







# Nano-copper sulfate mediated alleviation of salt stress: Optimizing elemental homeostasis and nutrient ratios in tomato

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## **Abstract**

Salt stress represents a critical constraint to agricultural productivity worldwide, necessitating innovative approaches for crop resilience enhancement. This study investigated the ameliorative effects of nano-copper sulfate (nano-CuSO<sub>4</sub>) on salt-stressed plants, examining elemental composition changes in two tomato ( $Solanum \, lycopersicum \, L$ .) varieties (Pant Tomato3: PT3 and Hisar Arun: HA) under graduated NaCl stress (25-100 mM) and nano-CuSO<sub>4</sub> treatments (5-100 ppm). Results demonstrated that nanoparticle treatments significantly improved plant performance under salt stress. Treatment  $T_{14}$  (100 mM NaCl + 10 ppm nano-CuSO<sub>4</sub>) emerged as the most effective for PT3 variety, enhancing Ca (+7.55 %), K (+11.57 %) and Cu (+3.14 %), along with improvement in K/Na (+6.60 %) and Ca/Na (+2.75 %) ratios. For HA variety,  $T_{17}$  (100 mM NaCl + 50 ppm nano-CuSO<sub>4</sub>) proved most effective, increasing Ca (+7.76 %), K (+11.79 %) and Cu (+3.09 %) with superior ratio improvements (K/Na +8.93 %, Ca/Na +4.89 %). The morphological analysis demonstrated that 10 ppm nano-CuSO<sub>4</sub> ( $T_{6}$ ) consistently optimized plant architecture, enhancing plant height by 20.4 % and 15.1 % in PT3 and HA respectively, while increasing branching by 7.2 % and 24.0 %. The reproductive performance data revealed that nano-CuSO<sub>4</sub> treatments accelerated flowering by up to 10.5 % in PT3 and 8.6 % in HA, counteracting salt-induced delays and promoting early reproductive development. Critical findings revealed that nano-CuSO<sub>4</sub> application effectively counteracted salt-induced ionic imbalances, with treatments  $T_{13}$ - $T_{17}$  working best. The study provides compelling evidence for nano-CuSO<sub>4</sub> as a precision agriculture tool for salt stress mitigation, offering variety-specific treatment protocols for optimized crop performance under saline conditions.

Keywords: copper sulfate; precision; salt stress; stress mitigation; tomato

## Introduction

Soil salinization affects approximately 20 % of irrigated agricultural land globally, causing annual crop losses exceeding \$30 billion worldwide (1). Saline soils accumulate excessive soluble salts which are detrimental to most plants by limiting plant growth and productivity. The escalating challenge of soil salinity, exacerbated by climate change and intensive agricultural practices, demands innovative solutions for sustainable crop production.

Salt stress induces multifaceted physiological disruptions in plants, primarily through osmotic stress, ionic toxicity and oxidative damage (2). The accumulation of toxic ions, particularly Na<sup>+</sup> and Cl<sup>-</sup>, disrupts cellular homeostasis and interferes with essential metabolic processes (3). These ionic imbalances manifest as altered K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios, which serve as critical indicators of plant salt tolerance (4).

Tomato (*Solanum lycopersicum* L.), a moderately salt-sensitive crop, exhibits reduced vegetative growth, impaired flowering and significant yield loss under saline conditions. High salinity leads to ionic toxicity and water deficit, ultimately suppressing fruit set and size.

At very high salinity (EC around 6 dS/m and above), total yield loss can approach 50 % compared to optimal conditions (5).

Recent advances in nanotechnology have opened new avenues for agricultural applications, with nanoparticles emerging as promising tools for stress mitigation. Accumulating evidence demonstrates that supplementation of nanoparticles to plants can significantly alleviate the injurious effects caused by various harsh conditions including salt stress and hence, regulate adaptive mechanisms in plants (6). Among various nanomaterials, copper-based nanoparticles have garnered particular attention due to their dual role as essential micronutrients and stressmitigating agents.

Copper plays crucial roles in plant physiology, serving as a cofactor for numerous enzymes involved in photosynthesis, respiration and antioxidant defense systems (7). Notably, both CuS NPs and ionic Cu mitigated the drought-induced inhibition of flower production, showing 41.7 and 33.3 % improvement (8). However, the application of nano-copper sulfate for salt stress alleviation remains underexplored, particularly regarding optimal dosing strategies and variety-specific responses. The rising

incidence of soil salinization driven by intensive irrigation, climate variability and inadequate drainage, salt stress has become a major abiotic constraint on global crop productivity. Tomato (*Solanum lycopersicum* L.), a globally cultivated horticultural crop, is highly sensitive to salinity, which significantly hampers its growth, fruit set and yield. Even moderate salt stress can disrupt physiological and biochemical functions in tomatoes, including ion homeostasis, water relations and reproductive development. Due to its economic importance and susceptibility to salinity, tomato serves as a model crop for evaluating potential nanotechnological interventions aimed at improving stress resilience. Thus, investigating the role of nano-copper sulfate in mitigating salt-induced damage in tomato is both timely and agronomically relevant.

The present study addresses this knowledge gap by systematically evaluating the effects of nano-CuSO<sub>4</sub> treatments on salt-stressed plants. We hypothesized that nano-CuSO<sub>4</sub> would enhance plant salt tolerance by improving essential element uptake and maintaining favorable ionic ratios.

## **Materials and Methods**

## **Experimental design**

The experimental setup was conducted at GBPUA&T, Pantnagar, situated in the foothills of the Himalayas in Uttarakhand, India. The location has coordinates of 29°02'60.00" N latitude and 79° 30'59.99" E longitude, with an elevation of 243.84 m above sea level. The experiment employed a completely randomized design with two salinity contrasting tomato varieties (PT3: salinity susceptible and HA: salinity tolerant) subjected to 20 different treatments. Treatments were designed to evaluate individual and combined effects of salt stress and nano-CuSO<sub>4</sub> application (Supplementary Table 1).

## **Material and growth conditions**

Seeds of varieties PT3 and HA were obtained from Vegetable Research Center, GBPUA&T (Pantnagar) and HAU, Hisar respectively. Plants were grown under polyhouse conditions at temperature 18-32 °C with relative humidity 65-85 %.

## **Treatment application**

Seedlings were transplanted into pots @ 20 Days After Sowing (DAS) and salt stress was given @ 10 Days After Transplantation (DAT). Nano-CuSO $_4$  were applied as foliar sprayed @15 DAT. Combined treatments involved sequential application of salt stress followed by nano-CuSO $_4$  treatment @15 DAT.

## **Elemental analysis**

At least three biological replicates were used per treatment, with leaf samples pooled from the third fully expanded leaf of each replicate plant to ensure representativeness and consistency. Leaf samples were collected 30 DAT, to assess the mid-phase physiological impact of treatments.

## Sample preparation

0.5 g of leaf sample were weighed and 10 mL of Milli Q water was added in a beaker. To it, 8 mL HNO $_3$  and 1 mL HCl were added. These were then kept on hot plate for digestion till clear solutions were attained (30-45 min). After digestion, volume was made up to 50 mL with Milli Q water.

## Sample analysis

Elemental analysis for Ca, K, Na and Cu was performed using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) at Ohms laboratory, New Delhi.

## **Statistical analysis**

Data (in mg/kg dry weight) were analyzed for replications in triplicate using two-way analysis of variance (ANOVA) with treatment and variety as fixed factors. Percent changes were calculated relative to the  $T_1$  (control) for each variety. K/Na and Ca/Na ratios were computed and analyzed for treatment effects. Statistical significance was determined at p value  $5\,\%$  or less.

## Morphological parameter measurement

Morphological traits such as plant height, average number of branches and days to 50 % flowering were recorded for both tomato varieties (PT3 and HA) under all treatment combinations. Plant height was measured from the base of the stem to the topmost growing point using a measuring scale. Number of branches was recorded by counting all primary branches emerging from the main stem at the flowering stage. Days to 50 % flowering was noted when 50 % of the plants in a treatment group had initiated flowering. All measurements were carried out on minimum of ten biological replicates per treatment and mean values were used for statistical analysis. Data was analyzed for replications in triplicate using two-way analysis of variance (ANOVA) and Tuckey's HSD test with treatment and variety as fixed factors. Statistical significance was determined at p value 5 % or less.

## **Results**

## Effects of salt stress on elemental composition

Salt stress treatments (T<sub>2</sub>-T<sub>4</sub>) induced significant alterations in plant elemental composition compared to control (T<sub>1</sub>). In PT3 variety, increasing NaCl concentration progressively reduced Ca content (T<sub>1</sub>: 452.384 mg/kg) by -2.95 %, -5.4 % and -7.29 % and K content (T<sub>1</sub>: 9837.261 mg/kg) by -1.07 %, -1.36 and -2.18 % while substantially increasing Na accumulation (T<sub>1</sub>: 592.141 mg/kg) by +5.48 %, +26.41 and +58.23 % for 25 mM, 50mM and 100 mM NaCl concentrations, respectively. These trends demonstrate a clear inverse relationship between salt concentration and the levels of Ca and K, likely due to competitive ion uptake and osmotic imbalance induced by excess Na<sup>+</sup> ions. The marked accumulation of Na<sup>+</sup> with increasing salt stress underscores its disruptive influence on nutrient homeostasis and plant ion selectivity mechanisms. For HA variety (T1: Ca-478.575 mg/kg; K-10076.24 mg/kg and Na-632.512 mg/kg), Ca (-2.74 %, -5.2 and -7.09 %) and K (-0.87 %, -1.16 % and -1.98 %) showed similar trends as of PT3. In contrast to PT3, HA showed insignificant increase in Na content (0.4 %, 1.09 % and 1.35 %) for the same salt concentrations, respectively (Table 1, Fig. 1-3).

The K/Na ratio, a critical indicator of salt tolerance, declined dramatically under salt stress. In PT3, the K/Na ratio ( $T_1$ : 16.61 mg/kg) decreased by 6.20 %, 21.98 % and 38.16 % for  $T_2$ ,  $T_3$  and  $T_4$  treatments, respectively. HA variety ( $T_1$ : 15.93) showed greater resilience, with corresponding decrease of 1.22 %, 2.24 % and 3.25 % respectively (Table 1, Fig. 4).

Table 1. Percent change in mineral content and mineral ratios across different treatments for PT3 and HA tomato varieties

Treatments	Ca %		K %		Na %		Cu %		K/Na %		Ca/Na %	
	PT3	НА	PT3	НА	PT3	НА	PT3	НА	PT3	НА	PT3	HA
T <sub>1</sub>	0	0	0	0	0	0	0	0	0	0	0	0
T <sub>2</sub>	-2.95	-2.74	-1.07	-0.87	5.48	0.40	1.72	1.54	-6.20	-1.22	-7.98	-3.17
T <sub>3</sub>	-5.40	-5.20	-1.36	-1.16	26.41	1.09	-3.25	-2.91	-21.98	-2.24	-25.13	-6.21
T <sub>4</sub>	-7.29	-7.09	-2.18	-1.98	58.23	1.35	-5.08	-4.55	-38.16	-3.25	-41.40	-8.33
T <sub>5</sub>	0.67	0.87	0.79	0.99	0.69	0.09	18.73	15.57	-0.11	0.94	0.00	0.79
T <sub>6</sub>	1.22	1.42	1.19	1.38	0.23	0.03	19.03	15.81	0.96	1.37	1.05	1.32
T <sub>7</sub>	1.61	1.82	1.64	1.84	-0.62	0.22	20.93	17.51	2.31	1.65	2.23	1.59
T <sub>8</sub>	1.75	1.95	1.72	1.91	24.17	0.28	23.09	19.45	-18.06	1.67	-18.08	1.59
T <sub>9</sub>	1.84	2.05	1.99	2.19	1.68	0.06	2.60	2.31	0.31	2.14	0.13	1.98
T <sub>10</sub>	2.92	3.12	4.55	4.75	3.45	0.13	2.87	2.55	1.07	4.62	-0.52	2.91
T <sub>11</sub>	3.45	3.66	5.65	5.85	4.34	0.42	2.95	2.64	1.28	5.44	-0.78	3.17
T <sub>12</sub>	3.87	4.08	6.48	6.67	4.52	0.80	4.73	4.23	1.87	5.85	-0.66	3.17
T <sub>13</sub>	4.66	4.87	9.83	10.04	4.64	1.07	2.99	2.66	4.98	8.91	0.00	3.70
T <sub>14</sub>	7.55	5.58	11.57	10.13	4.68	1.42	3.14	2.80	6.60	8.56	2.75	3.96
T <sub>15</sub>	6.22	6.42	10.64	10.85	4.80	1.75	3.36	3.00	5.58	8.93	1.31	4.49
T <sub>16</sub>	6.93	7.14	11.13	11.33	4.87	2.27	5.67	5.08	6.00	8.85	1.96	4.63
T <sub>17</sub>	5.39	7.76	9.93	11.79	4.99	2.64	3.45	3.09	4.68	8.93	0.39	4.89
T <sub>18</sub>	-0.93	2.64	-2.94	-2.74	11.29	3.00	3.95	3.54	-12.78	-5.55	-11.00	-0.40
T <sub>19</sub>	-2.93	1.87	-5.80	-5.60	12.24	3.49	4.10	3.66	-16.06	-8.82	-13.49	-1.58
T <sub>20</sub>	-3.42	1.39	-7.00	-6.80	54.78	3.99	6.01	5.38	-39.91	-10.37	-37.55	-2.51

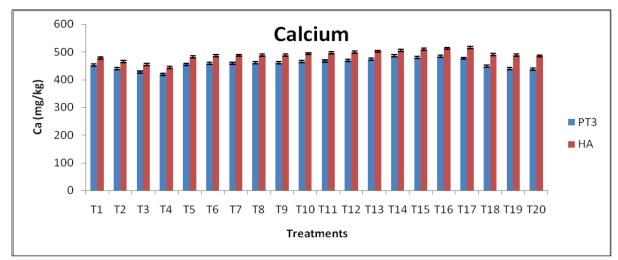


Fig. 1. Calcium content (mg/kg) in different treatments comparing PT3 and HA tomato varieties.

Data are presented as mean ± standard error. Blue bars represent PT3 (salinity susceptible variety) and red bars represent HA (salinity tolerant variety). Error bars indicate standard error of the mean. Statistical significance was determined at p < 0.05 level

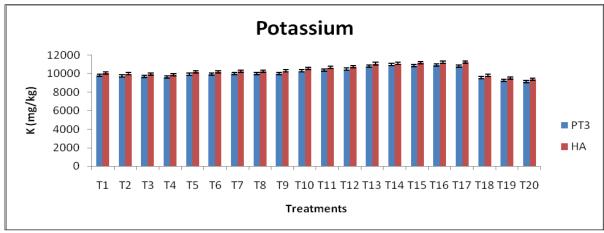


Fig. 2. Potassium content (mg/kg) in different treatments comparing PT3 and HA tomato varieties.

Data are presented as mean ± standard error. Blue bars represent PT3 (salinity susceptible variety) and red bars represent HA (salinity tolerant variety). Error bars indicate standard error of the mean. Statistical significance was determined at p < 0.05 level

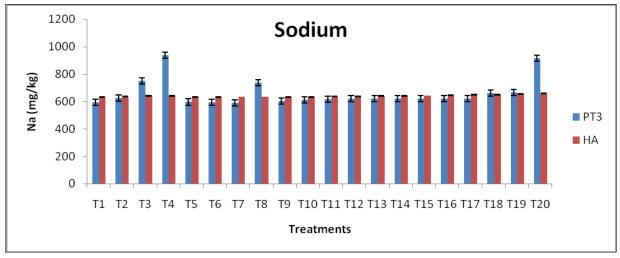


Fig. 3. Sodium content (mg/kg) in different treatments comparing PT3 and HA tomato varieties.

Data are presented as mean ± standard error. Blue bars represent PT3 (salinity susceptible variety) and red bars represent HA (salinity tolerant variety). Error bars indicate standard error of the mean. Statistical significance was determined at p < 0.05 level

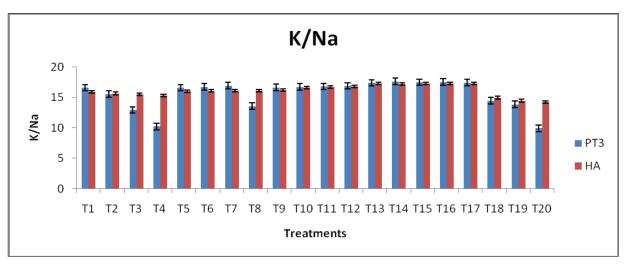


Fig. 4. Potassium to sodium (K/Na) ratio in different treatments comparing PT3 and HA tomato varieties.

Data are presented as mean ± standard error. Blue bars represent PT3 (salinity susceptible variety) and red bars represent HA (salinity tolerant variety). Error bars indicate standard error of the mean. Statistical significance was determined at p < 0.05 level

## Effect of nano-CuSO<sub>4</sub> on unstressed plants

Nano-CuSO $_4$  treatments alone ( $T_5$ - $T_8$ ) generally promoted beneficial changes in elemental composition. Low concentrations enhanced Ca ( $T_5$ -PT3:0.6% and HA: 0.87%;  $T_6$ -PT3:1.2% and HA:1.42%) and K ( $T_5$ -PT3:0.7 and HA:1 %;  $T_6$ -PT3:1.19 and HA:1.38) content in both varieties respectively (Table 1, Fig. 1, 2), while maintaining stable Na levels. Higher concentrations showed diminishing returns, with  $T_8$  (100 ppm) causing slight decrease in performance metrics.

The PT3 variety demonstrates superior copper accumulation capacity, with percentage increases ranging from +18.73% to +23.09%, while the HA variety shows more moderate increases from +15.57% to +19.45% (Table 1, Fig. 5).

## **Combined treatment effects**

The most significant findings emerged from combined salt and nano -CuSO<sub>4</sub> treatments (T<sub>9</sub>-T<sub>20</sub>). Sequential application of nano-CuSO<sub>4</sub> following salt stress effectively counteracted many adverse effects of salinity.

In PT3 variety, treatment  $T_{14}$  emerged as optimal, demonstrating enhanced Ca content (+7.55 %), increased K content (+11.57 %), improved Cu uptake (+3.14 %), favorable K/Na ratio improvement 6.60 % and positive Ca/Na ratio ( $T_{1}$ : 0.764) change by

 $2.75\,\%$  (Table 1, Fig. 4, 6). Other high-performing treatments included  $T_{16}$  (+6.93 % Ca, +11.13 % K) and  $T_{13}$  (+4.66 % Ca, +9.83 % K), indicating a treatment window of optimal effectiveness (Table 1, Fig. 1-3).

Moreover, in case of HA variety,  $T_{17}$  proved most effective with maximum Ca enhancement (+7.76 % vs control), highest K improvement by 11.79 %, substantial Cu increase by 3.09 %, superior K/Na ratio enhancement by 8.93 % and best Ca/Na ratio improvement by 4.89 % as compared to control (Table 1, Fig. 6).

 $T_{15}$  and  $T_{16}$  showed comparable performance, confirming the  $T_{13}$ - $T_{17}$  range as optimal for both varieties. Treatments ( $T_{18}$ - $T_{20}$ ) exhibited declining performance, with  $T_{20}$  showing particularly poor outcomes due to excessive Na accumulation (+54.78% in PT3).

# Impact on plant growth and reproductive performance

## Vegetative growth parameters

Plant height responded dramatically to the imposed treatments, with distinct varietal differences observed (Fig. 7). PT3 plants under control conditions reached an average height of 124.2 cm, while HA plants achieved 128.4 cm. Salt stress severely impacted PT3, with progressive reductions observed as NaCl concentrations increased from 25 mM ( $T_2$ : 104.4 cm) to 100 mM ( $T_4$ : 83.0 cm),

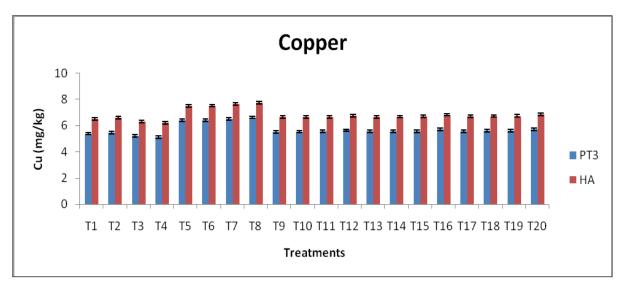


Fig. 5. Copper content (mg/kg) in different treatments comparing PT3 and HA tomato varieties.

Data are presented as mean  $\pm$  standard error. Blue bars represent PT3 (salinity susceptible variety) and red bars represent HA (salinity tolerant variety). Error bars indicate standard error of the mean. Statistical significance was determined at p < 0.05 level

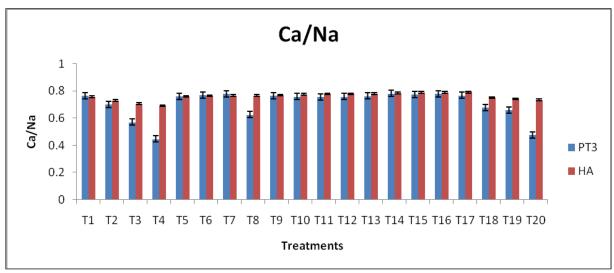


Fig. 6. Calcium to sodium (Ca/Na) ratio in different treatments comparing PT3 and HA tomato varieties.

Data are presented as mean  $\pm$  standard error. Blue bars represent PT3 (salinity susceptible variety) and red bars represent HA (salinity tolerant variety). Error bars indicate standard error of the mean. Statistical significance was determined at p < 0.05 level

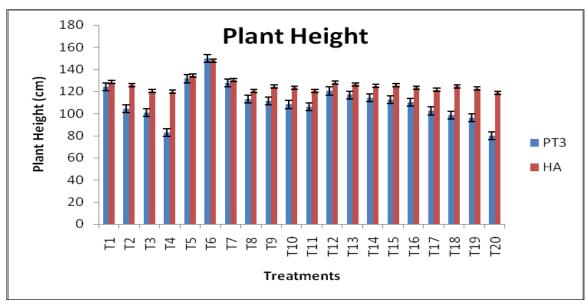


Fig. 7. Plant height (cm) of tomato varieties under different salt stress and nano-CuSO<sub>4</sub> treatments.

Blue bars represent PT3 (salinity-susceptible variety) and red bars represent HA (salinity-tolerant variety). Values represent mean  $\pm$  standard error. Error bars indicate standard error of the mean. Statistical significance was determined at p < 0.05 level

representing a 33.2 % reduction from the control. In contrast, HA showed greater resilience, maintaining heights of 125.83 cm, 120.18 cm and 119.54 cm under 25, 50 and 100 mM NaCl treatments respectively, with the maximum reduction being only 6.9 % under the highest salt concentration.

The application of nano-CuSO $_4$  at 10 ppm (T $_6$ ) produced remarkable growth enhancement in both varieties, with plant heights reaching 149.6 cm in PT3 and 147.8 cm in HA, representing increase of 20.4 % and 15.1 % over their respective controls. This suggests that nano-CuSO $_4$  not only mitigated salt stress effects but also provided growth-promoting benefits under non-stressed conditions. Higher nano-CuSO $_4$  concentrations showed diminishing benefits, with T $_8$  (100 ppm) reducing heights to 113.2 cm in PT3 and 120.4 cm in HA. Combined treatments generally maintain moderate plant heights ranging from 80.0-120.4 cm in PT3 and 118.76-127.8 cm in HA, indicating partial recovery from salt-induced growth inhibition.

Average number of branches followed similar trends to plant height, with significant implications for plant architecture and potential yield (Fig. 8). PT3 produced 22.5 mean number of branches under control conditions, while HA generated 25.0 branches, establishing a 11.1 % baseline advantage for the tolerant variety. Salt stress progressively reduced branching as NaCl concentrations increased, with 100 mM NaCl (T<sub>4</sub>) reducing average number of branches to 14.2 and 20.8 in PT3 and HA respectively, representing reductions of 36.9 % and 16.8 %.

The most remarkable response was observed with 10 ppm nano-CuSO<sub>4</sub> treatment ( $T_6$ ), which increased branching to 24.12 branches in PT3 and 31.0 branches in HA, surpassing control values by 7.2 % and 24.0 % respectively. This enhanced branching pattern suggested improved resource allocation and plant vigor under nano-CuSO<sub>4</sub> application. Combined treatments produced intermediate average number of branching ranging from 13.74-19.69 branches in PT3 and 17.8-24.6 branches in HA, confirming the stress-mitigating properties of nano-CuSO<sub>4</sub>, though complete restoration to control levels was not achieved under the most severe stress combinations.

# Reproductive performance

Salt stress significantly delayed flowering in both varieties, with PT3 showing greater susceptibility than HA (Fig. 9). Under control

conditions ( $T_1$ ), PT3 required 38 days to reach 50 % flowering, while HA achieved this in 35 days. The application of increasing NaCl concentrations caused progressive delays in flowering, with 100 mM NaCl ( $T_4$ ) extending the period to 49 days in PT3 compared to 43 days in HA, representing delays of 28.9 % and 22.9 % respectively. This differential response highlighted the superior stress tolerance mechanisms in HA, enabling more stable reproductive timing under saline conditions.

Nano-CuSO $_4$  only treatments ( $T_5$ - $T_8$ ) demonstrated ameliorative effects on flowering time in both varieties. The optimal concentration was 10 ppm ( $T_6$ ), which reduced flowering time to 34 days in PT3 and 32 days in HA, representing improvements of 10.5 % and 8.6 % over control conditions respectively. Even at 50 ppm ( $T_7$ ), flowering occurred at 35 days in PT3 and 32 days in HA, maintaining superior performance compared to controls. Combined treatments ( $T_9$ - $T_{20}$ ) generally produced intermediate effects, with flowering times ranging from 36-46 days in PT3 and 33-41 days in HA, suggesting that nano-CuSO $_4$  partially mitigated the adverse effects of salt stress on reproductive development, though the degree of amelioration decreased with increasing salt stress severity.

## **Discussion**

## Mechanisms of nano-CuSO<sub>4</sub> mediated salt tolerance

The ameliorative effects of nano-CuSO $_4$  on salt-stressed plants likely involve multiple mechanisms. Plant salinity resistance results from a combination of responses at the physiological, molecular, cellular and metabolic levels (9). Copper nanoparticles may enhance antioxidant enzyme activity, reducing oxidative stress induced by salt exposure (10). Additionally, nano-CuSO $_4$  may improve membrane integrity and selective ion transport, helping maintain favorable K $^+$ /Na $^+$  ratios.

The observed improvements in K and Ca uptake under nano-CuSO<sub>4</sub> treatment suggest enhanced nutrient acquisition mechanisms. Plants experience diverse abiotic stresses, encompassing low or high temperature, drought, water logging and salinity and nanoparticles may help plants cope with these challenges by improving cellular functions.

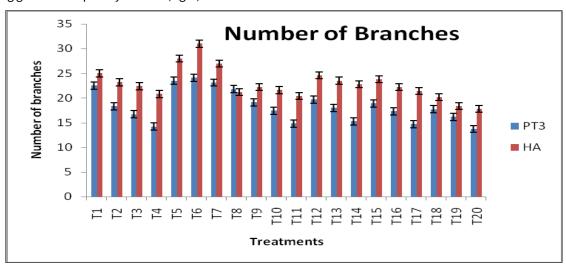


Fig. 8. Average number of branches of tomato varieties under different salt stress and nano-CuSO<sub>4</sub> treatments.

Blue bars represent PT3 (salinity-susceptible variety) and red bars represent HA (salinity-tolerant variety). Values represent mean  $\pm$  standard error. Error bars indicate standard error of the mean. Statistical significance was determined at p < 0.05 level

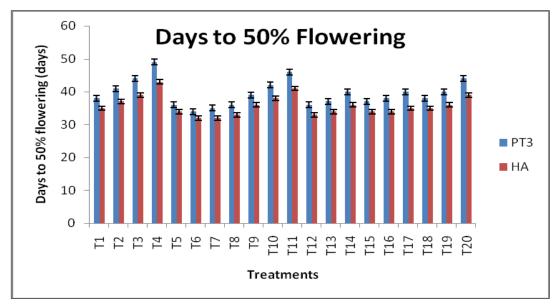


Fig. 9. Days to 50 % flowering of tomato varieties under different salt stress and nano-CuSO<sub>4</sub> treatments.

Blue bars represent PT3 (salinity-susceptible variety) and red bars represent HA (salinity-tolerant variety). Values represent mean ± standard error. Error bars indicate standard error of the mean. Statistical significance was determined at p < 0.05 level

# **Optimal treatment strategies**

The concentration-dependent effects of nano-CuSO₄ revealed an optimal application of 10 ppm (T<sub>6</sub>) for maximizing plant performance in both varieties. Higher concentrations (50-100 ppm) showed diminishing returns or potential phytotoxic effects, emphasizing the importance of precise dosage in nano-fertilizer applications. These morphological responses provide crucial insights into the mechanisms by which nano-CuSO₄ influences plant development under saline conditions and establish the foundation for understanding the physiological processes underlying these observed changes. Our results demonstrate clear treatment windows for maximum effectiveness. The superior performance of T<sub>13</sub>-T<sub>17</sub> treatments suggests that moderate salt combined with stress low-to-moderate nano-CuSO<sub>4</sub> concentrations provides optimal conditions for stress mitigation. This finding shows that mild stress combined with protective agents can enhance plant performance beyond control levels. Specifically, treatment with copper nanoparticles in tomato under moderate salinity (50mM NaCl) improved Na<sup>+</sup>/K<sup>+</sup> balance, resulting in enhanced physiological performance (11). Nanopriming under mild salinity stress showed enhanced abiotic stress tolerance, evoked antioxidant defense and improved growth and yield in wheat, especially with low-to-moderate nanoparticle doses (12). The declining performance of treatments (T<sub>18</sub>-T<sub>20</sub>) highlights the importance of treatment concentration. Excessive Na accumulation in these treatments may overwhelm the protective capacity of nano-CuSO<sub>4</sub>, emphasizing the need for preventive rather than corrective application strategies. Overdosing nanoparticles can lead to phytotoxicity, including oxidative damage and membrane disruption, underscoring the importance of dosage control (13).

# **Variety-specific considerations**

The distinct response patterns between PT3 and HA varieties underscore the importance of genotype-specific treatment protocols. The higher volatility observed is likely reflecting narrow tolerance range of PT3 variety to salt stress. Throughout all measured parameters, HA consistently demonstrated superior performance under salinity stress conditions compared to PT3,

validating its classification as a salinity-tolerant variety. The variety maintained more stable growth and reproductive performance across all treatment combinations, suggesting more efficient physiological mechanisms for coping with ionic and osmotic stress (Supplementary Fig. 1).

# Nano-CuSO4 on unstressed plants

Calcium and potassium show more modest improvements (1-2 %). This indicated that these essential macronutrients may have tighter homeostatic regulation.

Across all treatments  $T_5$ - $T_8$ , PT3 consistently outperforms HA variety by approximately 3-4 %, suggesting better genetic predisposition or physiological mechanisms for copper uptake and accumulation.  $T_8$  proves to be the most effective treatment for both varieties, achieving the highest copper enhancement (PT3: 23.09 %, HA: 19.45 %). The data indicated that treatments  $T_5$ - $T_8$  are highly effective for copper biofortification.

## Implications for sustainable agriculture

Global climate change and the decreasing availability of high-quality water led to an increase in the salinization of agricultural lands (14). Our findings offer practical solutions for this growing challenge. The identification of optimal nano-CuSO $_4$  concentrations provides a foundation for developing precision agriculture protocols for salt-affected soils.

## **Conclusion**

This study provides compelling evidence for nano-CuSO $_4$  as an effective tool for salt stress mitigation in tomato.  $T_{14}$  for PT3 variety and  $T_{17}$  for HA variety emerged as most effective, providing balanced improvements in elemental composition and ionic ratios. Distinct response patterns between varieties emphasize the need for genotype-specific treatment protocol. Nano-CuSO $_4$  effectively counteracts salt-induced ionic imbalances by enhancing beneficial element uptake while maintaining favorable K/Na and Ca/Na ratios. The morphological analysis further demonstrated that nano-CuSO $_4$  at optimal concentrations consistently improved plant architecture, significantly enhancing

both plant height and branching patterns in both varieties. Salt stress caused severe growth restrictions in the susceptible PT3 variety while the tolerant HA variety maintained relatively stable growth parameters, confirming distinct varietal responses to saline conditions. Nano-CuSO<sub>4</sub> treatments effectively accelerated flowering time, counteracting salt-induced reproductive delays and promoting earlier developmental transitions. Combined treatments demonstrated partial restoration of morphological parameters under moderate stress conditions, though complete recovery was not achieved under severe saline stress, indicating both the potential and limitations of nano-CuSO<sub>4</sub> intervention strategies. These findings contribute significantly to our understanding of nano-enabled salt stress mitigation and offer promising avenues for enhancing crop resilience in the face of increasing soil salinization challenges.

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## **Authors' contributions**

RA performed the experiments and did result analysis. RA wrote the manuscript and AP revised it, in the light of available literature, under the guidance of SA. The work was conceptualized and designed by SA. All authors read and approved of the final manuscript.

# **Compliance with ethical standards**

**Conflict of interest:** Authors do not have any conflict of interests to declare.

Ethical issues: None

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