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Responses of radish (*Raphanus sativus* L. var. Saxa) to salt stress during early vegetative development

Running title: Radish Responses to Salt Stress during Early Growth

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ABSTRACT

Increased soil salinity is a threat to plant growth and development. Plant species, including different genotypes within the same species, vary in their tolerance to stress. By monitoring quantitative morphological growth parameters under stress conditions, insight can be gained into plant stress resistance. In this context, the present study examined the effects of two types of salts, NaCl and K_2SO_4, at concentrations ranging from 0.025 M to 0.25 M, on the germination and early vegetative development of radish (*Raphanus sativus*, variety Saxa). Results showed that the highest biomass yield was recorded in plants grown under non-stress conditions, indicating optimal growth. In contrast, the lowest biomass was observed under severe sodium salt stress (0.2 M NaCl), although the smallest seedlings were found under strong potassium salt stress (0.15 M K_2SO_4). Notably, plant growth appeared more sensitive to salt-induced stress than seed germination. Under the highest stress intensity for both salts, germination was completely inhibited.

Keywords: salt stress, Raphanus sativus, germination, seedling growth

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Introduction

During ontogeny, plants are continuously exposed to a range of environmental factors, which can become stressful when their intensity exceeds species-specific optimal thresholds. The impact of these stressors on plant growth and development depends on their intensity and duration. Plant responses vary by species and can manifest resistance, tolerance, or varying degrees of sensitivity. Sensitivity levels also fluctuate with developmental stage and species-specific traits. When adverse conditions persist, stress factors can inhibit growth, delay development, and ultimately reduce yield (Stikić & Jovanović, 2012).

Soil salinization represents a major environmental challenge, with over 955 million hectares globally affected and approximately 20% of irrigated land classified as saline (Metternicht & Zinck, 2003). Saline soils are present across all climate zones and are characterized by high concentrations of soluble salts such as NaCl, Na_2SO_4, $NaHCO_3$, Na_2CO_3, $MgCl_2$, $MgSO_4$, K_2SO_4, $CaCO_3$ and $CaSO_4$, which exert osmotic and ionic stress on plants. These stresses negatively impact various physiological and biochemical processes, thereby inhibiting plant growth and development (Metternicht & Zinck, 2003; Cuartero et al., 2006). Projections indicate that by 2050, up to 50% of arable land may become saline, posing a substantial threat to global crop production and food security. Plant responses to salinity are highly variable and species-specific, influenced by salt type, concentration, and developmental stage. Tolerance mechanisms vary across species, underscoring the need for targeted studies on the effects of salinity and other abiotic stressors to inform sustainable agricultural practices and enhance crop resilience in agroecosystems. Therefore, the action of each stress factor should be given special attention to each plant species.

Raphanus sativus L. (Brassicaceae) is a root vegetable with a long-standing role in human nutrition, valued for its distinctive pungent flavor and high nutritional content. The edible roots and leaves are commonly consumed fresh or cooked and represent a rich source of calcium, potassium, phosphorus, carbohydrates, and vitamins A, C, and B (Larry, 1977). In addition to its dietary relevance, radish exhibits medicinal potential, further underscoring its agronomic importance (Ghosh et al., 2014). The presence of bioactive compounds—particularly phenolics, flavonoids, and antioxidants—has been associated with various health benefits, including confirmed anticancer properties of certain secondary metabolites (Cartea & Velasco, 2007; Barilliari2008).

This study investigates the effects of salt stress induced by different concentrations of NaCl and K_2SO_4 on seed germination and early seedling growth of Raphanus sativus. The cultivar 'Saxa', characterized by its round red roots and broad adaptability, was selected due to its suitability for cultivation under diverse environmental conditions.

Experimental

Commercial seeds were surface sterilized by immersion in 25% sodium hypochlorite solution containing two drops of liquid detergent for 30 minutes, followed by three rinses with sterile distilled water. Germination tests were conducted using the standard Petri dish method. Seeds were placed on two layers of filter paper, moistened with either distilled water (control) or saline solutions representing salt-stress treatments. Two salt types, NaCl and K_2SO_4, were applied at five concentrations (0.025 M, 0.05 M, 0.1 M, 0.15 M and 0.2 M). Petri dishes were incubated in a growth chamber under controlled conditions at 20 °C, with a photoperiod of 16 hours of light and 8 hours of dark.

Seed germination was monitored over a period of ten days, after which the germination percentage (GP) and sensitivity index (SI) were calculated. The germination percentage was determined using the formula $GP = (N/S) \times 100$, where N is the number of germinated seeds, and S is the total number of seeds sown. The sensitivity index (SI) was calculated as $SI = e/d$, where e represents the germination percentage in the control, and d the germination percentage under salt treatment. After the tenth day, growth parameters were assessed, including root length (mm), shoot length (mm), and seedling fresh and dry weight (g).

The experiment was conducted in triplicate, and all obtained data were statistically analyzed using analysis of variance (ANOVA), followed by the LSD (Least Significant Difference) test at a significance level of $p < 0.05$. Statistically significant differences between means are indicated by different letters.

Results and Discussion

The germination percentage was evaluated ten days after the experiment was initiated. In the control treatment (distilled water), radish seeds exhibited the highest germination rate (86.6%) (Table 1). Exposure to salt stress resulted in a concentration-dependent decline in germination, with more pronounced effects observed under NaCl treatment. At a concentration of 0.15 M, germination in NaCl solution was approximately 2.5 times lower compared to K_2SO_4. In a 0.2 M NaCl solution, germination remained equally low, whereas in the corresponding K_2SO_4 solution, no germination occurred despite visible seed imbibition. At the highest tested concentration (0.25 M), seed germination was completely inhibited under both salt treatments.

Table 1. Seed germination and sensitivity index under salt stress conditions

Treatment		GP	SI
Control	dH_2O	86.6	
NaCl (M)	0.025	33.3	2.6
	0.05	60	1.4
	0.1	43.3	2
	0.15	6.66	13.03

	0.2	6.66	13.03
	0.25	0	-
K_2SO_4 (M)	0.025	70	1.2
	0.05	80	1.08
	0.1	60	1.44
	0.15	16.66	5.19
	0.2	0	-
	0.25	0	-

The germination percentage (GP) is calculated as GP = (N/S) × 100, where N is the number of germinated seeds and S is the total number of seeds sown. The sensitivity index (SI) is calculated as SI = e/d, where e is the germination percentage in the control treatment and d is the germination percentage under salt treatment.

Different radish varieties such as *R. sativus* L. var. Red Bombay, Tasakisan Mula-1, and Druti also showed that germination was inhibited as the NaCl concentration increased (Ghosh et al. 2014). *R. sativus* L. var. *longipinnatus* Bailey also showed a significantly low percentage of germination at the highest NaCl concentration (Jungklang, 2018).

Comparison of the sensitivity index (SI) for seed germination under salt stress revealed that germination is more adversely affected by NaCl than by K_2SO_4 (Table 1). The lowest SI value was observed under low-intensity potassium salt stress (0.05 M), whereas the highest SI value (13.03) was recorded under high NaCl stress. These results indicate that radish seeds exhibit approximately 60% greater sensitivity to NaCl-induced stress compared to potassium salt stress.

Seedlings exhibited the greatest growth, with the longest root and shoot lengths under control conditions (Table 2). Exposure to salt stress significantly reduced root length, even at the lowest NaCl concentration tested. A pronounced inhibition of root growth was observed under high NaCl stress (0.2 M). Although mild K_2SO_4 stress also negatively affected root elongation, its impact was less severe than that of NaCl stress. However, under higher potassium salt concentrations, seedlings developed the shortest roots, measuring approximately 11 mm.

The greatest shoot length (Table 2) was observed in seedlings exposed to 0.025 M K_2SO_4, slightly exceeding that of the unstressed control. In contrast, NaCl at the same concentration caused a 19.6% reduction in shoot length, indicating that salt tolerance or sensitivity varies with salt type. Under higher potassium salt stress, shoot length was significantly reduced, reaching a minimum of 9.5 mm.

Table 2. Seedling growth under salt stress condition

Treatment		Root length (mm)	Shoot length (mm)	Fresh weight (mg)	Dry weight (mg)
Control	dH_2O	79.67 ± 7.38^{d}	37.54 ± 2.53^{d}	78.0 ± 9.0^{e}	13.3 ± 0.2^{e}
NaCl (M)	0.025	33.37 ± 7.53^{b}	30.17 ± 3.94^{cd}	25.0 ± 3.0^{c}	1.0 ± 0.8^{a}
	0.05	42.05 ± 4.76^{c}	28.44 ± 2.47^{c}	25.0 ± 3.0^{c}	8.7 ± 0.5^{b}
	0.1	$43.50 \pm 3,72^{c}$	32.93 ± 3.65^{d}	40.0 ± 6.0^{d}	12.8 ± 0.8^{d}
	0.15	44.00 ± 4.05^{c}	18.36 ± 2.23^{b}	18.0 ± 2.0^{b}	10.3 ± 0.6^{c}
	0.2	18.50 ± 4.50^{a}	13.50 ± 0.65^{a}	14.0 ± 2.0^{a}	8.5 ± 0.6^{b}

K_2SO_4 (M)	0.025	66.61 ± 7.63^{c}	39.84 ± 2.91^{d}	56.0 ± 8.0^{d}	11.0 ± 0.1^{a}
	0.05	49.17 ± 4.69^{c}	28.88 ± 2.63^{c}	53.0 ± 6.0^{d}	12.0 ± 0.1^{ab}
	0.1	27.33 ± 5.45^{b}	18.30 ± 2.88^{b}	24.0 ± 4.0^{c}	1.3 ± 0.3^{b}
	0.15	11.00 ± 1.00^{a}	9.50 ± 2.50^{a}	17.0 ± 2.0^{b}	1.2 ± 0.1^{ab}
	0.2	-	-	-	-

Values represent means ± SE, n = 3. Means within the same column marked with different letters are significantly different according to Tukey's HSD test at $P \leq 0.05$.

The highest biomass accumulation was recorded in seedlings grown under non-stress conditions, reflecting optimal growth. Although the smallest seedlings were observed under severe potassium salt stress (0.15 M K_2SO_4), the lowest biomass values were found under intense sodium salt stress (0.2 M NaCl). Water uptake by plants occurs when the root system's water potential is lower than that of the surrounding substrate.

Excessive concentrations of dissolved salts in the substrate decrease the water potential at the root-soil interface, thereby restricting water uptake by the roots (Zelm et al., 2020). This salt-induced osmotic stress results in a water deficit within the plant, leading to reduced cell turgor pressure, impaired hydraulic conductivity, and decreased relative water content (RWC). Consequently, water-use efficiency (WUE) is negatively affected (Jia et al., 2001; Huang et al., 2017). Collectively, these physiological disruptions contribute to a significant decline in plant biomass accumulation.

When comparing seedlings subjected separately to potassium and sodium salt stress, the highest biomass yield was observed under low potassium salt stress, whereas under sodium salt stress, the highest biomass was recorded at moderate stress intensity (0.1 M NaCl). Literature reports on Raphanus sativus L. var. longipinnatus Bailey indicate that exposure to 0.1 M NaCl stress also resulted in maximal fresh biomass, aligning with our findings. Jungklang (2018) further documented significant reductions in all measured growth parameters at 0.2 M NaCl, consistent with our results. Despite using different radish varieties, the concordance between these results suggests that, notwithstanding genetic variability, similar physiological and biochemical mechanisms govern the response to salt stress of equal intensity across varieties.

Radish seed germination exhibited greater resilience to salt stress compared to seedling growth. Salt-induced osmotic stress increases the osmotic pressure of the soil solution, leading to water deficit that adversely impacts cellular processes such as cell division and elongation, photosynthesis, transpiration, and dry matter allocation (Lu et al., 2023; Zhao et al., 2021; Arif et al., 2020). Additionally, ion accumulation within cells disrupts nutrient uptake, further inhibiting growth. Collectively, these physiological disturbances under saline conditions negatively affect plant development and ultimately reduce crop yield.

Conclusion

Salt stress negatively influences *R. sativus* seedlings, especially at high concentrations. Measured morphological parameters indicate that plant growth is more severely reduced as salt stress intensity increases. Low doses of salt may be stimulative for seedling development, depending on the type of salt. Seed germination is less susceptible to the presence of salt than the growth of sprouts and roots. The inhibitory effect of salt on early vegetative development affects biomass production, and a decrease in yield would negatively affect agroeconomic profit.

Radish seed germination and seedling growth are adversely affected under saline conditions. Varying concentrations of sodium and potassium salts reduce overall plant growth, with the inhibitory effects most pronounced at higher salt concentrations. While seed germination exhibits a relatively lower sensitivity to salinity compared to seedling growth, it is more susceptible to the effects of sodium salts than to those of potassium salts.

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Conflict-of-Interest Statement

The authors declare no conflict of interest.

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