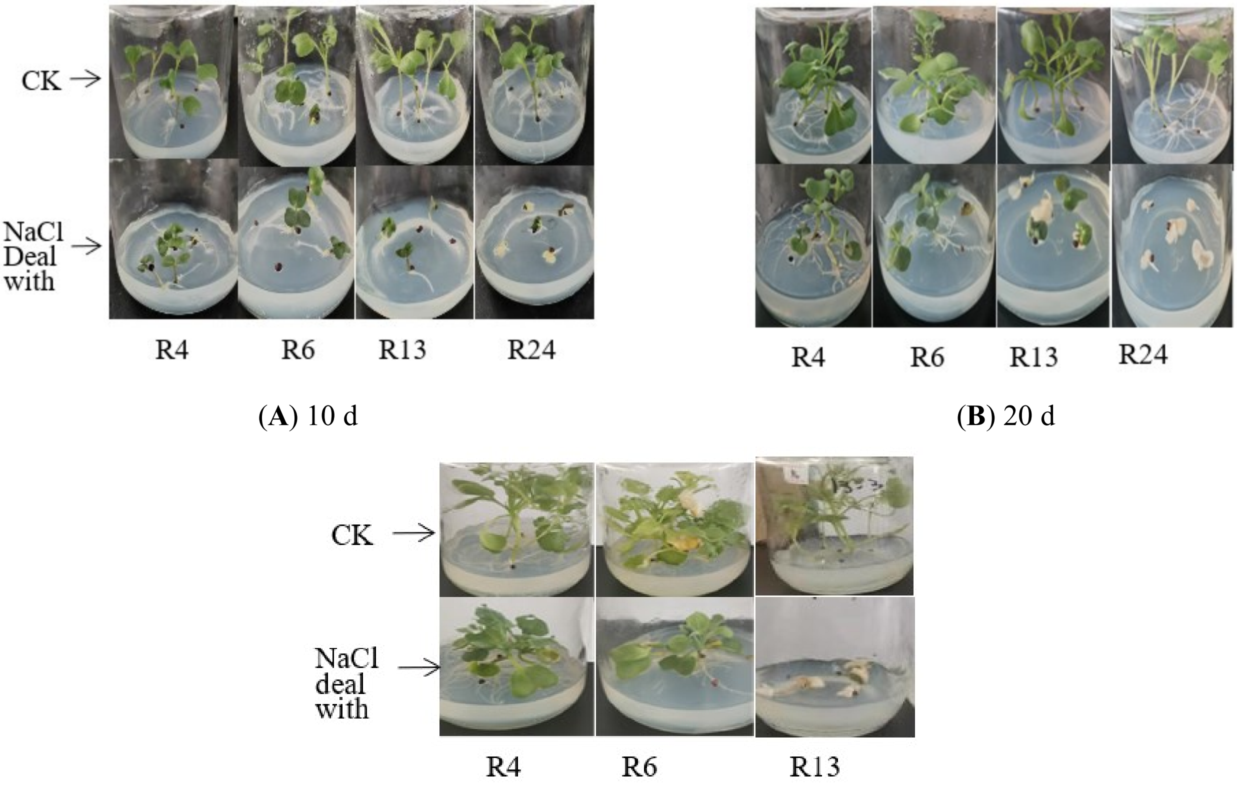
3.2. Effects of NaCl stress on the seedling rate and salt damage index of different kale varieties

It can be seen from **Figure 1A–C** that the salt tolerance of kale under different NaCl stress days can be continuously observed on the medium.

It can be seen from **Table 2** that under NaCl stress for 20 d and 40 d, the relative seedling rate and salt damage index of different kale varieties were significantly different. Under NaCl stress for 20 d, the relative seedling rate of the tested varieties was less than or equal to 50.00%, including R10, R13 and R24, whose values were 50.00%, 37.50% and 0.00%, respectively; the relative seedling rate of the remaining varieties were all greater than 50.00. %, and the relative seedling rate of R4, R7, R14, R20, R25, R32 and R35 reached 100.00%. Under NaCl stress for 40 d, the relative seedling rate of R22 decreased the smallest, which was 3.70%; R13, R33 and R34 had a larger decline, and their values were all above 50.00%. Among them, the relative seedling rate of R34 decreased the most, which was 100.00%; the relative seedling rate of R20 reached 100.00% under salt stress for 20 d. The relative seedling rate of R25 and R25 decreased by 50.00%, and the remaining varieties decreased slightly, ranging from 13.33% to 22.22%. It can be seen that R4, R7, R14, R22, R32 and R35 have strong salt tolerance.

Under NaCl stress for 20 d, the salt damage index of R24 was the largest among the tested varieties, reaching 100.00%; the salt damage index of the remaining varieties were all small, and the salt damage indexes of R4, R9, R14, R22, R23, R30 and R32 were all 20.00%. Under NaCl stress for 40 d, the salt damage index of R34 was 100.00%, which was 48.00% higher than that of salt stress for 20 d.

Correlation analysis was made based on the relative salt damage rate of seeds germination period of different kale varieties and the salt damage index of seedling stage (20 d, 40 d), the results showed that the relative salt damage rate and the salt damage index of 20 d and 40 d were not correlated, r is 0.1123 and 0.2431 ($a = 0.05$, $r = 0.3882$).



(**A**) 10 d

(**B**) 20 d

(**C**) 40 d

Figure 1. Growth of Chinese kale on medium (10 d; 20 d and 40 d).

Table 2. Comparison of seedling growth rate and salt damage index of different Chinese kale varieties under 20 d and 40 d NaCl stress (%).

Variety	Relative seedling rate Relative seedling rate			Salt damage index Salt injury index		
	20d	40d	Descend range	20d	40d	Ascensional range
R1	80.00 ± 20.00abc	44.44 ± 12.83bcd	44.45	38.00 ± 18.00c	68.00 ± 6.93bcdef	44.12
R3	75.00 ± 25.00abc	60.00 ± 11.55abc	20.00	46.67 ± 6.67bc	50.00 ± 5.77cdefghij	6.66
R4	100.00 ± 0.00a	86.67 ± 13.33a	13.33	20.00 ± 0.00c	33.33 ± 9.62hij	39.99
R6	80.00 ± 20.00abc	46.67 ± 13.33abcd	41.66	32.00 ± 8.00c	50.67 ± 17.02cdefghij	36.85
R7	100.00 ± 0.00a	83.33 ± 8.33ab	16.67	22.00 ± 2.00c	44.44 ± 5.67efghij	50.50
R9	87.50 ± 12.50ab	46.67 ± 13.33abcd	46.66	20.00 ± 0.00c	54.44 ± 9.44cdefghij	63.26
R10	50.00 ± 30.00bc	41.67 ± 8.33cd	16.66	55.00 ± 30.00bc	65.33 ± 5.33bcdefg	15.81
R11	60.00 ± 0.00abc	40.00 ± 23.09cd	33.33	32.00 ± 12.00c	74.00 ± 15.01abc	56.76
R12	80.00 ± 0.00abc	70.00 ± 0.00abc	12.50	25.00 ± 0.00c	40.00 ± 0.00fghij	37.50
R13	37.50 ± 37.50cd	16.67 ± 16.67de	55.55	78.00 ± 22.00ab	89.00 ± 8.62ab	12.36
R14	100.00 ± 0.00a	86.67 ± 13.33a	13.33	20.00 ± 0.00c	26.67 ± 6.67j	25.01
R18	62.50 ± 12.50abc	50.00 ± 28.87abcd	20.00	50.00 ± 23.33bc	70.00 ± 17.32bcde	28.57
R19	80.00 ± 0.00abc	73.33 ± 6.67abc	8.34	22.50 ± 2.50c	33.33 ± 7.06hij	32.49
R20	100.00 ± 0.00a	50.00 ± 5.77abcd	50.00	25.00 ± 0.00c	56.00 ± 2.31cdefghi	55.36
R21	70.00 ± 10.00abc	60.00 ± 0.00abc	14.29	46.00 ± 2.00bc	46.00 ± 8.08defghij	0.00
R22	90.00 ± 10.00ab	86.67 ± 6.67a	3.70	20.00 ± 0.00c	38.67 ± 9.62ghij	48.28
R23	70.00 ± 10.00abc	50.00 ± 5.77abcd	28.57	20.00 ± 0.00c	60.00 ± 4.62cdefgh	66.67

Table 2. (*Continued*).

Variety	Relative seedling rate Relative seedling rate			Salt damage index Salt injury index		
	20d	40d	Descend range	20d	40d	Ascensional range
R24	0.00 ± 0.00d	0.00 ± 0.00e	0.00	100.00 ± 0.00a	100.00 ± 0.00a	0.00
R25	100.00 ± 0.00a	50.00 ± 17.32abcd	50.00	24.00 ± 0.00c	54.00 ± 8.08cdefghij	55.56
R28	81.67 ± 1.67abc	53.33 ± 13.33abcd	34.70	25.00 ± 5.00c	60.00 ± 10.07cdefgh	58.33
R29	90.00 ± 10.00ab	80.00 ± 11.55abc	11.11	28.00 ± 8.00c	34.67 ± 8.11ghij	19.24
R30	90.00 ± 10.00ab	80.00 ± 0.00abc	11.11	20.00 ± 0.00c	36.00 ± 0.00hij	44.44
R32	100.00 ± 0.00a	86.67 ± 6.67a	13.33	20.00 ± 0.00c	30.67 ± 5.33ij	34.79
R33	87.50 ± 12.50ab	41.67 ± 8.33cd	52.38	36.00 ± 16.00c	74.67 ± 2.67abc	51.79
R34	60.00 ± 0.00abc	0.00 ± 0.00e	100.00	52.00 ± 4.00bc	100.00 ± 0.00a	48.00
R35	100.00 ± 0.00a	77.78 ± 6.42abc	22.22	24.00 ± 4.00c	46.00 ± 3.46defghij	47.83

3.3. The physiological indicators of different kale varieties

It can be seen from **Table 3** that the relative value of SPAD, relative content of soluble protein, relative content of soluble sugar, relative conductivity and relative content of malondialdehyde in leaves of different kale varieties under NaCl stress for 20 d were significantly different.

The variation range of the relative value of leaf SPAD in the tested varieties was 94.60%–167.81%, and the higher range of leaf SPAD value was 127.59%–167.81%, including 16 varieties, of which the SPAD value of R14 was significantly higher than the remaining 9 varieties. In the relative content of soluble protein, the relative content of soluble protein of R4, R6, R14, R22, R25, R30, R32 and R35 is relatively high, ranging from 192.17% to 289.60%, and the relative content of soluble protein of R25 is the highest, and its value is significant higher than the remaining 17 varieties. The varieties with relatively high soluble sugar content are R4, R14, R25, R30, R32 and R35, ranging from 1250.92% to 1774.75%, among which R30 has the highest relative soluble sugar content, and its value is significantly higher than that of R4, R14, R25, Varieties other than R32 and R35.

The variation range of relative conductivity is 36.57%~369.81%, and there are 13 varieties with low relative conductivity, ranging from 36.57% to 135.56%, among which R25 has the lowest relative conductivity, and its value is significantly lower than the remaining 12 varieties. The varieties with relatively low MDA content ranged from 25.02% to 350.92%, including 18 varieties, of which R6 had the lowest MDA content, which was significantly lower than that of R1, R3, R10, R13, R18, R21 and R34.

Table 3. Effects of NaCl stress on physiological indexes of different Varieties of Chinese kale (%).

Variety	Leaf relative SPAD value	Relative conductivity value	Relative soluble protein content	Relative soluble sugar content	Relative malondialdehyde content
R1	110.94 ± 1.90cdefg	183.98 ± 85.30cdefgh	90.50 ± 4.50fgh	649.32 ± 93.73efgh	380.46 ± 27.53bcd
R3	105.48 ± 2.69efg	369.81 ± 0.00a	64.99 ± 0.00h	323.32 ± 22.25gh	583.86 ± 0.00b
R4	164.69 ± 0.54ab	69.56 ± 19.52hi	237.48 ± 41.10abcd	1264.00 ± 244.00abcd	109.84 ± 24.16de
R6	164.15 ± 0.00ab	85.98 ± 13.03fghi	205.41 ± 0.00abcde	1045.89 ± 2.11bcdef	25.02 ± 14.85e
R7	130.92 ± 19.32abcdefg	116.19 ± 56.37defghi	121.34 ± 1.66efgh	683.18 ± 68.31defgh	276.45 ± 55.69bcde

Table 3. (*Continued*).

Variety	Leaf relative SPAD value	Relative conductivity value	Relative soluble protein content	Relative soluble sugar content	Relative malondialdehyde content
R9	127.59 ± 8.47abcdefg	128.09 ± 0.00defghi	138.38 ± 0.38defgh	468.17 ± 178.19fgh	234.23 ± 10.90cde
R10	94.60 ± 26.69g	222.61 ± 53.18bcd	53.18 ± 14.54h	268.10 ± 0.90h	949.80 ± 50.68a
R11	146.48 ± 28.75abcde	164.39 ± 13.95cdefgh	138.64 ± 29.27defgh	570.50 ± 47.50fgh	315.90 ± 203.16bcde
R12	140.74 ± 6.72abcdef	156.25 ± 10.54cdefgh	178.82 ± 32.58bcdefg	547.00 ± 39.00fgh	306.66 ± 15.39bcde
R13	100.09 ± 10.46fg	252.19 ± 0.00bc	77.73 ± 0.27gh	318.15 ± 0.85gh	1012.99 ± 327.34a
R14	167.81 ± 9.57a	74.94 ± 4.97ghi	264.18 ± 49.99ab	1558.50 ± 121.66ab	140.66 ± 1.66de
R18	106.77 ± 4.83defg	299.07 ± 58.22ab	88.38 ± 0.00fgh	416.17 ± 60.05gh	491.62 ± 63.83bc
R19	139.65 ± 18.93abcdef	169.29 ± 9.73cdefgh	158.05 ± 17.15cdefgh	701.88 ± 187.12defgh	286.16 ± 1.84bcde
R20	150.82 ± 1.26abcd	103.84 ± 58.46efghi	180.68 ± 6.18bcdefg	921.74 ± 0.00cdefg	194.00 ± 7.00cde
R21	114.06 ± 0.00cdefg	175.04 ± 0.00cdefgh	137.67 ± 71.77defgh	436.57 ± 95.16fgh	377.11 ± 135.88bcd
R22	163.21 ± 2.78ab	89.19 ± 4.97fghi	234.16 ± 32.92abcd	847.11 ± 134.34defgh	168.12 ± 0.88cde
R23	135.67 ± 8.03abcdefg	186.34 ± 0.00cdefg	150.85 ± 35.26cdefgh	745.54 ± 41.34defgh	334.39 ± 33.61bcde
R25	165.73 ± 6.03ab	36.57 ± 0.00i	289.60 ± 0.00a	1561.41 ± 594.87ab	97.82 ± 17.00de
R28	121.76 ± 6.35bcdefg	135.56 ± 36.76defghi	159.39 ± 19.99bcdefgh	592.61 ± 78.42fgh	308.00 ± 7.00bcde
R29	131.04 ± 17.31abcdefg	134.59 ± 44.99defghi	132.10 ± 20.91	419.60 ± 41.54	350.92 ± 85.94bcde
R30	163.69 ± 31.83ab	112.50 ± 12.68defghi	192.17 ± 60.94abcdef	1774.75 ± 25.89a	217.24 ± 34.09cde
R32	159.96 ± 12.27ab	94.23 ± 16.58fghi	249.91 ± 58.65abc	1505.14 ± 427.51abc	192.70 ± 0.00cde
R33	121.25 ± 1.28bcdefg	192.18 ± 22.07bcdef	93.76 ± 30.66fgh	488.12 ± 59.06fgh	300.49 ± 158.60bcde
R34	110.22 ± 0.00cdefg	218.09 ± 53.99bcde	81.68 ± 29.27gh	343.00 ± 22.00gh	426.14 ± 106.69bcd
R35	152.80 ± 5.46abc	86.21 ± 0.64fghi	224.87 ± 14.11abcde	1250.92 ± 303.24abcde	180.93 ± 20.67cde

3.4. Salt tolerance indexes of different kale varieties

Seedling rate and salt damage index are relatively intuitive identification indicators to measure salt stress. It can be seen from **Table 4** that the relative content of soluble protein, the relative value of electrical conductivity, the relative content of soluble sugar, the relative content of malondialdehyde, the relative value of leaf SPAD, the relative seedling rate and the salt damage index are significantly correlated. The relative content of soluble protein, the relative content of soluble sugar, the relative value of SPAD in leaves and the relative seedling rate were all significantly positively correlated, with r values of 0.7172, 0.6816 and 0.7228, respectively. The relative seedling rates were all significantly negatively correlated, with r values of −0.8372, −0.7202 and −0.8232, respectively. The relative conductivity, the relative content of malondialdehyde and the salt damage index were all significantly positively correlated, with r values of 0.7258 and 0.8557, respectively; while the relative content of soluble protein, the relative content of soluble sugar, the relative value of SPAD in leaves and the salt damage index were all extremely significant. Negative correlation, r is −0.7118, −0.6148 and −0.7774, respectively. Relative lateral root number, relative plant height, relative root length, relative shoot fresh weight and relative root fresh weight were not significantly correlated with relative seedling rate and salt damage index.

3.5. Cluster analysis of salt tolerance of different kale varieties under NaCl stress

Cluster analysis was performed on the mean values of 14 indexes, including relative seedling rate, salt damage index, growth index and physiological index of the tested materials (the reciprocal of the low and excellent indexes was taken). It can be seen from **Figure 2** that the tested materials can be divided into three groups at the Euclidean distance of 50.9: R14, R25, R30, R32 are the first category, which are highly salt-tolerant varieties; R4, R6, R20, R22, R35 It belongs to the second category and belongs to the moderate salt-tolerant varieties; other varieties belong to the third category and belong to the low-salt-tolerant varieties. Since the seedlings of R24 have died under NaCl stress for 20 d, R24 is classified into the fourth category and belongs to the salt-sensitive variety, see **Table 4**.

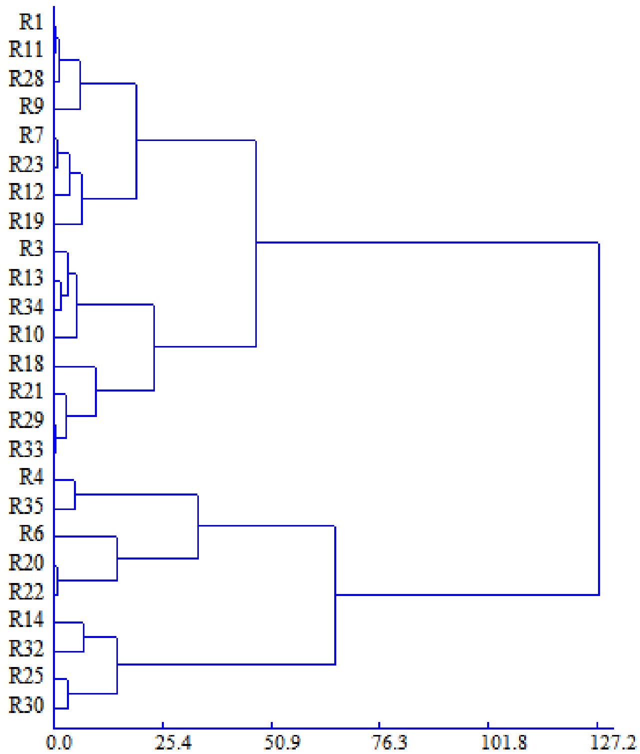


Figure 2. Cluster analysis of salt tolerance of different Chinese kale varieties at seedling stage.

Table 4. Correlation analysis table of salt tolerance indexes of different Chinese kale varieties.

Index	Relative lateral roots	Relative plant height	Relative root length	Relative shoot fresh weight	Relative root fresh weight	20 d relative seedling rate	20 d salt injury index	Relative soluble protein content	Relative conductivity value	Relative soluble sugar content	Relative malondialdehyde content
Relative plant height	0.2055										
Relative root length	0.7614	0.4177									
Relative aboveground fresh weight	0.0712	0.4118	0.0358								
Relative root fresh weight	0.4676	0.2020	0.4056	0.4276							
20d relative seedling rate	0.2169	0.1782	0.3303	-0.0638	−0.1240						
20d salt damage index	−0.2481	−0.2486	−0.2666	−0.0817	0.1111	−0.8372					
Soluble protein relative content	0.5259	0.1411	0.5599	−0.1157	0.1649	0.7172	−0.7118				
Conductivity relative value	−0.4460	−0.2441	−0.4754	−0.0403	−0.2261	−0.7202	0.725	−0.8495			
Soluble sugar relative content	0.5204	0.1913	0.5767	−0.2431	−0.0127	0.6816	−0.6148	0.8611	−0.7279		
The relative content of malondialdehyde	−0.3372	−0.1548	−0.3802	−0.0498	0.0558	−0.8232	0.8557	−0.7673	0.7481	−0.6539	
Relative value of blade SPAD	0.5218	0.2240	0.4611	−0.0771	0.0561	0.7228	−0.7774	0.9302	−0.8345	0.8575	−0.8294

Note: Critical value of correlation coefficient, a = 0.05, r = 0.3961, a = 0.01, r = 0.5052.

4. Discussion and conclusions

Seedlings are most sensitive to NaCl stress during growth and are affected by many factors, such as ion toxicity, osmotic stress and nutrient deficiency, and different varieties have different tolerances to salt stress [16–18]. Literature [19] found that the plant height, leaf area and dry matter of different sugar beet varieties showed significant differences under NaCl stress, and this index can be referred to evaluate the salt tolerance of varieties; In the study of literature [20], it was also found that the root length, hypocotyl and dry and fresh weight of different celery celery showed significant differences under salt stress, which can be used as an identification index of salt tolerance in the seedling stage. The results of this experiment were consistent with the above research results. There were significant differences in the number of lateral roots, shoot length, root length, shoot fresh weight and root fresh weight among different kale varieties under salt stress. Reference [21] used the salt damage index to identify the salt tolerance of strawberry seedlings. Reference [22] used the leaf salt damage index to test the salt tolerance of holly seedlings and achieved good results. Reference [23] showed that the salt tolerance of different small watermelon varieties There are different degrees of differences in the damage index, and it is considered that the salt damage index is suitable as one of the indicators for the identification of salt tolerance of varieties. This experimental study believes that the salt damage index of different kale varieties under salt stress can be used as an indicator for salt tolerance identification, which is intuitive and convenient, and increases the accuracy of salt tolerance identification.

The level of malondialdehyde content and the change of relative conductivity are important physiological indicators used to reflect the salt tolerance of vegetable crops [24–26]. Literature [27] showed that with the increase of salt concentration, the relative conductivity and malondialdehyde content of seedlings of different tomato varieties showed a significant upward trend. The literature [28] showed that with the increase of salt concentration, the electrical conductivity of different sugar beet varieties showed a significant upward trend. Literature [29] found that the relative conductivity and malondialdehyde content of leaves of salt-tolerant pepper varieties were lower than those of non-salt-tolerant varieties. This experiment confirmed this, the results showed that the relative conductivity and malondialdehyde content in the leaves of kale varieties with strong salt tolerance were lower than those with weak salt tolerance, and the relative conductivity and malondialdehyde content could be used as screening varieties for salt tolerance index of. Under salt stress, plants will accumulate some osmotic regulators, such as soluble proteins and soluble sugars, which can reduce the water potential of cells and promote cells to absorb water [19]. Under NaCl stress, the increase of soluble sugar and soluble protein is beneficial to delay the damage of membrane and reduce the water potential of cells. In the literature [30], it was found that the soluble sugar, soluble protein, and proline of leaves of two sugar beet lines tended to increase under salt stress. The literature [31] showed that with the prolongation of salt stress time, both soluble sugar and soluble protein showed an upward trend. In this experiment, the soluble protein and soluble sugar contents of kale varieties with strong salt tolerance were higher than those with weak salt tolerance.

Chlorophyll is the basis of photosynthesis, and chlorophyll content is one of the important indicators to measure the salt tolerance of vegetable crops. Under salt stress, the chloroplast structure of plants is destroyed, resulting in a decrease in chlorophyll content. Literature [30] and Liu [32] found in their studies that the chlorophyll content of salt-sensitive varieties under salt stress was significantly lower than that of salt-tolerant varieties. The literature [33] showed that the chlorophyll content in forage leaves decreased with the increase of salt concentration. However, other research results are contrary to this. The increase of chlorophyll content under salt stress is due to the destruction of the permeability of the cell membrane, and the large amount of chlorophyll molecules penetrates. The literature [34] showed that under salt stress, the content of photosynthetic pigments in leaves of salt-tolerant varieties of winter rapeseed was higher than that of salt-sensitive varieties. In this study, the SPAD values of 25 kale varieties under salt stress were all higher than those of the control, and only R10 was lower than that of the control, which may be due to the decrease of chlorophyll content due to the enhanced activity of chlorophyll-degrading enzymes.

An in-depth discussion of how salt stress affects the root system across different kale varieties would provide a clearer understanding of salt tolerance. Roots are the first to encounter saline conditions, and analyzing changes in root architecture, such as lateral root formation and root length, could explain why certain varieties display better nutrient uptake and water retention, leading to higher salt tolerance.

The effect of salt stress on photosynthesis can be explored further, particularly through the lens of chlorophyll content (SPAD values) and stomatal regulation. The study could examine how salt-tolerant varieties maintain higher chlorophyll levels or display more efficient photosynthetic rates under stress, contrasting with salt-sensitive varieties where chlorophyll degradation and impaired photosynthesis may occur.

Salt stress often induces oxidative stress, and a detailed analysis of antioxidant responses such as enzyme activities (e.g., superoxide dismutase, peroxidase) or non-enzymatic antioxidants (e.g., proline, soluble proteins) would provide insight into how different kale varieties mitigate cellular damage. By exploring how certain varieties exhibit stronger antioxidant responses, the study could better explain the mechanistic basis of their superior salt tolerance.

The current research results show that a single index cannot fully and objectively reflect the salt tolerance of plants, and multiple indicators need to be integrated to evaluate the salt tolerance [35–37]. In this experiment, the growth and physiological indicators of different kale varieties were used. 14 indicators of salt tolerance, such as seedling rate, seedling rate and salt damage index, were used as the basis for evaluating the salt tolerance of kale varieties in the seedling stage. Through cluster analysis, 26 kale varieties were clustered into high salt tolerance, moderate salt tolerance and low salt tolerance. There are four types of salt tolerance and salt sensitivity. The varieties with high salt tolerance are R14, R25, R30 and R32, and the salt sensitive variety is R24.

Based on the current analysis of salt tolerance in kale varieties, some specific limitations and improvements for future research could be outlined as follows:

Limitations:

The study primarily conducted experiments under controlled laboratory conditions, which may not fully reflect the complexity of salt stress in field environments with different soil types and climatic conditions. The applicability of laboratory findings in natural settings remains uncertain.

While the study effectively identified physiological differences in salt tolerance, it did not delve deeply into the molecular mechanisms and genetic factors that contribute to these differences.

Future Directions and Improvements:

Future research should verify the applicability of laboratory results by conducting experiments in different saline soil types and under various field conditions to better simulate real-world scenarios and stress environments.

In-depth molecular studies, such as gene expression profiling and transcriptomic analysis, should be conducted to identify the genetic pathways involved in salt tolerance and discover potential biomarkers for breeding salt-tolerant varieties.

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Data availability: The experimental data used to support the findings of this study are available from the corresponding author upon request.

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References

1. Zhu, Y., Wang, Q., Guo, W., Gao, Z., Wang, Y., Xu, Y., ... & Li, J. Screening and identification of salt-tolerance genes in Sophora alopecuroides and functional verification of SaAQP. Planta. 2021; 254(4), 1-22.
2. Peng, Z., Jiang, X., Wang, Z., Wang, X., Li, H., He, S., ... & Du, X. Identification of Raf-Like Kinases B Subfamily Genes in Gossypium Species Revealed GhRAF42 Enhanced Salt Tolerance in Cotton. International Journal of Molecular Sciences. 2021; 22(23), 12649.
3. Zhang, J., Wang, P., Tian, H., Jiang, H., Wang, Y., & Yan, C. Identification of interior salt-tolerant bacteria from ice plant Mesembryanthemum crystallinum and evaluation of their promoting effects. Symbiosis. 2018; 76(3), 243-252.

4. Nounjan, N., & Theerakulpisut, P. Physiological evaluation for salt tolerance in green and purple leaf color rice cultivars at seedling stage. Physiology and Molecular Biology of Plants. 2021; 27(12), 2819-2832.
5. Zhao, L., Zhang, F., Liu, B., Yang, S., Xiong, X., Hassani, D., & Zhang, Y. CmRAV1 shows differential expression in two melon (Cucumis melo L.) cultivars and enhances salt tolerance in transgenic Arabidopsis plants. Acta Biochimica et Biophysica Sinica. 2019; 51(11), 1123-1133.
6. He, L., Li, J., Shi, L., Zhao, Q., Wu, Z., Zeng, S., ... & Gao, J. Exogenous metabolites spray, which identified from metabolomics analysis and transcriptomic analysis, can improve salt tolerance of Chinese cabbages (Brassica rapa L. ssp pekinensis). Journal of Plant Interactions. 2021; 16(1), 452-461.
7. Ahmad, W., Ayyub, C. M., Shehzad, M. A., Ziaf, K., Ijaz, M., Sher, A., ... & Shafi, J. Supplemental potassium mediates antioxidant metabolism, physiological processes, and osmoregulation to confer salt stress tolerance in cabbage (Brassica oleracea L.). Horticulture. 2019; Environment, and Biotechnology, 60(6), 853-869.
8. Abd Al Galil, Fahd Mohammed, Sureshchandra Popat Zambare, Fahd A. Al-Mekhlafi, and Lamya Ahmed Al-Keridis. "Effect of dimethoate on the developmental rate of forensic importance Calliphoridae flies." Saudi Journal of Biological Sciences 28, no. 2 2021): 1267-1271.
9. Altay, A., & Mirici, İ. H. Efl Instructors' Implementations of 21st Century Skills in Their Classes. International Journal for Housing Science and Its Applications. 2024; 45(2), 37-46.
10. Al-Mekhlafi, Fahd A., Reem A. Alajmi, Zainab Almusawi, Fahd Mohammed Abd Al GAlil, Pawandeep Kaur, Muhammad Al-Wadaan, and Mohammed S. Al-Khalifa. "A study of insect succession of forensic importance: Dipteran flies (diptera) in two different habitats of small rodents in Riyadh City, Saudi Arabia." Journal of King Saud University-Science 32, no. 7 2020): 3111-3118.
11. Linić, I., Šamec, D., Grúz, J., Vujčić Bok, V., Strnad, M., & Salopek-Sondi, B. Involvement of phenolic acids in short-term adaptation to salinity stress is species-specific among Brassicaceae. Plants. 2019; 8(6), 155.
12. Linić, I., Mlinarić, S., Brkljačić, L., Pavlović, I., Smolko, A., & Salopek-Sondi, B. Ferulic acid and Salicylic acid foliar treatments reduce short-term salt stress in Chinese cabbage by increasing phenolic compounds accumulation and photosynthetic performance. Plants. 2021; 10(11), 2346.
13. Zou, L., Yu, B., Ma, X. L., Cao, B., Chen, G., Chen, C., & Lei, J. Cloning and expression analysis of the BocMBF1c gene involved in heat tolerance in Chinese kale. International journal of molecular sciences. 2019; 20(22), 5637.
14. dos Santos, B. D., Silva, P. F. D., Matos, R. M. D., D Neto, J., de Lima, V. L., Bonou, S. I., ... & Melo, Y. L. Induction of salt stress tolerance in chives by priming with H2O2 in hydroponic cultivation. Chilean journal of agricultural research. 2021; 81(3), 317-325.
15. Li, D. H., Shen, F. J., Li, H. Y., & Li, W. Kale BoRACK1 is involved in the plant response to salt stress and Peronospora brassicae Gaumann. Journal of plant physiology. 2017; 213, 188-198.
16. Boonnoi, N., Nuntagij, I., & Koohakan, P. Growth and yield of Chinese kale grown in dynamic root floating technique (DRFT) by reused nutrient solution. International Journal of Agricultural Technology. 2017; 13(7), 1467-1477.
17. Sun, X., Xu, L., Wang, Y., Yu, R., Zhu, X., Luo, X., ... & Liu, L. Identification of novel and salt-responsive miRNAs to explore miRNA-mediated regulatory network of salt stress response in radish (Raphanus sativus L.). BMC genomics. 2015; 16(1), 1-16.
18. Elakkiya, A. T. A Note on Decomposition of Tensor Product of Complete Multipartite Graphs into Gregarious Kite. Journal of Combinatorial Mathematics and Combinatorial Computing, 120, 295-299.
19. Miao, H. Y., Wang, M. Y., Chang, J. Q., Tao, H., Sun, B., & Wang, Q. M. Effects of glucose and gibberellic acid on glucosinolate content and antioxidant properties of Chinese kale sprouts. Journal of Zhejiang University-SCIENCE B. 2017; 18(12), 1093-1100.
20. Kaplan, M., Baser, M., Hasan, K. A. L. E., Irik, H. A., Ulger, I., & Unlukara, A. Change in yield and chemical composition of tall fescue (festuca arundinacea schreb.) plants under salt stress. Turkish Journal of Field Crops. 2017; 22(2), 204-210.
21. Zhou, C. Q., Lu, C. H., Mai, L., Bao, L. J., Liu, L. Y., & Zeng, E. Y. Response of rice (Oryza sativa L.) roots to nanoplastic treatment at seedling stage. Journal of Hazardous Materials. 2021; 401, 123412.
22. Rasel, M., Tahjib-Ul-Arif, M., Hossain, M. A., Hassan, L., Farzana, S., & Brestic, M. Screening of salt-tolerant rice landraces by seedling stage phenotyping and dissecting biochemical determinants of tolerance mechanism. Journal of Plant Growth Regulation. 2021; 40(5), 1853-1868.
23. Ma, X., Deng, X., Qi, L., Jiang, Y., Li, H., Wang, Y., & Xing, X. Fully convolutional network for rice seedling and weed image segmentation at the seedling stage in paddy fields. PloS one. 2019; 14(4), e0215676.
24. Chunthaburee, S., Dongsansuk, A., Sanitchon, J., Pattanagul, W., & Theerakulpisut, P. Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage. Saudi Journal of Biological Sciences. 2016; 23(4), 467-477.
25. Li, J., Cang, Z., Jiao, F., Bai, X., Zhang, D., & Zhai, R. Influence of drought stress on photosynthetic characteristics and protective enzymes of potato at seedling stage. Journal of the Saudi Society of Agricultural Sciences. 2017; 16(1), 82-88.
26. Li, R., Zeng, Y., Xu, J., Wang, Q., Wu, F., Cao, M., ... & Lu, Y. Genetic variation for maize root architecture in response to drought stress at the seedling stage. Breeding science. 2015; 65(4), 298-307.
27. Zhou, C. Q., Lu, C. H., Mai, L., Bao, L. J., Liu, L. Y., & Zeng, E. Y. Response of rice (Oryza sativa L.) roots to nanoplastic treatment at seedling stage. Journal of Hazardous Materials. 2021; 401, 123412.

28. Rasel, M., Tahjib-Ul-Arif, M., Hossain, M. A., Hassan, L., Farzana, S., & Brestic, M. Screening of salt-tolerant rice landraces by seedling stage phenotyping and dissecting biochemical determinants of tolerance mechanism. Journal of Plant Growth Regulation. 2021; 40(5), 1853-1868.
29. Rehman, A., Wang, N., Peng, Z., He, S., Zhao, Z., Gao, Q., ... & Du, X. Identification of C2H2 subfamily ZAT genes in Gossypium species reveals GhZAT34 and GhZAT79 enhanced salt tolerance in Arabidopsis and cotton. International Journal of Biological Macromolecules. 2021; 184, 967-980.
30. Mazumder, A., Rohilla, M., Bisht, D. S., Krishnamurthy, S. L., Barman, M., Sarma, R. N., ... & Mondal, T. K. Identification and mapping of quantitative trait loci (QTL) and epistatic QTL for salinity tolerance at seedling stage in traditional aromatic short grain rice landrace Kolajoha (Oryza sativa L.) of Assam. 2020; India. Euphytica, 216(5), 1-18.
31. Wang, J., Zhu, J., Zhang, Y., Fan, F., Li, W., Wang, F., . & Yang, J. Comparative transcriptome analysis reveals molecular response to salinity stress of salt-tolerant and sensitive genotypes of indica rice at seedling stage. Scientific reports. 2018; 8(1), 1-13.
32. Liu, D., Dong, S., Bo, K., Miao, H., Li, C., Zhang, Y., ... & Gu, X. Identification of QTLs controlling salt tolerance in cucumber (Cucumis sativus L.) seedlings. Plants. 2021; 10(1), 85.
33. Xu, P., Guo, Q., Meng, S., Zhang, X., Xu, Z., Guo, W., & Shen, X. Genome-wide association analysis reveals genetic variations and candidate genes associated with salt tolerance related traits in Gossypium hirsutum. BMC genomics. 2021; 22(1), 1-14.
34. Zhang, H., Xu, W., Chen, H., Chen, J., Chen, X., & Yang, S. Evaluation and QTL mapping of salt tolerance in yardlong bean [Vigna unguiculata (L.) Walp. Subsp. unguiculata Sesquipedalis group] seedlings. Plant Molecular Biology Reporter. 2020; 38(2), 294-304.
35. Soren, K. R., Madugula, P., Kumar, N., Barmukh, R., Sengar, M. S., Bharadwaj, C., ... & Varshney, R. K. Genetic dissection and identification of candidate genes for salinity tolerance using Axiom® CicerSNP array in chickpea. International journal of molecular sciences. 2020; 21(14), 5058.
36. Zahra, A. Evaluation of sugar beet monogerm O-type lines for salinity tolerance at vegetative stage. African Journal of Biotechnology. 2020; 19(9), 602-612.
37. Bolton, A., & Simon, P. Variation for salinity tolerance during seed germination in diverse carrot [Daucus carota (L.)] germplasm. HortScience. 2019; 54(1), 38-44.