MITIGATING SALINITY STRESS IN MUNG BEAN [*Vigna radiate* (L.) R. WILCZEK] USING POTASSIUM NITRATE

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Abstract. Soil salinity is one of the serious environmental threat causing significant reduction in crop growth and productivity in different parts of the globe. *Vigna radiata* (L.) R. Wilczek is considered sensitive to salt stress. Potassium (K^+), a major essential nutrient of plants ensures growth and quality of crops under salinity stress. In view of that, experiments were designed to assess the impact of various doses of potassium nitrate (KNO_3) application (K_1- 0.24 g, K_2- 0.40 g and K_3- 0.48 g) under three salinity levels (S_1- 50 mM, S_2- 75 mM, and S_3- 100 mM) of NaCl on growth attributes, physiological and biochemical traits, antioxidant activities of mungbean variety (MH 2-15). Salinity stress had adverse effects on the growth and physiological responses of *V. radiata*. Plants treated with KNO_3 (0.48 g) under 50 mM salt stress produced the best growth characteristics *viz.* length of shoot and root, fresh and dry weight of shoot and root, while the least performance in respect of above traits were observed without KNO_3 under stressful conditions (at 100 mM). Application of KNO_3 significantly improved various biochemical components such as different photosynthetic pigments, proline and total soluble proteins contents, thereby restoring the activity of various antioxidant enzymes counteract the oxidative stress under salinity stress. The results revealed that the adequate application of KNO_3 not only improves the performance of all traits but also diminish the negative effect of salt stress and contributes to accomplishing sustainable productivity.

Key words: mitigation; antioxidant enzymes; mungbean (MH 2-15); potassium nitrate; salt tolerance.

INTRODUCTION

Pulses are widely recognized for being a valuable source of high-quality proteins, essential for fulfilling the nutritional requirements of the rapidly expanding global population. They are also regarded as alternatives to animal proteins and hold a special position in the vegetarian diet. Their role in enhancing soil health is significant as they host *Rhizobia* in their root system, allowing plants to independently fulfil their nitrogen needs by converting atmospheric nitrogen, consequently decreasing reliance on chemical fertilizers [40].

Vigna radiata (L.) Wilczek, also referred to as mungbean and green gram, is a short-term leguminous pulse crop widely cultivated in dry land agriculture during the summer season due to its high-quality seed protein that is easily digestible. With a protein content of 23%, along with phosphorus, carbohydrates, minerals, and provitamin A, it plays a vital role in the Indian diet [23]. In terms of production in India, it holds the third position after chickpea and pigeonpea. Additionally, it serves as effective green manure and can be utilized as a cover crop before or after cereal crops. Furthermore, it can be used as fodder, and even the seed husk can be soaked in water to serve as cattle feed [25, 37, 38].

Soil salinity is a critical problem for mungbean production in many countries due to its impact on various plant processes. Excessive salts in the soil solution or irrigation water currently affect about twenty percent of the world's cultivated land, with this number expected to rise to fifty percent by 2050, particularly in arid and semiarid regions [39]. The accumulation of sodium in the soil can lead to decreased soil porosity, reduced soil aeration, and impaired water conductance, resulting in physiological drought conditions that hinder plant water absorption [18]. The presence of high salt concentration causes osmotic and ionic stresses, which disrupt the normal uptake of nutrients and lead to the generation of reactive oxygen species (ROS), causing a decrease in photosynthetic performance [20]. This initially results in reduced plant growth, dry weight, and chlorophyll content, ultimately impacting the productivity and quality of crops by affecting various bio-physiological activities. Additionally, it interferes with essential nutrient levels (N, P, K, S) and N_2 fixation in legumes [13, 14].

However, the degree of salinity tolerance also depends plant cultivar and appropriate fertilizer management. Utilization of salt-tolerant crops does not eliminate salt but they also accumulate salts. Alternative agronomic practices, such as phytoremediation for salt removal, are expensive and labor-intensive [19]. The external application of different potassium compounds like potassium nitrate (KNO_3) in crop plants has emerged as another significant strategy for mitigating salinity stress. Potassium, an essential nutrient, significantly contributes to enhancing plant tolerance to saline conditions by averting cellular damage [31]. Nitrate is a critical component of plant nutrition, serving as a primary source of nitrogen for plant growth and development. It not only increases protein content in plants and also improves plant tolerance to stress [6]. But it is also essential to optimize nitrate supply and monitor nitrate accumulation in seeds to ensure plant nutrition benefits while minimizing potential health risks especially for infants and young children, due to their immature digestive systems and higher water intake relative to their body weight [10].

Considering the importance of mung bean as a source of high protein, the present study was aimed to

assess the impact of varying salinity levels on various traits of the *Vigna radiata* (L.) Wilczek variety (MH 2-15). To counter the effects of salinity stress on plant characteristics, KNO_3 treatments were also applied.

MATERIALS AND METHODS

Experimental setup: The seeds of *V. radiata* (variety MH2-15) were obtained from Chaudhary Charan Singh Haryana Agricultural University, Hisar (India). The healthy seeds were surface sterilized with 0.01% (v/v) mercuric chloride ($HgCl_2$) solution for 2-5 minutes followed by washing with double distilled water (DDW). A pot experiment was conducted at Department of Botany, Kurukshetra University, Kurukshetra, Haryana (29°57'32.0"N 76°48'46.5"E) under anti-insect net house during the summer season (April, 2022-2023). The soil characteristics were as follows: total N, 0.042%; available P, 0.017%; and organic carbon, 0.06%. Earthen pots (25 × 25 cm) were filled with mixture of soil with farmyard manure (6:1) were organized in a simple randomized block design (RBD). The experiment was classified into thirteen groups, each group include five replicates and seven seeds were sown at 2 cm depth in each pot. For maintaining 5 plants of uniform size in each pot thinning was done after 15 days of sowing (DAS). Sodium chloride (NaCl) solution at three different concentrations S_1= 50 mM, S_2= 75mM, S_3= 100 mM was applied to the pots after sowing. The control group was without salt stress i.e. irrigated with only normal water up to maturity. The salt treated plant were subjected with three different KNO_3 treatment i.e. K_1= 0.24 g, K_2= 0.40 g, and K_3= 0.48 g) as given in Table 1. No potassium was added to the control group.

Table 1. Treatment schedule of KNO_3 and NaCl

Treatment	Code	Details
T1	C	Control (only irrigation with tap water)
T2	S_1	NaCl (50 mM)
T3	K_1+S_1	KNO_3 (0.24 g) + NaCl (50 mM)
T4	K_2+S_1	KNO_3 (0.40 g) + NaCl (50 mM)
T5	K_3+S_1	KNO_3 (0.48 g) + NaCl (50 mM)
T6	S_2	NaCl (75 mM)
T7	K_1+S_2	KNO_3 (0.24 g) + NaCl (75 mM)
T8	K_2+S_2	KNO_3 (0.40 g) + NaCl (75 mM)
T9	K_3+S_2	KNO_3 (0.48 g) + NaCl (75 mM)
T10	S_3	NaCl (100 mM)
T11	K_1+S_3	KNO_3 (0.24 g) + NaCl (100 mM)
T12	K_2+S_3	KNO_3 (0.40 g) + NaCl (100 mM)
T13	K_3+S_3	KNO_3 (0.48 g) + NaCl (100 mM)

A pot experiment was conducted to study the effects of potassium nitrate on growth, photosynthetic pigments, biochemical parameters and antioxidant activities of mungbean [*Vigna radiata* (L.) Wilczek] variety (MH2-15) under different level of NaCl.

Growth and morphological parameters. The plants under each treatment were uprooted carefully from the pots and washed with distilled water to remove the sand adhering to roots. The measurements of the plant's height (cm), root length (cm) and number of branches per plant were recorded. After that, the shoots were separated from the roots and wrapped in filter paper in order to remove moisture from their surface. Then these were placed on the digital balance for the calculation of fresh weights. The shoot and root systems were dried in labelled paper envelopes in an oven at 60 °C till a constant weight was attained and then the weight of the dried plants was calculated by a digital scale and expressed as gram plant^{-1}.

Biochemical characteristics. The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (mg g^{-1} FW) were determined in primary leaves using standard equations given by Arnon [3]. Grind 0.1 g of fresh leaf sample using a mortar and pestle using small quantity pre-chilled acetone solution (80%) with a pinch of $CaCO_3$ and kept under dark conditions. The absorbance was read spectrophotometrically at 663, 645 and 480 nm in UV-Spectrophotometer (Systronics 2205, India). Total chlorophyll equals the sum of chlorophylls *a* and *b*.

The estimation of total soluble protein (TSP) content (mg·g^{-1} FW) was measured using the Bradford Assay [8] and quantification was carried out in a UV-Vis spectrophotometer. In this experiment 100 mg of fresh leaf sample from each treatment were taken and warmed in 80% ethanol (10 mL) in a hot waterbath for 2-5 minutes. It was then cooled at 25 °C and homogenized with ethanol and centrifuged at 5000 rpm for about five minutes. The residue was then re-extracted with 5 mL of perchloric acid (5%) and then centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the residue was re-extracted with 5 mL of 1 N NaOH by keeping it in warm water at 40-50 °C for 20-30 minutes.

The accumulation of proline was assessed using the procedure outlined by Bates *et al.* [7]. Take 0.5 mL of aliquot and add 1 mL of ninhydrin acid and and 1 mL of glacial acetic acid, and incubated for one hour in boiling water at 100 °C, reaction. After cooling, the blend was then disturbed rigorously by applying toluene (3 mL) to the cyclomixer. The coloured layer was isolated from the aqueous phase and the organic phase was read at 520 nm. Proline content was expressed as μmol·g^{-1} FW.

The extent of lipid peroxidation was measured in terms of malondialdehyde (MDA) content according to Heath and Packer [22]. Leaf sample (200 mg) was homogenized in 3 mL of 50 mM phosphate buffer (ph 7.0) and then centrifuge at 15000 rpm for 15 minutes. Then, 2 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA solution was added to 0.5 mL of remaining supernatant. The mixture was heated at 90 °C for 30 minutes on the water bath, followed by rapid cooling in an ice bath. The absorbance of the supernatant was recorded spectrophotometrically at 532 nm. The value for nonspecific absorption of each sample at 600 nm was also recorded and subtracted from the absorbance recorded at 532 nm. MDA concentration was estimated with 155 mM^{-1}·cm^{-1} extinction coefficient.

Antioxidant enzymes. Superoxidase dismutase (SOD) activity was analysed by the method of

Giannopolitis and Ries [11]. Fresh leaves (50 mg) were crushed in 2 mL of 0.1 M EDTA-phosphate buffer (pH 7.8) and centrifuged at 15000 rpm. The supernatant was used as crude extract. The reaction mixture was prepared by adding 0.1 mL of crude extract followed by 0.9 mL of double distilled water (DDW), accompanied by 0.5 mL of 300 mM Na_2CO_3 (pH 10.2), 0.5 mL of 378 µM p-nitrobluetetrazolium chloride (NBT), 0.5 mL of 78 mM L-methionine and 0.5 mL of 7.8 µM riboflavin to get a total reaction mixture. The reaction was carried out for 15 minutes under fluorescent light (100 µmol photon $m^{-2} \cdot s^{-1}$ PFD) at 25 °C. The initial reaction rate was determined by measuring the difference in absorbance (560 nm) between the reaction mixture with and without the extract. SOD activity was calculated as the amount of enzyme required to inhibit the reaction by 50%.

The method of Maehly [27] was used for evaluating Peroxidase (POD) activity. Plant material (0.1 g) was homogenized with 2 mL of ice-cold distilled water, centrifuged at 6000 rpm for 10 minutes, and the supernatant used as the enzyme source. The reaction mixture (2 mL enzyme source + 2 mL phosphate buffer, pH 7.0) was incubated for 10 minutes, and absorbance recorded at 420 nm. Protein was estimated using Bradford's method (1976), and POD activity expressed as $mol \cdot mg^{-1}$ protein.

Catalase (CAT) activity was analysed by using the Aebi method [1]. The same supernatant used for APOX activity was used as the crude extract. The reaction mixture consisted of 30 µL plant extract, 1.2 mL 150 mM H_2O_2, and 1.5 ml 50 mM HEPES (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid) buffer. A blank mixture without enzyme was prepared by replacing the plant extract with 50 µL of 50 mM HEPES buffer. Absorbance change was recorded at 490 nm using a UV-VIS spectrophotometer. Catalase activity was expressed as $mol \cdot mg^{-1}$ protein $\cdot min^{-1}$. Protein was estimated from the same extract by Bradford [8].

Ascorbate peroxidase (APX) activity was determined by using the method of Janda *et al.* [24]. Plant leaf material (100 mg) was crushed in 1 mL of 100 mM HEPES/NaOH buffer (pH 7.6) containing 8.8 mg ascorbic acid. The supernatant was obtained by centrifuging at 10,000 rpm for 5 minutes at 4 °C, and used as the crude extract. The reaction mixture consisted of 50 µL plant extract and 100 µL 3 mM hydrogen peroxide, with a total volume of 1200 µL. A blank mixture without enzyme was prepared by replacing the plant extract with 50 µL 50 mM HEPES buffer. Absorbance change was recorded at 290 nm using a UV-Vis spectrophotometer. Specific activity of APOX was expressed as $mol \cdot mg^{-1}$ protein $\cdot min^{-1}$.

Statistical Analysis. From every replication, a mean of three reading was taken. Statistical analysis was done using SPSS (ver. 16, Chicago, IL). Data were analyzed for significance using one-way and two-way analysis of variance (ANOVA). Differences between the mean values were evaluated using Duncan's multiple range test (DMRT) at $P \leq 0.05$.

RESULTS

Salt stress poses a significant challenge, strongly impacting most of the physiological and biochemical pathways within plants. According to the results, there were significant differences ($P < 0.05$) among the effects of salt stress and potassium nitrate, which led to changes in different plant growth characteristics, photosynthetic pigments, biochemical attributes including enzymatic antioxidants.

Growth parameters. Plant height serves as a significant quantitative trait that can influence crop yield. These results presented in Table 2. showed that shoot and root length of mungbean usually decreases as a consequence of the osmotic extraction of intracellular water subjected to varying levels of salt stress. Of the different salt treatments (50, 75, and 100 mM), the highest NaCl concentration (100 mM) resulted in the most substantial reduction in the shoot and root length of the plants. The application of potassium nitrate mitigated the destructive effects of sodium chloride. The results indicated that the highest shoot length (21.10 cm) was achieved with the K_3+S_1 application, followed by K_3+S_2 (19.03 cm), while the lowest shoot length (17.50 cm) was observed in the K_3+S_3 treatment. The root length of the crop also increased with potassium treatment at various salt concentrations, and K_3+S_1, K_2+S_1, and K_3+S_2 showed better results compared to the control.

The experimental treatments resulted in varying interactions that impacted the fresh and dry weights of the shoot of the mungbean variety (MH2-15) (Table 2). Among the treatments, the unstressed plants treated with 0.48 g potassium concentration at 100 mM NaCl exhibited the highest fresh and dry shoot weights compared to the control. Conversely, there was minimal increase exhibited by K_1 at all salinity levels at 45 DAS. The fresh and dry weight of the root decreased gradually as the salt concentration increased. However, using potassium nitrate led to an increase in both the fresh and dry weight of the root. The most significant increase in both fresh and dry weight of the root was observed in the K_3+S_1 treatment, followed by K_3, especially at 75- and 100-mM salt treatments (Table 2).

Photosynthetic pigments. The rate of photosynthesis depends on the amount of chlorophyll content in plants. The results indicated that as the NaCl concentration increased, there was a significant decline in the levels of various photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a*+*b*), and carotenoids), with the highest reduction occurring at the S_3 level (Table 3). Plants treated with KNO_3 treatment exhibited an increased levels of chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents even under conditions of severe salinity stress. The highest chlorophyll a content was observed with the K_3+S_1

treatment, followed by K_2+S_1 and K_3+S_2. Similarly, the K_3 treatment at 50 mM salinity level exhibited the highest value for chlorophyll *b*, followed by K_2, and K_3 at 75mM. The total chlorophyll content exhibited a significant increase with potassium nitrate treatment at K_3+S_1, followed by K_2+S_1 and K_3+S_2. The addition of potassium combined with salt also showed significant results in terms of carotenoid concentration. Carotenoid levels gradually increased as the stress levels increased. Plants treated with KNO_3 showed comparatively less increase in carotenoid content (Table 3).

Biochemical Parameters. The data related to the total soluble protein, proline and lipid peroxidation of mungbean (MH2-15) under both control and salt stress conditions are given in Table 4. There was a significant decrease in total soluble protein due to salt stress. The most significant reduction occurred in saline conditions without the application of KNO_3. In general, the K_3+S_2 treatment showed the best performance, followed by K_3+S_1 and K_3+S_3.

The proline content was higher in plants exposed to salinity compared to the control, as shown in Table 4. Non-stressed plants had the lowest proline content, while the highest level was observed in response to 100 mM salinity followed by S_2 treatment. KNO_3 application helped regulate proline content and enhance salt stress tolerance. Compared to the control, there was a slight increase in proline content at S_1 (by approximately 37.32%), S_2 (by 39%), and S_3 (by 39.25%).

The application of KNO_3 and subsequent exposure to NaCl led to a decrease in lipid peroxidation and the maintenance of membrane integrity, as indicated by lower MDA levels. The MDA content significantly increased with NaCl treatment compared to the control in this study, and the highest lipid peroxidation occurred with the S_3 (100 Mm NaCl) treatment (Table 4). Higher potassium concentrations counteracted the negative effects of severe salt stress and led to a significant decrease in MDA levels, with the most pronounced effect seen in the K_3+S_3 treatment, followed by K_3+S_2 and K_3+S_1.

Table 2. Effect of KNO_3 treatment on growth parameters of mungbean (MH2-15) grown under different level of salt stress at 45 DAS (days after sowing).

Treatments	Shoot Length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	No. of branches/plant
C	23.56±0.218^{a}	6.5±0.866^{ef}	0.479±0.001^{b}	0.181±0.001^{b}	0.227±0.017^{b}	0.083±0.005^{a}	6.76±0.240^{a}
S_1	19.03±0.466^{cd}	5.7±0.435^{g}	0.405±0.003^{e}	0.163±0.003^{e}	0.214±0.002^{bc}	0.051±0.002^{gf}	5.30±0.115^{ed}
K_1+S_1	19.33±0.202^{bcd}	6.0±2.227^{ed}	0.445±0.006^{c}	0.173±0.003^{c}	0.227±0.004^{b}	0.057±0.003d^{ef}	5.50±0.231d^{ef}
K_2+S_1	20.50±0.251^{bc}	7.2±2.088^{b}	0.471±0.004^{b}	0.181±0.002^{b}	0.245±0.003^{b}	0.067±0.004^{cde}	6.03±0.176^{b}
K_3+S_1	21.10±0.251^{b}	10.6±1.417^{a}	0.503±0.003^{a}	0.191±0.006^{a}	0.287±0.015^{a}	0.088±0.004^{a}	6.43±0.120^{a}
S_2	17.53±0.296^{de}	6.3±0.701^{h}	0.345±0.002^{i}	0.147±0.002^{f}	0.101±0.006^{f}	0.041±0.005^{gh}	4.33±0.202^{gh}
K_1+S_2	17.70±0.461^{de}	7.1±1.178^{fg}	0.379±0.001^{g}	0.159±0.002^{d}	0.121±0.006ϵ^{f}	0.047±0.004f^{gh}	4.76±0.133^{ef}
K_2+S_2	18.20±0.351^{de}	8.1±0.556^{de}	0.394±0.003^{f}	0.173±0.005^{c}	0.173±0.004^{cd}	0.055±0.006^{efg}	4.90±0.450^{efg}
K_3+S_2	19.03±0.437^{cd}	8.7±0.173^{c}	0.416±0.001^{d}	0.187±0.002^{a}	0.314±0.023^{a}	0.079±0.205^{abc}	5.70±0.115^{b}
S_3	14.13±1.351^{g}	4.6±1.311^{h}	0.291±0.002^{k}	0.123±0.002^{h}	0.095±0.004^{f}	0.034±0.026^{h}	3.40±0.173^{i}
K_1+S_3	15.16±0.726^{fg}	6.2±0.556^{h}	0.315±0.002^{j}	0.136±0.003^{g}	0.143±0.033^{de}	0.048±0.003^{fgh}	4.10±0.115^{h}
K_2+S_3	16.66±0.440^{de}	7.3±0.854^{g}	0.341±0.005^{i}	0.144±0.003^{f}	0.145±0.003^{de}	0.057±0.004^{def}	4.76±0.088^{fg}
K_3+S_3	17.50±0.346^{ef}	8.1±0.173^{ef}	0.364±0.003^{h}	0.153±0.002^{e}	0.199±0.016^{bc}	0.071±0.001^{bcd}	4.90±0.115^{efg}
F value $_{(12,26)}$	18.985	16.758	400.765	127.237	23.691	12.917	10.447
Salinity	67.468	2.982	0.011	0.005	0.026	0.002	9.077
Treatment	9.751	21.622	0.046	0.002	0.028	0.002	2.961
Salinity×treatment	1.269	3.785	0.001	5.800	0.008	4.783	0.231

C-control, S_1.50 mM NaCl, S_2-75 mM NaCl, S_3, 100 mM NaCl, K_1- 0.24g, K_2-0.40g, K_3-0.48g.
Mean value followed by different letter within a column do not differ significantly according to Duncan's multiple range test (P<0.05)

Table 3. Effect of KNO_3 treatment on photosynthetic pigments of mungbean (MH2-15) grown under different level of salt stress at 45 DAS.

Treatments	Parameters (mg × g^{-1} FW)			
	Chlorophyll-*a*	Chlorophyll-*b*	Total Chlorophyll	Carotenoids
C	0.763±0.007^{a}	0.425±0.008^{c}	1.188±0.009^{b}	0.622±0.001^{d}
S_1	0.381±0.003^{e}	0.271±0.003^{e}	0.655±0.004^{e}	0.611±0.001^{f}
K_1+S_1	0.431±0.004^{d}	0.292±0.002^{d}	0.729±0.009^{d}	0.643±0.002^{c}
K_2+S_1	0.647±0.006^{b}	0.437±0.003^{b}	1.084±0.006^{c}	0.655±0.002^{b}
K_3+S_1	0.769±0.007^{a}	0.508±0.002^{a}	1.277±0.009a	0.666±0.001^{a}
S_2	0.283±0.003^{h}	0.155±0.005^{g}	0.442±0.002^{h}	0.531±0.001^{i}
K_1+S_2	0.308±0.004^{g}	0.211±0.004^{f}	0.519±0.007^{g}	0.544±0.001^{h}
K_2+S_2	0.341±0.007^{f}	0.258±0.001^{e}	0.598±0.006^{f}	0.561±0.002^{g}
K_3+S_2	0.451±0.007^{c}	0.267±0.001^{e}	0.719±0.007^{d}	0.575±0.001^{gf}
S_3	0.149±0.004^{l}	0.089±0.006^{j}	0.242±0.008^{l}	0.405±0.002^{m}
K_1+S_3	0.186±0.003^{k}	0.107±0.003^{i}	0.293±0.006^{k}	0.422±0.001^{l}
K_2+S_3	0.211±0.004^{j}	0.117±0.001^{i}	0.328±0.004^{j}	0.435±0.007^{k}
K_3+S_3	0.242±0.006^{i}	0.132±0.003^{h}	0.376±0.007^{i}	0.464±0.002^{j}
F value $_{(12,26)}$	1.330	1.083	2.336	1.048
Salinity	0.438	0.191	1.204	0.098
Treatment	0.082	0.032	0.217	0.003
Salinity×treatment	0.034	0.016	0.094	0.001

C-control, S_1.50 mM NaCl, S_2-75 mM NaCl, S_3, 100 mM NaCl, K_1-0.24g, K_2-0.40g, K_3-0.48g
Mean value followed by different letter within a column do not differ significantly according to Duncan's multiple range test (P<0.05)

Table 4. Effect of KNO_3 treatment on biochemical parameters of mungbean (MH2-15) grown under different levels of salt stress at 45 DAS.

Treatments	Biological Parameters		
	TSP (mg · g^{-1} FW)	MDA (nmol·g^{-1} FW)	Proline (µmol·g^{-1} FW)
C	9.06±0.024^{a}	1.78±0.020j	3.79±0.030^{m}
S_1	6.19±0.015^{h}	1.99±0.010^{i}	4.95±0.022^{h}
K_1+S_1	6.86±0.020^{g}	2.21±0.002h	4.78±0.018^{j}
K_2+S_1	7.85±0.024^{e}	2.23±0.030g	4.58±0.037^{k}
K_3+S_1	8.33±0.026^{b}	2.84±0.013b	3.96±0.039^{l}
S_2	5.62±0.037^{j}	2.33±0.002^{i}	5.52±0.026^{e}
K_1+S_2	6.07±0.034^{i}	2.47±0.003^{g}	5.36±0.027^{f}
K_2+S_2	7.18±0.029^{g}	2.55±0.005e	5.17±0.014^{g}
K_3+S_2	8.02±0.026^{d}	2.92±0.031a	4.86±0.026^{i}
S_3	5.22±0.026^{k}	2.57±0.001e	7.43±0.034^{a}
K_1+S_3	6.22±0.018^{h}	2.62±0.002d	6.96±0.011^{c}
K_2+S_3	6.79±0.023^{g}	2.73±0.009^{c}	6.41±0.015^{d}
K_3+S_3	7.47±0.032^{e}	2.93±0.006a	5.83±0.032^{e}
F value$_{(12,26)}$	1.789	1.574	576.030
Salinity	10.198	0.520	12.685
Treatment	8.873	0.597	1.950
Salinity×treatment	0.169	0.070	0.287

C-control, S_1.50 mM NaCl, S_2-75 mM NaCl, S_3, 100 mM NaCl, K_1-0.24g, K_2-0.40g, K_3-0.48g
Mean value followed by different letter within a column do not differ significantly according to Duncan's multiple range test (P<0.05)

Antioxidant Enzymes. The activity of antioxidant enzymes can be one of the important indicators that help to determine the tolerance of plants to salt stress conditions. Under control conditions, the activity of different antioxidant enzymes (SOD, POD, APX, and CAT) in mungbean leaf showed suppressed levels compared to other treatments (Figs. 1-4). Our findings revealed that KNO_3 had the maximum positive on the activity of antioxidant enzymes at varying levels of salt stress. The plant treated with 0.48g of KNO_3 at 100 mM salinity level showed the highest activity of different antioxidant enzymes. The application of KNO_3 (K_3 treatment) increased the activity of salt stressed SOD by 63%, 60.62%, and 59.78%; POD by approximately 52.92%, 43.55%, and 33.68%; APX by about 52.91%, 48%, and 43.65%; and CAT by approximately 74.71%, 71.76%, and 60.99% at salinity levels of 100 mM, 75 mM, and 50 mM, respectively followed by K_2 (Figs. 1-4).

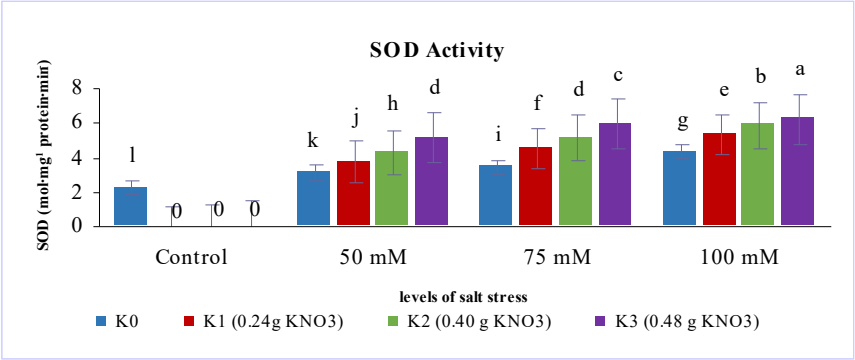

Figure 1. Effect of KNO_3 treatment on SOD activity of mungbean (MH2-15) grown under different level of salt stress at 45 DAS. (values are means ± SE of three replicates). Mean values followed by different letters are significantly different at $p \leq 0.05$ according to DMRT test. Vertical bars represent means ± SD ($n = 3$).

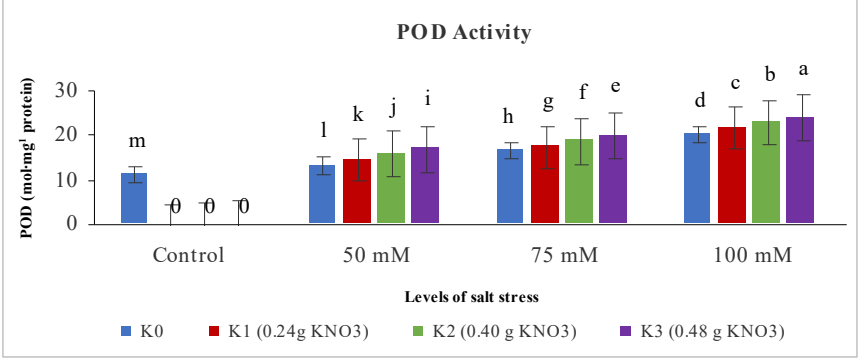

Figure 2. Effect of KNO_3 on POD activity of mungbean (MH2-15) grown under different level of salt stress at 45 DAS. (values are means ± SE of three replicates). Mean values followed by different letters are significantly different at $p \leq 0.05$ according to DMRT test. Vertical bars represent means ± SD ($n = 3$).

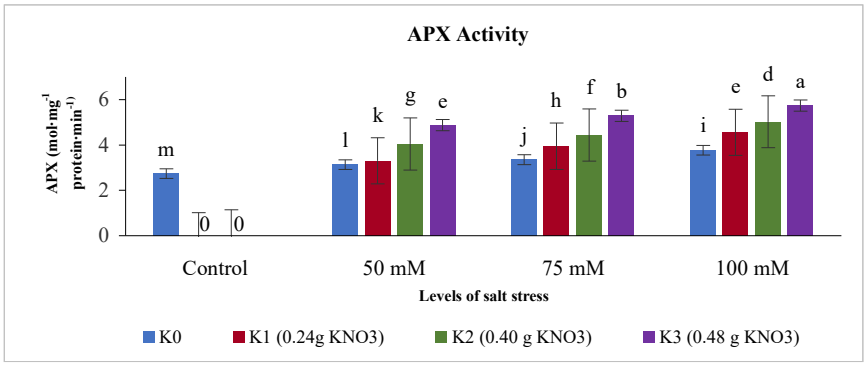

Figure 3. Effect of KNO_3 on APX activity of mungbean (MH2-15) grown under different level of salt stress at 45 DAS. (values are means ± SE of three replicates). Mean values followed by different letters are significantly different at $p \leq 0.05$ according to DMRT test. Vertical bars represent means ± SD ($n = 3$).

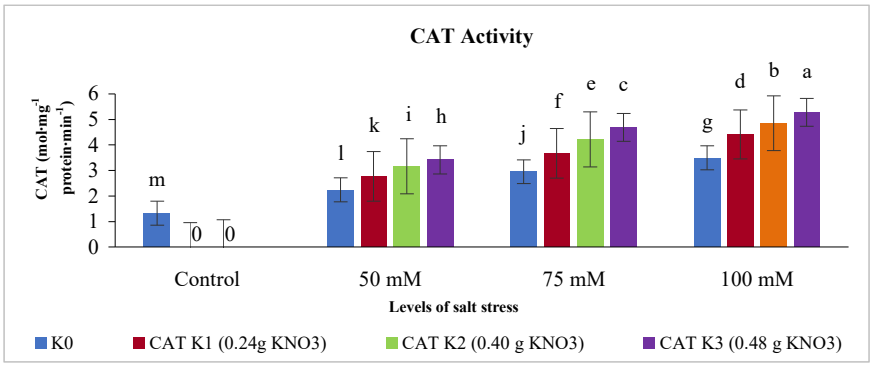

Figure 4. Effect of KNO_3 on CAT activity of mungbean (MH2-15) grown under different level of salt stress at 45 DAS. (values are means ± SE of three replicates). Mean values followed by different letters are significantly different at $p \leq 0.05$ according to DMRT test. Vertical bars represent means ± SD ($n = 3$).

DISCUSSION

The presence of elevated levels of salt in the soil hinders the ability of plant roots to absorb water, thus disrupting the plant's water balance. It is evident that soil salinity has detrimental effects on all morphological and other growth-related characteristics [39]. Increased concentrations of NaCl are harmful to plant cells and result in damage to the cellular membrane, potentially causing ion loss. These alterations were associated with a reduction in relative water content and K^+ concentration [2]. The similarities in physicochemical properties and common transport mechanism between Na^+ and K^+ could lead to competition for major binding sites in key metabolic processes. As a result, Na^+ could take over K^+ binding sites led to a disruption in cellular ionic balance, resulting in increased oxidative stress and membrane damage, as evidenced by the increased levels of MDA and electrolyte leakage. This ionic imbalance may have contributed to the observed decline in chlorophyll content, likely due to the degradation of chlorophylls within the chloroplasts. These findings align with previous results that also confirmed that this condition ultimately causes a significant reduction in cytosolic K^+ levels, thereby hindering plant growth attributes [5].

The roots' ability to absorb nutrients is compromised by excessive salinity stress, which in turn disrupts the plant's metabolic and cellular functions. Studies indicate that maintaining cell turgor and osmotic balance in salt-stressed plants relies significantly on K^+ ions [33, 34, 42]. Furthermore, high levels of Na^+ and Cl^- ions in salt-stressed plants can also interfere with the absorption and processing of other essential nutrients. The decrease in plant biomass under severe salinity stress may be attributed to limitations in nitrate assimilation, caused by reduced activity of the cytosolic NADH nitrate reductase enzyme, which is a key regulatory step in the nitrate assimilation pathway [28]. Applying appropriate concentrations of KNO_3 can lead to the mitigation of Na accumulation, which otherwise leads to decrease in MDA and electrolyte leakage. Qu *et al.* [35, 36] also noted that a deficiency of K^+ significantly inhibited nitrogen and photosynthetic carbon assimilation and disrupts the light reaction pathways of PS I and PS II in maize under salt stress. The photosynthetic rate of plants is a critical factor for growth and final biomass production. Salinity negatively affects essential physiological characteristics of plants, such as leaf chlorophyll content and stomatal activity, leading to increased necrosis and chlorosis [15].

When salt stress persists, it leads to oxidative damage caused by reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radical, ion toxicity, and K-deficiency. The excessive production of ROS in chloroplasts increases lipid peroxidation, protein breakdown, and DNA mutation.

A low K^+ status may stimulates the accumulation of ROS and related cell damage under saline conditions, leading to programmed cell death [26].

Using potassium nitrate in combination with NaCl has been found to enhance the ability of plants to tolerate salt stress by improving potassium absorption. It is involved in the metabolism of carbohydrates, the production of proteins, and the activation of enzymes. Enzymes are a type of protein, and the production of proteins relies on efficient nitrogen metabolism, which is disrupted by high salinity [41]. Applying potassium using appropriate methods facilitates the photosynthesis process and contributes to chlorophyll synthesis and stomata movement. Increased rates of CO_2 assimilation can lead to enhanced growth and higher crop yield. Potassium is also crucial for moving photoassimilates during root growth. The negative impacts of NaCl stress were significantly reduced with the use of K_2SO_4, which promotes the production of organic osmolytes and reduces the levels of Na^+ and Cl^- while increasing the levels of K^+. Moreover, excessive production of reactive oxygen species (ROS) can be regulated by applying potassium, which helps to maintain electron transport and photosynthetic efficiency, thereby reducing oxidative stress. Additionally, potassium can mitigate the effects of NADPH oxidase, a key enzyme involved in ROS production, thereby protecting plants from oxidative damage [29].

The tolerance of plants to salt stress conditions can be assessed by examining the activity of antioxidant enzymes. Plants have established a defense system against oxidative stress to neutralize reactive oxygen species (ROS), which involves non-enzymatic antioxidants such as tocopherols and carotenoids, as well as enzymatic antioxidants like SOD, POD, and CAT, to safeguard cell structures and organelles from ROS during salt stress [17, 20]. The application of potassium was found to enhance the scavenging of ROS through the improvement of SOD, CAT, and GPX activities in the scavenging system. Applying the appropriate quantity of KNO_3 effectively boosted the activity of antioxidant enzymes, thereby reducing the formation of reactive oxygen species in plant cells by improving the flow of electrons in photosynthesis. However, under K deficiency, the activity of NADPH oxidase increased significantly, leading to higher production of NADPH-dependent O_2 and the synthesis of ABA in the roots [18].

Potassium is also referred to as the "policeman nutrient" due to its interactive regulatory functions with other biomolecules. Over sixty enzymes, RuBisCO, sucrose phosphate synthase, α-amylase, starch synthase, invertase, phosphofructokinase, and pyruvate kinase, rely on K as a cofactor for activation, controlling essential metabolic processes in plants [16]. K^+ enhances the antioxidant system in plants, strengthening their growth under salinity stress. In response to salinity stress conditions, soluble sugars like fructose and glucose play a central role in osmotic adjustment in almost all plants. Researchers have repored that plants accumulate sucrose under drought or salinity stress [30]. K has a significant interactive relationship with various large biomolecules such as cellulose, starch, and protein. Amino acids like arginine, glycine, serine, alanine, leucine, valine, and proline also play a crucial role as a solute in osmotic adjustment of plant cells [4]. The primary response of plants under salinity stress is the accumulation of proline content as an osmoprotective agent and a radical scavenger in plant cells [21]. As a result, the number of small molecules like amino acids, organic acids, and amides are reduced in the cell while the biosynthesis of phenolic compounds is stimulated to counteract high levels of reactive oxygen species (ROS) and enhance tolerance to various environmental stresses. Stress leads to the accumulation of polyamines in plants at elevated levels, and these polyamines play a crucial role in regulating the plasma membrane K^+ channel of guard cells, which in turn modulates stomatal function [9]. The root systems of plants are able to detect changes in potassium availability in the soil. Additionally, research has shown that potassium is involved in various plant signaling pathways, aiding in defense mechanisms against stress through the activation of antioxidant systems [12]. The application of potassium presents an environmentally friendly, cost-efficient, and practical approach for mitigating salt stress [32]. The application of KNO_3 not only provides potassium but also increases the nitrate supply to plants. While nitrate is essential for plant growth, excessive levels can have detrimental effects on plant metabolism and human health. Elevated nitrate concentrations can disrupt the nitrate assimilation pathway, leading to the accumulation of toxic compounds. Moreover, excessive nitrate uptake can cause an imbalance in nutrient acquisition, potentially impacting plant growth and development. Therefore, careful management of nitrate supply through KNO_3 applications is crucial to avoid potential negative consequences [6, 10].

In conclusion, our results showed that KNO_3 (0.48 g) under 50 mM salt stress significantly enhanced growth parameters, pigment content, and antioxidant enzyme activity in mungbean plants under salt stress conditions. Moderate NaCl and optimal KNO_3 levels helped mitigate the adverse effects of salt stress by improving the K^+: Na^+ ratio, reducing oxidative stress, and promoting nitrate assimilation. These findings suggest that KNO_3 can be a valuable tool in alleviating salt stress in mungbean plants. Furthermore, the importance of careful nitrate management when using KNO_3 should be highlighted to avoid potential negative consequences. Overall, our results provide valuable insights into the development of effective strategies for evaluating and mitigating salinity stress in crops, thereby securing future food production.

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