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Mitigating Salinity Stress in *Brassica campestris* (L.) Varieties by Rhizospheric Application of Potassium Nitrate

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ABSTRACT

The motive of the study was to evaluate the effect of Potassium Nitrate (KNO_3) on the plants which are under different levels of salt stress. Salt-induced stress is a common abiotic constraint hindering agricultural productivity, especially in salt-sensitive crops such as *Brassica campestris* L, used for extraction of valuable oil and biodiesel. Potassium Nitrate (KNO_3) has potential to alleviate the adverse effects of salinity, particularly in the presence of Sodium Chloride stress is discussed in this paper. In a controlled pot trial, two different *Brassica campestris* cultivars Tori-7 (V1) and yellow mustard (V2), were applied with six experimental treatments with different concentrations of NaCl and KNO_3. The parameters studied included secondary metabolite profiles that were fully evaluated in the study included alkaloids, flavonoids and phenolic compounds, in addition to yield attributes and measures of plant growth. As per the results, the accumulation of biomass, root and shoot elongation, vegetative development, and reproductive performance were significantly reduced by NaCl stress; the outcomes became worse with the increase in the amount of NaCl. In salty conditions, however, growth and yield parameters were augmented by the application of KNO_3 in the rhizosphere, especially in V2 that was much more salt-tolerant than V1. Notably, the concentration of secondary metabolites showed a slightly different pattern; plants subjected to salt stress contained more of such compounds, and the content of alkaloid decreased after the addition of KNO_3. The results support the notion that KNO_3 enhances the capacity of *Brassica campestris* to resist salt and that it may be a viable agricultural management technique to reduce salinity stress and increase crop production in salty soils.

INTRODUCTION

Salinity is a ubiquitous and dominant abiotic causal factor of stress with enormous adverse effects on agricultural production capacity in the world, particularly in semi-arid and arid regions. The presence of soluble salts in the soil matrix disrupts important physiological plant functions leading to low crop yields and impairments on metabolic functions. This is because, sooner or later, osmotic stress, ionic toxicity and oxidative damage will all contribute to the inhibition of water absorption, nutrient absorption and cell homeostasis (Kamran et al., 2019). The total area of the terrestrial surface which is adversely affected by salinity and, therefore, influences food security on Earth is estimated to be approximately one-fifth of the total irrigated terrestrial land worldwide (FAO, 2024). As salt stress is known to impair the major plant processes, including seed germination, vegetative growth, and overall production, one of the largest economic crops, *Brassica campestris* L. (mustard), is especially vulnerable to saline conditions (Bassegio et al., 2020; Zhang et al., 2011). Secondly, mustard is the second-largest oil-bearing crop in Pakistan; therefore, it represents approximately 17 percent of the total production of edible oil in the nation and demonstrates its economic importance (Pakissan, 2017). This is similarly simplified when the salt stress effect is a negative contributor on flowering, seed set, and seed yield development (Shah et al., 2021).

One of the main causes of salt to lead to dysfunction in cells is excess sodium ions (Na^+) in the root cells. According to Munns and Tester (2008), these ions interfere with enzymatic processes necessary to maintain metabolism in a plant, slow nutrient mobility, and adjust water potential. Alongside these direct physiological alterations, salinity may also lead to the synthesis of the so-called secondary metabolites (e.g flavonoids and phenolic compounds), which also predetermines the occurrence of oxidative stress and is also regarded as a component of the adaptive response to abiotic stress in the plant (Yuenyong et al.,

2018).

A broad spectrum of agronomic interventions has been suggested to minimize adverse effects of salt, including the prudent utilization of fertilizer. Among these, which involve Potassium Nitrate (KNO_3), has attracted attention as an effective intervention to strengthen the halotolerance of plants. Even though the ionic balance and activity of the enzymes and cell membrane integrity require the presence of potassium (K^+), nitrate (KNO_3) is a precondition to ensure the synthesis of nitrogen and proteins in the stressful conditions (Aslam et al., 2023; Sardan et al., 2021). The study did not investigate it indepth, so today, there is a significant knowledge gap concerning the role of rhizospheric KNO_3 injection in alleviating salt stress on *Brassica campestris,* although evidence shows that potassium supplements can offer a variety of agronomic advantages to a variety of crops (Hasanuzzaman et al., 2018).

The primary hypothesis of the current work is that addition of KNO_3 to the rhizosphere will enhance growth and yield, and also, impact the productivity of secondary metabolites which will counteract the inhibitory effect of salt stress exerted by NaCl on *Brassica campestris*. The vegetative growth and crop salt tolerance induced by KNO_3 supplementation developed by previous researchers has been shown to be effective at a fraction of the cost of genetic engineering, or traditional breeding methods (Zheng et al., 2010; Siringam et al., 2023; Chele et al., 2021). Not only will the expected results of the study make mustard more salinity resistant, but will also enhance the resilience of other oil seed crops against salinity in the salinity prone areas, which would boost agricultural activities in the salinity prone areas.

MATERIALS AND METHODS

Chemical Used

All the chemicals used in this study were of analytical grade and purchased from reputed supplier including Sigma Aldrich (USA) and Merck (Germany).

Experimental Site and Design

A pot experiment was conducted at the Botanical Garden of Bahauddin Zakariya University, Multan, Pakistan, from October 2023 to February 2024. The soil used was loamy in texture with a pH of 6.9, which is considered ideal for mustard cultivation. The experiment was arranged in a Completely Randomised Design (CRD) comprising six treatment groups, each replicated three times, for a total of 18 pots. Each pot was filled with 5 kg of air-dried, sieved loamy soil. The following treatments were applied:

T1: Control
T2: 50 mM NaCl
T3: 100 mM NaCl
T4: 50 mM KNO_3
T5: 50 mM NaCl + 50 mM KNO_3
T6: 100 mM NaCl + 50 mM KNO_3

Planting and Treatment Application

Seeds were sown directly into the pots, and irrigation was carried out with deionized water until seedlings reached the three-leaf stage. Approximately ten days after germination, treatments were initiated through rhizospheric irrigation. Each pot received 100 mL of the respective treatment solution to ensure uniform soil moisture and solute distribution.

Monitoring and Maintenance

The experiment was monitored regularly to maintain optimal plant health and ensure consistent application of treatments. Special care was taken to avoid excessive salt accumulation and to maintain adequate soil moisture throughout the growing period. All cultural practices, including thinning, weeding, and pest management, were uniformly applied across treatments.

Growth Parameters Measurement

Several plant growth parameters were recorded 45 days after sowing (DAS), including shoot length (SL), root length (RL), shoot and root fresh weight (FW), and dry weight (DW). Shoot and root lengths were measured in centimeters using a calibrated measuring scale after carefully uprooting the plants and washing off the soil from the root system. Fresh biomass was determined immediately after harvest by cutting the base of the stems and weighing the material using an analytical balance (Science Buddies, n.d.). Dry weight was assessed by placing the fresh plant material in a hot air oven at a constant temperature of 60 °C for 72 hours until the weight stabilized, indicating complete dehydration. Biomass yield for each treatment was then calculated based on the dry weight measurements (Wilke & Boughton, 2024).

Yield Estimation

Yield attributes of *Brassica campestris* were evaluated approximately 90 days after sowing to assess fertility, reproductive potential, and overall productivity under the different treatment regimes, following the methodology of Barick et al. (2020). Yield parameters included the number of branches (N. Br) per plant, number of seeds (N.Seeds) per plant, number of siliquae (N.Sil) per plant, and dry weight of siliquae (DW.Sil). All counts (branches, siliquae, and seeds) were performed manually, and siliquae were then weighed after oven-drying.

Biochemical and Phytochemical Analysis

To assess the biochemical responses to salt stress and potassium nitrate treatment, dried root tissue samples were analyzed for secondary metabolite content nine weeks after sowing. The concentration of alkaloids was estimated using the method described by Harborne (1998), which involves extraction with acidified methanol followed by spectrophotometric measurement at 780 nm. Flavonoid content was determined using the aluminum chloride colorimetric method, as described by Bohm and Kocipai-Abyazan (1994), where the aluminum-flavonoid complex forms a colored product measured by spectrophotometry. Total phenolic content was measured using the Folin–Ciocalteu method (Singleton & Rossi, 1965), where phenolic compounds in the plant extract react with the Folin–Ciocalteu reagent to form a blue complex. The absorbance was measured spectrophotometrically, and results were expressed in gallic acid equivalents (GAE) per kilogram of dry weight.

Statistical Analysis

Data from each experiment were subjected to analysis of variance (ANOVA) to determine whether statistically

significant differences ($p < 0.05$) existed among treatment groups. To further evaluate treatment effects on growth, yield, and biochemical parameters, post hoc comparisons of means were conducted using the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Effects of Salinity on Growth Parameters

Different concentrations of NaCl had significant, concentration-dependent effects on the growth of *Brassica campestris*. As shown in Table 1, salinity caused a reduction in shoot length, root length, and shoot and root fresh and dry weights. These reductions were more pronounced at 100 mM NaCl compared to 50 mM NaCl. Variety V1 (Tori-7) exhibited greater sensitivity to salt stress than V2 (Yellow Sarson), showing more drastic declines in growth parameters.

Impact of Potassium Nitrate (KNO_3) on Salt-Stressed Plants

Application of 50 mM KNO_3 alone and in combination with NaCl partially alleviated the negative effects of salinity. Growth parameters showed slight improvement, especially under 50 mM NaCl plus KNO_3 treatment. This improvement was more evident in V2 than in V1, indicating a positive role of potassium nitrate in mitigating mild salt stress.

Comparison with Previous Studies

The observed growth inhibition due to salinity aligns with previous reports that salt stress limits plant growth by impairing nutrient and water uptake (Zhang et al., 2014; Shah et al., 2021). The greater reduction in shoot growth compared to root length is consistent with known shoot sensitivity to water-deficit conditions (Munns & Tester, 2008; Blumwald, 2000; Kapoor & Pande, 2015; Debnath et al., 2020). The partial mitigation of salinity effects by KNO_3 supports prior findings that potassium and nitrate contribute to osmotic regulation and nitrogen metabolism under stress (Hasanuzzaman et al., 2018; Sardans & Peñuelas, 2021; Aslam et al., 2023; Wahid et al., 2025). However, the improvement did not fully reverse the impact of high salinity, consistent with previous observations (Siringam et al., 2013; Zheng et al., 2010).

Table 1

Effect of different treatments on various growth parameters (Shoot Length (SL), Root Length (RL), Shoot Fresh Weight (FW), Root Fresh Weight (FW), Shoot Dry Weight (DW), and Root Dry Weight (DW)) for two varieties (V1 and V2).

Treatments	Control	50mM NaCl	100mM NaCl	50mM KNO_3	50mM NaCl+50mM KNO_3	100mM NaCl+50mM KNO_3
SL (cm) V1 % Difference	72.33±1.43	59.34±1.52 -19.73%	48.69±1.23 -39.07%	87.6±2.34 19.9%	61.29±2.14 16.52%	50.2±0.34 -36.12%
SL (cm) V2 % Difference	72.66±2.11	62.62±2.13 -14.84%	49.33±1.09 -38.24%	89.66±2.12 20.94%	63.03±2.25 -14.19%	51.3±0.37 -34.46%
RL (cm) V1 % Difference	18.60±0.23	16.30±1.75 -12.37%	15.92±2.35 -14.41%	19.63±2.15 5.54%	16.40±1.88 -11.83%	15.93±0.89 -14.35%
RL (cm) V2 % Difference	18.26±0.5	15.92±1.67 -12.81%	15.43±2.67 -10.02%	17.2±2.10 5.81%	16.16±0.92 -11.50%	16.16±0.92 -11.50%
Shoot FW (g) V1 %Difference	18.60±0.23	9.30±1.7 -66.66%	6.92±2.35 -91.53%	19.63±2.15 7.403%	13.40±1.88 -32%	8.93±0.89 -79.27%
Shoot FW (g) V2 % Difference	18.62±0.51	9.43±2.67 -63.77%	6.92±1.67 -90.07%	19.89±3.01 8.5%	13.2±2.10 -32.16%	8.16±0.92 -78.02%
Root FW (g) V1 % Difference	6.23±0.07	6.11±0.62 -1.93%	5.78±0.08 -7.22%	6.32±0.44 1.44%	6.14±0.25 -1.44%	5.82±0.4 -6.58%
Root FW (g) V2 % Difference	6.59±0.04	6.42±0.46 -2.58%	6.32±0.09 -4.10%	6.60±0.13 0.15%	6.40±0.37 -2.88%	6.40±0.52 -2.88%
Shoot DW (g) V1 % Difference	3.20±1.23	1.91±0.98 -49.60%	0.99±0.03 -105.48%	4.39±1.13 31.35%	2.14±1.06 -39.70%	1.47±0.56 -78.08%
Shoot DW (g) V2 % Difference	3.17±1.11	1.95±0.99 -47.65%	1.09±0.05 -97.16%	4.37±1.11 31.83%	2.15±1.08 -38.49%	1.46±0.45 -73.86%
Root DW (g) V1 % Difference	2.06±0.12	2.03±0.12 -1.46%	1.84±0.01 -10.68%	2.60±0.02 26.21%	2.01±0.56 -2.43%	1.92±0.68 -6.80%
Root DW (g) V2 % Difference	2.10±0.09	2.06±0.14 -1.90%	1.94±0.01 -7.62%	2.68±0.04 27.62%	2.1±0.67 0%	2.01±0.85 -4.29%

Yield Response to Salinity and Potassium Nitrate

Yield parameters exhibited trends similar to the growth traits. Salinity significantly reduced the number of branches, seeds, siliquae per plant, and the dry weight of siliquae, indicating a marked decline in reproductive capacity. This reduction is consistent with ion toxicity interfering with nutrient distribution and translocation, which adversely affects reproductive development (Flowers et al., 2015; Jamil et al., 2011). As presented in Table 1, all yield parameters declined with increasing salt concentration from 50 mM to 100 mM NaCl in both varieties V1 and V2, underscoring the detrimental effects of salinity on plant yield. Treatment with KNO_3, alone and in combination with both low and high salt concentrations, resulted in slight increases in yield parameters in both varieties, partially alleviating the loss caused by salt stress. These findings support the hypothesis that potassium supplementation enhances ion uptake and reproductive growth (Gorham et al., 1985). However, the addition of KNO_3 did not fully restore yield under severe salinity stress, indicating that complete recovery under harsh conditions is unlikely (Kaya et al., 2003).

Table 2

Effect of different treatments on various yield parameters (Number of Branches (N. Br) per plant, Number of Seed (N.Seeds) per plant, Number of Siliquae (N.Sil) per plant and Dry Weight of Siliquae (DW.Sil)) for two varieties (V1 and V2).

Parameters	Control	50mM NaCl	100mM NaCl	50mM KNO_3	50mM NaCl+50mM KNO_3	100mM NaCl+50mM KNO_3
N.Br/plant V1	7.56±0.02	5.23±0.01	3.33±0.01	9.56±0.03	6.23±0.02	5.00±0.01
% Difference		-23.59%	-77.68%	23.59%	-19.23%	-40.76%
N.Br/plant V2	6.56±0.01	4.90±0.03	3.43±0.03	8.90±0.03	6.50±0.02	4.46±0.02
% Difference		-28.97%	-62.66%	3.27%	-0.91%	-38.11%
N.Seeds/plant V1	268±4.0	214±4.2	176±7.6	281±8.1	238±6.5	208±11
% Difference		-22.40%	-41.44%	4.73%	-11.85%	-25.21%
N.Seed/plants V2	270±8.3	216±4.3	181±6.1	293±5.3	241±4.5	218±10
% Difference		-22.22%	-39.46%	8.17%	-11.35%	-21.38%
N.Sil/plant V1	17±2.13	13.66±0.25	10.33±0.25	19±0.64	15.33±1.11	13.33±1.3
% Difference		-21.78%	-48.81%	11.11%	-10.33%	-24.20%
N.Sil/plant V2	16.66±3.14	13±0.34	11±0.36	18±0.82	15.66±1.44	13±1.42
% Difference		-19.78%	-40.92%	7.7%	-6.13%	-24.67%
DW.Sil (g) V1	18.20±0.23	14.93±0.34	12.73±0.13	22.23±0.34	17.13±0.71	15.76±0.41
% Difference		-20.06%	-34.93%	19.60%	-6.3%	-14.69%
DW.Sil (g) V2	17.50±0.32	15.21±0.04	11.2±0.44	20.6±0.54	16.23±0.12	15.7±0.31
% Difference		-14.23%	-44.06%	16.10%	-7.70%	-11.01%

Changes in Secondary Metabolites under Salinity and KNO_3 Treatments

During NaCl stress and all KNO_3 treatments, secondary metabolites—including alkaloids, flavonoids, and phenols—exhibited patterns opposite to those observed for growth and yield parameters. While growth and yield declined under salt stress, the concentrations of these phytochemicals increased with rising salt levels. The elevated phytochemical levels likely protect plant cells from oxidative damage by neutralizing reactive oxygen species (ROS) generated during salt stress (Buchanan et al., 2015). As shown in Table 3, alkaloid, flavonoid, and phenol concentrations increased in both shoots and roots of varieties V1 and V2 at low and high NaCl concentrations. The increase was more pronounced under high NaCl stress, with roots showing a greater accumulation than shoots. Treatment with KNO_3 alone or combined with different salt concentrations led to a slight decrease in phytochemical levels in both varieties, indicating that potassium reduces ROS and enhances antioxidant defenses (Hasanuzzaman et al., 2018; Ruiz and Blumwald, 2002). Although the combination of high salt and KNO_3 reduced phytochemical accumulation compared to salt alone, it did not completely inhibit their increase. Interestingly, V2 showed higher flavonoid and phenolic content than V1 under stress, potentially preventing excessive buildup seen in V1. Overall, these results demonstrate that potassium nitrate significantly influences phytochemical levels in response to salinity stress, and these compounds serve dual roles as protective agents and stress indicators (Harborne, 1998; Singleton and Rossi, 1965).

Table 3

Effect of different treatments on various secondary metabolites (Alkaloids, Flavonoids and Phenols in Shoot and Root) for two varieties (V1 and V2).

Parameters	Control	50mM NaCl	100mM NaCl	50mM KNO_3	50mM NaCl+50mM KNO_3	100mM NaCl+50mM KNO_3
Alkaloids in shoot (g/kg)V1	29±0.91	31.3±0.12	35.1±0.23	25.2±0.16	30.1±0.14	33.6±0.24
% Difference		7.93%	21.03%	-13.10%	3.79%	15.86%
Alkaloids in shoot (g/kg)V2	28.7.3±0.4	31.3±0.13	35.6±0.31	27.4±0.14	29.8±0.28	30.6±0.25
% Difference		9.06%	24.04%	- 4.53%	3.83%	6.62%
Alkaloids in root (g/kg)V1	17.33±0.13	24±0.24	28.66±0.09	14.66±0.13	20±0.23	22±0.24
% Difference		32.27%	49.27%	-16.69%	14.30%	23.74%
Alkaloids in root (g/kg)V2	17.33±0.14	24±0.23	29.3±0.10	15.33±0.15	19.6±0.23	23.6±0.25
% Difference		32.27%	51.34%	-12.24%	12.59%	30.63%
Flavonoids in shoot (g/kg)V1	354±3.4	356.7±5.5	360.8±5.2	350.6 ±3.7	355.3 ±3.3	360.6 ±4.1
% Difference		0.76%	1.92%	-0.96%	0.37%	1.86%
Flavonoids in shoot (g/kg)V2	369.4±3.2	370.0±4.3	381.9%4.2	367.5 ±4.0	369.1 ±3.6	371.7 ±4.2
% Difference		0.16%	3.38%	-0.51%	0.08%	0.62%
Flavonoids in root (g/kg)V1	97.1±1.5	94.3±0.8	82.6± 1.2	97.5±1.5	95.1±0.8	90.4±1.5
% Difference		2.88%	14.93%	-0.41%	2.06%	6.90%
Flavonoids in root (g/kg) V2	99.9±1.1	97.6±1.5	84.5±0.6	99.8±0.9	97.5±1.7	92.4±0.8
% Difference		2.30%	15.42%	-0.10%	2.40%	7.51%
Phenolics in shoot (g/kg)V1	20.9±0.26	28.34±0.51	38.01±1.2	19.03±0.36	23.4±0.61	32.9±0.85
% Difference		31.21%	58.08%	-9.36%	11.28%	44.60%
Phenolics in shoot (g/kg)V2	21.2±0.45	29.3±1.04	37.3±1.5	18.07±0.42	23.06±0.56	31.8±0.96
% Difference		32.07%	55.04%	-15.94%	8.40%	40%
Phenolics in root (g/kg)V1	4.77±0.33	8.68±0.62	12.5±0.65	3.76±0.15	6.27±0.54	9.2±1.76
% Difference		56%	89.5%	-23.68%	27.17%	63.42%
Phenolics in root (g/kg) V2	5.37±0.45	9.0±1.66	12.3±0.91	3.6±0.21	6.2±0.31	9.5±0.99
% Difference		69.25%	95.14%	-19.32%	34.62%	73.9%

CONCLUSION

This study demonstrates that the application of Potassium Nitrate (KNO_3) within the rhizosphere mitigates the adverse effects of salinity on *Brassica campestris* by

enhancing morphological attributes, reproductive performance, and the synthesis of stress-related secondary metabolites. While it does not provide a comprehensive solution to salinity stress, KNO_3 represents a pragmatic and viable approach for augmenting crop productivity under saline conditions. Salinity reduced the growth parameters like fresh and dry weight and lengths of shoot and root. But application of KNO_3 alone and along with salt reduced the effect of salinity and made the plant grow better. Yield parameters which were count of branches, seeds and siliquae and dry weight of seeds and siliquae were also decreased due to salt stress and the application of KNO_3 improved all the yield traits and increased the yield. Secondary metabolites increased as the salt concentration increased. To protect the plant from the harmful effects of salt stress, that increase was made. The level of various secondary metabolites dropped after KNO_3 therapy was applied because it lessened the effects of salinity stress, which meant that the secondary metabolites were no longer required and returned to normal. Although not entirely eliminated, the impacts of salinity on growth, yield, and secondary metabolites were reduced to a higher degree. Therefore, farmers may utilize it to address the salinity issue.

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