

EFFECTS OF HYDROPRIMING, HALOPRIMING, AND HORMOPRIMING SEED TREATMENTS ON THE SUBSEQUENT SALT STRESS TOLERANCE OF QUINOA (*Chenopodium quinoa* WILLD.) IN ALGERIA

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ABSTRACT

Quinoa, a valuable halophyte, plays a crucial role in ensuring food and nutritional security under climate change. However, high salinity levels can hinder seed germination and subsequent plant growth. The current study aimed to enhance the salinity tolerance of pre-optimized quinoa seeds through various priming techniques: hydropriming (distilled water, 25 °C, 12 hours), halopriming (75 mM CaCl₂, KNO₃, and MgSO₄ at 25 °C for 12 hours), and hormoprimering with gibberellic acid (GA₃ 25 mM, 25 °C, 12 hours). The seed germination parameters: germination percentage, relative salt tolerance and salt tolerance index, as well as growth parameters of seedlings as plant height, leaf number, plant fresh weight, root length, root fresh weight, plant height/root length ratio, plant height stress tolerance index, root length stress tolerance index, and chlorophyll content, were evaluated in Giza 02 and Q102 *Chenopodium quinoa* varieties. Halopriming with KNO₃, hormoprimering with GA₃, followed by halopriming with MgSO₄, effectively mitigated the negative impacts of salinity. This priming approach shows promise for enhancing quinoa crop resilience in saline soils and could serve as a model for other salt-sensitive crops.

Keywords: priming techniques, salinity, seed germination, growth parameter, chlorophyll

INTRODUCTION

High salinity poses a significant threat to agricultural productivity, particularly in arid and semi-arid locations worldwide [Lallouche et al. 2017]. According to an estimate by Wicke et al. [2011], approximately 1.128 million hectares (Mha) of land are affected by salinity globally. Out of this total, around 100 Mha has become saline as a result of using brackish irrigation, as reported by FAO [2008]. Various strategies have been implemented to address the challenges associated with salt stress. However, the most straightforward and environmentally sustainable approach is the cultiva-

tion of halophytic crops, such as quinoa (*Chenopodium quinoa* Willd.) in saline conditions.

Halophytes thrive in environments with high salt concentrations, particularly with 50 mM NaCl for monocots and 100–200 mM NaCl for dicots [Flowers and Colmer 2008]. Quinoa, a facultative halophyte, is notable for its ability to withstand high salinity, making it a promising crop for nutritional and food security in changing climates. The World Health Organization recognizes quinoa as a significant contributor to these goals. However, salt stress can impede seed germi-

nation and growth, particularly in Chilean varieties [Hariadi et al. 2011, Ruiz-Carrasco et al. 2011]. Seed priming is an effective strategy to mitigate the negative effects of salt stress and sustain metabolic activities in plants [Yan 2016]. This technique enhances the seeds' inherent ability to cope with unfavorable conditions, such as high salinity, by treating them with specialized solutions at an early stage. This treatment activates a stress-responsive mechanism, making the seeds more resilient to salinity as they grow. Bradford et al. [1990] found that seed priming can improve germination rate in glycophytes that tolerate low salinity and require adequate moisture when exposed to high salt levels. In this context, seed priming serves as a pre-germination treatment that enhances the germination rate and overall seed performance in glycophytes. According to Bradford et al. [1990], the technique typically involves soaking seeds in water or solutions containing specific nutrients or growth regulators for a set period, followed by drying. However, the effectiveness of seed priming in improving seed germination and alleviating salt stress, specifically in halophytes, remains unclear.

Hydropriming is an economical and beneficial method that involves soaking seeds in water and then drying them before planting. This technique allows hydroprimed seeds to rapidly absorb significant moisture, which accelerates germination, growth, and overall plant development. This is achieved by regulating physiological responses and influencing the synthesis of proteins and genes across various plant genotypes [Yan 2016, Karalija and Selović 2018].

Additionally, seed priming with hormone solutions, such as gibberellic acid (GA₃), can alter biochemical pathways, enabling plants to better tolerate stressful environments [Chauhan et al. 2019]. This process enhances plant growth, function, and chemical processes, ultimately helping to mitigate the effects of salt stress [Jiang et al. 2020].

Halopriming is a seed-priming technique that enables plants to better cope with the effects of salinity and drought by promoting the accumulation of stress-responsive proteins, enhancing antioxidant systems, and increasing the levels of Na⁺, K⁺, and Ca²⁺. These processes collectively support plant growth and improved photosynthesis [Mamedi et al. 2022, Paul et al. 2024].

Despite the potential benefits of seed priming for halophytes, research on this topic remains limited. This

scarcity of studies highlights the need for further investigation into the effects of halopriming on plants that naturally thrive in saline environments [Mamedi et al. 2022, Abdulmajeed 2023, Paul et al. 2024].

The objective of the current study was to investigate the effects of seed priming on germination in quinoa, a model crop, under high salinity conditions. Specifically, the research aimed to achieve three primary goals: first, to assess the response of two quinoa varieties to salt during germination; second, to evaluate the influence of seed priming on germination rates in salty conditions; and third, to identify the most effective seed priming agents and optimal germination conditions for each variety. Additionally, the study examined the impacts of various priming agents under salt stress and sought to determine the most significant interaction between the priming agents and the quinoa varieties.

MATERIAL AND METHODS

Plant materials and experimental design

The present study focuses on two quinoa varieties (*C. quinoa* Willd.), specifically Giza 02 and Q102, provided by the Technical Institute for the Development of Saharan Agronomy (ITDAS) in Ain Ben Naoui, Biskra, Algeria. The seeds were sourced from the United States Department of Agriculture (USDA). Prior to priming, the seeds were disinfected by soaking them in a 1% sodium hypochlorite solution for five minutes, followed by rinsing with distilled water three to five times. The seeds were then primed using various priming agents for 12 hours at room temperature, as detailed in Table 1. After priming, the seeds underwent a drying process at room temperature (25 °C), ensuring that any surface moisture was removed beforehand.

The treated seeds were then sown in plastic pots (1 L) filled with a mixture of sand, agricultural soil, and potting soil in a 1:1:1 ratio. These pots were arranged in a completely randomized design, on a slab, in a controlled greenhouse, maintained at a temperature of 22 °C during the day and 15 °C at night. The greenhouse operated on a 16-hour light/8-hour dark cycle, with a relative humidity of 50%. Immediately after sowing the seeds, the pots were manually irrigated with either tap water or a saline solution containing 200 mM NaCl diluted in tap water. These irrigation solutions were

Table 1. Seed priming methods used in the experimental research

Priming	Agent	Dose	Duration
No priming	–	–	–
Hydropriming	distilled water	200 mL	12 h
Hormoprimer	gibberellic acid (GA ₃)	25 mM	12 h
Halopriming	ZnSO ₄	75 mM	12 h
	CaCl ₂	75 mM	12 h
	KNO ₃	75 mM	12 h
	MgSO ₄	75 mM	12 h

Table 2. Specific calculation method employed in this study

Trait	Unit	Calculation method
Germination percentage (GP)	%	$GR = \text{total germinated seeds} / \text{total seeds} \times 100\%$
Relative salt tolerance (RST)	%	$RST = (\text{GP of primed seeds} / \text{GP of non-primed seeds}) \times 100\%$
Salt tolerance index (STI)	%	$STI = \text{GP under salt stress} \times \text{GP of non-primed seeds} / (\text{average GP of two quinoa variety non-primed seeds})^2$

Table 3. A description of the salinity tolerance evaluation traits used in this study

Trait	Unit	Formula
Plant height (PL)	cm	after 40 days of germination
Root length (RL)	cm	after 40 days of germination
Leaf fresh weight (LFW)	g	measured with a precision balance (max: 600 g d = 0.01 g, EMB 600-2, KERN & Sohn GmbH)
Root fresh weight (RFW)	g	measured with a precision balance (max: 600 g d = 0.01 g, EMB 600-2, KERN & Sohn GmbH)
Plant height / Root length Ratio (PRR)	–	the ratio of PH to RL
Plant height stress tolerance index (PLHSTI)	%	$PHLSTI = (\text{SL under primed conditions} / \text{SL under non-primed conditions}) \times 100$
Root length stress tolerance index (RLSTI)	%	$RLSTI = (\text{RL under primed conditions} / \text{RL under non-primed conditions}) \times 100$

specified as follows: tap water (EC = 1.55 ms/cm; pH = 7.58), salt solution: tap water + 200 mM NaCl (EC = 20.2 ms/cm; pH = 8.18).

Each pot was manually irrigated with 200 mL of the respective irrigation solution (tap water and salt) every three days for the first 20 days to maintain field capacity. After this period, the irrigation frequency was increased to every other day for the subsequent 40 days.

Each treatment was replicated three times, with 30 seeds included in each replication. The number of germinated seeds was counted on the seventh day to calculate the germination rate. Additionally, various morpho-physiological traits were assessed 40 days after sowing to evaluate how the different quinoa varieties coped with salt stress. Samples of plant material, including the whole plants, leaves and roots, were collected for further analysis.

Estimation of germination and growth parameters

The seed germination parameters were assessed using the formulas outlined in Table 2. At the vegetative stage, the following parameters: plant height, number of leaves, and length of root were measured. Additionally, the root fresh weight (RFW) and plant fresh weight (PFW) were recorded, as detailed in (Table 3).

To conduct the pigment analysis, the content of total chlorophyll, chl a, and chl b of the leaves was quantified using the protocol described by Lichtenthaler and Buschmann [2001].

Statistical analysis

The control (no primed) and primed samples were subjected to statistical analysis to identify significant differences between various groups. This was conducted using a two-tailed test and ANOVA, with the latter performed using StatBox Pro. The Newman-Keuls test was employed to compare means at a significance level of $\alpha = 5\%$. The correlations among all parameters were assessed using multivariate analysis. PCA (principal component analysis) and HCA (hierarchical cluster analysis) were performed using XLSTAT Version 2014.05.03 software (Addinsoft, www.xlstat.com). A cluster analysis was performed to categorize the priming agents based on 14 parameters, including germination, growth and chlorophyll content. The Ward method was employed to facilitate the grouping of these agents.

RESULTS

Effect of salinity and priming on seed germination

Statistical analysis revealed a significant interaction between the priming and salt treatments for all germination parameters, including germination percentage (GP), salt tolerance index (STI) and relative salt tolerance (RST) (Table 4).

Among these parameters, STI was found to be the most discriminative (F value = 140.837), followed by RST (F value = 92.728) and GP (F value = 15.135).

The STI parameter was positively affected by the priming treatments under salt stress in both of the studied varieties (Table 4). The correlations matrix revealed a strong positive correlation between the salt tolerance index and relative salt tolerance ($r = 0.993$).

All priming treatments resulted in a significantly higher percentage of germination compared to the control seeds. The highest GP was recorded in seeds primed with KNO_3 for both varieties. Additionally, seeds primed with MgSO_4 showed a substantial increase in germination, with a rise of 45.16% for the Giza 02 variety and 39.98% for Q102 compared with seeds without priming (Table 4). Moreover, seeds that underwent hydropriming exhibited a significantly higher germination percentage for both Giza 02 and Q102 varieties, compared to no-priming and priming with CaCl_2 and ZnSO_4 treatments (Table 4). Data demonstrate that various priming agents have a beneficial impact on germination, with significant differences observed among the quinoa varieties. Giza 02 exhibited a considerable improvement in GP when treated with halopriming using KNO_3 and hormoprimer with GA_3 . Conversely, Q102 showed favorable responses when treated with halopriming using KNO_3 and MgSO_4 (Table 4).

Additionally, both the STI and RST were found to be positively influenced by the priming treatments under salt stress. Table 4 demonstrates that the Giza 02 variety exhibited higher tolerance to saline stress compared to Q102. Seed priming significantly enhanced quinoa tolerance to saline conditions by increasing the percentage of germination, which, in turn, had a positive impact on RST. The beneficial effects of seed priming with MgSO_4 , CaCl_2 , hydropriming, and ZnSO_4 were observed under salinity stress, resulting in an increase in the STI of 1.07 to 1.48 times compared to no-primed seeds. Notably, KNO_3 and GA_3 seed priming led to an even higher increase in STI, approximately doubling the value compared to non-primed seeds. It can be reasonably inferred that halopriming with KNO_3 and hormoprimer with GA_3 significantly enhanced the STI and RST percentage of seeds stressed with NaCl (Table 4). Overall, the tested quinoa varieties displayed differential responses to salt exposure, with Giza 02 demonstrating higher tolerance than Q102, as indicated by the higher GP observed with Giza 02.

Effect of priming on growth parameters

Table 5 presents the data on growth parameters recorded at 40 days after sowing salinity-stressed plants from both no-primed (control) and primed seeds. The

Table 4. Germination parameters for two *Chenopodium quinoa* varieties under different types of priming in salt stress conditions

Varieties	Seed priming agent	Parameter under 200 mM NaCl		
		GP%	RST%	STI%
Giza 02	No priming	62 ±2 ef	–	–
Q102		59 ±2.1 f	–	–
Giza 02	ZnSO ₄	66 ±1.8 e	106 ±1.76 i	67.63 ±2.2 e
Q102		66 ±2.5 e	111 ±0.5 h	64.36 ±3 f
Giza 02	CaCl ₂	65 ±4.6 e	104 ±3 i	66.61 ±0.6 e
Q102		66 ±1.2 e	111 ±1.2 h	64.36 ±1.5 f
Giza 02	GA ₃	90 ±2.4 a	150 ±0.5 cd	95.3 ±1.4 ab
Q102		73 ±3 d	123 ±1.5 f	71.19 ±1 e
Giza 02	Hydropriming	83 ±2.3 c	133 ±2.5 g	85 ±2 g
Q102		80 ±0.4 c	135 ±0.4 d	78 ±2 d
Giza 02	KNO ₃	96 ±1.6 a	154 ±1 b	98.3 ±0.5 a
Q102		93 ±4 b	152 ±3 a	87.76 ±4 c
Giza 02	MgSO ₄	90 ±0.5 b	145 ±4 e	92.23 ±0.6 b
Q102		82.5 ±1 c	138 ±1.5bc	79.96 ±0.5 d
F value	–	15.135	92.728	140.837
P	–	0	0	0
S	–	***	***	***
G × T	–	yes	yes	yes

germination rate – GP, salt tolerance index – STI, relative salt tolerance – RST, significance – S, interaction between varieties, priming, and salt stress – GXT, probability – P; $P \leq 0.001$ very highly significant (***)

analysis of variance indicates a significant interaction between salinity and priming across all growth-related traits, including plant height, leaf number, plant fresh weight, root length, root fresh weight, and plant height/root length ratio (PRR).

Plants subjected to salinity stress from primed seeds demonstrated greater plant height than those from no-primed seeds. The analysis showed that plant height was statistically significant for both varieties, while plant fresh weight and leaf number emerged as the most discriminative parameters (F value = 12.798; F value = 10.761, respectively).

Although plant height, leaf number, root fresh weight and PRR also exhibited genotype-by-treatment effects, their F values were comparatively lower (Table 5). Notably, the most discriminative parameter, PFW, was positively influenced by salinity in the primed varieties. Among the priming agents tested, KNO₃ yielded the highest results (6.61 ±0.53; 5.40 ±0.07 for both varieties), while hydropriming produced the lowest values. This trend was consistent for leaf number as well.

In this respect, the plant height stress tolerance index (PLHSTI) and root length stress tolerance index (RLSTI) obtained from primed quinoa seeds

(hydroprimed, GA₃, MgSO₄, ZnSO₄, CaCl₂, KNO₃) seeds were found to be statistically highly significant (Table 5). The correlations matrix revealed a strong positive correlation between PLHSTI and STI ($r = 0.973$), and RST ($r = 0.964$).

In response to salt exposure, both Giza 02 and Q102 exhibited improved performance when subjected to hormonal and halopriming treatments, which significantly mitigated the adverse effects of salinity on growth parameters compared to seedlings derived from non-primed seeds (Table 5).

Effect of priming on chlorophyll content

The data shown in Table 6 reveals a significant interaction between the effects of salinity and priming on the levels of total chlorophyll, Chl *a*, and Chl *b*. Additionally, the correlation matrix highlights a strong positive correlation between total chlorophyll content and Chl *a* ($r = 0.965$) and Chl *b* ($r = 0.900$).

In other words, the increased salinity leads to osmotic stress and ion toxicity, which can inhibit the biosynthesis of chlorophyll. This results in lower Chl *a* levels, affecting the plants' overall photosynthetic ca-

Table 5. Growth parameters for two *Chenopodium quinoa* varieties under different types of priming in salt stress conditions (200 mM NaCl)

Variety	Priming agent	PH (cm)	LN	PFW (g)	RL (cm)	RFW (g)	PRR	PLHSTI (%)	RLSTI (%)
Giza 02	No priming	20.1 ± 0.76e	16.9 ± 1.05h	1.32 ± 0.52h	8.63 ± 0.1 f	1.31 ± 0.08c	2.22 ± 0.2abc	–	–
		22.2 ± 0.3de	17.96 ± 1h	2.3 ± 0.02 fg	10.0 ± 0.6 f	2.22 ± 0.2 b	2.2 ± 0.2 abc	–	–
Giza 02	ZnSO ₄	24.1 ± 0.8de	24.2 ± 1.07f	4.2 ± 1.07cd	16 ± 1 cd	2.17 ± 0.16b	1.22 ± 0.22d	119.5 ± 2.7i	185.39 ± 4.61a
		24.3 ± 1.5de	24.33 ± 1.5f	3.8 ± 0.1ede	14.2 ± 1cd	2.16 ± 0.2 b	1.11 ± 0.1 d	109.5 ± 1.1 j	141.21 ± 3.79e
Giza 02	CaCl ₂	26.3 ± 3.04d	28 ± 2e	4.2 ± 0.26cd	13 ± 1.0de	2.20 ± 0.22b	1.43 ± 0.4 d	130.5 ± 2.3h	152.95 ± 2.9 d
		24.03 ± 2de	21.2 ± 0.6g	3.4 ± 0.5cde	14.03 ± 1 d	2.23 ± 0.25b	1.59 ± 0.4cd	108.6 ± 1.6j	141 ± 2 e
Giza 02	GA ₃	38 ± 2b	35 ± 1c	5.56 ± 0.41b	18.9 ± 2ab	2.28 ± 0.37b	2.75 ± 0.31 a	188.4 ± 1.5 c	123.3 ± 0.67 f
		33 ± 1c	33 ± 1.07c	4.46 ± 0.55c	17.16 ± 1bc	2.2 ± 0.37 b	1.8 ± 0.2 bcd	148.3 ± 1.5f	151 ± 2 d
Giza 02	Distilled water	32.66 ± 2.5c	23.11 ± 1fg	1.9 ± 0.06gh	11.3 ± 1.5of	2.3 ± 0.3 b	2.47 ± 0.5 ab	165 ± 3.40 d	150.7 ± 2.52 d
		29.36 ± 2.5c	23.11 ± 1fg	2.9 ± 0.1 ef	11.0 ± 1 of	2.5 ± 0.1 b	2.93 ± 0.11 a	137.3 ± 1.4g	110.22 ± 2.3 g
Giza 02	KNO ₃	44.1 ± 1.01a	44 ± 1b	5.40 ± 0.07b	19 ± 1.0ab	3.21 ± 0.22a	1.7 ± 0.2 bcd	218.1 ± 3.4 a	166.22 ± 2.03c
		43.3 ± 1.52a	49 ± 1a	6.61 ± 0.53a	21.1 ± 1.8 a	3.23 ± 0.21a	2.87 ± 0.15 a	194.4 ± 1.5b	146.1 ± 5.16de
Giza 02	MgSO ₄	31.55 ± 2.5c	30.22 ± 1d	3.2 ± 0.3 def	15 ± 0.5 cd	2.2 ± 0.25 b	2.4 ± 0.4 ab	156.4 ± 1.5 e	175.29 ± 1.1 b
		32.0 ± 1.07c	33.1 ± 1.01c	3.3 ± 0.3 def	15 ± 1.5cd	2.43 ± 0.05b	1.8 ± 0.1 bdc	145.6 ± 4.5 f	174.15 ± 4.0 b
F value		9.952	10.761	12.798	8.74	3.298	7.422	114.69	32.022
P		0.01	0	0	0.00002	0.01	0.00009	0	0
S		**	***	***	***	**	***	***	***
G × T		yes	yes	yes	yes	yes	yes	yes	yes

plant height – PH, leaf number – LN, plant fresh weight – PFW, root length – RL, root fresh weight / root length ratio – RFR, plant height stress tolerance index – PLHSTI, root length stress tolerance index – RLSTI, significance – S, interaction between varieties, priming, and salt stress – GXT, probability – P; P ≤ 0.01 highly significant (**); P ≤ 0.001 very highly significant (***)

Table 6. Total chlorophyll, chlorophyll *a*, and chlorophyll *b* contents for two *Chenopodium quinoa* varieties under different types of priming in salt stress conditions

Variety	Priming agent	TChl µg 0.1g ⁻¹ FW	Chl <i>a</i> µg 0.1g ⁻¹ FW	Chl <i>b</i> µg 0.1g ⁻¹ FW
Giza 02 Q102	No priming	5.09 ±0.83h	3.25 ±0.1i	1.84 ±0.12c
		5.32 ±1.01h	3.32 ±0.2i	1.957 ±0.5c
Giza 02 Q102	ZnSO ₄	8.717 ±0.4ef	6.357 ±0.3def	2.36 ±0.2bc
		7.813 ±0.24fg	5.84 ±0.06fg	1.973 ±0.1c
Giza 02 Q102	CaCl ₂	8.527 ±0.5ef	6.19 ±0.1ef	2.337 ±0.12bc
		8.723 ±0.24ef	6.523 ±0.1de	2.2 ±0.06bc
Giza 02 Q102	GA ₃	10.553 ±0.5bc	7.98 ±0.25ab	2.573 ±0.45bc
		9.467 ±0.45df	6.963 ±0.59cd	2.503 ±0.23bc
Giza 02 Q102	Distilled water	7.233 ±0.25g	5.23 ±0.1h	2.003 ±0.6c
		9.89 ±0.25cd	6.96 ±0.3cd	2.93 ±0.28ab
Giza 02 Q1028	KNO ₃	11.653 ±1.01a	8.217 ±0.28a	3.437 ±0.1a
		11.167 ±0.51ab	7.567 ±0.5bc	3.633 ±0.75a
Giza 02 Q102	MgSO ₄	7.607 ±0.46fg	5.427 ±0.3gh	2.18 ±0.12bc
		10.633 ±0.46bc	7.25 ±0.29c	3.383 ±0.3a
F value	–	17.36	21.938	4.412
P	–	0	0	0.002
S	–	***	**	***
G × T	–	yes	yes	yes

total chlorophyll content – TChl, chlorophyll *a* – Chl *a*, chlorophyll *b* – Chl *b*, interaction between varieties, priming, and salt stress – G×T, significance – S, probability – P, P ≤ 0.001 very highly significant (***)

capacity. However, in plants derived from seeds primed with KNO₃, the amount of Chl *a* was 2.52-fold higher in Giza 02 and 2.27-fold higher in Q102 than in seedlings from non-primed seeds. Similarly, the results for total Chl and Chl *b* exhibited a similar pattern to that observed for Chl *a* (Table 6).

Environmental stresses that affect one type of chlorophyll will likely influence the total chlorophyll content, underscoring the importance of both components in plant function. The average lower level of total chlorophyll in stressed plants grown from non-primed seeds underlines the adverse effects of salinity and highlights the importance of seed priming in improving plant resilience. Plants that grew from KNO₃-treated seeds exposed to salt stress showed a 1.97-fold rise in total chlorophyll in Giza 02 and a 2.09-fold increase in Q102 variety. Consequently, the application of priming agents led to a notable reduction in the negative impacts of high salt concentration on the biosynthesis of photosynthetic components in comparison to non-primed seeds. Specifically, the application of KNO₃ through halopriming, and to a lesser extent, the use of GA₃ through hormoprimer

ing, along with MgSO₄ through halopriming, resulted in a considerable augmentation in the biosynthesis of photosynthetic components (Table 6) in both varieties.

Principal component analysis (PCA) and hierarchical cluster analysis (HCA)

A principal component analysis was performed to explore the relationship between quinoa varieties and priming treatments under salt stress. The analysis revealed that the first and second principal components accounted for 72.11% and 11.83% of the total variation observed in the varieties and priming treatments, respectively. These data were utilized to create a score plot, as illustrated in Figure 1.

Hormoprimer with GA₃ and halopriming with KNO₃ and MgSO₄ were positioned on the right side of the score plot, while treatments without priming, hydropriming, and halopriming with ZnSO₄ and CaCl₂ were located on the left side (Fig. 1). All priming treatments localized on the right side of the score plot were positively correlated with several growth parameters, including germination percentage, relative salt tolerance, plant height, salt tolerance index, leaf num-

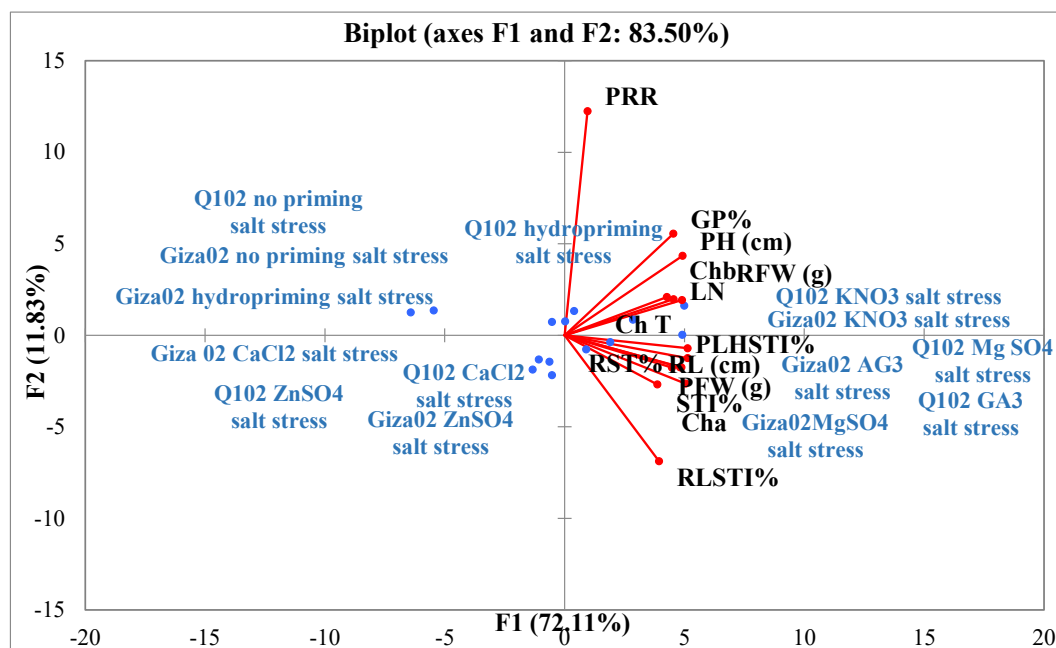


Fig. 1. Principal component analysis of all traits in the quinoa varieties Giza 02 and Q102, subjected to six priming treatments in the presence of 200 mM NaCl

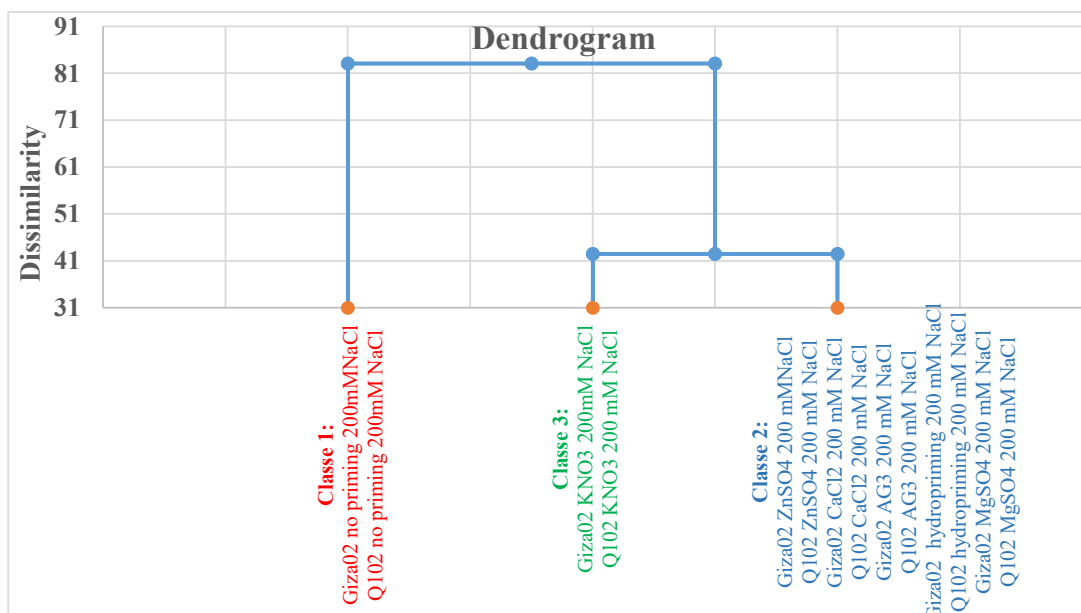


Fig. 2. Hierarchical classification of two *Chenopodium quinoa* varieties using six priming treatments in the presence of 200 mM NaCl

ber, plant fresh weight, root length, root fresh weight, and chlorophyll content. The PCA plots indicated that the PLHSTI was the most discriminative trait, with halopriming using KNO_3 identified as the most effective priming treatment for enhancing the germination and growth of quinoa seeds and seedlings under salt stress (Fig. 1).

The cluster analysis resulted in three distinct categories. Class C1 represented the control group (non-primed seeds). Class C2 highlighted halopriming with KNO_3 , which was identified as the most effective treatment for enhancing the germination of quinoa seeds and the growth of seedlings under salinity stress. Class C3 included other treatments such as halopriming with $ZnSO_4$, $CaCl_2$, and $MgSO_4$, hydropriming with distilled water, and hormoprimer with GA_3 . The results indicated that these priming treatments with moderate efficacy effectively promoted the establishment and growth of quinoa seeds and seedlings under salt stress (Fig. 2).

DISCUSSION

Researchers have established that abiotic stressors, such as salinity, significantly diminish crop yield in both agricultural and horticultural contexts, posing a considerable challenge to the sustainability of global crop production [Zörb et al. 2019].

Cultivating halophyte crops like quinoa, which are nutritionally rich, offers a promising strategy to combat the escalating food insecurity crisis. Quinoa demonstrates the ability to tolerate salt stress and drought, conditions that are increasingly prevalent due to climate change. However, this capacity is often lacking during the critical early stages of seed germination and growth. Various researchers have documented this limitation in quinoa [Hariadi et al. 2011, Ruiz-Carrasco et al. 2011, Causin et al. 2020, Bourhim et al. 2022, Abdulmajeed 2023]. Accordingly, Gómez-Pando et al. [2010] conducted research indicating that the germination capacity of quinoa germplasm in saline conditions varies based on the presence of specific genes. Their findings revealed that certain genes confer higher resilience to salt stress than others. The studies found that higher salinity levels led to a decline in seed germination, significantly affecting seedling emergence [Chauhan et al. 2019, Alam et al. 2021, Bourhim et

al. 2022]. In addition, research by Causin et al. [2020] has demonstrated that high saline levels slow down the rate and speed of quinoa seed germination rather than affecting seedling growth.

However, seed priming is a cost-effective and straightforward technique that can improve plant establishment and growth in saline environments. Research has shown that seed priming can also enhance plants' tolerance to salt stress [Afzal et al. 2017, Bouallègue et al. 2019, Devika et al. 2021]. The present study demonstrates that the optimized priming treatments, including halopriming with KNO_3 , $CaCl_2$, $MgSO_4$, and $ZnSO_4$, hormoprimer with GA_3 , and hydropriming with distilled water, significantly improved seed germination quality under salt stress at a concentration of 200 mM NaCl. These treatments enhanced stress-tolerance responses within biological systems, alleviating the inhibitory effects of high salinity and promoting physiological processes associated with germination. The results indicated an increased percentage of seed germination, elevated relative salt tolerance, and a higher salt tolerance index. A study by Alam et al. [2021] suggested that KNO_3 application facilitates seed germination and promotes growth by protecting membrane integrity and detoxifying excess reactive oxygen species. Various studies indicate that both halopriming and hormoprimer are excellent methods for enhancing germination, growth, and yield in saline conditions [Salehi et al. 2018, Mamedi et al. 2022, Bourhim et al. 2022, Jahantighi and Roshandel 2023].

The observed enhancements can be attributed to changes at the seed level, particularly the increased water absorption [Mamedi et al. 2022]. This increase leads to the accumulation of metabolites that help protect cell membranes, reduce oxidative compounds, maintain ion balance, and regulate osmotic pressure within cells. Mamedi et al. [2022] noted that physiological responses to salt stress are maintained during the post-priming stages. These outcomes can be explained by the phenomenon known as "embryo memorization events", which occur during the priming and rehydration processes. Moreover, Kinoshita and Seki [2014] demonstrated that plants can retain information from stressful experiences and utilize this memory to enhance their responses to similar stressors in the future. Furthermore, Mamedi et al. [2022] found that this priming memory in seeds can mitigate the toxic,

osmotic, and oxidative effects of salinity during early seed development.

In the present study, under saline conditions, seed priming had a significant effect on the growth parameters of quinoa seedlings as plant height, leaf number, root length, plant fresh weight, plant height/root length ratio, root fresh weight, plant height tolerance index and root length stress tolerance index when compared with non-primed seeds. Haider et al. [2020] and Feghhenabi et al. [2020] have reported comparable results in wheat (*Triticum aestivum* L). In the present study, all priming agents effectively mitigated the negative impacts of salinity. However, priming with halopriming (KNO_3 and MgSO_4) and hormoprimer (GA_3) resulted in beneficial effects under salt stress, with the most pronounced effect observed after the application of KNO_3 . Of the priming agents tested, KNO_3 was the most efficacious. Seed priming is recognized as an effective technique for promoting more robust seedling growth in stressful circumstances [Feghhenabi et al. 2020, Jahantighi and Roshandel 2023]. The application of KNO_3 significantly enhances plant development under salinity stress by protecting cellular membrane integrity, enhancing the uptake of K^+ and Na^+ exclusion in *Zea mays* [Ashraf and Rauf 2001] and *T. aestivum* [Jafar et al. 2012].

This study also found that seed priming with halopriming, hydropriming, and hormoprimer resulted in higher chlorophyll contents in seedlings under salt stress. Other studies have shown similar conclusions that seed priming improved the leaf chlorophyll contents in *Brassica rapa* [Hussain et al. 2024], in five canola genotypes [Iqbal et al. 2022] and quinoa [Raza et al. 2024]. The findings revealed that seedlings primed under similar salinity conditions showed increased growth potential and germination performance. Moreover, it was found that priming canola genotypes [Iqbal et al. 2022], quinoa cultivar Giza 1 [Abdulmajeed 2023] and *B. rapa* [Hussain et al. 2024] seeds increased the production of chlorophyll (total, a and b) under different salt stress conditions.

The use of KNO_3 protects plant chlorophyll under salt stress by down-regulating the chlorophyll degradation genes [Rehman et al. 2024]. Jahantighi and Roshandel [2023] reported that quinoa plants can thrive in saline environments by mitigating the detrimental effects of oxidative stress caused by salinity.

They accomplish this by preserving their photosynthetic pigments. The current study also demonstrated that KNO_3 reduces chlorophyll degradation in quinoa plants under saline conditions. Seed priming emerges as a promising technique for enhancing salt tolerance in quinoa when exposed to high salinity levels.

CONCLUSIONS

On the basis of the findings, it is concluded that priming ameliorated the inhibitory effects of salinity on the seed germination and plant seedling growth of quinoa. Seed priming with potassium nitrate (KNO_3) and gibberellic acid (GA_3) proved effective for the induction of salt tolerance through increasing seedling vigor, improving seed germination rates, and enhancing chlorophyll synthesis in quinoa seedlings, thereby contributing to their salt tolerance. However, further research is needed to elucidate the underlying mechanisms by which KNO_3 and GA_3 confer resistance to salt stress during seed germination.

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REFERENCES

- Abdulmajeed, A. (2023). Salinity stress amelioration and morpho-physiological growth stimulation by silicon priming and biochar supplementation in *Chenopodium quinoa*. *Not. Bot. Horti Agrobot. Cluj-Napoca*, 51(2), 13043. <https://doi.org/10.15835/nbha51213043>
- Afzal, I., Rahim, A., Qasim, M., Younis, A., Nawaz, A., Bakhtavar, M. A. (2017). Inducing salt tolerance in French marigold (*Tagetes patula*) through seed priming. *Acta Sci. Pol. Hortorum Cultus*, 16(3), 109–118. <https://doi.org/10.24326/asphc.2017.3.11>
- Alam, A., Ullah, H., Cha-um, S., Tisarum, R., Datta, A. (2021). Effect of seed priming with potassium nitrate on growth, fruit yield, quality and water productivity of cantaloupe under water-deficit stress. *Sci Hortic.*, 288, 110354. <https://doi.org/10.1016/j.scienta.2021.110354>
- Ashraf, M., Rauf, H. (2001). Inducing salt tolerance in maize (*Zea mays* L.) through seed priming with chloride salts: Growth and ion transport at early growth stages. *Acta Physiol. Plant.*, 23(4), 407–414. <https://doi.org/10.1007/s11738-001-0050-9>

- Bouallègue, A., Souissi, F., Nouairi, I., Souibgui, M., Abbes, Z., Mhadhbi, H. (2019). Physiological and biochemical changes modulated by seeds' priming of lentil (*Lens culinaris* L.) under salt stress at germination stage. *Acta Sci. Pol. Hortorum Cultus*, 18(5), 27–38. <https://doi.org/10.24326/asphc.2019.5.3>
- Bourhim, M.R., Cheto, S., Qaddoury, A., Hirich, A., Ghoulam, C. (2022). Chemical seed priming with zinc sulfate improves quinoa tolerance to salinity at germination stage. *Environ. Sci. Proc.*, 16(23), 23. <https://doi.org/10.3390/environsciproc2022016023>
- Bradford, K.J., Steiner, J.J., Trawatha, S.E. (1990). Seed priming influence on germination and emergence of pepper seed lots. *Crop Sci.*, 30(3), 718–721. <https://doi.org/10.2135/cropsci1990.0011183X003000030049x>
- Causin, H.F., Bordón, D.A.E., Burrieza, H. (2020). Salinity tolerance mechanisms during germination and early seedling growth in *Chenopodium quinoa* Willd. genotypes with different sensitivity to saline stress. *EEB.*, 172, 103995. <https://doi.org/10.1016/j.envexpbot.2020.103995>
- Chauhan, A., AbuAmarah, B.A., Kumar, A., Verma, J.S., Ghramh, H.A., Khan, K.A., Ansari, M.J. (2019). Influence of gibberellic acid and different salt concentrations on germination percentage and physiological parameters of oat cultivars. *Saudi J. Biol. Sci.*, 26(6), 1298–1304. <https://doi.org/10.1016/j.sjbs.2019.04.014>
- Devika, O.S., Singh, S., Sarkar, D., Barnwal, P., Suman, J., Rakshit, A. (2021). Seed priming: a potential supplement in integrated resource management under fragile intensive ecosystems. *Front. Sustain. Food Syst.*, 5, 654001. <https://doi.org/10.3389/fsufs.2021.654001>
- FAO, (2008). International Fund for Agricultural Development (IFAD). *Gender in agriculture. Sourcebook*. World Bank Publications.
- Feghhenabi, F., Hadi, H., Khodaverdiloo, H., van Genuchten, M.Th. (2020). Seed priming alleviated salinity stress during germination and emergence of wheat (*Triticum aestivum* L.). *Agric Water Manag.*, 231, 106022. <https://doi.org/10.1016/j.agwat.2020.106022>
- Flowers, T.J., Colmer, T.D. (2008). Salinity tolerance in halophytes. *New Phytol.*, 179(4), 945–963. <https://doi.org/10.1111/j.1469-8137.2008.02531.x>
- Gómez-Pando, L.R., Álvarez-Castro, R., Eguiluz-de La Barra, A. (2010). Effect of salt stress on peruvian germplasm of *Chenopodium quinoa* Willd. A promising crop. *J. Agron. Crop Sci.*, 196(5), 391–396. <https://doi.org/10.1111/j.1439-037X.2010.00429.x>
- Haider, I., Raza, M.A.S., Iqbal, R., Aslam, M.U., Habibur-Rahman, M., Raja, S., Khan, M.T., Aslam, M.M., Waqas, M., Ahmad, S. (2020). Potential effects of biochar application on mitigating the drought stress implications on wheat (*Triticum aestivum* L.) under various growth stages. *J. Saudi Chem. Soc.*, 24(12), 974–981. <https://doi.org/10.1016/j.jscs.2020.10.005>
- Hariadi, Y., Marandon, K., Tian, Y., Jacobsen, S.-E., Shabala, S. (2011). Ionic and osmotic relations in quinoa (*Chenopodium quinoa* Willd.) plants grown at various salinity levels. *J. Exp. Bot.*, 62(1), 185–193. <https://doi.org/10.1093/jxb/erq257>
- Hussain, S., Ahmed, S., Akram, W., Ahmad, A., Yasin, N.A., Fu, M., Li, G., Sardar, R. (2024). The potential of selenium to induce salt stress tolerance in *Brassica rapa*: Evaluation of biochemical, physiological and molecular phenomenon. *Plant Stress.*, 11, 100331. <https://doi.org/10.1016/j.stress.2023.100331>
- Iqbal, W., Afridi, M.Z., Jamal, A., Mihoub, A., Saeed, M.F., Székely, Á., Zia, A., Khan, M.A., Jarma-Orozco, A., Pompelli, M.F. (2022). Canola seed priming and its effect on gas exchange, chlorophyll photobleaching, and enzymatic activities in response to salt stress. *Sustainability*, 14(15), 9377. <https://doi.org/10.3390/su14159377>
- Jafar, M.Z., Farooq, M., Cheema, M.A., Afzal, I., Basra, S.M.A., Wahid, M.A., Aziz, T., Shahid, M. (2012). Improving the performance of wheat by seed priming under saline conditions. *J. Agron. Crop Sci.*, 198(1), 38–45. <https://doi.org/10.1111/j.1439-037X.2011.00485.x>
- Jahantighi, M., Roshandel, P. (2023). The effect of seed priming of *Chenopodium quinoa* L. var. Giza 1 with ascorbic acid on increasing salt tolerance. *Iran J. Seed Sci. Res.*, 10(3), 81–93. <https://doi.org/10.22124/jms.2023.7676>
- Jiang, X.-W., Zhang, C.-R., Wang, W.-H., Xu, G.-H., Zhang, H.-Y. (2020). Seed priming improves seed germination and seedling growth of isatis indigotica fort. under salt stress. *HortSci.*, 55(5), 647–650. <https://doi.org/10.21273/HORTSCI14854-20>
- Karalija, E., Selović, A. (2018). The effect of hydro and proline seed priming on growth, proline and sugar content, and antioxidant activity of maize under cadmium stress. *Environ. Sci. Poll. Res. Int.*, 25(33), 33370–33380. <https://doi.org/10.1007/s11356-018-3220-7>
- Kinoshita, T., Seki, M. (2014). Epigenetic memory for stress response and adaptation in plants. *Plant Cell Physiol.*, 55(11), 1859–1863. <https://doi.org/10.1093/pcp/pcu125>
- Lallouche, B., Boutekrabet, A., Hadjkouider, B., Riahi, L., Lamine, S., Zoghalmi, N. (2017). Use of physio-biochemical traits to evaluate the salt tolerance of five opuntia species in the Algerian steppes. *Pak. J. Bot.*, 49(3), 837–845.
- Lichtenthaler, H.K., Buschmann, C. (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Curr. Prot. Food Anal. Chem.*, 1(1), <https://doi.org/10.1002/0471142913.faf0403s01>

- Mamedi, A., Sharifzadeh, F., Maali-Amiri, R., Divargar, F., Rasoulnia, A. (2022). Seed osmopriming with Ca²⁺ and K⁺ improves salt tolerance in quinoa seeds and seedlings by amplifying antioxidant defense and ameliorating the osmotic adjustment process. *Physiol. Mol. Biol. Plants*, 28(1), 251–274. <https://doi.org/10.1007/s12298-022-01125-3>
- Paul, A., Mondal, S., Chakraborty, K., Biswas, A.K. (2024). Moving forward to understand the alteration of physiological mechanism by seed priming with different halo-agents under salt stress. *Plant Mol. Biol.*, 114(2), 24. <https://doi.org/10.1007/s11103-024-01425-0>
- Raza, M.A.S., Aslam, M.U., Valipour, M., Iqbal, R., Haider, I., Mustafa, A.E.-Z.M.A., Elshikh, M.S., Ali, I., Roy, R., Elshamly, A.M.S. (2024). Seed priming with selenium improves growth and yield of quinoa plants suffering drought. *Sci. Rep.*, 14(1), 886. <https://doi.org/10.1038/s41598-024-51371-6>
- Rehman, B., Zulfiqar, A., Attia, H., Sardar, R., Saleh, M. A., Alamer, K. H., Mehmood, F. (2024). Seed priming with potassium nitrate can enhance salt stress tolerance in maize. *Phyton*, 93(8), 1819–1838. <https://doi.org/10.32604/phyton.2024.048780>
- Ruiz-Carrasco, K., Antognoni, F., Coulibaly, A.K., Lizardi, S., Covarrubias, A., Martínez, E.A., Molina-Montenegro, M.A., Biondi, S., Zurita-Silva, A. (2011). Variation in salinity tolerance of four lowland genotypes of quinoa (*Chenopodium quinoa* Willd.) as assessed by growth, physiological traits, and sodium transporter gene expression. *Plant Physiol. Biochem.*, 49(11), 1333–1341. <https://doi.org/10.1016/j.plaphy.2011.08.005>
- Salehi, M., Soltani, V., Dehghani, F. (2018). Effect of salt stress and seed priming methods on emergence and seedling characteristics of quinoa (*Chenopodium quinoa* Willd.). *Environ. Stress. Crop Sci.*, 11(2), 381–391. <https://doi.org/10.22077/escs.2017.595.1127>
- Wicke, B., Smeets, E., Dornburg, V., Vashev, B., Gaiser, T., Turkenburg, W., Faaij, A. (2011). The global technical and economic potential of bioenergy from salt-affected soils. *Energy Environ. Sci.*, 4(8), 2669–2681. <https://doi.org/10.1039/C1EE01029H>
- Yan, M. (2016). Hydro-priming increases seed germination and early seedling growth in two cultivars of Napa cabbage (*Brassica rapa* subsp. *pekinensis*) grown under salt stress. *J. Hortic. Sci. Biotechnol.*, 91(4), 421–426. <https://doi.org/10.1080/14620316.2016.1162031>
- Zörb, C., Geilfus, C.M., Dietz, K.J. (2019). Salinity and crop yield. *Plant Biol.*, 21(S1), 31–38. <https://doi.org/10.1111/plb.12884>