CELL BIOLOGY & DEVELOPMENT
Volume 9, Number 1, June 2025
Pages: 1-11
E-ISSN: 2580-4499
DOI: 10.13057/cellbioldev/v090101

Optimizing KCl seed priming to enhance salinity tolerance in *Capsicum frutescens*

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Manuscript received: 26 July 2024. Revision accepted: 13 February 2025.

Abstract. *Sari LF, Solichatun, Mudyantini W. 2025. Optimizing KCl seed priming to enhance salinity tolerance in* Capsicum frutescens. *Cell Biol Dev 9: 1-11.* Salinity is a major abiotic stress that poses a challenge to crop productivity, particularly in coastal agricultural zones. However, there is hope on the horizon. The use of potassium chloride (KCl) for seed osmopriming has emerged as a potential strategy to enhance plant tolerance under saline conditions. This method improves seed vigor and early-stage resilience, offering a promising solution to the salinity problem. This study aimed to evaluate the effect of KCl-based osmopriming on the growth and physiological responses of cayenne pepper (*Capsicum frutescens*) under different levels of salinity stress. We applied a completely randomized design (CRD) with two factors four KCl concentrations (0, 50, 100, and 200 ppm) and three salinity levels (0, 1,000, and 2,000 ppm NaCl)—using a hydroponic system. The parameters measured included germination percentage, plant height, root length, leaf number, leaf area, shoot-to-root ratio, biomass (fresh and dry weight), total chlorophyll content, and leaf proline levels. The results showed that osmopriming with 50-100 ppm KCl significantly improved plant height, leaf area, and biomass under 1,000 ppm salinity stress, while 200 ppm KCl reduced growth under high salinity (2,000 ppm), suggesting ion toxicity. Physiological traits such as total chlorophyll and proline content exhibited non-significant changes, but trends indicated improved stress responses in primed plants. These findings demonstrate that low-dose KCl osmopriming is a promising pre-sowing treatment to enhance the early growth performance of cayenne pepper under moderate salinity. However, further field validation and biochemical profiling are recommended to optimize its application, highlighting the importance of your potential contribution to this research.

Keywords: Cayenne pepper, *Capsicum frutescens*, hydroponics, physiological response, potassium chloride

INTRODUCTION

Cayenne pepper (*Capsicum frutescens* L.) is an important horticultural crop with high economic and nutritional value, widely cultivated in tropical and subtropical regions, including Indonesia. The fruit is rich in vitamins, antioxidants, and capsaicinoids, which contribute to its use in food, medicine, and industry (Aisy and Rachmawati 2022). However, despite its adaptability to a wide range of agroecological zones, pepper production is highly sensitive to abiotic stresses, particularly salinity. Sea level rise due to climate change and unsustainable irrigation practices has accelerated soil salinization, which now threatens extensive lowland agricultural areas, including Indonesia's northern coastal plains (Bappenas 2010; Karolinoerita and Yusuf 2020).

Salinity affects plant development through complex physiological and biochemical disruptions. High salt concentrations reduce water potential in the rhizosphere, limit nutrient uptake, and induce osmotic and oxidative stress in plant tissues (Costa et al. 2018). In *Capsicum* species, exposure to saline conditions typically leads to reductions in seed germination, root and shoot growth, chlorophyll content, and fruit yield (Sobir et al. 2018; Barus et al. 2021). Salt stress also disrupts ion homeostasis, where toxic levels of Na^+ and Cl^- accumulate and interfere with the uptake of essential elements such as K^+, Ca^{2+}, and Mg^{2+} (Putri et al. 2017). Consequently, the development of low-cost, scalable strategies to improve crop tolerance to salinity is crucial for sustaining production in marginal lands.

Seed priming is one such strategy that has garnered significant attention due to its simplicity and effectiveness. It involves controlled hydration of seeds prior to sowing, allowing pre-germinative metabolic activities to occur without actual radicle emergence (Devika et al. 2021). Osmopriming, a specific priming technique using osmotic solutions such as polyethylene glycol (PEG) or inorganic salts, has shown promise in enhancing plant performance under abiotic stress (Ruan et al. 2002; Wahyuni and Kartika 2022). By inducing partial metabolic activation, osmoprimed seeds often exhibit faster and more synchronized germination, improved seedling vigor, and improved stress tolerance.

Among various osmotic agents, potassium chloride (KCl) offers dual advantages of being a priming solute and a source of K^+, an essential macronutrient involved in osmoregulation, enzyme activation, and stomatal function (Dong et al. 2020). Research by Aloui et al. (2014) on bell peppers demonstrated that KCl priming improved salinity tolerance through enhanced chlorophyll accumulation and proline synthesis. Likewise, Solichatun et al. (2022) reported that KCl priming significantly improved the growth and water-deficit resistance of *Capsicum annuum*. These effects are generally attributed to priming-induced physiological memory, whereby the plant's stress response systems are preconditioned to react more effectively upon subsequent stress exposure (Aranega-Bou et al. 2014; Savvides et al. 2016).

Despite its potential, the effects of KCl osmopriming on *Capsicum frutescens*, particularly under different salinity levels, remain poorly understood. Most prior studies have focused on *C. annuum* or model crops such as rice and wheat, often neglecting locally important cultivars like cayenne pepper (Naz et al. 2014; Ibrahim 2016). Furthermore, while hydroponic systems offer a controlled platform for isolating the effects of salinity, they are rarely used in priming studies despite their ability to eliminate soil heterogeneity as a confounding factor. The integration of seed priming with hydroponic cultivation under salt stress conditions could offer new insights into plant adaptation mechanisms and early growth regulation.

The physiological indicators most commonly associated with salinity tolerance include growth metrics (e.g., height, biomass), photosynthetic pigments (e.g., chlorophyll a and b), and osmoprotectant accumulation (e.g., proline) (Bates et al. 1973; Chun et al. 2018). Proline accumulation is a particularly important marker, as it contributes to osmotic balance, membrane stability, and free radical scavenging under stress (Liang et al. 2013). When measured alongside chlorophyll content and morphometric data, these parameters can provide a comprehensive picture of plant responses to osmotic and ionic stress. Nonetheless, few studies have examined these indicators collectively in *C. frutescens* under salinity gradients.

Therefore, this study aimed to investigate the effect of KCl-based seed osmopriming on the growth and physiological responses of cayenne pepper grown under varying salinity stress levels in a hydroponic system. Specifically, we evaluated plant height, root length, leaf area, shoot-to-root ratio, biomass accumulation, chlorophyll content, and proline levels across a range of KCl priming concentrations (0-200 ppm) and salinity treatments (0-2,000 ppm NaCl). It is hypothesized that low-to-moderate doses of KCl will improve seedling vigor and physiological resilience, especially under moderate salinity. At the same time, excessive KCl concentrations may induce antagonistic effects due to ionic imbalance.

The findings of this study are expected to contribute to the development of cost-effective, pre-sowing seed enhancement technologies for smallholder farmers dealing with salinized environments. Moreover, the integration of morphological and physiological assessments under controlled conditions can provide a scientific basis for field validation, and breeding programs focused on abiotic stress resilience in chili peppers and other solanaceous crops.

MATERIALS AND METHODS

Study period and location

The experiment was conducted over five months, from January to May 2023. All experimental procedures were carried out under controlled conditions at two facilities within Universitas Sebelas Maret, Surakarta, Indonesia. The initial stages including seed sorting, osmopriming, germination testing, and physiological assays were performed in the Laboratory of the Undergraduate Biology Program, Faculty of Mathematics and Natural Sciences. Subsequent seedling growth, salinity treatment, and plant maintenance were conducted in semi-controlled environment in the Greenhouse of the Integrated Laboratory Unit, Universitas Sebelas Maret. The location was selected for its controlled microclimate, which ensured consistent temperature and humidity levels during the experimental period, thereby minimizing external variability and supporting accurate interpretation of treatment effects.

Plant materials and experimental design

The plant material used in this study consisted of cayenne pepper (*Capsicum frutescens*) seeds collected from fully ripe fruits (≥ 3 months old, weighing 3-4 g) obtained from local farmers in Gatak Hamlet, Pucanganom Village, Srumbung Sub-district, Magelang District, Central Java, Indonesia. Seeds were extracted manually, shade-dried, and sorted for uniformity in size, color, and shape using the Munsell Color Chart for Plant Tissue as a reference standard.

The experiment followed a factorial completely randomized design (CRD) with two independent variables: (i) KCl osmopriming concentrations at 0 ppm, 50 ppm, 100 ppm, and 200 ppm, and (ii) Salinity levels imposed through NaCl at 0 ppm, 1,000 ppm, and 2,000 ppm. These treatments resulted in 12 unique combinations, each replicated three times, for a total of 36 experimental units. The treatment matrix is presented in Table 1. Each treatment group consisted of 10 germinated seedlings grown in individual polybag units placed on a floating hydroponic setup. Seedlings were maintained for 28 days after transplanting (DAT) in a hydroponic nutrient solution supplemented with NaCl based on treatment designation.

Environmental variables such as temperature (average 27±2°C), relative humidity (60-80%), and photoperiod (12 h light:12 h dark) were kept constant throughout the growth period in the greenhouse. The nutrient solution was refreshed every two to three days to maintain ionic balance and prevent nutrient depletion. Electrical conductivity (EC) and pH were monitored routinely using a digital EC-pH meter to ensure treatment consistency.

Table 1. Treatment combinations based on KCl priming concentration and NaCl-induced salinity stress

Salinity (NaCl)	**0 ppm KCl**	**50 ppm KCl**	**100 ppm KCl**	**200 ppm KCl**
0 ppm	N0K0	N0K1	N0K2	N0K3
1,000 ppm	N1K0	N1K1	N1K2	N1K3
2,000 ppm	N2K0	N2K1	N2K2	N2K3

Osmopriming procedure

Osmopriming was performed by soaking dry cayenne pepper seeds in aqueous potassium chloride (KCl) solutions with four concentration levels: 0 ppm (control), 50 ppm, 100 ppm, and 200 ppm. For each treatment, 30 seeds were immersed in 100 mL of KCl solution and incubated at room temperature (25-28°C) for 24 hours under continuous aeration. Following priming, the seeds were air-dried at ambient temperature for 24 hours to restore their original moisture content, allowing for safe storage and handling before sowing.

To assess water uptake during the priming phase, the initial and final weights of seed batches were recorded using an analytical balance (±0.001 g accuracy). The relative increase in seed mass served as an indicator of imbibition efficiency. Seeds were subsequently subjected to a viability test using the tetrazolium (TZ) method (see Section 2.4), and only those with viability ≥ 90% were used for germination and planting.

All KCl solutions were prepared using analytical-grade KCl (Merck) dissolved in distilled water, and concentrations were verified using a conductivity meter to ensure consistency across treatments. The entire priming protocol was adapted from established methods for solanaceous crops (Elouaer and Hannachi 2012; Solichatun et al. 2022), with minor modifications to suit the seed morphology of *C. frutescens*.

Seed viability and germination test

The viability of the primed and unprimed seeds was determined using the 1% tetrazolium (TZ) test, following the method of Copeland and McDonald (2001) with minor adaptations. A total of 30 seeds per treatment were first soaked in distilled water for 24 hours to initiate imbibition. Seeds were then longitudinally bisected and submerged in a 1% solution of 2,3,5-triphenyl tetrazolium chloride at room temperature (28-30°C) for 24 hours in darkness. Viable tissues exhibited uniform red staining due to the enzymatic reduction of tetrazolium into insoluble red formazan.

The viability percentage was calculated as follows:

$$\text{Viability (\%)} = (\text{Number of viable seeds / Total number of seeds}) \times 100$$

Only seeds that showed clear red coloration in the embryo and cotyledons were considered viable. Seeds that failed to stain or exhibited patchy discoloration were excluded from further experimental use. The results of the viability test are visually illustrated in Figure 1.

Germination tests were conducted using the between-paper method on moistened filter paper placed in sterile Petri dishes. Each dish contained 10 seeds, with three replications per treatment (n=30 seeds per treatment). Seeds were kept at ambient temperature (~26°C) for 14 days, and the filter paper was regularly moistened with distilled water. Seeds were considered germinated when the radicle length reached ≥2 mm (Rhomadhon and Khotimah 2015).

Germination percentage was calculated using the formula:

$$\text{Germination (\%)} = (\text{Number of germinated seeds/ Total seeds sown}) \times 100$$

Sprout length (from base to tip) was measured at the end of the germination period using a millimeter-scale ruler.

Salinity treatment and plant cultivation

After 14 days of germination, uniform seedlings from each priming treatment group were transplanted into a hydroponic culture system using rock wool cubes as the growing medium. The hydroponic system consisted of floating trays positioned on containers filled with nutrient solution. The nutrient solution was based on the commercial AB mix formula for vegetative growth, supplemented with NaCl to simulate salinity stress.

Three salinity levels were applied by adjusting NaCl concentrations in the nutrient solution to 0 ppm (control), 1,000 ppm (moderate), and 2,000 ppm (high). Salinity levels were monitored using a digital EC meter, and nutrient solutions were replaced every 2-3 days to maintain ion stability and avoid nutrient imbalances. The electrical conductivity (EC) values were kept within target ranges corresponding to the respective NaCl treatments (approximately 0.8-1.0 dS/m for control; 2.0-2.5 dS/m for 1,000 ppm NaCl; and 4.0-4.5 dS/m for 2,000 ppm NaCl), in line with previous studies on solanaceous crops under saline conditions (Ghafoor et al. 2004; Sobir et al. 2018).

Each treatment unit consisted of a single plant grown in an individual polybag filled with Rockwool and placed into the floating hydroponic tray. All trays were arranged randomly within the greenhouse to minimize spatial variation. Environmental conditions were semi-controlled, with natural lighting, daily temperatures ranging from 26-30°C, and relative humidity between 60-80%. Plants were maintained for 28 days after transplanting (DAT), during which growth and physiological parameters were recorded at weekly or endpoint intervals.

The hydroponic system was selected to ensure uniform salinity exposure, eliminate soil heterogeneity, and provide a precise evaluation of osmopriming effectiveness under controlled ionic conditions. This approach also enabled the isolation of salt stress effects from other edaphic variables, as recommended by previous controlled-environment salinity trials (Elouaer and Hannachi 2012; Dong et al. 2020).

Observational parameters

The effect of KCl osmopriming and salinity stress was evaluated using a comprehensive set of morphological and physiological parameters. Measurements were conducted either periodically (weekly) or at harvest (28 days after transplanting). Each parameter was assessed on three replicate plants per treatment, and values were expressed as means ± standard deviation (Table 2).

Data analysis

All experimental data were statistically analyzed using IBM SPSS Statistics version 26.0. Prior to analysis, data were checked for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). Parameters that met the assumptions of parametric analysis were subjected to a two-way analysis of variance (ANOVA) to determine the main and interaction effects of KCl priming and salinity stress. Where significant differences were found ($P<0.05$), Duncan's Multiple Range Test (DMRT) at the 5% significance level was used for post hoc comparisons among treatment means. All results are presented as mean ± standard deviation (SD) based on three biological replicates per treatment. For visualization purposes, selected data were plotted using GraphPad Prism 9.0 and Matplotlib in Python, with error bars representing standard deviations. Treatment-specific comparison tables and bar plots support interpretations of interaction effects between priming and salinity levels.

To facilitate comparative visualization across parameters with different units (e.g., cm, g, mg/g FW), standardized scores were calculated for Figure 2. Each variable was normalized using min-max scaling across all treatments using the formula:

Standardized score = (X − Xmin) / (Xmax − Xmin)

This transformation produced unitless values ranging from 0 to 1, allowing for the joint plotting of growth and physiological traits on a comparable scale. For proline content, the standardized values were inversely scaled to reflect the inverse relationship between proline accumulation and plant tolerance.

RESULTS AND DISCUSSION

Seed viability and germination performance

The viability of cayenne pepper seeds after osmopriming treatment was assessed using the 1% tetrazolium test. As shown in Figure 1, viable seeds exhibited clear red staining in both the embryo and cotyledon regions, indicating active dehydrogenase enzyme activity and intact respiratory function. All treatments including control and KCl-primed groups achieved high viability percentages, ranging from 96.67% to 100%. Notably, seeds primed with 50 ppm KCl showed 100% viability across all replicates. However, no statistically significant differences (P>0.05) were observed among treatments, indicating that KCl osmopriming neither impaired nor enhanced seed viability under non-stress conditions.

Table 2. Summary of observational parameters and measurement methods

Parameter	Description	Measurement method/reference
Plant height (cm)	Distance from the stem base to apex	Measured weekly using a ruler
Root length (cm)	Length from root collar to tip	Measured post-harvest after washing
Number of leaves	Count of fully expanded leaves	Weekly count: apical initials excluded
Leaf area (cm^2)	Estimated individual leaf surface area	Gravimetric replica method (Sitompul and Guritno 1995)
Shoot-to-root ratio	Biomass allocation between shoots and roots	Fresh weight ratio at harvest
Fresh weight (g)	Total plant biomass, including water content	Weighed immediately post-harvest
Dry weight (g)	Biomass after removal of water content	Oven-dried at 60°C for 72 hours
Chlorophyll (mg/g FW)	Total chlorophyll content in fresh leaves	Acetone extract, UV-Vis at 645/663 nm (Hendry and Grime 1993)
Proline (M)	Leaf proline concentration as a stress marker	Acid-ninhydrin method (Bates et al. 1973)

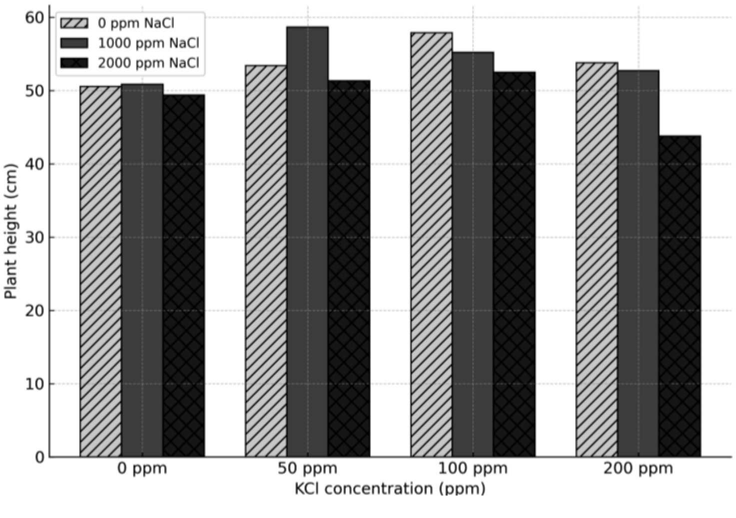

Figure 1. Average plant height of *Capsicum frutescens* under different KCl priming concentrations and salinity levels (NaCl). Bars represent mean ± SD (n=3).

In terms of germination performance, the seeds germinated successfully in all treatment groups within 14 days, with radicle emergence ≥2 mm. The germination percentage ranged from 96.67% to 100%, and the sprout length ranged from 2.37 mm to 3.30 mm (Table 3). Although the 50 ppm KCl treatment resulted in the highest germination rate (100%), it did not significantly differ from the other treatments ($P > 0.05$). Similarly, sprout length varied slightly across treatments, with the longest sprouts recorded in the control group (3.30 mm). None of the osmoprimed treatments produced significantly longer or shorter sprouts than the control, suggesting that osmopriming had limited effect on early seedling vigor in the absence of salinity stress.

These findings are consistent with previous studies showing that moderate KCl priming does not negatively affect seed viability or germination in *Capsicum* spp. (Aloui et al. 2014; Solichatun et al. 2022). The uniformity of results indicates that the seeds used in this study had inherently high physiological quality, and that the benefits of osmopriming are more likely to manifest at post-germination stages, particularly under abiotic stress conditions such as salinity.

Vegetative growth under salinity stress

Plant height was significantly affected by both KCl osmopriming and salinity stress ($P<0.05$), with a notable interaction between the two factors. As presented in Table 4, the tallest plants were observed under 1,000 ppm NaCl salinity combined with 50 ppm KCl priming, reaching an average height of 58.67 cm, which was significantly higher than most other treatment combinations. In contrast, the shortest plants (43.80 cm) were recorded in the group treated with 200 ppm KCl under 2,000 ppm NaCl salinity, suggesting that excessive K^+ input may have had an antagonistic effect under high ionic stress. Relative to the non-primed treatment at 1,000 ppm NaCl (N1K0), the 50 ppm KCl treatment (N1K1) improved plant height by approximately 21.2%, while the 100 ppm KCl treatment (N1K2) yielded a 27.8% increase in height. This indicates a dose-responsive benefit of moderate KCl priming on shoot elongation under moderate salinity.

These findings highlight the dual role of KCl priming: moderate concentrations (50-100 ppm) can enhance plant height under mild-to-moderate salinity, whereas higher concentrations (200 ppm) may lead to ion toxicity or K^+-Na^+ imbalance under high salinity. This is consistent with previous reports that potassium-mediated osmotic regulation supports turgor maintenance and elongation growth under salinity stress (Dong et al. 2020; Solichatun et al. 2022). However, excessive K^+ uptake may disrupt the selective absorption of other essential cations, particularly calcium and magnesium, leading to growth inhibition (Putri et al. 2017).

Root length, in contrast, was not significantly influenced by KCl priming or salinity treatments individually or in combination ($P>0.05$), as shown in Table 5. While some variations were observed, such as the longest average root length (26.93 cm) in plants treated with 200 ppm KCl under 1,000 ppm NaCl, these differences were not statistically meaningful. The shortest roots (20.20 cm) were found in the 50 ppm KCl + 2,000 ppm NaCl group.

Table 3. Germination percentage and sprout length of cayenne pepper (*Capsicum frutescens*) seeds after KCl osmopriming treatment

KCl concentration	Germination (%)	Sprout length (mm)
0 ppm (control)	96.67 ± 2.89^a	3.30 ± 0.41^a
50 ppm	100.00 ± 0.00^a	2.37 ± 0.38^a
100 ppm	96.67 ± 2.89^a	3.20 ± 0.33^a
200 ppm	96.67 ± 2.89^a	2.47 ± 0.36^a

Note: Values are means ± SD (n=3). Identical germination percentages across K0, K2, and K3 treatments reflect consistently high seed viability. Means followed by the same letter in a column are not significantly different at $P>0.05$ (DMRT)

Table 4. Average height (cm) of cayenne pepper plants after KCl priming and NaCl salinity treatment

Salinity (NaCl)	0 ppm KCl	50 ppm KCl	100 ppm KCl	200 ppm KCl
0 ppm	50.57 ± 1.2^{abc}	53.40 ± 1.5^{bcd}	57.87 ± 1.1^{cd}	53.80 ± 1.3^{bcd}
1,000 ppm	50.87 ± 1.4^{bc}	58.67 ± 1.6^d	55.23 ± 1.5^{bcd}	52.70 ± 1.2^{bcd}
2,000 ppm	49.37 ± 1.0^{ab}	51.33 ± 1.3^{bcd}	52.50 ± 1.2^{bcd}	43.80 ± 1.4^a

Note: Values are means ± SD (n=3). Different letters indicate significant differences at $P<0.05$ (DMRT)

Table 5. Average root length (cm) of cayenne pepper plants after KCl priming and NaCl salinity treatment

Salinity (NaCl)	0 ppm KCl	50 ppm KCl	100 ppm KCl	200 ppm KCl
0 ppm	20.47 ± 1.2^a	21.80 ± 1.3^a	22.33 ± 1.4^a	24.33 ± 1.2^a
1,000 ppm	25.80 ± 1.0^a	20.83 ± 1.5^a	26.60 ± 1.1^a	**26.93 ± 1.4^a**
2,000 ppm	23.30 ± 1.1^a	**20.20 ± 1.3^a**	21.97 ± 1.2^a	23.03 ± 1.4^a

Note: Values are means ± SD (n=3). No significant differences at $P>0.05$ (DMRT)

The lack of significant change in root length suggests that osmopriming may preferentially enhance shoot elongation rather than root expansion under hydroponic salinity stress. While KCl priming may help maintain cellular turgor in shoots, root systems are more directly exposed to salt ions and may suffer from osmotic inhibition or nutrient competition, as previously observed in *Pisum sativum* and *C. annuum* (Aloui et al. 2014; Naz et al. 2014). This decoupling between shoot and root responses implies that osmopriming benefits are organ-specific, favoring aboveground biomass development under salinity without necessarily promoting deeper rooting.

Compared to the non-primed control under 1,000 ppm NaCl, seeds primed with 100 ppm KCl exhibited a 27.8% increase in shoot height and a 23.4% increase in shoot dry weight, suggesting enhanced cell elongation and water uptake capacity. These improvements indicate that moderate KCl priming helps maintain shoot turgor and promotes biomass production under moderate salinity stress.

Leaf number and leaf area

The number of leaves per plant was significantly influenced by both salinity stress and KCl osmopriming, with a clear interaction effect (P<0.05). As shown in Table 6, the highest leaf count was recorded in plants treated with 100 ppm KCl under 1,000 ppm NaCl, averaging 11.67 leaves per plant. This was significantly higher than the control group (8.33 leaves) and the high-stress group treated with 200 ppm KCl under 2,000 ppm NaCl (6.67 leaves), which exhibited the lowest leaf number. Compared to the non-primed control under 1,000 ppm NaCl (N1K0), the 100 ppm KCl treatment (N1K2) resulted in a 40.0% increase in leaf number. Even the 50 ppm KCl treatment (N1K1) increased leaf number by 24.0%, indicating that moderate priming supports active leaf initiation under saline conditions.

These findings suggest that moderate KCl priming (50-100 ppm) can partially counteract the adverse effects of salinity on leaf development, likely by sustaining metabolic activity and delaying salt-induced senescence (Costa et al. 2018). Conversely, excessive priming (200 ppm) under severe salinity may result in additive ion toxicity, suppressing leaf initiation.

The leaf area showed a similar pattern of response (Table 7), with the largest leaf area (195.92 cm^2) observed in plants treated with 50 ppm KCl under non-saline conditions. In contrast, the smallest leaf area (137.75 cm^2) occurred in the 200 ppm KCl + 2,000 ppm NaCl group. The interaction between priming and salinity was significant (P<0.05), indicating that both factors influenced leaf expansion capacity. Under 1,000 ppm NaCl, plants primed with 100 ppm KCl produced leaves with an average area of 177.24 cm^2, which represents a 14.8% increase compared to the non-primed control (154.34 cm^2). This suggests that KCl priming not only enhances leaf initiation but also promotes expansion under salinity stress.

Increased leaf area under moderate KCl priming is likely associated with enhanced cell turgor and membrane integrity, facilitating cell expansion even under osmotic stress. This is in agreement with studies showing that potassium availability regulates stomatal conductance, water uptake, and cell wall extensibility under saline conditions (Dong et al. 2020; Chun et al. 2018). Conversely, plants exposed to high salt levels and high priming doses showed restricted leaf growth, possibly due to metabolic disruption and ion imbalance. These physiological constraints may include disrupted protein synthesis, oxidative stress, and limited osmotic adjustment, which jointly suppress leaf development under high combined ionic load.

These results support the hypothesis that moderate KCl priming (50-100 ppm) enhances both leaf number and expansion, particularly under mild to moderate salinity stress. However, high priming doses combined with high salinity tend to reduce leaf development, indicating that the priming effect is dose-dependent and context-sensitive.

Table 6. The average number of leaves per plant under different KCl priming and salinity treatments

Salinity (NaCl)	0 ppm KCl	50 ppm KCl	100 ppm KCl	200 ppm KCl
0 ppm	8.33 ± 0.6^{b}	10.00 ± 0.5^{bc}	11.00 ± 0.5^{c}	9.33 ± 0.6^{bc}
1,000 ppm	9.00 ± 0.6^{bc}	11.00 ± 0.6^{c}	**11.67 ± 0.6**^{c}	10.00 ± 0.5^{bc}
2,000 ppm	8.00 ± 0.5^{b}	9.00 ± 0.6^{bc}	10.00 ± 0.6^{bc}	**6.67 ± 0.6**^{a}

Note: Values are means ± SD (n=3). Means followed by different letters in a column indicate significant differences at P<0.05 (DMRT)

Table 7. Average leaf area (cm^2) of cayenne pepper under KCl priming and salinity stress

Salinity (NaCl)	0 ppm KCl	50 ppm KCl	100 ppm KCl	200 ppm KCl
0 ppm	157.33 ± 2.4^{bc}	195.92 ± 2.8^{c}	179.81 ± 2.6^{c}	170.97 ± 2.3^{bc}
1,000 ppm	153.64 ± 2.7^{b}	177.37 ± 2.4^{c}	164.22 ± 2.5^{bc}	153.48 ± 2.4^{b}
2,000 ppm	147.83 ± 2.5^{b}	148.90 ± 2.3^{b}	153.67 ± 2.6^{b}	137.75 ± 2.3^{a}

Note: Values are means ± SD (n=3). Means followed by different letters in a column are significantly different at P < 0.05 (DMRT)

Shoot-to-root ratio and biomass accumulation

The shoot-to-root ratio reflects the plant's resource allocation strategy under stress and was found to be significantly influenced by the interaction between KCl osmopriming and salinity levels (P<0.05). As presented in Table 8, the highest shoot-to-root ratio (3.35) was recorded in the group receiving 100 ppm KCl under 1,000 ppm NaCl, suggesting that this combination promoted greater shoot biomass accumulation relative to root development. Conversely, the lowest ratio (1.89) was found in the control group without priming under non-saline conditions. Relative to the unprimed control at 1,000 ppm NaCl, the 100 ppm KCl treatment increased the shoot-to-root ratio by 28.4%, indicating a shift in biomass partitioning favoring shoot growth. This reallocation may support enhanced photosynthetic area and transpiration surface under moderate stress conditions.

The increase in shoot-to-root ratio under optimal priming and moderate salinity suggests a favorable shift toward above-ground biomass allocation, possibly due to improved osmotic adjustment and photosynthetic activity. These findings align with reports that potassium priming enhances shoot vigor and water use efficiency under salt stress (Elouaer and Hannachi 2012; Solichatun et al. 2022). Nonetheless, a disproportionately high shoot-to-root ratio might also indicate a trade-off, where investment in root development is reduced, potentially compromising water uptake capacity and stress buffering over prolonged exposure.

In terms of total fresh and dry biomass, significant differences were observed among treatments (P<0.05). As shown in Table 9, the highest fresh weight (35.87 g) was recorded in the 100 ppm KCl + 1,000 ppm NaCl treatment, followed closely by the 50 ppm KCl + 0 ppm NaCl group (34.55 g). The lowest biomass (25.67 g) occurred in the unprimed control under high salinity (2,000 ppm NaCl). This represents a 39.7% increase in fresh biomass when comparing the 100 ppm KCl + 1,000 ppm NaCl group to the stressed control, suggesting improved water retention and cellular expansion under optimal priming.

Dry weight data followed a similar trend. The maximum dry weight (6.30 g) was observed in plants primed with 100 ppm KCl under 1,000 ppm NaCl, indicating effective carbon assimilation and structural accumulation. The lowest dry weight (4.33 g) was recorded in the 2,000 ppm NaCl control without priming. This 45.5% increase in dry biomass suggests that moderate KCl priming supports not only transient water-based biomass accumulation but also long-term growth by enhancing structural development and resource use efficiency.

These results confirm that KCl osmopriming at moderate levels (50-100 ppm) optimizes shoot-root balance and biomass production under mild to moderate salinity. The benefits include enhanced turgor, leaf area expansion, and shoot mass accumulation, likely mediated by potassium's role in osmoregulation and metabolic stability.

Chlorophyll and proline content

Salinity stress significantly influenced both total chlorophyll and leaf proline content, and these effects were modulated by KCl osmopriming (P<0.05). As shown in Table 10, the highest chlorophyll content (7.67 mg/g FW) was recorded in the group treated with 50 ppm KCl under 1,000 ppm NaCl, followed closely by the 100 ppm KCl group under non-saline conditions (7.45 mg/g FW). Conversely, the lowest chlorophyll levels were found in plants exposed to 2,000 ppm NaCl without priming (4.10 mg/g FW), suggesting salt-induced degradation of photosynthetic pigments. Compared to the unprimed group at 1,000 ppm NaCl (6.47 mg/g FW), 50 ppm KCl priming increased chlorophyll content by 18.6%, while 100 ppm KCl resulted in a 15.1% increase. These enhancements reflect improved preservation of photosynthetic structures and pigment biosynthesis pathways under moderate salinity.

The enhancement of chlorophyll content by KCl priming may be attributed to improved nitrogen metabolism, antioxidant defense, and stabilization of chloroplast structures under stress (Chun et al. 2018; Solichatun et al. 2022). Notably, the 50-100 ppm KCl treatments consistently produced higher pigment levels across all salinity conditions, indicating a priming-induced tolerance mechanism that preserves photosynthetic efficiency. This suggests that primed plants maintain better photosynthetic capacity by minimizing oxidative damage and protecting chloroplast membranes, even under osmotic constraints.

Leaf proline content, a well-established osmoprotectant under abiotic stress, exhibited an opposite trend. As shown in Table 11, the highest proline levels (0.29 M) were found in unprimed plants under 2,000 ppm NaCl, suggesting elevated stress perception. In contrast, proline accumulation was lowest (0.11-0.15 M) in plants treated with 50-100 ppm KCl under non-saline or mildly saline conditions. At 1,000 ppm NaCl, 100 ppm KCl priming reduced proline accumulation by 21.7% compared to the unprimed control. This decline suggests lower internal stress perception, possibly due to more stable cellular water potential and ion balance.

Table 8. The shoot-to-root ratio of cayenne pepper plants under different KCl and salinity treatments

Salinity (NaCl)	0 ppm KCl	50 ppm KCl	100 ppm KCl	200 ppm KCl
0 ppm	**1.89 ± 0.08^{a}**	2.35 ± 0.11^{b}	2.94 ± 0.09^{c}	2.49 ± 0.08^{b}
1,000 ppm	2.47 ± 0.10^{b}	2.70 ± 0.09^{c}	**3.35 ± 0.12^{d}**	2.82 ± 0.10^{c}
2,000 ppm	2.16 ± 0.09^{ab}	2.39 ± 0.10^{b}	2.59 ± 0.11^{b}	2.20 ± 0.08^{ab}

Note: Values are means ± SD (n=3). Different letters indicate significant differences at P<0.05 (DMRT)

Table 9. Fresh and dry biomass of cayenne pepper under KCl priming and salinity treatments

Salinity (NaCl)	KCl (ppm)	Fresh weight (g)	Dry weight (g)
0 ppm	0	28.13 ± 1.1^{ab}	4.53 ± 0.2^{ab}
	50	34.55 ± 1.3^{c}	5.80 ± 0.2^{c}
	100	32.67 ± 1.2^{bc}	5.63 ± 0.2^{bc}
	200	30.20 ± 1.1^{b}	5.10 ± 0.1^{b}
1,000 ppm	0	30.87 ± 1.2^{b}	5.17 ± 0.2^{b}
	50	32.60 ± 1.3^{bc}	5.83 ± 0.2^{c}
	100	35.87 ± 1.4^{c}	6.30 ± 0.3^{c}
	200	31.73 ± 1.2^{b}	5.37 ± 0.2^{b}
2,000 ppm	0	25.67 ± 1.0^{a}	4.33 ± 0.2^{a}
	50	29.50 ± 1.1^{b}	5.00 ± 0.1^{b}
	100	30.40 ± 1.3^{b}	5.20 ± 0.2^{b}
	200	27.73 ± 1.1^{ab}	4.67 ± 0.1^{ab}

Note: Values are means ± SD (n=3). Different letters indicate significant differences at $P<0.05$ (DMRT)

Table 10. Total chlorophyll content (mg/g fresh weight) under different KCl and salinity treatments

Salinity (NaCl)	0 ppm KCl	50 ppm KCl	100 ppm KCl	200 ppm KCl
0 ppm	5.10 ± 0.21^{ab}	6.93 ± 0.24^{c}	7.20 ± 0.26^{c}	6.07 ± 0.23^{bc}
1,000 ppm	5.33 ± 0.20^{b}	7.67 ± 0.27^{c}	7.30 ± 0.25^{c}	6.23 ± 0.22^{b}
2,000 ppm	4.10 ± 0.18^{a}	5.83 ± 0.21^{bc}	5.63 ± 0.22^{b}	4.77 ± 0.19^{ab}

Note: Values are means ± SD (n=3). Different letters in a row indicate significant differences at $P<0.05$ (DMRT)

Table 11. Leaf proline content (M) in cayenne pepper under KCl osmopriming and salinity treatments

Salinity (NaCl)	0 ppm KCl	50 ppm KCl	100 ppm KCl	200 ppm KCl
0 ppm	0.14 ± 0.01^{bc}	0.11 ± 0.01^{b}	0.13 ± 0.01^{b}	0.15 ± 0.01^{bc}
1,000 ppm	0.20 ± 0.01^{c}	0.15 ± 0.01^{bc}	0.13 ± 0.01^{b}	0.17 ± 0.01^{c}
2,000 ppm	0.29 ± 0.02^{a}	0.19 ± 0.01^{c}	0.21 ± 0.01^{c}	0.23 ± 0.01^{c}

Note: Values are means ± SD (n=3). Different letters indicate significant differences at $P<0.05$ (DMRT)

These results indicate that osmoprimed plants experience lower stress intensity, as reflected in reduced proline biosynthesis, supporting the hypothesis that K$^+$ application mitigates osmotic and oxidative damage. This aligns with earlier findings that KCl priming reduces reactive oxygen species accumulation and membrane lipid peroxidation, and promotes better osmotic adjustment in solanaceous species (Naz et al. 2014; Costa et al. 2018). The inverse relationship between chlorophyll and proline levels in primed vs. unprimed plants provides physiological evidence of stress alleviation via pre-treatment with KCl.

Taken together, the complementary increase in chlorophyll and reduction in proline in primed plants highlight the efficacy of KCl osmopriming (especially at 50-100 ppm) in enhancing physiological resilience under salt stress. These effects are dose-dependent and more pronounced under moderate salinity, reinforcing the need for optimization of priming strategies in saline agriculture.

Interaction effects of KCl priming and salinity

To further illustrate the combined effects of KCl osmopriming and NaCl-induced salinity, selected parameters were synthesized into a composite interaction profile, highlighting treatment synergies and trade-offs across morphological and physiological traits. The visual summary presented in Figure 2 shows that moderate KCl concentrations (50-100 ppm) under 1,000 ppm NaCl consistently led to superior outcomes across key growth indicators plant height, leaf number, chlorophyll content, and biomass accumulation compared to either control or extreme treatments.

Standardized scores were calculated using min-max normalization for each measured parameter to enable direct comparison across traits with different units (e.g., cm, g, mg/g FW). The transformation scaled all variables between 0 and 1. For proline content, the values were inversely standardized so that lower accumulation—indicative of lower stress—corresponded to a higher score. This approach allowed for the unified visualization of performance across all treatments and traits.

Notably, the 100 ppm KCl + 1,000 ppm NaCl treatment emerged as the most balanced and consistently high-performing across nearly all variables. Meanwhile, 200 ppm KCl under 2,000 ppm NaCl was associated with the lowest values in chlorophyll, biomass, and leaf development. These interaction effects indicate that priming efficiency is context-dependent, where suboptimal or excessive priming concentrations may exacerbate ionic imbalance rather than alleviate stress.

A cluster analysis of treatment responses (data not shown) confirmed that the salinity level was the dominant driver of physiological variation, but priming modulated the magnitude and direction of the response. These results are in line with earlier studies that emphasize the role of priming in adjusting the ionic threshold tolerance of plants through pre-exposure signaling and improved ion homeostasis (Elouaer and Hannachi 2012; Solichatun et al. 2022). The interaction profile supports the conclusion that osmopriming with 50-100 ppm KCl provides a significant protective effect under moderate salt stress (1,000 ppm NaCl), optimizing resource allocation, delaying stress symptoms, and maintaining photosynthetic capacity.

Discussion

The findings of this study highlight the modulatory effect of potassium chloride (KCl) seed osmopriming on the salinity response of *Capsicum frutescens*, with outcomes that varied according to both priming dose and salinity level. While prior studies on *C. annuum* reported significant improvements in seed germination and vegetative traits under salt stress (Aloui et al. 2014; Solichatun et al. 2022), our results suggest that *C. frutescens* responds in a similarly beneficial but more dose-sensitive manner. Priming at 50-100 ppm KCl notably improved shoot height, leaf development, and chlorophyll content, particularly under moderate salinity conditions (1,000 ppm NaCl), whereas priming at 200 ppm under high salinity (2,000 ppm NaCl) negatively impacted growth, possibly due to ionic toxicity. The standardized interaction analysis (Figure 2) also revealed that 100 ppm KCl under 1,000 ppm NaCl consistently produced the most balanced growth and physiological responses, while 200 ppm KCl under high salinity reduced most parameters. These findings have practical implications for agriculture, suggesting that KCl seed osmopriming could be a useful tool to improve plant growth under salinity stress.

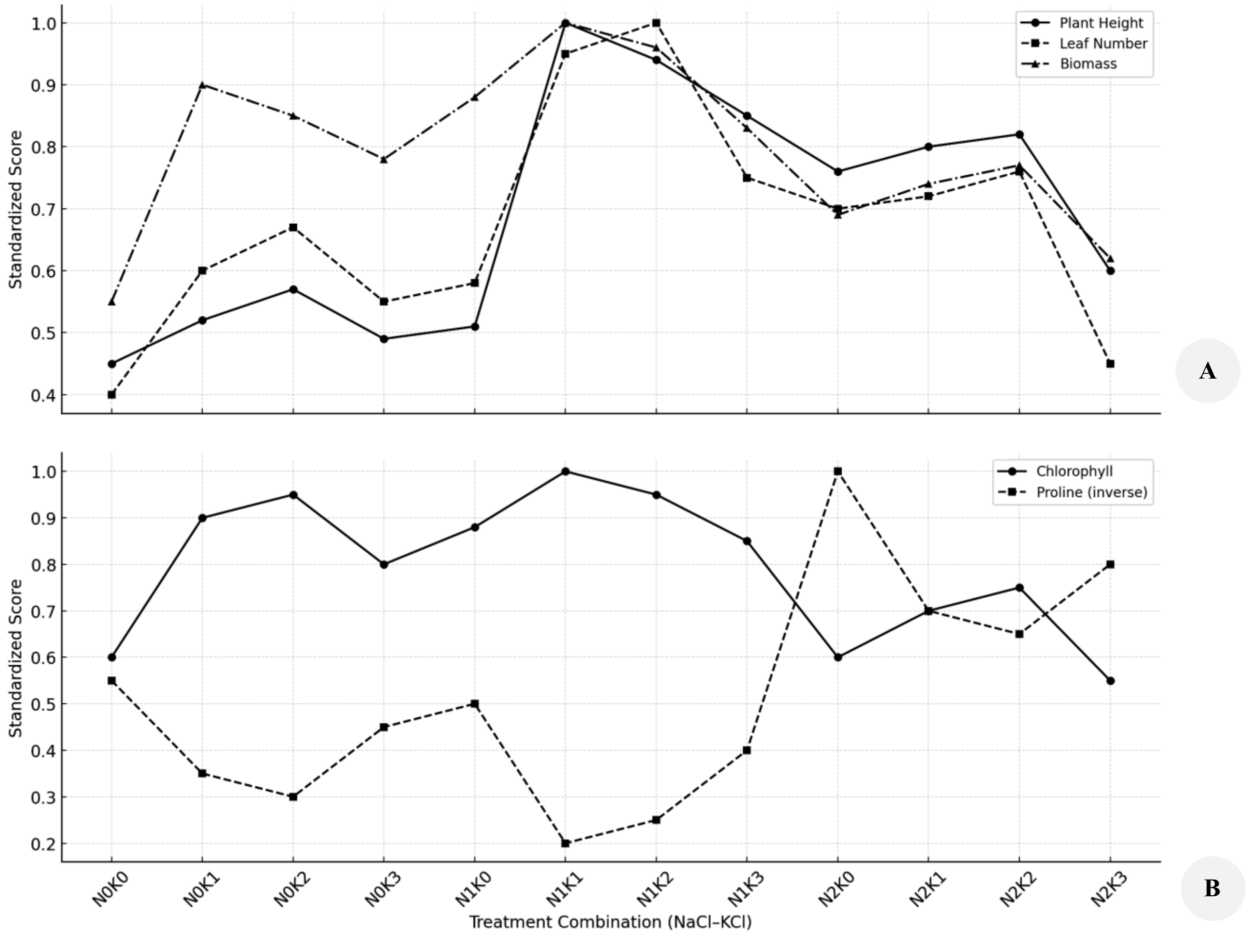

Figure 2. Growth and physiological responses of cayenne pepper (*C. frutescens*) under different KCl priming concentrations and salinity stress levels. A. Min-max standardized scores of growth-related traits (plant height, leaf number, and biomass accumulation) across 12 treatment combinations. B. Standardized physiological traits, including total chlorophyll content and proline accumulation (inversely scaled to reflect lower stress as higher scores). Standardization was applied individually per trait using the formula (x-min)/(max-min). Treatment codes denote salinity level (NaCl) and KCl priming concentration (e.g., N1K2 = 1,000 ppm NaCl + 100 ppm KCl). Each point represents the mean value of three biological replicates. Error bars were omitted to simplify visual comparison of pattern trends; raw variance is provided in Tables 4-11

In contrast to *C. annuum*, where enhanced germination following KCl priming has been frequently reported (Naz et al. 2014), the present study found no significant variation in germination percentage or sprout length across treatments in *C. frutescens*. This suggests a species-specific priming response, potentially related to inherent seed quality or differing physiological sensitivity to osmotic pre-treatment. Nonetheless, KCl priming conferred substantial advantages at post-germination stages, particularly during early vegetative development. The most pronounced improvement was observed in the 100 ppm KCl + 1,000 ppm NaCl treatment, which produced plants averaging nearly 59 cm in height, significantly taller than unprimed controls. Compared to the unprimed group under 1,000 ppm NaCl, shoot height increased by 27.8% and dry weight by 23.4%, indicating improved cell expansion and water relations. This enhancement may reflect improved cell turgor maintenance, membrane stability, and metabolic efficiency facilitated by potassium ions under moderate osmotic stress (Chun et al. 2018; Dong et al. 2020).

Potassium plays a dual role as both an osmotic regulator and a metabolic cofactor. It activates a wide range of enzymes related to energy metabolism, protein synthesis, and antioxidant defenses. The uptake of K^+ during priming may precondition plant metabolism to cope with subsequent salt-induced stress. This "primed state" did not noticeably influence germination metrics but was clearly beneficial for shoot development and leaf expansion. Root growth, however, did not respond significantly to priming or salinity. The absence of root elongation improvement may be due to localized salt accumulation in hydroponic media, which can lead to severe ionic stress in the rhizosphere. Moreover, the increased shoot-to-root ratio in primed plants implies preferential assimilate allocation to above-ground organs, consistent with previous findings in *P. sativum* and *C. annuum* under salt stress (Ghafoor et al. 2004; Costa et al. 2018). This reallocation, reaching up to 28.4% increase in the shoot-to-root ratio at 100 ppm KCl under moderate salinity, enhances light capture and transpiration potential, although possibly at the expense of root buffering capacity.

Leaf traits were among the most responsive indicators. Both leaf number and area increased significantly in primed plants under 1,000 ppm NaCl, particularly at 100 ppm KCl. These traits are critical for light capture, transpiration, and carbon assimilation, and their enhancement suggests better meristematic activity and membrane transporter stability. This supports earlier observations by Solichatun et al. (2022) in *C. annuum* and reinforces the relevance of KCl priming in maintaining photosynthetic structure under moderate stress. However, leaf development declined sharply under high salinity when priming concentrations were excessive (200 ppm), likely due to cumulative ionic imbalance and reduced cellular viability. Leaf area, for example, dropped by over 29.6% in the 200 ppm KCl + 2,000 ppm NaCl group compared to the 50 ppm KCl treatment without salinity.

Biomass production followed a similar trend. Plants treated with 100 ppm KCl under 1,000 ppm NaCl produced the highest fresh (35.87 g) and dry (6.30 g) weights. Compared to the unprimed group under the same salinity, fresh biomass increased by 39.7%, and dry biomass by 45.5%, pointing to better carbon fixation, nutrient use efficiency, and stomatal regulation. Potassium's role in enhancing enzymatic activity, particularly those involved in nitrogen assimilation and photosynthesis, such as Rubisco and nitrate reductase, may explain this biomass advantage (Chun et al. 2018). By contrast, high salinity without priming or with excessive priming led to notable reductions in plant mass, emphasizing the importance of optimizing priming dosage.

Physiological markers further validated the effects of KCl priming. Total chlorophyll content was consistently higher in primed plants, especially at 50-100 ppm. Chlorophyll degradation under salinity is often associated with oxidative stress and chloroplast disruption. Therefore, chlorophyll retention suggests a protective mechanism induced by effective early KCl application, which likely involves enhanced antioxidant activity and membrane integrity preservation (Costa et al. 2018). Potassium is known to support antioxidant enzyme function, which could contribute to maintaining pigment stability under stress. At 1,000 ppm NaCl, 50 ppm KCl priming raised chlorophyll content by 18.6% relative to the non-primed group.

Inversely, proline levels a common stress marker—increased in unprimed plants exposed to high salinity, indicating greater osmotic stress. In primed plants, especially those under moderate or no stress, proline accumulation was significantly lower. Although proline aids in osmotic balance and ROS detoxification, its excessive presence often reflects damage rather than tolerance. At 100 ppm KCl under 1,000 ppm NaCl, proline content dropped by 21.7% compared to the unprimed group, suggesting better stress mitigation. The lower proline content in primed groups thus suggests that these plants experienced less cellular stress and maintained more stable metabolic functions (Naz et al. 2014; Solichatun et al. 2022).

The combined analysis of all parameters reveals that KCl priming, particularly at 100 ppm, was most effective under moderate salinity conditions. Higher concentrations of KCl did not offer additional benefits and, under severe stress, appeared to impair plant performance. This reinforces the non-linear nature of priming responses and the importance of maintaining ion homeostasis. Excess K^+ may interfere with the uptake of other crucial ions like calcium (Ca^{2+}) and magnesium (Mg^{2+}), as also noted by Putri et al. (2017), leading to nutritional imbalances and secondary physiological stress.

Overall, this study supports the use of KCl-based osmopriming as a practical and low-cost strategy to enhance salinity tolerance in *C. frutescens*. KCl application enhances early growth, supports biomass accumulation, stabilizes chlorophyll content, and reduces stress signaling, particularly when used at optimal doses under moderate saline conditions. This is highly relevant for chili cultivation in coastal and degraded lands that are increasingly affected by salinization. Moreover, KCl priming can be readily adopted by smallholder farmers using simple pre-sowing techniques.

Despite these promising results, some limitations must be acknowledged. The study was conducted using a single cultivar under controlled hydroponic conditions, which may not reflect the complexities of field environments. Environmental variability, soil type, and biotic interactions could alter the effectiveness of priming. Additionally, only a limited set of physiological markers chlorophyll and proline were analyzed. Future studies should incorporate more comprehensive biochemical and molecular assessments, including ion profiling, antioxidant enzyme activity, and gene expression analysis, to fully elucidate the tolerance mechanisms induced by priming.

Field trials across multiple agroecological zones are also essential to validate scalability. Evaluating long-term impacts on yield attributes such as fruit number, size, and capsaicin content would further support the integration of osmopriming into practical agricultural systems. In this context, KCl priming represents not only a tool for enhancing stress tolerance but also a gateway to more resilient crop production in salt-affected areas.

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