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Barbara Hawrylak-Nowak;  
Department of Botany and Plant  
Physiology, University of Life  
Sciences in Lublin, Poland;  
<https://orcid.org/0000-0002-2935-1491>

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## ORIGINAL RESEARCH PAPER

# The efficacy of ammonium as seed priming agents for promoting tomato (*Solanum lycopersicum* L.) germination under salinity stress

Najam ul Sahar<sup>1</sup>, Nazakat Hussain Memon <sup>2\*</sup>,  
Muhammad Rafiq <sup>1\*</sup>, Majid Ali Abbasi <sup>2</sup>, Naseem Khatoon<sup>1</sup>,  
Arshad Hussain Laghari<sup>2</sup>, Dildar Ali Solangi<sup>3</sup>, Beena Naqvi<sup>4</sup>,  
Muhammad Idrees <sup>5</sup>

<sup>1</sup>Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro (76080), Sindh, Pakistan

<sup>2</sup>Department of Biochemistry, Ghulam Muhammad Mahar Medical College and Hospital, Sukkur @ SMBBMU Larkana, Sindh, Pakistan

<sup>3</sup>Department of Pathology, Diagnostic & Research Laboratory, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan

<sup>4</sup>PCSIR Laboratory Complexes, Karachi, Sindh, Pakistan

<sup>5</sup>College of Life Science, Neijiang Normal University, Neijiang 641000, Sichuan, PR China

\* To whom correspondence should be addressed. Emails: [nazakat.hussain@smbbmu.edu.pk](mailto:nazakat.hussain@smbbmu.edu.pk), [m.rafiq@usindh.edu.pk](mailto:m.rafiq@usindh.edu.pk)

## Abstract

The aim of the current study was to assess the effectiveness of  $\text{NH}_4^+$  priming to enhance tomato seed germination and plant growth under saline stress. In the absence of light at  $25 \pm 1$  °C, tomato seeds were primed with 50 and 100 mmol of  $\text{NH}_4\text{NO}_3$  and with 50 and 100 mmol of  $(\text{NH}_4)_2\text{SO}_4$  for 12 and 24 h, respectively. In the seed germination test, twenty primed seeds were used for each treatment, along with five replica plates and a control, and were all incubated at 25 °C with 25 mL of moistened water. In a second greenhouse experiment, primed seeds were planted in garden soil and watered with tap water, salt water, and a control. The concentration of saline water (50, 100, 150, and 200 mmol NaCl) was gradually raised after a 10-day break. The germination percentages in T5 (50 mmol  $(\text{NH}_4)_2\text{SO}_4$ , 12 hrs) were  $82 \pm 3.7\%$  and  $80 \pm 4.47\%$ , respectively, followed by T1 and T3 (50 mmol  $(\text{NH}_4)_2\text{SO}_4$ , 12 h and 100 mmol  $\text{NH}_4\text{NO}_3$ , 12 h). When compared to the unprimed tomato seeds, the  $\text{NH}_4^+$  priming with  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  improved plant height and other growth parameters. Furthermore, the chlorophyll and total flavonoid content were improved in both saline and non-saline treatments. In terms of salinity, the  $\text{NH}_4^+$  priming increased the proline content while decreasing the total protein content. It is concluded that further research will be needed to clarify the effect of  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  as  $\text{NH}_4^+$  priming in tomato plants because other factors and nutrition can play a role in seed germination and plant growth development.

## Keywords

abiotic stress; chlorophyll; growth parameters; NaCl; Nema cultivar; proline

## 1. Introduction

The most significant and susceptible phases of a crop cycle are seed germination and seedling emergence, as they require water, air, temperature, and light (Biswas et al., 2019). To obtain the amino acids and energy necessary for plant growth, a complex metabolic process oxidises the carbohydrates and lipids within the seed and separates protein molecules (Almansouri et al., 2001). Low quality of seed and planting

conditions affect crop productivity, as it influences seed germination and seedling development (Lamichhane et al., 2019). Poor germination and seedling establishment, which have an adverse effect on crop plant growth and development, are caused in part by soil salinity and are accountable for low agricultural productivity (Kooshkaki et al., 2023). In soils containing excessive sodium chloride, plant accessible water is limited, leading to partial hydration of the cell cytoplasm. The metabolism of cells and the activities of macromolecules are exaggerated by such plasmolysis, eventually leading to the termination of growth (Le Rudulier, 2005). Salt impacts seed germination by osmotic effects, ion toxicity, or a combination of the two. Salts absorb and store moisture to the point where it is no longer readily accessible in the soil and, eventually, the osmotic pressure of the soil solution rises. Plants absorb salt from the soil through transporters, which causes ion toxicity and disrupts mineral uptake and ion homeostasis. Salinity causes significant ion accumulation ( $\text{Na}^+$  and  $\text{Cl}^-$ ) and inhibits  $\text{K}^+$  and  $\text{Ca}^{2+}$  uptake, resulting in ionic imbalance (Isayenkov & Maathuis, 2019). Salinity can reduce the germination rate and final germination percentage (FG%), resulting in irregular growth and poor yields (Tsegay & Gebreslassie, 2014).

Mineral fertilizers are used by farmers in high production systems to boost crop output and reduce abiotic stress damage (nutrient deficiency, drought, and salinity). Excess fertilizers can negatively impact plant growth and nutritional value and contribute to soil deterioration (Li et al., 2018). The most extensively used fertilizers are nitrogen-based, and they are to blame for the huge nitrogen footprint in agriculture (Hessini et al., 2019). The roots of plants absorb both inorganic (nitrate and ammonium) and organic (urea, amino acids, peptides) forms of nitrogen, i.e. the most prevalent mineral element in plants. In the most natural and agricultural environments, ammonium is the main inorganic source of nitrogen for plants (Liu & von Wirén, 2017). Many halophytes, including *Salicornia bigelovii* (Kudo & Fujiyama, 2010), *Phragmites australis*, *Spartina alterniflora*, and *Glyceria maxima* (Munzarova et al., 2006), have been found to benefit from ammonium in low N and high NaCl conditions (Hessini et al., 2009).

Seed priming is a novel approach in plant stress physiology that increases plant tolerance to various biotic and abiotic adverse environments (Sheteiwy et al., 2017). A physiological strategy called “Seed priming” accelerates and synchronises seed germination (Wiszniewska, 2021). Controlled hydration treatments in aerated and diluted salt solutions have proven to benefit a wide range of species (Rehman et al., 1998). Recently, various commercial fertilizers (macro- and micro-nutrients) have been used as nutripriming agents to improve the crop yield. For example, nutripriming with  $\text{ZnSO}_4$  stimulated the development of young seedlings, increased seedling vigour, and increased Zn and B content in grain cowpea (Raj et al., 2020).  $\text{NH}_4^+$  feeding promoted the development of *S. alterniflora* in high salinity environments by increasing the activity of the key antioxidant enzymes (Hessini et al., 2013).  $\text{NH}_4\text{NO}_3$  nutripriming boosted the pre-germinative metabolism, emergence, and early development of rice seedlings. The most robust germinative efficacy was observed in seeds treated with 6 mM  $\text{NH}_4\text{NO}_3$ , whereas 2 mM  $\text{NH}_4\text{NO}_3$  provided greater root length and N- $\text{NH}_4^+$  content in roots and stimulated nitrogen metabolism in rice seedlings, which resulted in seedlings with superior growth and higher nitrogen content in their tissues (Pereira et al., 2022).

Tomato (*Solanum lycopersicum* L.) is the world's second most valuable fruit or vegetable crop from the Solanaceae family. The presence of various health-promoting chemicals, such as carotenoids, vitamins, and phenolic compounds, contribute significantly to their nutritional value (Li et al., 2018). Water shortages, soil salinization, and other abiotic factors all pose challenges to tomato production around the world (Zhou et al., 2019). Tomato seeds are extremely sensitive to saline stress, which can impact their germination and vigour (Giannakoula & Ilias, 2013). Salinity stress causes tomato plants to grow shorter and have a reduced leaf area. The most important indicators for tomato producers are fruit size and total yield per plant, and in saline conditions, leaf relative chlorophyll content, which indicates the amount of nitrogen, a vital factor for photosynthetic improvement, may also be lowered (Zhang et al., 2017). The aim of this study is to examine where  $\text{NH}_4^+$  nutrition enhances the salt tolerance of tomato plants through a priming effect. We investigated the effects of  $\text{NH}_4\text{NO}_3$

**Table 1** Priming agent name with concentration and soaking time.

Treatment	Chemical name	Concentration (mmol)	Duration in hours (h)
Control	Unprimed	Unprimed	Unprimed
T1 (treatment 1)	NH <sub>4</sub> NO <sub>3</sub>	50	12
T2 (treatment 2)	NH <sub>4</sub> NO <sub>3</sub>	50	24
T3 (treatment 3)	NH <sub>4</sub> NO <sub>3</sub>	100	12
T4 (treatment 4)	NH <sub>4</sub> NO <sub>3</sub>	100	24
T5 (treatment 5)	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	50	12
T6 (treatment 6)	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	50	24
T7 (treatment 7)	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	100	12
T8 (treatment 8)	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	100	24

and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> as NH<sub>4</sub><sup>+</sup> priming agents on seed germination, growth parameters, and chlorophyll, proline, total protein, total phenolic, and total flavonoid content in non-saline and saline environmental conditions.

## 2. Material and methods

### 2.1. Plant material and NH<sub>4</sub><sup>+</sup> priming treatment

Tomato seeds (*Solanum lycopersicum* L.) of the local variety NEMA were obtained from the plant protection department, Karachi, and the research was conducted in 2017–2020 at the PCSIR labs complex in Karachi, Pakistan, in collaboration with the Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan. The tomato seeds were surface-sterilized for 5 min with a 5% (w/v) NaClO solution. The disinfectant was then removed by rinsing three to four times with distilled water. The seeds were then placed on tissue paper to dry. For priming, the tomato seeds were immersed in 50 and 100 mmol concentrations of NH<sub>4</sub>NO<sub>3</sub> and 50 and 100 mmol concentrations of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, respectively. The seeds were primed in the absence of light for 12 and 24 h at 25 ± 1 °C, while dry and unprimed seeds were used as a control (Table 1). After conditioning, the seeds were thoroughly rinsed three times with distilled water and dried between two layers of tissue paper.

### 2.2. Experimental design and conditions

The research was conducted in a laboratory and a greenhouse experiment, and eight treatments were designed. The detailed design of the experiment is shown in Table 1. In the lab trial, twenty seeds were taken for each treatment, along with five replica plates and a control, and placed on four layers of filter paper. For tomato seed germination, all Petri plates moistened with 25 mL of distilled water were incubated at 25 °C. The germinated seedlings were counted every 24 h for 15 days (Association, 1985). The following parameters were assessed in this experiment:

The germination percentage (GP) was calculated on the 15th-day post sowing using the formula:

$$GP (\%) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

The mean germination time (MGT) in days was calculated according to the equation (Ellis & Roberts, 1981):

$$MGT = \frac{\sum Dn}{\sum n}$$

$n$  = number of germinated seeds on day, and  $D$  = days counted from the beginning of germination.

The germination rate (GR) provides a measure of the time course of seed germination. GR was calculated based on the equation created by Ellis and Roberts (1981).

**Table 2** Effects of  $\text{NH}_4^+$  priming on germination of tomato cv. Nema.

Treatment	GP %	MGT (day)	MGR (day)	T50 (day)
Control	54 ± 4.3b	7.13 ± 0.1a	0.14 ± 0.002b	6.47 ± 0.198a
T1 (treatment 1)	80 ± 4.47a	5.13 ± .07b	0.195 ± .002a	4.78 ± .11b
T2 (treatment 2)	78 ± 3.74a	5.09 ± 0.2b	0.198 ± .009a	4.68 ± .26b
T3 (treatment 3)	80 ± 5.47a	4.97 ± .07b	0.201 ± .002a	4.72 ± .11b
T4 (treatment 4)	74 ± 6ab	5.03 ± 0.1b	0.199 ± .006a	4.72 ± .12b
T5 (treatment 5)	82 ± 3.7a	4.92 ± .08b	0.203 ± .003a	4.56 ± .07b
T6 (treatment 6)	74 ± 6ab	5.07 ± 0.1b	0.198 ± .007a	4.69 ± .17b
T7 (treatment 7)	72 ± 2ab	4.83 ± 0.02b	0.207 ± .001a	4.36 ± .05b
T8 (treatment 8)	72 ± 2ab	4.86 ± 0.1b	0.20 ± .007a	4.38 ± .19b

Mean ± standard error, values with different letters are significantly different from each other.

GP - Germination percentage, MGT - mean germination time (day), MGR - mean germination rate, T50 - time to 50% germination.

**Table 3** Soil analysis before and after harvest of the plants.

	Before Seedling	Control	Primed	Primed Saline
Texture sandy loam				
pH	7.1	7.12	7.01	7.19
Electrical conductivity (EC) ( $\mu\text{s}/\text{cm}$ )	700	712	1690	9870
Sodium ( $\text{ppm}^*$ )	8	5	18	15
Potassium ( $\text{ppm}^*$ )	25	13	11	14
Calcium ( $\text{ppm}^*$ )	230	240	560	300
Magnesium ( $\text{ppm}^*$ )	972	972	3402	430
Sulphate ( $\text{ppm}^*$ )	1.57	1.646	0.962	1.25
Chloride ( $\text{ppm}^*$ )	1704	1065	852	294

\* Parts per million.

The germination rate is calculated by the formula:

$$\text{GR} = \sum N_i T_i$$

$N$  = germinated seed number at time  $t$  and  $T$  is days of experiment.

The second pot experiment was conducted in a greenhouse at the tissue culture laboratory, PCSIR labs complex in Karachi, Pakistan, to evaluate the impact of  $\text{NH}_4^+$  priming ( $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$ ) on growth parameters and biochemistry of tomato plants in saline and non-saline environments (Table 2). The soil used in the experiment was evaluated before and after the tomato seedlings were planted (Table 3). Tomato seeds were planted in pots with five replicates and treated with tap water after being primed with  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$ . The tomato seedlings were replanted in garden soil 40 days after sowing, according to RCBD.

### 2.3. Salinity treatments

The seedlings were divided into three groups after transplantation: control (unprimed) watered with regular tap water, primed seeds irrigated with regular tap water, and primed seeds irrigated with increasing concentrations of saltwater. The first application of saltwater (50 mmol) to the soil occurred 40 days after the seedling were transplanted. After a 10-day break, the concentration of saline water (50, 100, 150, and 200 mmol NaCl) was gradually raised.

### 2.4. Morphological variables

After seedlings were transplanted into new pots, the morphological characteristics were observed every seven days. All plants were measured for morphological characteristics such as number of leaves, plant height (cm), and leaf area. The leaf area in  $\text{cm}^2$

was calculated using a leaf area machine (C1-202-Area Meter USA). After the plant was harvested, the fresh and dry weight of the shoot and root was calculated. The fresh weight of the shoot and root was assessed using an Electronic Weighing Balance (g), followed by 72 h of drying in an oven at 72 °C, and finally, the dry weight of the shoot and root was determined.

## 2.5. Physiological variables

The leaf chlorophyll content was measured by the acetone extraction process. Approximately 0.1 g of leaf tissue was homogenized in 10 mL of 95% (v/v) acetone mixture, and tubes were incubated for 48 h at 4 °C. The concentrations of chlorophyll a, b, and carotenes (g/g FW) were determined by measuring the optical density of the extracted sample at 662, 644, and 470 nm, respectively, in new test tubes with replicates for each sample. The chlorophyll content was calculated using the following equation:

$$\begin{aligned} \text{Chl a} &= 9.784D_{662} - 0.99D_{644} \\ \text{Chl b} &= 21.42D_{644} - 4.65D_{662} \\ \text{T. Chl} &= \text{Chl a} + \text{Chl b} \\ \text{CX} + \text{C} &= \frac{1000D_{470} - 1.90\text{Chl a} - 63.14\text{Chl b}}{214} \end{aligned}$$

## 2.6. Determination of proline, total protein, total phenolic, and flavonoid contents

The contents of proline and total protein in tomato leaves from non-saline and saline conditions were estimated according to the detailed methodologies for proline (Bates et al., 1973) and total protein (Bradford, 1976), as shown in Table S1.

For the total phenolic assay, 0.1 g of leaf tissue was homogenized with 2 mL of absolute ethanol solution using a pestle and mortar. After homogenization, the plant mixture was centrifuged to collect the upper layer of each sample in a test tube. The reaction was carried out with 0.1 mL of supernatant, followed by 1.0 mL of Folin-Ciocalteu reagent and 0.3 mL NaCO<sub>3</sub> in the tube. For 30 min, the mixture was kept at room temperature after measuring the optical density at 756 nm against a blank. Gallic acid was used as standard phenolic acid to prepare a standard curve to quantify the total phenolic concentration (Singleton & Rossi, 1965). The total flavonoid content was measured using the methodology proposed by Kim et al. (2003). In a test tube, 0.1 mL of the sample was mixed with 0.3 mL of 5% NaNO<sub>2</sub> for the reaction. The reaction mixture was incubated at room temperature for 5 min. The samples were then treated with 0.3 mL of AlCl<sub>3</sub> for 5 min at room temperature. The volume of the reaction mixture was increased to 10.0 mL with distilled water by adding 2.0 mL of 1 mol NaOH. The intensity of the product was measured at 510 nm against a rutin standard to assess the flavonoid concentration.

## 2.7. Statistical analysis

All experiments were carried out five times, and the results were statistically analysed with ANOVA (SPSS 18.0; SPSS Inc., Chicago, USA). Using SPSS 18.0, Duncan's multiple range test was done to determine whether there were significant changes between the treatments. At  $p < 0.05$ , different letters indicate significant differences.

## 3. Results

### 3.1. Effects of NH<sub>4</sub><sup>+</sup> priming on germination

As shown in Table 2, the highest percentage of germination was obtained in treatment T5 (50 mmol (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 12 h), followed by T1 and T3 (50 mmol NH<sub>4</sub>NO<sub>3</sub>, 12 h & 100 mmol (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 12 h), both of which showed 80% germination. The lowest GP among all the treatments were recorded in T7 & T8 at 72%. The germination percentage increased by 51% in all primed treatments, compared to the control. The T7 (100 mmol (NH<sub>4</sub>)<sub>2</sub>SO<sub>3</sub>, 12 h) treatment gave the lowest value of MGT, followed by T5

(50 mmol  $(\text{NH}_4)_2\text{SO}_3$ , 12 h). The MGT of the tomato seeds was reduced in all primed treatments, compared to the control.

### 3.2. Soil analysis

In the field trials, the treated seeds were planted in soil inside a greenhouse and irrigated with two different methods: tap water and saltwater. Table 3 shows the results of a soil analysis performed prior to seed sowing and after plant harvesting.

### 3.3. Effect of $\text{NH}_4^+$ priming on growth parameters

#### 3.3.1. Plant height (cm)

The effect of the  $\text{NH}_4^+$  priming on seeds grown in the control conditions resulted in a favourable response of plant height, as shown in Table 4. The highest plant height was observed in treatment T6 (81.5 cm), followed by T3 (77.6 cm), compared to NC (45.8 cm), while the lowest plant height was noted in T7 (57.2 cm) among all the treatments. Overall, the primed treatment increased plant height by 77% more than in the Nema control. Using the various chemicals as an  $\text{NH}_4^+$  priming agent resulted in a significant stimulatory effect on plant height in the Nema cultivar in the saline conditions. When compared to the control (unprimed seeds), the uppermost plant height was noted in treatment T2 (67 cm), followed by treatment T1 (66.6 cm), and all the primed treatments yielded the lowest plant heights (T3 and T7), as shown in Table 4.

#### 3.3.2. Shoot fresh and dry weight

When compared to the control, the highest shoot fresh weight (112.7 g) was observed in T7, followed by T3, which was irrigated with normal water (41.9 g). The effect of the seed priming on the shoot fresh weight (SFW) (g/plant) of the tomato variety is shown in Table 4. However, when the  $\text{NH}_4^+$  primed seeds were grown in saline stress, the shoot fresh weight increased significantly. In comparison to the control Nema (41.9 g), the maximum shoot fresh weight (g/plant) was recorded in treatment T8 (71.5 g). In the normal conditions, the primed tomato seeds responded favourably in the shoot dry weight (SDW). When compared to the control, T1 (50 mM  $\text{NH}_4\text{NO}_3$ , 12 h) had the lowest SDW value (5.8 g), followed by T2 (8.6 g). However, the highest shoot dry weight (g/plant), i.e. 14.7 g, was observed in treatment T3 (100 mM  $\text{NH}_4\text{NO}_3$ ). The experiment findings revealed a variation among all the treatments carried out in saline conditions (Table 4). T5 (7.23 g) was found to exhibit the lowest dry biomass of shoot in freshwater *versus* the control (9.65 g).

#### 3.3.3. Root fresh and dry weight

The analysis of variance revealed that the  $\text{NH}_4^+$  priming caused a significant ( $p < 0.05$ ) variation in root fresh weight (RFW) in both non-saline and saline environments. The highest root fresh weight (g/plant) was noticed in treatment T7 (22.7 g), compared to the control (9.4 g), while the lowest root fresh weight (5.5 g) was noticed in treatment T2. Moreover, when the seed priming and saline conditions were compared to the controls, treatment T3 exhibited the highest root fresh weight value (11.1 g) (g/plant), while treatment T4 was observed to have the lowest root fresh weight (5.67 g). In comparison to the control, T2 (0.58 g) had the lowest RDW in the normal conditions. While in the saline conditions, the  $\text{NH}_4^+$  seed priming was observed to exert a significant effect on root dry weight. The maximum root dry weight (g/plant) was recorded in T7 (1.13 g) and the minimum root dry weight was observed in T5 (0.75 g) in cv, when grown in the control conditions (unprimed).

#### 3.3.4. Leaf number and leaf area

The primed tomato seeds responded positively in terms of the number of leaves per plant in the normal conditions. Table 4 demonstrated that the leaf number was higher in T1 (50 mmol  $\text{NH}_4\text{NO}_3$ , 12 h) (92.4 g/plant), followed by T4 (91.8 g/plant),

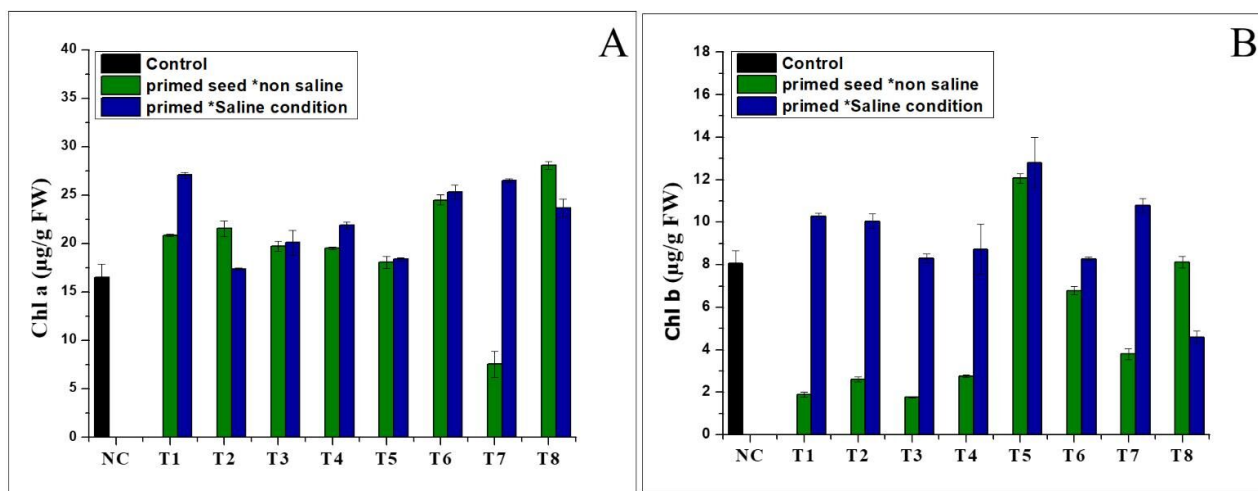


**Table 4** Effects of NH<sub>4</sub><sup>+</sup> priming on growth parameters.

Conditions →	Plant height		SFW (g)		SDW (g)		RFW (g)		RDW (g)		Leaves/plant		Leaf area	
	Non-Saline Condition	Saline Condition	Non-Saline Condition	Saline Condition	Non-Saline Condition	Saline Condition	Non-Saline Condition	Saline Condition	Non-Saline Condition	Saline Condition	Non-Saline Condition	Saline Condition	Non-Saline Condition	Saline Condition
<b>Primed Treatment ↓</b>														
Control	45.8±0.2g	45.8±0.2e	41.9±1.7cd	41.9±1.7bc	9.65±0.4bc	9.65±0.4bcd	9.42±0.4a	9.42±.4bc	1.09±.04bc	1.09±.04ab	38±1.9d	38±1.9d	14.3±.09e	14.3±.09d
T1 (treatment 1)	72±1.0cd	66.6±1.3ab	20.8±0.9e	33.4±1.6cd	5.8±0.2c	7.75±0.6cd	6.8±0.3bcd	8.1±1c	0.83±.09bc	0.9±.01abcd	92.4±1.1a	82.2±3a	18.5±0.4a	15.5±0.16c
T2 (treatment 2)	65.7±1.1e	67±1.8a	35.8±1.4de	52±2b	8.6±0.6bc	9.2±2.2bcd	5.5±0.2d	11.1±0.3a	0.58±.04c	0.77±0.04d	86.6±1.8a	75.4±1.6abc	17.7±0.2b	18±0.14a
T3 (treatment 3)	77.6±0.5b	57.2±1.7d	59.5±0.9b	40±3.7bc	14.8±0.5a	9.16±0.3bcd	7.75±0.4bcd	6.5±0.41d	1.1±0.1bc	0.8±0.02cd	77 ±6.4b	67±2.0c	18.±0.1ab	15.6±0.14c
T4 (treatment 4)	68.5±1.0e	59.3±2.2bc	52.6±1.3bc	37±1.3c	10.6±0.7b	14.5±1.5a	8.07±0.2bcd	5.67±0.7d	1.04±0.1bc	0.83±.08bcd	91.8±1.0a	74.8±6abc	16.6±0.1c	15.7±0.2c
T5 (treatment 5)	71±1.1d	58.6±2.2bc	20±0.2e	22.5±2.7d	7.75±1.9bc	7.23±0.3d	6.19±1.5cd	6.22±0.3d	0.65±0.15c	0.75±.02d	76±1.3b	72.6±3abc	17.5±0.1b	16.8±0.3b
T6 (treatment 6)	81.5±1.0a	57.1±1.8cd	58±6.6bc	68±2.8a	10.6±0.6b	9.85±0.3bcd	9.29±0.5bc	8.4±0.2c	1.57±0.14b	1.12±0.09a	85.8±2.0a	72±2.9bc	15.6±0.1d	17.5±0.3a
T7 (treatment 7)	57.2±1.6f	59.9±2.1bc	112.7±6.3a	41.5±1.6bc	10.6±.03b	10.4±.6bc	22.7±2.5a	9.4±0.4bc	2.55±0.7a	1.13±0.1a	66.8±1.8c	66.2±2.9c	18.5±0.1a	11±0.06e
T8 (treatment 8)	75±1.3bc	59±6.8bc	33.2±2.6de	71.5±5.3a	10±0.9b	11.5±0.7b	8.43±0.6bcd	10±0.3ab	1.2±0.1bc	1.06±.06abc	72.±2.3bc	80.4±3.4ab	16.2±0.2c	17±0.07

Values are represented as mean ± standard error. Values with different letters are significantly different from each other, as determined by the LSD test.

SFW - shoot fresh weight, SDW - shoot dry weight, RFW - root fresh weight, RDW - root dry weight.



**Figure 1** Effects of  $\text{NH}_4^+$  priming on chlorophyll a and b.

compared to the control. In contrast, in the saline environments, the  $\text{NH}_4^+$  seed priming showed a progressive effect on the number of leaves, with the optimum leaf number per plant recorded in treatment T1 (82.2/plant), compared to the unprimed variant. Table 4 indicates the effect of seed priming and salinity on leaf area in the analyzed tomato variety. The highest leaf area was found in both T1 and T7 (18.5 cm<sup>2</sup>), followed by T3 (18 cm<sup>2</sup>), which was irrigated with normal water, compared to the control (14.3 cm<sup>2</sup>). However, when the primed tomato seedlings were grown in the saline conditions, the maximum leaf area (cm<sup>2</sup>) was recorded in T2 (18 cm<sup>2</sup>), compared to the control (unprimed).

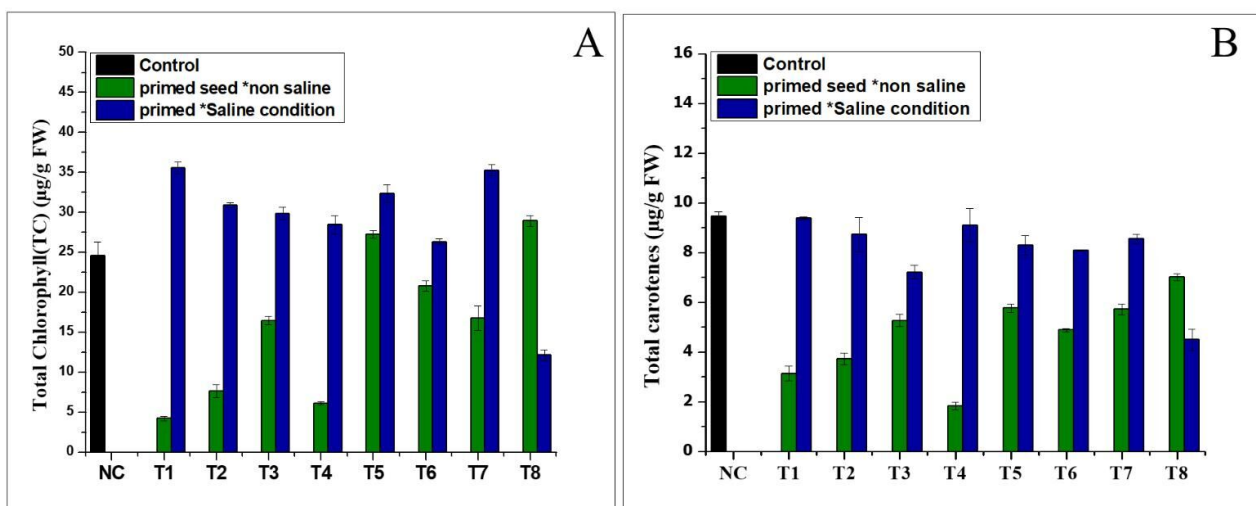
### 3.4. Effects of $\text{NH}_4^+$ priming on chlorophyll and carotene content

The results (Figure 1A) show that the chlorophyll a (Chl a) levels were higher in all the treatments than in the Nema control. In the normal conditions, the treatment produced the highest amounts of chlorophyll a (28.1 µg/g FW), followed by T4 (21.54 µg/g FW) in the saline conditions. Furthermore, the results revealed that the chlorophyll b levels increased only in a few treatments grown in the different conditions (normal and saline), compared to the control (Figure 1B). Treatment T5 was characterised by the higher chlorophyll b levels (12.06 and 12.8 µg/g FW) under seed priming in the normal and saline conditions, respectively. As shown in Figures 2A and 2B,  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  significantly increased the total chlorophyll (TC) content in the different (normal and saline) conditions. Treatment T1 exhibited the highest amount of total chlorophyll (35.56 µg/g FW) in the saline conditions, followed by treatment T8 (28.9 g/g FW) in the normal conditions (Figure 2A). The findings shown in Figure 2B indicate that the total carotene was positively enhanced in the seed priming treatment in the normal and saline conditions, but the maximum total carotene was found in T4 (9.108 µg/g FW) in the seed priming treatment in the saline conditions (Figure 2B).

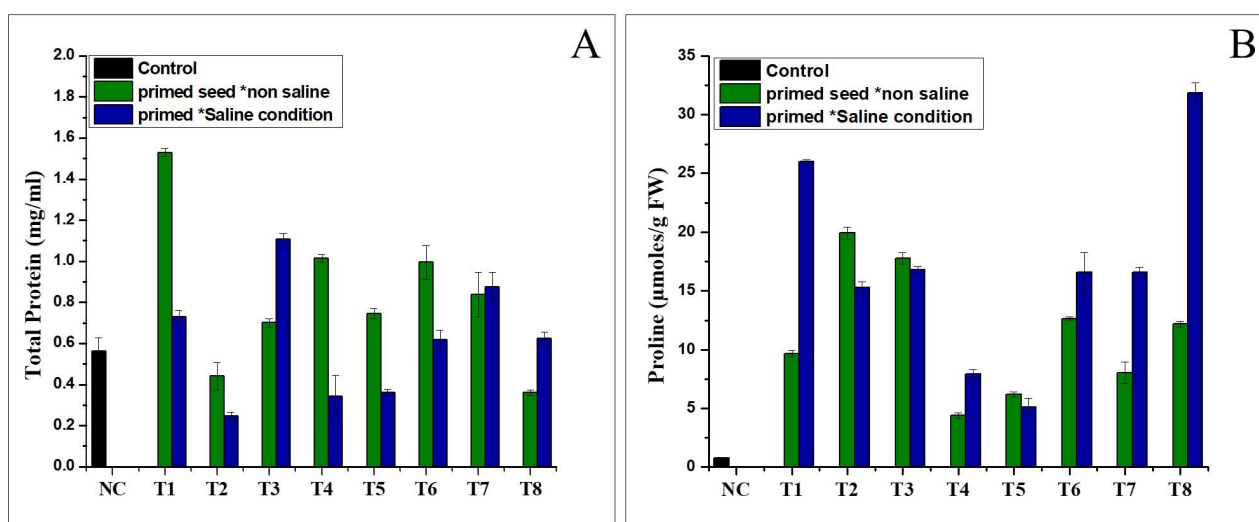
### 3.5. Effect of $\text{NH}_4^+$ priming on proline and total protein content

The result showed that the  $\text{NH}_4^+$  priming without saline and under saline stress had a positive effect on the total protein content (Figure 3A). As shown in Figure 3A, the highest total protein concentration was found in treatment T1 (1.5299 mg/mL) in the normal water conditions, followed by T3 (100 mmol  $\text{NH}_4\text{NO}_3$ , 12 h). Under various abiotic stresses, including salinity, proline is the most frequently accumulated endogenous osmolyte. Proline improves crop tolerance to various abiotic stresses, particularly by protecting against harmful ROS effects. The content of free proline in the varieties varied significantly ( $p < 0.05$ ). The proline content in the tomato leaves differed significantly between the  $\text{NH}_4^+$  priming treatments. In the Nema variety, the





**Figure 2** Effects of  $\text{NH}_4^+$  priming on total chlorophyll and carotene content.

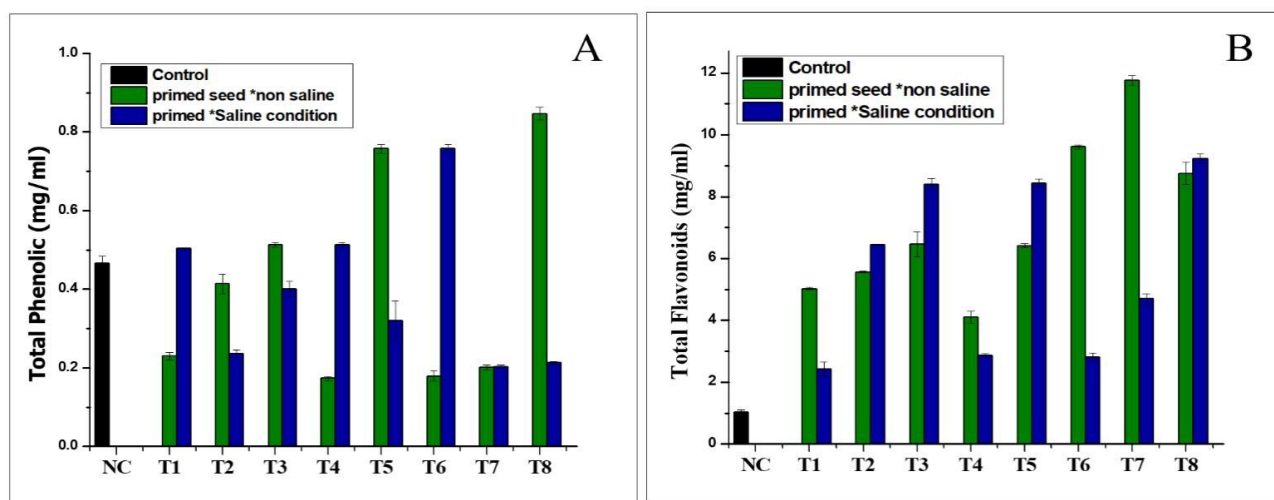


**Figure 3** Effect of  $\text{NH}_4^+$  priming on proline and total protein content.

treatments caused variable changes in the proline content (Figure 3B). Treatment T2 of cv. Nema (19.93 mol/g FW) in the saline conditions exhibited the highest proline content, compared to the control (Figure 3B).

### 3.6. Effect of $\text{NH}_4^+$ priming on total flavonoids and total phenolic content

The total flavonoid content in the Nema variety was improved by the  $\text{NH}_4^+$  priming treatment grown in the saline conditions, compared to the control (Figure 4A). The highest concentration was found in seed priming treatment T7 (11.7 mg/mL) in the normal conditions, followed by seed priming treatment T8 (9.2 mg/mL), which increased the total value of flavonoid content in the primed seeds in the saline conditions, compared to the control (1.03 mg/mL), as shown in Figure 4A. The statistical analysis of the data revealed that the seed priming treatment had essentially little effect on total phenolic contents in the non-saline (normal) and saline environments, as shown in Figure 4B. The results demonstrated that the total phenolic content was higher in treatments T8 (0.84 mg/mL) in the non-saline medium, while the total phenolic content in the seed priming treatment T5 was 0.75 mg/mL in the saline conditions, as shown in Figure 4B.



**Figure 4** Effect of  $\text{NH}_4^+$  priming on total flavonoid content and total phenolic content.

#### 4. Discussion

The current research demonstrated that two different ammonium sources ( $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$ ) were employed as priming agents for seeds. This led to an enhancement in seed germination while simultaneously reducing the mean germination time (MGT) in tomato plants (Table 2). It is clear that the  $\text{NH}_4^+$  priming with T5 increased the percentage of germination, whereas the  $\text{NH}_4^+$  priming with T7 decreased the MGT in the tomato plant. In comparison to the unprimed seeds, the  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  priming increased the germination percentage. According to a previous study (Pereira et al., 2022), nutripriming with 6 mmol  $\text{NH}_4\text{NO}_3$  improved emergence synchronization (ES) caused by a decrease in the mean germination time in rice seedlings, and nutripriming with 2 mmol  $\text{NH}_4\text{NO}_3$  improved total rice seedling growth. Vaktabhai and Kumar (2017) investigated tomato seedling vigour against salt stress. According to the findings, the halo-priming of tomato seeds with 1% or 2%  $\text{KNO}_3$  for 24 or 48 h could protect tomato seedlings from salt stress. Tomato seeds primed with 0.75%  $\text{KNO}_3$  were successful in enhancing seedling germination and vigour, as well as physiological and biochemical attributes in growth chamber and greenhouse conditions. As revealed in a study conducted by Hessini et al. (2013),  $\text{NH}_4^+$  is more beneficial for the growth of *S. alterniflora* under high salinity than  $\text{NO}_3^-$ . Changes in leaf area and leaf number were associated with changes in plant biomass. Hydro-priming and osmo-priming with Calcium Ammonium Nitrate (CAN) at 0.5% for 24 h stimulates maize crop development and vigour in aggressive environments, as reported by Khan et al. (2016). Based on the current findings, the leaf area and leaf number were improved in the different variants of  $\text{NH}_4^+$  priming in the non-saline conditions. The positive responses to  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  were also observed in the salinity conditions, but a better response was observed in 50 mmol  $\text{NH}_4\text{NO}_3$ , 24 h. Furthermore, in the saline conditions, the  $\text{NH}_4^+$  priming induced a favourable response of the tomato plant biomass; when the  $\text{NH}_4\text{-N}/\text{total-N}$  ratio in the nutrient solution was greater than 0.1, both vegetative development and fruit outputs in tomatoes were limited (Akl et al., 2003). On the other hand, an increase in total and fruit dry weight was shown when the ammonium percentage was 0.25 (Dong et al., 2004). According to Heeb et al. (2005), providing 0.1% of total-N in the form of  $\text{NH}_4^+$  can improve fruit flavour by increasing glutamine and glutamate levels. Similarly, the current results showed that the fruit number and weight increased with the different  $\text{NH}_4^+$  priming agent concentrations in the non-saline conditions, compared to the unprimed variant, whereas the  $\text{NH}_4^+$  priming in the saline conditions gave the same positive response by increasing the fruit number and weight, but with a better response was shown in 50 mmol and  $(\text{NH}_4)_2\text{SO}_4$  for 12 h.

The present result (Figure 1, Figure 2) indicated that the photosynthetic pigments of the tomato plant were improved in the primed seeds in the normal conditions,

compared to the unprimed seeds, and that chlorophyll b and total carotenes significantly increased under salt stress. Previous studies showed that  $\text{NH}_4^+$  feeding on *Spartina alterniflora* in saline environments was associated with increased salt gland production, which led to a decrease in leaf mesophyll salt concentrations and enhanced photosynthetic activity (Hessini et al., 2011). Plant development and production are significantly impaired by salt stress. The salinity response to nitrogen nutrition types was studied in two major grass C4 species (sorghum and maize). Salt-stressed sorghum plants showed unchanged  $\text{CO}_2$  absorption when fed  $\text{NH}_4^+$ , a response strongly linked to photosystem II efficiency maintenance. The results clearly showed that grass species react differently to salt and different N types, demonstrating the vulnerability of salt-stressed maize plants to  $\text{NH}_4^+$  feeding and an opposite behaviour of sorghum (de Souza et al., 2021).

Proline is synthesized in large quantities during salt stress and plays an important role in osmotic regulation, subcellular structure stabilization, and enzyme activities. It can also maintain cellular turgor pressure, which is responsible for cell growth (Sairam & Tyagi, 2004). Under growth chamber and greenhouse settings, tomato seeds primed with 0.75%  $\text{KNO}_3$  were successful in enhancing seedling establishment and vigour, as well as physiological and biochemical properties (Moazz et al., 2020). The present study suggested that the proline content was raised in the  $\text{NH}_4^+$  primed seeds grown in the non-saline and saline conditions (Figure 3). *Spartina alterniflora*, a halophyte, performed better when grown with  $\text{NH}_4^+$  as an N source, and  $\text{NH}_4^+$  advantages were correlated with improved antioxidant enzyme activity (Hessini et al., 2013).

The current research indicated that the primed seeds grown in the saline and non-saline stress had higher flavonoid content than the control seeds, and the phenolic content was higher in some treatments grown in the non-saline conditions. Nutripriming has been demonstrated to promote germination and seedling growth in some plant species; however, it is unclear whether this is simply due to the presence of nitrogen or if it might be attributed to the addition of supplementary nutrients such as magnesium and potassium.

## 5. Conclusions

The current study demonstrated that using an ammonium priming agent favoured tomato seedling growth and development in both non-saline and saline environments. The 50 mmol  $(\text{NH}_4)_2\text{SO}_4$ , 12 h priming resulted in the highest seed germination performance; however, all the treatments performed better than the unprimed variant. In addition, the growth parameter in tomato seeds was improved in both  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  used at different concentration as  $\text{NH}_4^+$  priming in the non-saline and saline environment. This study justifies the use of  $\text{NH}_4^+$  priming to solve the poor tomato seedling and emergence problem, but more research is needed to clarify the effect of  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  as  $\text{NH}_4^+$  priming in tomato plants because other factors and nutrition can play a role in seed germination and development.

## 6. Supplementary material

The following supplementary material is available for this article:

**Table S1.** Extraction of proline and total protein procedure.

## Acknowledgement

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