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Evaluate the salt tolerance of Algerian carrot (*Daucus carota* L.) germplasm using germination, biochemical parameters and plant growth potential

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Abstract

The objective of the current study was to determine the behaviour of six carrot varieties of Algerian origin (Muscad d'Alger, Super Muscad, Touchon, Nantaise, Nantaise améliorée and Breclium) regarding five germination traits, nine seedling growth parameters and three biochemical traits (proline, sugar and chlorophyll). In this, four concentrations of NaCl were used when inducing salt stress (control, 25 mM, 50 mM and 75 mM). At germination stage, the percentage of germination was the highest discriminatory trait for all the salt concentrations (25, 50 and 75 mM NaCl). Three different groups of varieties were clustered whereby Muscade d'Alger developed the highest NaCl tolerance during germination. The ANOVA indicated that at the maturity stage, root length and total chlorophyll were the most significant for the biochemical and growth traits. In the biplot analysis, Muscade d'Alger was identified as the maximum salt-tolerant variety. Although Nantaise améliorée and Breclium were identified as the most sensitive, it can be concluded that it is possible to use germination %, germination stress index, root length, root and leaf length stress index as marker traits for the identification of salt (NaCl) tolerance in carrot variety.

Keywords: Salt stress, Algerian Carrot, Germination traits, Growth traits, biochemical traits

INTRODUCTION

Soil is an invaluable natural resource that is often underestimated, particularly by decision-makers and political leaders. The depletion of high-quality soil poses a serious threat to the availability of clean, fresh water, food, and fiber, as well as to the overall health of ecosystems. Moreover, it undermines the soil's critical role in the global carbon cycle. Despite its profound economic and environmental significance, soil continues to be lost across the globe due to various degradation processes, with salinity being one of the most prominent factors. This trend is evident in the steady increase of saline land, which is expanding at a rate of 10% per year. It is projected that by 2050, more than 50% of the world's arable land could become saline (Kumar and Sharma, 2020). This phenomenon is closely associated with high evapotranspiration rates, which lead to insufficient rainfall to flush out salts from the soil, resulting in the accumulation of salts over time (Rozema and Flowers, 2008). Most crops, including cultivated carrots (*Daucus carota* L.), are classified as glycophytes, meaning they are sensitive to saline conditions.

The productivity and growth of glycophytes are markedly hindered in saline soils, as previously reported (Lallouche et al., 2017). Under such challenging conditions, significant reductions in root yield (Jahan et al., 2019) and changes in protein composition, enzyme activity, photosynthetic efficiency, carotenoid levels, and total chlorophyll content have been observed in both carrot roots and leaves (Gibberd, 2002; Bano et al., 2014). Despite these detrimental effects, certain carrot varieties grown in parts of Asia have demonstrated an ability to tolerate elevated soil salinity. However, the underlying mechanisms of this adaptation remain poorly understood (Munns, 2005).

One effective strategy to mitigate the adverse effects of salt stress in sensitive crops is to explore new genetic sources of tolerance and adopt efficient morphological approaches to develop salt-tolerant cultivars (Munns, 2005). The tolerance of a crop to salt stress is significantly influenced by its germination and early developmental stages (Jahan et al., 2019; Lallouche and Hadj kouider, 2024; Tarchoun et al., 2022).

To identify the most tolerant varieties, it is crucial to evaluate plants throughout their entire life cycle, from germination to reproduction. Such comprehensive assessments are essential to pinpoint varieties that demonstrate consistent tolerance across all stages of carrot development. Despite the importance of this issue, global carrot production remains heavily constrained by the crop's high sensitivity to salt stress. Unfortunately, there is a notable scarcity of scientific studies dedicated to testing genetic salt stress tolerance in carrots. Furthermore, the limited availability of genetic material poses a significant challenge for plant breeders striving to enhance salt (NaCl) tolerance in carrots.

The objective of this study was to assess the response of various carrot varieties to salt stress, identify salt-tolerant genetic resources that could be valuable for breeding programs, and establish reliable classification markers for evaluating salt tolerance in carrots during the germination and seedling stages.

MATERIALS AND METHODS

Plant Material

This study examines six distinct carrot varieties: Muscad d'Alger, Super Muscad, Touchon, Nantaise, Nantaise améliorée, and Breclium. These varieties are widely cultivated, produced, and consumed in Algeria, making them well-adapted to the arid and semi-arid regions of the country. The samples used in this investigation were sourced from the local market. The experiment was carried out at an experimental station located in M'sila, Algeria (latitude 35º74' N, longitude 04º55' E).

Germination

To begin, each carrot variety was sterilized by immersing twenty seeds in 70% ethanol for 15 seconds, followed by disinfection with a 6% sodium hypochlorite solution for 10 minutes. The seeds were then thoroughly washed three times with distilled water, with each wash lasting 15 minutes.

The seeds were sown in plastic pots (41 cm high, 40 cm in diameter) filled with a mixture of organic substrate and fine sand in a 1/2 (v/v) ratio, and were placed under natural growth conditions. The experiment was designed as a completely randomized trial, with fifteen replications for each variety and salt treatment (NaCl) level.

Over the course of 145 days, all pots were irrigated weekly with saline solutions. The treatments included four different concentrations of sodium chloride: 0, 25, 50, and 75 mM. These concentrations were prepared by dissolving 1.46, 2.92, and 4.38 g of NaCl in 1 liter of distilled water.

A total of 40 samples were analyzed for each variety, with 15 samples per treatment level. The study evaluated the variability in salt stress tolerance among the different carrot varieties, using both morphological and biochemical parameters. After 145 days of exposure to salt stress, samples of leaves and roots were collected for further analysis.

Germination Parameters

The salt tolerance of the carrot seeds was assessed by examining several germination-related characteristics under saline conditions. These parameters were recorded daily until no additional seed germination was observed, as outlined in Table 1.

Germination was considered successful when the radicle emerged, typically reaching a length of approximately 2 mm. The indicators of relative salt tolerance (RST), inhibitory index (II), absolute decrease (AD) in germination due to salt, and the salt tolerance index (STI) were used to quantify the reduction in seed performance under salt stress, compared to the control group (0 mM NaCl). The detailed calculation methods for these indices are provided in Table 1.

Table 1. Specific calculation methods of salt stress tolerance evaluation were used in these experiments.

Parameter	calculation method	Reference
Germination percentage (GP) (%)	$GP = \frac{\text{total germinated seeds}}{\text{total seeds}} \times 100$	(Ravelombola vd., 2017)
Relative salt tolerance (RST) (%)	RST = germination percentage under NaCl stress conditions /germination percentage under control conditions × 100%.	
Absolute decrease due to salt (AD) (%)	AD = Percentage of seed germination under control conditions– germination percentage of salt treatment	
Salt tolerance index (STI) (%)	STI = (germination percentage under NaCl stress conditions * germination percentage under control conditions) / average percentage of germination for all carrot varieties tested under control conditions)^2 "PG Average represents the mean percentage of germination for all carrot varieties examined under control conditions".	
Inhibition index (II) (%)	II = 100 * (germination percentage under control conditions – germination percentage under NaCl stress conditions) / (germination percentage under control conditions)	

Growth Parameters at Maturity

After 145 days of germination, five seedlings were randomly selected for measurement of plant, leaf, and root lengths (LL and RL) using a standard ruler. The fresh weights of the whole plant (PFW), roots (RFW), and leaves (LFW) were recorded using analytical balances, as detailed in Table 2.

Table 2. Specific calculation methods for salt stress tolerance evaluation were used in these experiments.

parameter	Method	Reference
Leaf length (LL) (cm)	after 145 days of germination	
Root length (RL) (cm)	after 145 days of germination	
Leaf fresh weight (LFW) (g)	after 145 days of germination	
Root fresh weight (RFW) (g)	after 145 days of germination	
Leaf length/Root length Ratio (SRR)	LRR= LL /RL	
Leaf length reduction (SLR) (cm)	LLR = LL under control conditions − LL under NaCl stress conditions	(Partheeban vd., 2017)
Root length reduction (RLR) (cm)	RLR = RL under control conditions − RL under NaCl stress conditions stress plants	(Thabet vd., 2018)
Leaf length stress tolerance index (LLSTI) (%)	$LLSTI = \frac{\text{LL under NaCl stress conditions}}{\text{LL under control conditions}} \times 100$	(Partheeban vd., 2017)
Root length stress tolerance index (RLSTI) (%)	$RLSTI = \frac{\text{RL under NaCl stress condtions}}{\text{RL under control conditions}} \times 100$	

Estimation of Chlorophyll Content at Maturity

The total chlorophyll content (LCh *t*), as well as the individual chlorophyll *a* (LChl *a*) and chlorophyll *b* (LChl *b*) concentrations, were analyzed following the protocol developed by

Lichtenthaler and Buschmann (2001), with modifications by Gugliuzza et al. (2003). The chlorophyll contents were determined using the methods outlined below.

Leaf chlorophyll *a* = 12.25a 663 - 2.79b 647;

Leaf chlorophyll *b* = 21.50b 647 - 5.10a 663;

Total leaf chlorophyll = 20.29b 647 + 8.02a 663

Estimation of root soluble sugar and proline content at maturity under salt stress

The root soluble sugar content was measured following the protocol adopted by DuBois et al. (1956), with results expressed as μgg-1FW. The proline content in the roots was determined using the protocol outlined by Monneveux and Nemmar (1986), and the results were also expressed as μgg-1FW.

Statistical Analysis

The data were analyzed using ANOVA tests (StatBox_Pro.6. V4; $p \leq 0.05$), based on the experimental design that included six carrot varieties and four NaCl concentrations. Mean differences were compared using the Duncan Multiple Range Test (DMRT). Hierarchical cluster analysis (HCA) was performed using XLSTAT software.

RESULTS

Effect of variety and salt stress concentration on all germination-related parameters

Statistical analysis revealed significant differences among the carrot varieties Muscade d'Alger, Super Muscade, Touchon, Nantaise, Nantaise améliorée, and Breclium 145 days after treatment with 25 mM, 50 mM, and 75 mM NaCl ($p < 0.001$) (Table 3).

The analysis of variance presented in Table 3 demonstrated that NaCl stress had a significant impact on all germination-related parameters: relative salt tolerance, germination percentage, absolute decrease due to salt, salt tolerance index, and inhibitory index. GP was the most distinguishing factor (F value = 1338.547), followed by AD (F value = 599.26), II % (F value = 438.573), RST % (F value = 370.469), and STI (F value = 355.947), all of which were significantly influenced by salinity across the tested varieties (Table 3).

Super Muscade, Touchon, and Nantaise showed a decrease in germination percentage of 20%, 2%, and 19.4%, respectively. In contrast, Nantaise améliorée and Breclium exhibited a significant reduction in germination percentage, with decreases of 90% and 80%, respectively. Muscade d'Alger, however, demonstrated the highest germination percentage at all salt concentrations, with full germination observed at all NaCl levels (100%, 100%, 100%, and 100% at control, 25, 50, and 75 mM NaCl, respectively), indicating exceptional salt tolerance.

The relative salt tolerance of Nantaise, Nantaise améliorée, and Breclium decreased substantially, by more than 10%, 19%, and 20%, respectively. In contrast, Muscade d'Alger, Super Muscade, and Touchon showed notable increases in RST, with improvements of 100%, 80%, and 80%, respectively (Table 3).

Furthermore, a significant effect of NaCl stress concentration on the germination stress tolerance index was observed ($p<0.001$). The results showed a clear, proportional relationship between salt stress concentration and STI, with the index decreasing as the level of salt stress increased (from 20% to 10%). This suggests that higher NaCl concentrations have a more pronounced negative impact on seed germination capacity (Table 3).

To further analyze the data, the Ward method and cluster analysis (CA) were applied to group the varieties based on five key characteristics related to seed germination. The analysis revealed three distinct groups at the germination stage: Group 1 (G1), which includes the high salt-tolerant (HST) varieties, such as Muscade d'Alger; Group 2 (G2), representing salt-tolerant (ST) varieties like Super Muscade and Touchon; and Group 3 (G3), which includes the most salt-sensitive (SS) varieties, such as Nantaise, Breclium, and Nantaise améliorée (Figure 1).

Table 3. Response of carrot varieties to different salt stress concentrations (0, 25, 50 and 75 mM NaCl) in relation to seed germination at maturity.

Varieties	[NaCl]	GP (%)	RST (%)	AD (%)	STI (%)	II (%)
Muscad d'Alger	Control	100±0 a				
Super Muscad		100±0 a				
Touchon		100±0 a				
Nantaise		100±0 a				
Nantaise améliorée		100±0 a				
Breclium		100±0 a				
Muscad d'Alger	25 mM	100±0 a	100±0 a	0±0 e	100±0 a	0±0 e
Super Muscad		100±0 a	100±0 a	0±0 e	100±0 a	0±0 e
Touchon		100±0 a	100±0 a	0±0 e	100±0 a	0±0 e
Nantaise		60± 1,4 c	60±1,42 c	40± 2,82 c	60± 2,67 c	40±1,41 c
Nantaise améliorée		100±0 a	100±0a	0±0 e	100±0 a	0±0 e
Breclium		81±0,71b	81±1,41 b	20± 2,79 d	20± 4,2 d	20± 1,4 d
Muscad d'Alger	50 mM	100±0 a	100±0 a	0±0 e	100±0 a	0±0 e
Super Muscad		100±0 a	100±0 a	0±0 e	100±0 a	0±0 e
Touchon		100±0 a	100±0a	0±0 e	100±0 a	0±0 e
Nantaise		59±0,70 c	59±1,5 c	42,5±0,71 c	59±2,34 c	41,5±3,53 c
Nantaise améliorée		81,5±1,5 b	81±0 b	19±1,39 d	81±1,42 b	19± 2,82 d
Breclium		80,75±1,39 b	80,75±0 b	20±1,5 d	80±2,82 b	20±1,41 d
Muscad d'Alger		100±0 a	100±0 a	0±0 e	100±0 a	0±0 e

Super Muscad	75 mM	80±1,5 b	80±1,42 b	20±1,41 d	80± 2,43 b	20±0 d
Touchon		80±0,69 b	80±2,82 b	0±0 e	80± 1,39 b	20±2,82 d
Nantaise		19,5±1,41 d	19,4±1,41 d	80,6±1,41b	19,4±1,43 d	19,4±1,2 d
Nantaise améliorée		10±0 e	10±0 e	90±0 a	10±0 e	90±0 a
Breclium		20±1.41 d	20±4,24 d	80±1,51b	20±4,23 d	80±2,82 b
GXS	F value	1338,547***	370,469***	599,26***	355,947***	438,573***

GP: germination percentage; RST: relative salt tolerance; AD: Absolute decrease due to salt; STI: salt tolerance index); II: inhibition index; G × S genotype and salinity interaction

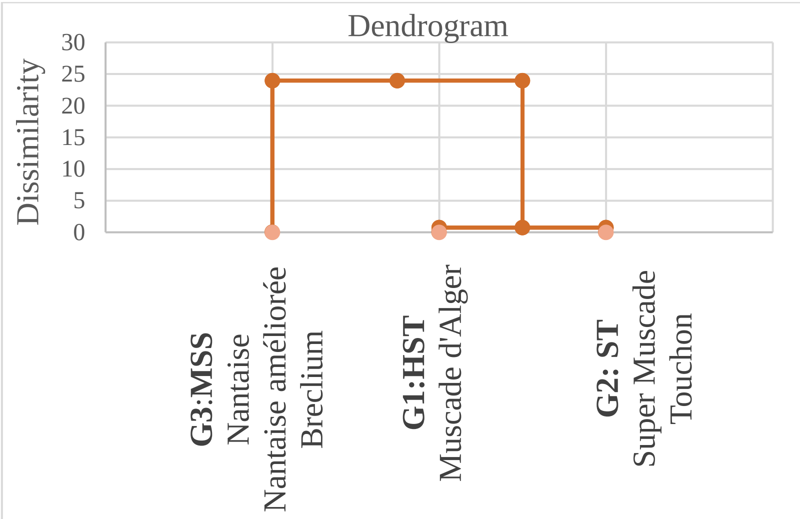

Figure 1. Cluster analysis based on all germination-related parameters: G1: highly salt tolerant (HST), G2: salt tolerant (ST), G3: most salt-sensitive (MSS).

Effect of Variety and Salt Stress Concentration on Growth Parameters at Maturity

Statistical analysis revealed significant differences between the carrot varieties Muscade d'Alger, Super Muscade, Touchon, Nantaise, Nantaise améliorée, and Breclium 145 days after treatment with control, 25 mM, 50 mM, and 75 mM NaCl ($p < 0.001$) (Table 4).

As shown in Table 1, the analysis of variance indicated a significant effect of NaCl stress on all growth-related parameters: LL, RL, LFW, RFW, LRR, LLR, RLR, LLSTI, and RLSTI.

Among these parameters, root length was the most discriminating characteristic, with an F value of 112.376. Other parameters, including RLSTI, LLSTI, LL, LRR, RFW, RLR, LLR, and LFW, also showed significant GXT effects; however, the F values for these traits were comparatively lower (Table 4).

The most discriminatory parameter, RL, was significantly affected by salt stress across all tested varieties at the different salt concentrations (Table 4). The greatest effects were observed under the highest stress level (75 mM NaCl) (Table 4). Root length decreased significantly in all varieties, with the most pronounced and rapid reductions seen in Breclium and Nantaise améliorée. In these varieties, RL decreased from 12.19 ± 1.42 in control plants to

6.14 ± 1.47 in stressed plants for Breclium, and from 8.70 ± 0.32 in control plants to 3.49 ± 1.33 cm under stress for Nantaise améliorée.

As a result, the leaf/root ratio was significantly influenced by the salt stress concentration, showing a progressive increase with higher salt levels. This increase was more pronounced and occurred more rapidly in Breclium and Nantaise améliorée. For instance, the LRR increased from 1.18 ± 0.39 in control plants to 1.61 ± 0.38 in stressed plants for Breclium, and from 1.40 ± 0.99 in control plants to 1.89 ± 0.45 in stressed plants for Nantaise améliorée. These changes suggest that the roots were more adversely affected than the leaves under salt stress conditions (Table 4).

Furthermore, the effects of salt stress were similarly pronounced for both RFW and LFW, although the impact was generally more significant in the leaves than in the roots (Table 4). Specifically, Muscade d'Alger, Super Muscade, Nantaise, and Touchon showed the highest values for both parameters, while the lowest values were recorded for Nantaise améliorée and Breclium (Table 4).

As a result, both the root length stress tolerance index (RLSTI) and leaf length stress tolerance index (LLSTI) displayed a downward trend with increasing salt stress concentration. The effects of salt stress on root length were more pronounced at higher NaCl concentrations, with the most significant differences observed at 50 mM and 75 mM NaCl. The data for leaf and root fresh weight also aligned with the trends seen for RLSTI and LLSTI. This is consistent with the observation that salinity has a more pronounced impact on root length, as reflected in the lower values of the RLSTI index compared to the LLSTI (Table 4).

Cluster analysis, employing Ward's method, was performed to categorize the varieties based on nine growth-related characteristics. This analysis identified three distinct clusters at maturity: Cluster 1 (C1) encompassed HST (Muscade d'Alger), Cluster 2 (C2) included ST (Super Muscade, Nantaise, and Touchon), and Cluster 3 (C3) comprised MSS (Nantaise améliorée and Breclium) (Figure 2).

The results indicate that seed germination is more vulnerable to the detrimental effects of salt stress compared to plant growth.

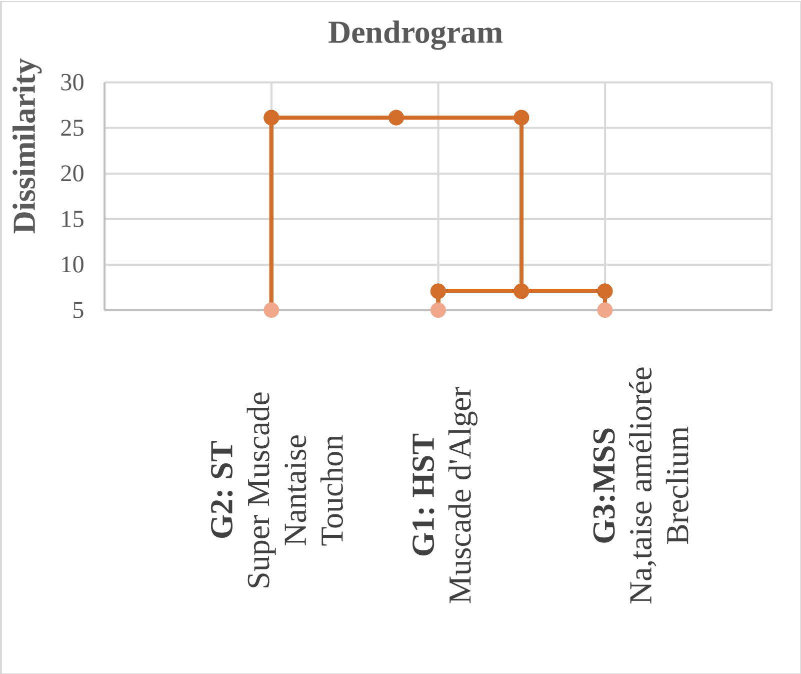

Figure 2. Cluster analysis based on growth parameters at maturity: G1: highly salt tolerant (HST), G2: salt tolerant (ST), G3: most salt-sensitive (MSS).

Table 4. Response of carrot varieties subjected to diverse salt stress conditions (0, 25, 50, 75 mM NaCl) in relation to biochemical traits at maturity.

Varieties	[NaCl]	LL (cm)	LFW (g)	RL (cm)	RFW (g)	LRR (cm)	LLR	RLR (cm)	LLSTI (%)	RLSTI (%)
Muscad d'Alger	Control	18,5±0,5 a	13,5±1,2 bcd	12± 0,4 f	12,6±1 efghi	1,5±1,3 cde				
Super Muscad		15,9±1,3 c	11,7±1,3 cd	13,5±1,4 d	16±1,3 cdef	1,1±1,3 def				
Touchon		17,0±1,4 b	17,8±1,3 a	14,4±1,4 b	24,5±1,3 a	1,2±1,4 def				
Nantaise		15±1,5 de	17,3±1,5 a	15,0±1,3 a	22,4±1,3 ab	0,9±0,1 ef				
Nantaise améliorée		15±0,5 de	14,9±1,4 abc	8,7± 0,3 l	16±1,3 cdef	1,4±1 cdef				
Breclium		14±1,3 def	11,4±0,6 cd	12,1±1,4 f	17,2±1,4 cde	1,2±0,3 def				
Muscad d'Alger	25 mM	15,7±0,4 c	16,7±1,4 ab	7,5±1,4 n	11,6±1 fghi	2,0±1,4 bc	3±1,4 def	4,5±1,3 abcd	0,8±0,2 abc	0,6±0,1 d
Super Muscad		14±1,5 def	10,4±1,3 cde	11,1± 0,4 h	12,5±1 efghi	1,3±1,4 def	1,7±1,4 ef	2,4±0,2 cdef	0,9±0,2 abc	0,8±0,3 abc
Touchon		16,6±1,3 b	12,8±1,3 bcd	11,4±1,4 hi	19,1±1,3 bcd	1,4±0 cdef	0,3±0,1 f	3±1,4 bcdef	0,9±0,2 abc	0,8±0,3 bcd
Nantaise		13±1,4 gh	12,5±1,2 bcd	13,3±1,4 de	18,7±1,3 bcd	0,9±0,7 ef	1,6±0,3 ef	1,8±0,2 ef	0,9±0,2 abc	0,9±0,2 a
Nantaise améliorée		13,5±1fg	9,9±1,4 cde	5,8± 0,5 o	15,4±1,4 def	2,3±1,3 b	1,4±0,4 ef	3±0,4 bcdef	0,9±0,3 ab	0,6±0,4 cd
Breclium		13±1,3 gh	12,8±1,4 bcd	8,5± 0,5 l	16±1,4 cdef	1,5±1cdef	1,3±0,2 ef	4±0,2abcde	0,9±0,2 ab	0,7±0,2 bcd
Muscad d'Alger	50 mM	13±1,4 gh	9,8±1,4 de	10,4±1,4 j	10,6±1,4 ghi	1,3±1,4 def	5,6±0,6 bcd	1,6±0,1 ef	0,7±0,1 e	0,9±0,2 a
Super Muscad		15,7±0,4 c	10,3±1,4 cde	11,5±1,4 g	13,4±1 efghi	0,8±0,7 ef	0,3±0,1 f	2±0,2 def	0,9±0,1 a	0,8±0,42 a
Touchon		15±0,3 cd	13,1±1,4 bcd	13,5±1,3 d	21,4±1,4 ab	1,1±0,7 def	1,9±0,1 ef	0,9±0,2 f	0,9±0,2 abc	0,9±0,2 a
Nantaise		14±1,3 ef	9,3±1,4 de	13,9±0,4 c	20,1±1,3 bc	1±0,2 ef	0,6±0,2 f	1,0±0,2 ef	0,9±0,2 a	0,9±0, 28 a
Nantaise améliorée		12,4±1 ghi	10,5±1,2 cde	3,5±1,3 q	11,7±1,4 fghi	3,5±1,4 a	2,3±1,5 ef	5,2±1 ab	0,9±0,1 abc	0,4±0,1 f
Breclium		11,1±0,4 j	9,4±1,4 de	10,0±1,3 k	15±1,3 defg	1,1±1,4 def	3,0±1,4 def	2,1±0,3 cdef	0,8±0,2 bcde	0,8±0,1 ab
Muscad d'Alger	75 mM	9,8±0,3 kl	8,8±1,3 de	8,1±1,4 m	9,9±1,4 hij	1,2±1def	8,8±1,4 a	4±0,3 abcde	0,5±0,2 f	0,6±0,1 abcd
Super Muscad		11,9±1,2 i	8,9±1,5 de	10,7±1,2 ij	10,3±1,3 ghi	0,6±0,1f	4,0±1,4 cde	3±1,4 bcdef	0,7±0,3 cde	0,8±0,2 abcd
Touchon		10±1,1 jk	8,2±1,4 de	13±1,4 e	8,9±0,7 jk	0,8±0,02 ef	6,4±0,2 bc	1,4±0,4 ef	0,6±0,1 e	0,9±0,1 a
Nantaise		10,6±1 jk	8,8±1,4 de	13±1,3 d	14±1,3 efg	0,8±0,1 ef	3,8±0,2 cde	1,6±0,2 ef	0,7±0,2de	0,9±0,3 a
Nantaise améliorée		7,3±0,4 m	5,9±1,4 e	3,9±1,4 p	8,9±1,3 k	1,9±0,4 bcd	7,4±1 ab	4,7±1,4 abc	0,5±0,2 f	0,4±0,2 ef
Breclium		9,7±0,4 l	5,8±1,4 e	6,1±1,4 o	8,9±1,3 ijk	1,6±0,3 cde	4,5±0,2 cde	6±1,4a	0,7±0,1 de	0,5±0,1 ef
GXS	F value	28,16***	2,466***	112,376***	6,881***	7,325***	3,354***	4,87***	33,43***	36,48***

LL: leaf length, RL: root length, LFW: leaf fresh weight, RFW: Root fresh weight, LRR: leaf length/root length ratio, LLR: Leaf length reduction, RLR: root length reduction, LLSTI: leaf length stress tolerance index, RLSTI: root length stress tolerance index; G × S genotype and salinity interaction

Effect of Variety and Salt Stress Concentration on Chlorophyll, Proline, and Soluble Sugar Content at Maturity

Statistical analysis demonstrated significant differences among the varieties Muscade d'Alger, Super Muscade, Touchon, Nantaise, Nantaise améliorée, and Breclium, measured 145 days after treatment with varying salt concentrations (0 mM, 25 mM, 50 mM, and 75 mM) ($p < 0.001$) (Table 5).

Analysis of variance, as summarized in Table 5, revealed a significant impact of salinity on total chlorophyll content, chlorophyll a and b content in leaves, as well as proline and soluble sugar content in roots (Table 5). Among the evaluated traits, LCht and LChlb were identified as the most discriminative characteristics (F-value = 4482.74; F-value = 1964.347). These were followed by root soluble sugar content, root proline content, and leaf chlorophyll *a* content (LChla) (Table 5).

The most discriminating traits, leaf total chlorophyll content and leaf chlorophyll *b* content, were significantly affected by salinity across all varieties (Table 5). A notable reduction in LCht was observed in all varieties, with the decline being more pronounced and rapid in Nantaise améliorée and Breclium. Specifically, LCht decreased from 1.183 ± 0.002 at 0 mM NaCl to 0.494 ± 0.006 at 75 mM NaCl for Nantaise améliorée and from 1.095 ± 0.001 at 0 mM NaCl to 0.494 ± 0.006 at 75 mM NaCl for Breclium.

Cluster analysis using Ward's method was performed to group the varieties based on five biochemical characteristics at the maturity stage. This analysis identified three distinct clusters: C1 included highly salt-tolerant varieties (Muscade d'Alger and Nantaise), C2 comprised salt-tolerant varieties (Super Muscade and Touchon), and C3 encompassed the most salt-sensitive varieties (Nantaise améliorée and Breclium) (Figure 3).

Table 5. Response of carrot varieties subjected to diverse salt stress conditions (0, 25, 50 and 75 mM NaCl) in relation to biochemical traits at maturity

Varieties	[NaCl]	Ch a (µgg-1FW)	Ch b (µgg-1FW)	Ch totale (µgg-1FW)	RSS (µgg-1FW)	RP (µgg-1 FW)
Muscad d'Alger	Control	0,89±0,001 b	0,22±0,001 k	1,53±0,004 a	0,50±0,001 l	0,08±0,003 o
Super Muscad		0,64±0,001 i	0,20±0,001 m	0,84±0,001 l	0,42±0,001 s	0,17±0,002 l
Touchon		0,66±0,003 h	0,40±0,001 c	1,06±0,007 f	0,48±0,002 n	0,11±0,003 n
Nantaise		0,95±0,004 a	0,41±0,006 b	1,36±0,001 b	0,40±0,001 t	0,10±0,001 n
Nantaise améliorée		0,80±0,011 d	0,36±0,001 d	1,18±0,002 c	0,52±0,002 k	0,16±0,002 l
Breclium		0,67±0,005 g	0,42±0,001 b	1,09±0,001 e	0,50±0,001 m	0,11±0,014 n
Muscad d'Alger	25 mM	0,82±0,011 c	0,70±0,001a	1,12±0,004 d	0,57±0,001 i	0,21±0,001 j
Super Muscad		0,58±0,004 j	0,27±0,001 g	0,85±0,003 k	0,44±0,001 r	0,29±0,001 i
Touchon		0,69±0,001 f	0,23±0,003 j	0,93±0,001 i	0,63±0,004 f	0,229±0,001 j
Nantaise		0,71±0,002 e	0,34±0,001 e	1,05±0,001 g	0,43±0,001 s	0,21±0,003 j
Nantaise améliorée		0,52±0,004 l	0,21±0,001 lm	0,73±0,004 n	0,53±0,001 j	0,14±0,001 m
Breclium		0,58±0,005 j	0,30±0,001 f	0,89±0,001 j	0,59±0,001 g	0,18±0,001 k
Muscad d'Alger	50 mM	0,57±0,004 j	0,26±0,001 h	0,84±0,001 l	0,59±0,001 g	0,35±0,003 g
Super Muscad		0,49±0,001 m	0,19±0,003 n	0,68±0,001 p	0,45±0,003 q	0,40±0,001 e
Touchon		0,52±0,002 l	0,25±0,003 i	0,77±0,001 m	0,68±0,004 c	0,37±0,001 f
Nantaise		0,58±0,005 j	0,36±0,001 d	0,94±0,001 h	0,46±0,004 p	0,34±0,028 h
Nantaise améliorée		0,40±0,009 n	0,25±0,001 i	0,66±0,004 q	0,59±0,001 h	0,29±0,028 i
Breclium		0,34±0,001 o	0,19±0,006 no	0,53±0,001s	0,69±0,001 b	0,40±0,028 e
Muscad d'Alger	75 mM	0,53±0,004 k	0,19±0,002 no	0,72±0,005 o	0,63±0,003 e	0,51±0,028 c
Super Muscad		0,27±0,006 p	0,16±0,001 p	0,49±0,006 u	0,47±0,004 o	0,56±0,003 a
Touchon		0,34±0,006 o	0,34±0,014 e	0,68±0,007 p	0,65±0,004 d	0,39±0,001 e
Nantaise		0,41±0,008 n	0,23±0,001 jk	0,64±0,004 r	0,49±0,001 m	0,51±0,002 c
Nantaise améliorée		0,28±0,004 p	0,18±0,001 o	0,49±0,006 u	0,68±0,002 c	0,43±0,003 d
Breclium		0,28±0,003 p	0,21±0,001 l	0,49±0,006 t	0,73±0,004 a	0,53±0,002 b
GXS	F value	218,708 ***	1964,347***	4482,74***	440,911***	47,42***

LlChla: (leaf chlorophyll a); LChlb: (leaf chlorophyll b); LCht: (leaf total chlorophyll content); RSS: (root soluble sugar content); RP: (root proline content); G × S Genotype and salinity interaction

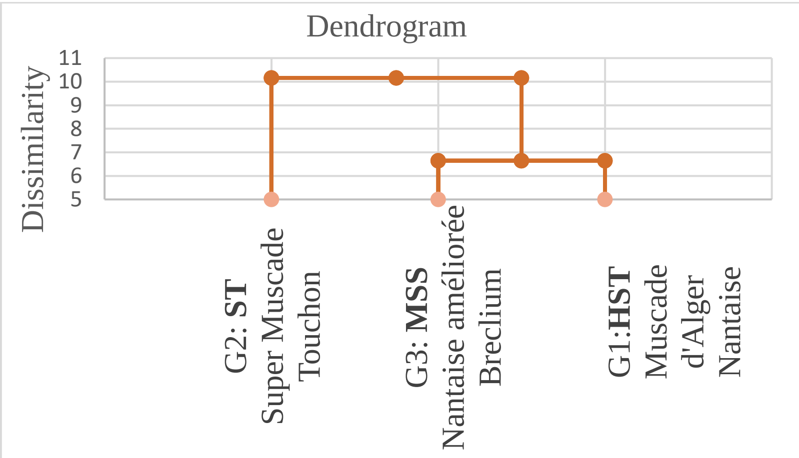

Figure 3. Cluster analysis based on biochemical parameters at maturity: HST (highly salt tolerant), ST (salt tolerant), MSS (most salt sensitive).

DISCUSSION

The selection of salt-stress-tolerant genotypes is crucial for efficiently identifying promising genotypes and optimizing their conservation and management strategies. Salt stress significantly impairs seed germination, root length, leaf length, and seedling fresh weight, primarily due to the toxic effects of sodium chloride ions and osmotic stress. This paper sought to evaluate the germination and seedling growth responses of various carrot varieties under salt stress conditions. Furthermore, it aimed to identify the most salt-sensitive and salt-tolerant varieties and to share these findings with the scientific community.

The findings of this study revealed that salt stress significantly influenced all traits related to germination, seedling growth, and biochemical responses, with variations depending on the intensity of the stress applied. Seed germination was notably delayed as salt concentration increased, confirming the adverse effects of salinity on carrot germination. The observed reduction in germination traits can be attributed to limited energy availability and reduced water uptake caused by elevated salt levels (Lallouche and Hadj kouider, 2024; Tarchoun et al., 2022).

These results are consistent with findings in other species, including lettuce, safflower, *Flueggea suffruticosa* genotypes, and Turnip (*Brassica rapa* L. sub sp. rapa), where salinity led to a significant decline in germination rates (Jia et al., 2020; Tonguç et al., 2021; Adhikari et al., 2022; Xu et al., 2023). Plant sensitivity to salt stress varies across different growth and developmental stages (Jahan et al., 2019). During germination, the effects of stress are amplified by restricted seed water uptake, which impairs the germination process (Ayaz et al., 2000).

The findings of the present study demonstrate that the severity of salt stress effects increases with higher stress concentrations. Similar trends have been reported in pumpkin (Tarchoun et al., 2022), Flueggea suffruticosa (Xu et al., 2023), and *Carthamus tinctorius* L. genotypes (Tonguç et al., 2021). All varieties exhibited significant responses to salt stress, particularly at the highest concentration (75 mM NaCl). The variability in stress responses among the varieties suggests substantial intraspecific variation in salt tolerance.

Muscade d'Alger emerged as the most resilient variety across all stress levels. In contrast, Nantaise, Nantaise améliorée, and Breclium showed no germination at any stress concentration, despite their relatively high germination capacity.

In addition to its impact on germination, salt stress caused a pronounced reduction in tissue elongation, observed as decreased leaf and root length with increasing salt concentrations. This reduction can be attributed to the toxicity of sodium chloride, as well as restricted water absorption and nutrient uptake due to osmotic stress (Bárzana and Carvajal, 2020; Ma et al., 2020). These factors collectively impair plant tissue development and hinder cell elongation (Tenhaken, 2015; Van Zelm et al., 2020).

The findings of this study align with those of Shin et al. (2020), who identified leaf and root length as critical traits for evaluating salt tolerance. Leaves play a vital role in water supply to aerial tissues, while roots are essential for water uptake, making both traits integral to plant adaptation under saline conditions.

The investigation revealed that seedling growth was inhibited at all salt stress concentrations, with root length being the most adversely affected, followed by a lesser reduction in leaf length. These findings are consistent with previous reports of significant salt stress effects on carrot roots (Jahan et al., 2019) and Tunisian pumpkin (*Cucurbita maxima* Duchesne) roots (Tarchoun et al., 2022).

Although salinity stress resulted in a considerable decrease in seedling growth across all studied varieties, significant variability was observed between the varieties. Under all salt stress concentrations, Muscade d'Alger exhibited the highest root and leaf length, demonstrating its superior salt tolerance. In contrast, Nantaise améliorée and Breclium displayed the lowest values, indicating a strong susceptibility to salt stress.

The results also showed a progressive decrease in leaf and root fresh weight with increasing salt stress, a trend primarily attributed to the ionic effects associated with elevated sodium ion (Na^+) concentration. Muscade d'Alger demonstrated the greatest growth potential, as reflected by its higher LFW and RFW under both salt stress and control conditions. Conversely, Nantaise améliorée and Breclium exhibited the most significant reductions in leaf and root fresh weight, respectively.

The aforementioned findings were further validated by the observation that elevated concentrations of salt stress had a significant impact on all the morphological parameters evaluated. Previous research has shown that salt stress adversely affects the germination stress tolerance index, root length stress tolerance index, and leaf length stress tolerance index across various crop species (Alom et al., 2016). The results of the present study align with the notion that the impact of salt stress varies depending on the specific varieties examined. Consequently, the variety "Muscade d'Alger" was identified as the most resilient based on LLSTI and GSTI, while "Super Muscade," "Nantaise," and "Touchon" were recognized as tolerant varieties based on RLSTI. Conversely, the combined data of LLSTI, GSTI, and RLSTI indicated that "Nantaise améliorée" and "Breclium" are susceptible varieties.

The biochemical response at the maturity stage, assessed through the synthesis of proline, soluble sugars, chlorophyll a, chlorophyll b, and total chlorophyll content in the varieties Muscade d'Alger, Super Muscade, Touchon, Nantaise, Nantaise améliorée, and Breclium under three salt stress concentrations (25, 50, and 75 mM NaCl), revealed that these compounds are accumulated in varying amounts across the different varieties. The extent of accumulation was influenced by both the variety and the salt concentration applied. The observed reduction in chlorophyll content (a, b, and total) with increasing salt concentration is a complex phenomenon that affects the forces maintaining the pigment-protein-lipid complex within the chloroplast (Lallouche et al., 2017). According to Shu et al. (2013), the stability of chloroplasts is contingent upon the integrity of the membrane, which is compromised under high salinity conditions. The decrease in chlorophyll content (total, a, and b) under saline conditions reflects a multi-trait response, not attributable to a single factor, but rather the combined effect of all plant traits (Lallouche et al., 2017).

The present study demonstrated that the concentrations of proline and soluble sugars increased with the rise in salt concentration (25 mM, 50 mM, and 75 mM NaCl) across all carrot varieties. The accumulation of proline and soluble sugars was notably higher in the roots under salt stress compared to normal conditions. Lallouche et al. (2015, 2017) observed a similar increase in proline and soluble sugar accumulation in five Opuntia species from the Algerian steppe under 600 mM NaCl stress. Both proline and soluble sugars, as key stress metabolites, play crucial roles in stabilizing proteins and regulating osmotic pressure. The accumulation of soluble sugars in the root appears to facilitate the gelation of cellular contents by saturating the internal environment of the cells. Consequently, a strong positive correlation was observed between proline levels and soluble sugar content, indicating a significant relationship between the two in the different carrot varieties.

CONCLUSIONS

This study investigated the response of six carrot varieties to four different sodium chloride concentrations (control, 25, 50, and 75 mM NaCl). Key traits, including germination characteristics, seedling growth parameters, and biochemical markers (proline, soluble sugars, and chlorophyll content), were analyzed. The varieties "Muscade d'Alger" and "Touchon" were found to be the most tolerant to salt stress, demonstrating superior performance compared to the other varieties. These results suggest that these varieties may serve as valuable genetic resources for breeding programs aimed at improving salt tolerance. The "Super Muscade" and

"Nantaise" varieties exhibited moderate tolerance to salt stress, while "Nantaise améliorée" and "Breclium" were highly susceptible to 75 mM NaCl stress. Additionally, the study highlights that germination percentage, germination stress index, root length, and the leaf and root length stress indices are reliable markers for assessing salt tolerance in carrot varieties.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

AUTHORS' CONTRIBUTION

Bahia Lallouche, Data collected, analyzed research data and wrote draft manuscript, designed research and provided suggestions regarding data analysis. Boubakr Hadj kouider, Data collected, edited draft and final manuscript. All authors read and approved the final manuscript.

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