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Salt stress responses in *Chenopodium quinoa* Willd.: A comparative analysis of germination and early seedling growth across cultivars

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Abstract

This study investigated the impact of salt stress on seed germination and early seedling growth in five quinoa (*Chenopodium quinoa* Willd.) cultivars. Seeds were subjected to sodium chloride (NaCl) concentrations ranging from 0 to 205 mM. The Blanca de Junin cultivar exhibited superior performance, maintaining 100 % germination even at 205 mM NaCl, while Amarilla Sacaca showed the lowest germination percentage (63.75 %) at this concentration. Germination energy at 205 mM NaCl ranged from 92.5 % in Blanca de Junin to 56.25 % in Amarilla Sacaca. Seedling length reductions at 205 mM NaCl varied from 44.76 % in Blanca de Junin to 71.29 % in Salcedo. Radicle length decreased by 31.68-73.16 % under severe salt stress. The results highlight significant variability in salt tolerance among quinoa cultivars, with Blanca de Junin demonstrating robust salt tolerance during germination and seedling stages. These findings provide valuable insights for breeding salt-tolerant quinoa varieties and expanding cultivation to salt-affected regions, contributing to food security and sustainable agriculture in marginal lands.

Key words: Quinoa, seed germination, seedling growth, salt stress, salt tolerance, cultivar variation

Introduction

Agricultural productivity and development face numerous environmental challenges globally, with water scarcity and soil salinity being critical factors limiting productivity, especially in arid and semi-arid regions (Golla, 2021). Selecting vegetation well-suited to these conditions is crucial for enhancing local production and achieving food security (Bazile *et al.*, 2016; Koyro and Eisa, 2007).

Quinoa (*Chenopodium quinoa* Willd.) emerges as a promising crop, demonstrating remarkable tolerance to diverse abiotic stresses, including high temperatures, drought, and salt stress (Hinojosa *et al.*, 2018; Maamri *et al.*, 2022; Oustani *et al.*, 2023). Widely cultivated in Andean regions, quinoa is a high-quality, gluten-free pseudo-cereal with rich protein content, making it an ideal food source for regions plagued by water scarcity and salinity (Ceyhun Sezgin and Sanlier, 2019; Lutz and Bascuñán-Godoy, 2017). Moreover, quinoa cultivation can help revitalize marginal and degraded lands, offering a sustainable option for agriculture (Iqbal *et al.*, 2018; Nanduri and Shahid, 2016; Radhouane, 2018).

Identifying salt-tolerant genotypes within plant species is key to improving agricultural production in salt-affected areas (Ashraf *et al.*, 2012; Iqbal *et al.*, 2018). The response of plants to salt stress is complex, influenced by factors such as species, variety, salt concentration, growing conditions, and developmental stage (Acila and Allioui, 2019; Cocozza *et al.*, 2012). Germination, a fundamental process in crop establishment, is particularly vulnerable to salt stress and other environmental factors (Finch-Savage and Bassel, 2016; Yamuna Devi *et al.*, 2022).

Salt stress affects various vital germination processes, including water absorption by seeds, hydrolase enzyme activity, and seed reserve conversion (Brakez *et al.*, 2014; Causin *et al.*, 2020). High concentrations of salt ions can be toxic to embryonic cells, causing oxidative stress and damage to metabolic mechanisms (Ali and Elozeiri, 2017). This damage results in reduced growth of embryonic axes and hinders the establishment of young seedlings (Causin *et al.*, 2020; Stoleru *et al.*, 2019).

In this context, our study aimed to investigate the inhibitory effects of NaCl on the germination and growth of young seedlings belonging to five cultivars of quinoa (*Chenopodium quinoa* Willd.). This research seeks to enhance our understanding of how these cultivars respond to salt stress, providing insights into their potential for promoting sustainable agriculture in challenging environments. The specific objectives were to: 1) Evaluate the germination performance of five quinoa cultivars under varying NaCl concentrations. 2) Assess the early seedling growth parameters of these cultivars in response to salt stress. 3) Identify potential salt-tolerant quinoa cultivars for further breeding or cultivation in salt-affected areas.

Materials and methods

Plant material: Five quinoa genotypes (*Chenopodium quinoa* Willd.) were studied: Amarilla Marangani (Q101), Amarilla Sacaca (Q102), Blanca de Junin (Q103), Salcedo (Q105), and Santa Maria. Seeds were sourced from the Technical Institute for Saharan Agricultural Development (ITDAS) in Aghfyan, Djamaa, El Oued, southeastern Algeria, from the 2020/2021 campaign.

Germination test and saline treatment: Seeds were surface-sterilized with 12% sodium hypochlorite for 10 minutes, then

rinsed with distilled water. Following the International Seed Testing Association (ISTA) guidelines, four replications of 50 seeds each (200 seeds total) per cultivar and treatment were used. Seeds were germinated in 10 cm diameter Petri dishes on filter paper moistened with 7 mL of either distilled water (control) or NaCl solutions at concentrations of 51 mM, 102 mM, 154 mM, and 205 mM. These concentrations corresponded to osmotic potentials of approximately -0.25 MPa, -0.51 MPa, -0.77 MPa, and -1.03 MPa, respectively. Petri dishes were incubated in darkness at 25 ± 1°C and monitored daily for 7 days. Germination was defined as the emergence of a 2 mm long radicle (Causin *et al.*, 2020).

Germination percentage (GP%): Calculated on the seventh day as (Number of germinated seeds / Total number of seeds) × 100 (Kandil *et al.*, 2012).

Germination energy (GE%): Determined as the percentage of seeds germinated on the third day (Ruan, 2002).

Germination kinetics: Monitored over time according to Sharma and Vimala (2016).

Seed vigor index (SVI): Calculated as [Seedling length (cm) × GP (%)] (Kandil *et al.*, 2012).

Relative salt injury rate (RSIR): Expressed as (GP% in control – GP% in NaCl Treatment) / GP% in control (Li, 2008).

Embryonic axis growth characteristics: After 7 days, 40 seedlings were randomly selected from each treatment and cultivar for growth analysis. Seedling length (SL), hypocotyl length (HL), and radicle length (RL) were measured using a millimeter ruler. Total seedling length (SL) was calculated as the sum of RL and HL. The HL/RL ratio was also determined for each seedling.

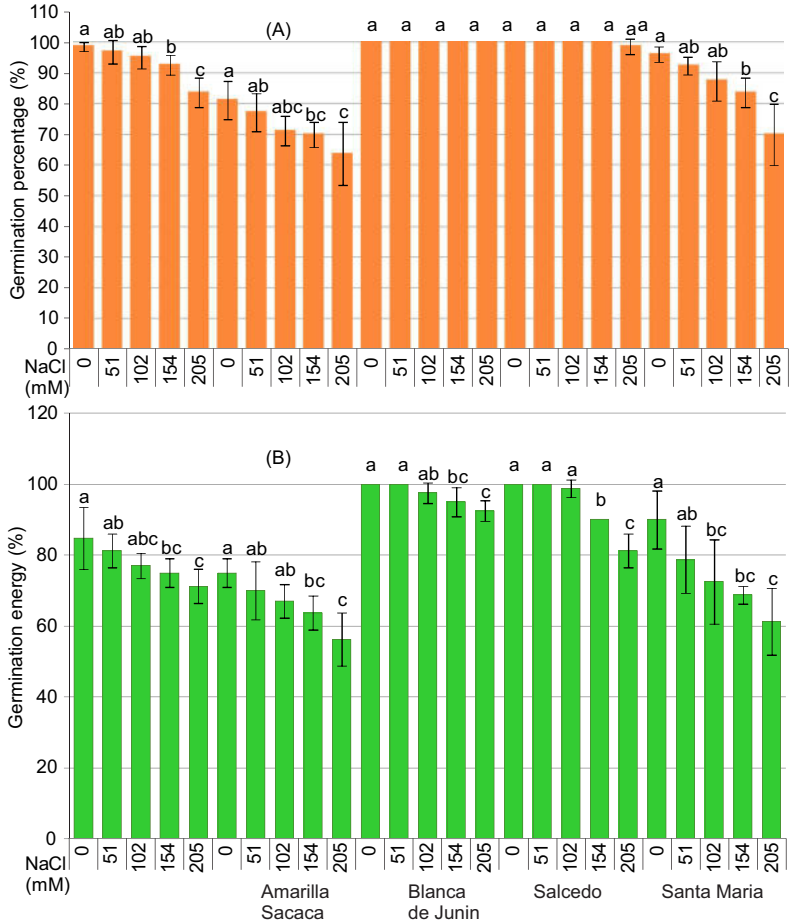

Fig. 1. Germination percentage (GP%) (A) and germination energy (GE%) (B) of five *Chenopodium quinoa* Willd. cultivars under various NaCl concentrations. Values are means ± SD (n=4). Different letters indicate significant differences (P<0.05, Fisher's LSD).

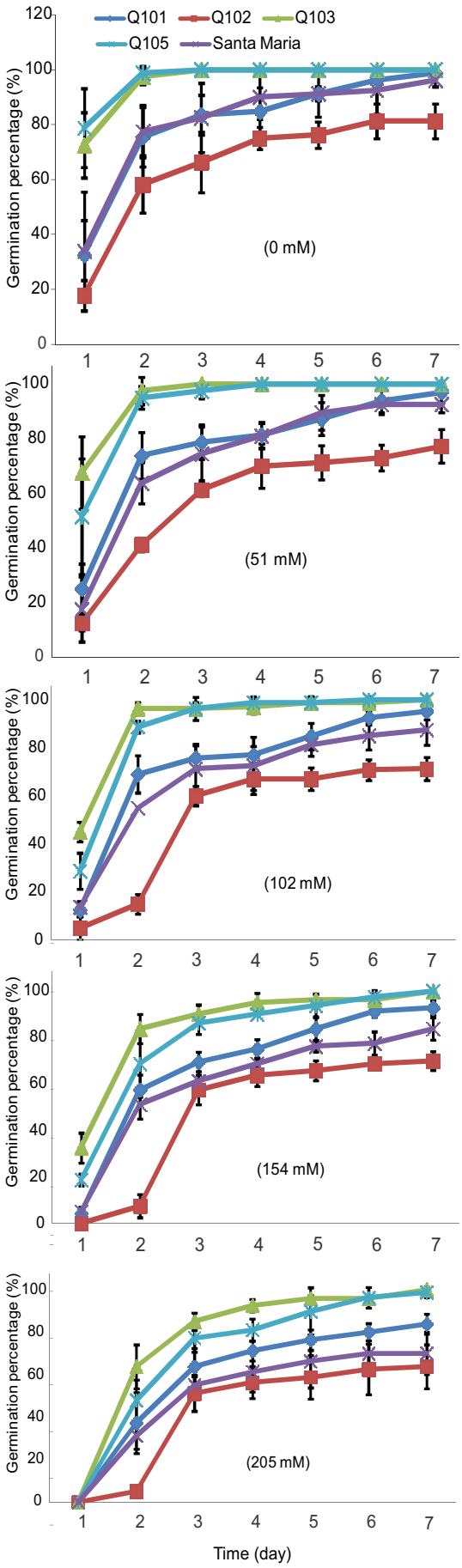

Fig. 2. Germination kinetics of five *Chenopodium quinoa* Willd. cultivars under different NaCl concentrations over time. Values are means ± SD (n=4).

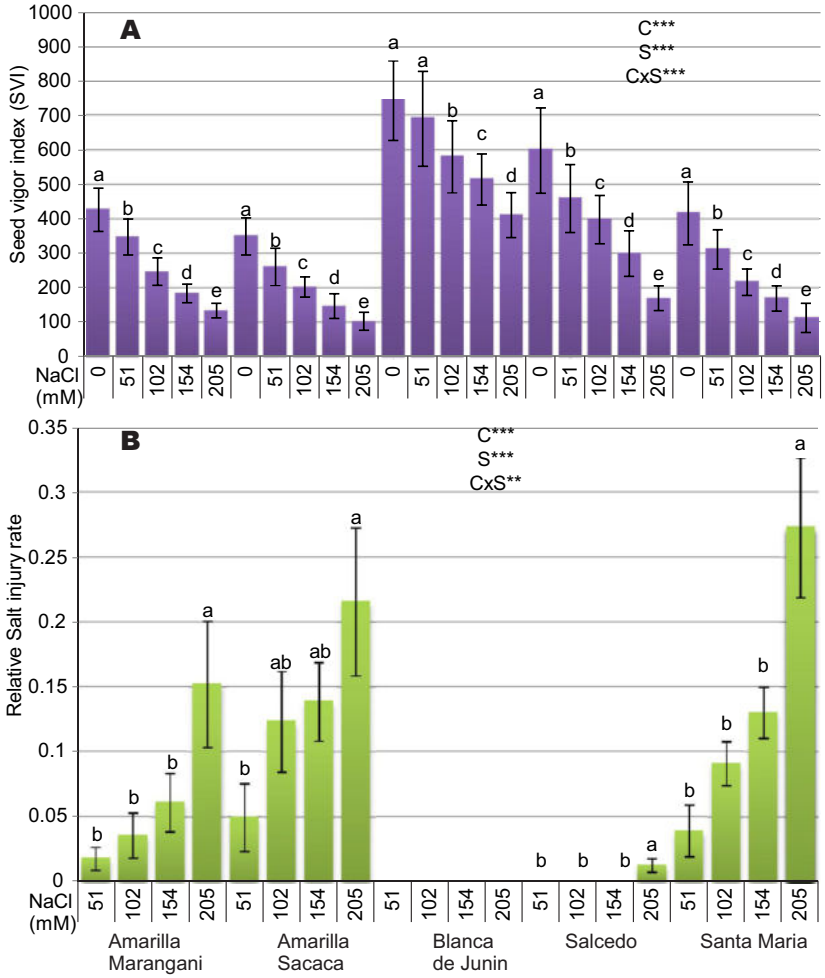

Fig. 3. Seed vigor index (SVI) (A) and relative salt injury rate (RSIR) (B) of five *Chenopodium quinoa* Willd. cultivars under different NaCl concentrations. Values are means ± SD (n=40 for SVI, n=4 for RSIR). Different letters indicate significant differences (P<0.05, Fisher's LSD).

Data analysis: One-way and two-way ANOVA were conducted at α = 0.05 using MiniTab 16. Linear correlations between growth parameters and NaCl concentration were tested, and histograms were created in Microsoft Excel 2010.

Results

Seed germination criteria

Germination percentage (GP%) and germination energy (GE%): Germination rates varied among quinoa cultivars under different NaCl concentrations (Fig. 1A). Q103 maintained 100 % germination across all salt concentrations, while Q105 achieved full germination at lower salt levels (51, 102, and 154 mM). Q101, Q102, and Santa Maria exhibited decreasing germination rates with increasing salt concentrations, with Q102 showing the lowest rate (63.75 %) at 205 mM NaCl. Germination energy (GE %) decreased with rising NaCl concentrations across all cultivars (Fig. 1B). Q103 and Q105 maintained higher GE% even at 205 mM (92.5 % and 81 % respectively), while Q102 recorded the lowest rate (56.25 %) at this concentration. ANOVA revealed highly significant effects (P < 0.001) for cultivar and salinity factors on both GP% and GE%. The cultivar*salinity interaction was significant for GP% but not for GE%.

Germination kinetics: Germination rates generally decreased over time with increasing NaCl concentrations (Fig. 2). Q103 and Q105 consistently achieved 100% germination rates from the third day at 51 mM NaCl, with Q103 maintaining full germination even at higher concentrations. Q101, Q102, and Santa Maria showed variable germination rates over time, with Q102 exhibiting the slowest rates.

Seed vigour index (SVI) and relative salt injury rate (RSIR): SVI values decreased significantly with increasing NaCl concentrations across all cultivars (Fig. 3A). Q103 maintained the highest SVI values (744 for control, 411 at 205 mM), while Q102 showed the lowest (349.4 for control, 102.16 at 205 mM NaCl). RSIR increased significantly in Q101, Q102, and Santa Maria with rising NaCl concentrations (Fig. 3B), reaching maximum values between 0.0177 and 0.2727. Q103 and Q105 exhibited minimal salt damage, with Q105 showing a minor RSIR value (0.0125) at 205 mM NaCl. ANOVA indicated highly significant effects (P < 0.001) for cultivar, salinity, and their interaction on both SVI and RSIR.

Seedling growth parameters: Early growth traits of quinoa seedlings declined in a dose-dependent manner with increasing NaCl salinity across all cultivars (Table 1). At 205 mM NaCl, seedling length decreased by 44.76 % to 71.29 %, while radicle length was reduced by 31.68 % to 73.16 %, depending on the cultivar. Q103 consistently maintained the longest radicle and seedling lengths across all NaCl concentrations. Hypocotyl growth appeared more sensitive to salinity than radicle growth overall. Strong negative linear correlations were observed between NaCl level and seedling length (R^2: 0.963 to 0.989), radicle length (R^2: 0.634 to 0.985), and hypocotyl length (R^2: 0.844 to 0.992) in all cultivars. ANOVA analysis revealed highly significant differences (P < 0.001) for all studied growth parameters, including cultivar, salinity, and their interaction.

Discussion

This study provides insights into the germination patterns of various quinoa cultivars under different NaCl concentrations. The high germination rates observed in Blanca de Junin (Q103) and Salcedo (Q105) cultivars, even at high salinity levels, align with previous research on quinoa by Panuccio *et al.* (2014) and Hariadi *et al.* (2011). The significant reduction in germination rates for Amarilla Marangani (Q101), Amarilla Sacaca (Q102), and Santa Maria cultivars at higher salt concentrations is consistent with findings from studies on other crops (Attri, 2018; Kalhori *et al.*, 2018).

The observed decline in germination rates can be attributed to the osmotic and toxic effects of sodium chloride salts (Brakez *et al.*, 2014; Hariadi *et al.*, 2011). High salt concentrations impede water absorption by seeds, reducing the activity of essential enzymes like amylase and protease, thus hampering the conversion of endospermic reserves for embryonic growth (Rajpar *et al.*, 2014). Furthermore, the accumulation of ions within seed cells disrupts cell division and elongation, inhibiting germination processes (Causin *et al.*, 2020; Stoleru *et al.*, 2019).

Table 1. Seedling growth parameters of five *Chenopodium quinoa* Willd. cultivars under different NaCl concentrations, seven days after sowing.

Variety	NaCl (mM)	Hypocotyl length (HL) (mm)	Radicle length (RL) mm)	hypocotyl / Radicule (HL/RL)	Seedling length (SL) (cm)	Decrease (%)
Amarilla Marangani (Q101)	dH_2O	22.20 ± 4.15^{a}	20.95 ± 3.61^{a}	1.08 ± 0.25^{a}	4.32 ± 0.64^{a}	-
	51	17.10 ± 3.89^{b}	18.75 ± 3.86^{b}	0.95 ± 0.31^{ab}	3.59 ± 0.54^{b}	16.90
	102	12.80 ± 3.75^{c}	13.10 ± 2.57^{c}	1.03 ± 0.41^{ab}	2.59 ± 0.42^{c}	40.05
	154	8.55 ± 1.73^{d}	11.25 ± 3.18^{cd}	0.85 ± 0.35^{b}	1.98 ± 0.29^{d}	54.17
	205	5.55 ± 1.85^{e}	10.40 ± 2.30^{d}	0.58 ± 0.27^{c}	1.60 ± 0.25^{e}	62.96
Linear correlation equation and R^2 value		Y = -1.395x + 21.61 (R^2 = 0.9923)	y = -0.9533x + 20.61 (R^2 = 0.927)	y = -0.0367x + 1.118 (R^2 = 0.7723)	y = -0.227x + 4.154 (R^2 = 0.9783)	-
Amarilla Sacaca (Q102)	dH_2O	23.90 ±5.78^{a}	19.10 ± 4.05^{a}	1.34 ± 0.56^{a}	4.30 ± 0.66^{a}	-
	51	11.50 ± 3.82^{b}	21.75 ± 5.60^{a}	0.55 ± 0.19^{b}	3.38 ± 0.71^{b}	21.40
	102	7.65 ± 2.82^{c}	20.70 ± 4.86^{a}	0.42 ± 0.27^{bc}	2.84 ± 0.41^{c}	33.95
	154	6.05 ± 2.44^{c}	14.85 ± 3.36^{b}	0.45 ± 0.26^{bc}	2.09 ± 0.52^{d}	51.40
	205	2.98 ± 0.73^{d}	13.05 ± 2.07^{b}	0.25 ± 0.09^{c}	1.60 ± 0.41^{e}	62.79
Linear correlation equation and R^2 value		y = -1.5767x + 19.875 (R^2 = 0.844)	y = -0.6333x + 21.69 (R^2 = 0.6341)	y = -0.076x + 1.058 (R^2 = 0.7146)	y = -0.223x + 4.18 (R^2 = 0.9895)	-
Blanca de Junin (Q103)	dH_2O	37.3 ± 7.93^{a}	37.10 ± 8.61^{a}	1.07 ± 0.33^{a}	7.44 ± 1.16^{a}	-
	51	32.95 ± 4.45^{b}	36.20 ± 9.43^{ab}	0.97 ± 0.24^{ab}	6.92 ± 1.37^{a}	6.99
	102	23.6 ± 5.89^{c}	35.50 ± 9.67^{ab}	0.82 ± 0.46^{bc}	5.81 ± 1.05^{b}	21.91
	154	21 ± 5.13^{c}	30.45 ± 6.88^{bc}	0.80 ± 0.42^{bc}	5.15 ± 0.74^{c}	30.78
	205	15.65 ± 2.76^{d}	25.45 ± 4.92^{c}	0.65 ± 0.19^{c}	4.11 ± 0.65^{d}	44.76
Linear correlation equation and R^2 value		y = -1.8417x + 37.15 (R^2 = 0.9727)	y = -0.9683x + 38.75 (R^2 = 0.8719)	y = -0.0337x + 1.064 (R^2 = 0.9671)	y = -0.281x + 7.572 (R^2 = 0.989)	-
Salcedo (Q105)	dH_2O	29.35 ± 5.3^{a}	30.55 ± 8.51^{a}	1.04 ± 0.31^{ab}	5.99 ± 1.25^{a}	-
	51	21.65 ± 5.98^{b}	24.30 ± 4.49^{b}	0.95 ± 0.37^{b}	4.59 ± 0.99^{b}	23.37
	102	18.8 ± 5.81^{b}	20.95 ± 7.58^{b}	1.10 ± 0.57^{ab}	3.98 ± 0.70^{c}	33.56
	154	14.25 ± 4.55^{c}	15.65 ± 4.07^{c}	0.93 ± 0.30^{ab}	2.99 ± 0.67^{d}	50.08
	205	9 ± 2.36^{d}	8.2 ± 2.94^{d}	1.23 ± 0.52^{a}	1.72 ± 0.36^{e}	71.29
Linear correlation equation and R^2 value		y = -1.6033x + 28.23 (R^2 = 0.9804)	y = -1.7783x + 30.6 (R^2 = 0.9854)	y = 0.012x + 0.978 (R^2 = 0.2182)	y = -0.338x + 5.882 (R^2 = 0.9867)	-
Santa Maria	dH_2O	25.00 ± 5.74^{a}	18.25 ± 4.97^{a}	1.42 ± 0.36^{a}	4.33 ± 0.95^{a}	-
	51	18.30 ± 3.85^{b}	15.35 ± 4.23^{b}	1.25 ± 0.33^{a}	3.36 ± 0.62^{b}	22.40
	102	10.25 ± 3.9^{c}	14.6 ± 3.23^{bc}	0.74 ± 0.33^{c}	2.47 ± 0.45^{c}	42.96
	154	7.70 ± 3.08^{cd}	12.50 ± 3.38^{c}	0.66 ± 0.27^{c}	2.02 ± 0.44^{d}	53.35
	205	7.55 ± 3.34^{d}	8.45 ± 3.74^{d}	0.98 ± 0.43^{b}	1.60 ± 0.60^{e}	63.05
Linear correlation equation and R^2 value		y = -1.52x + 22.87 (R^2 = 0.8842)	y = -0.7483x + 18.32 (R^2 = 0.9482)	y = -0.049x + 1.304 (R^2 = 0.5121)	y = -0.227x + 4.116 (R^2 = 0.9629)	-
Source of variation		*P* values of tow-way ANOVA				
Cultivar		0.000***	0.000***	0.000***	0.000***	-
Salinity level		0.000***	0.000***	0.000***	0.000***	
Cultivar*Salinity		0.000***	0.000***	0.000***	0.000***	

Each value is the mean ± SD of 40 replicate measurements. Means that do not share a letter are significantly different. using Fisher LSD Method and 95% confidence. *** (extremely significant).

The strong positive correlation between germination energy (GE%) and germination rates (GP%) observed in our study supports findings by Ologundudu *et al.* (2014) and Hakim *et al.* (2010). As Asch and Wopereis (2001) noted, cultivars with higher GE% rates may have a competitive advantage in exploiting environmental resources.

The consistent decrease in seed germination kinetics with increasing NaCl concentrations, varying among cultivars, aligns with studies by Causin *et al.* (2020) on quinoa and Sagar *et al.* (2018) on sorghum genotypes. The observed reduction in seed vigor index (SVI) with increasing salinity corroborates findings by Kalhori *et al.* (2018) on rice. Interestingly, the SVI values for Blanca de Junin and Salcedo cultivars did not precisely correspond with their germination rates, echoing observations by Bina and Bostani (2017) on zucchini. This suggests that salt stress impacts on seed vigor can be independent of germination rates, possibly due to reduced respiration and the availability of reserve respiratory metabolites (Smiri *et al.*, 2009).

The varying responses in embryonic axis growth under salt stress highlight the genetic diversity within quinoa germplasm. The superior performance of Blanca de Junin (Q103) across all NaCl levels suggests its potential as a salt-tolerant cultivar. The observed greater sensitivity of hypocotyl growth to salinity compared to radicle growth aligns with findings by Adolf *et al.* (2013) and may represent an adaptive strategy to promote root growth under osmotic stress.

In conclusion, this study examined five quinoa cultivars' responses to NaCl stress during germination and early growth. Blanca de Junin and Salcedo showed superior salt tolerance, maintaining high germination rates and seedling growth under stress, while Amarilla Sacaca, Amarilla Marangani, and Santa Maria exhibited greater sensitivity. Hypocotyl growth was more sensitive to salinity than radicle growth across cultivars. These findings highlight the importance of genotypic selection in quinoa breeding programs, providing valuable genetic resources for developing salt-tolerant varieties. This research contributes to expanding potential quinoa cultivation areas and emphasizes the need for further investigation into salt tolerance mechanisms and field performance of promising cultivars to promote sustainable agriculture in challenging environments.

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