

Effects of chemical seed priming on germination performance and seedling growth of *Lycopersicon esculentum* (Mill.) under salt stress

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Abstract. As an important economic plant, *Lycopersicon esculentum* (Mill.) faces salinity stress from germination to all growth stages. The aim of this study is to ride salt-induced agriculture difficulties of tomato by applying different chemical seed priming: ascorbic acid (ASA), potassium nitrate (KNO₃) and calcium nitrate (CaNO₃) during two time periods which are 24 and 48 hours. In the current case, the seeds were pre-treated with previously mentioned chemicals for varying periods of time before germination in a salt solution (100 mM NaCl). The treatments were replicated three times. For no primed seeds, salt treatment decreased germination parameters as well as seedling growth parameters (fresh weight, epicotyl and root length and chlorophyll content). Different chemical seed priming alleviated the salt harmful effect on germination and growth parameters. In saline conditions, the priming agents, had more significant effect in comparison with normal conditions. Significantly, the treatment including CaNO₃-48-h priming, had high efficacy in promoting germination and plant growth and is associated with reduced levels of leaf proline and malondialdehyde (MDA) content.

Key words: ascorbic acid, calcium nitrate, potassium nitrate, tomato, priming, salinity, seed germination.

INTRODUCTION

Salt exposure is one of major environmental stress that affect plant growth and agricultural crop (Mbarki et al., 2020; Shahid et al., 2020). Like several arid regions, in Saudi Arabia, tomato growth suffered from sub-optimal conditions, especially salinity (Al-Harbi et al., 2017). Germination stage is one of the most sensitive stages of plant growth to salinity, and as shown by Rajabi Dehnavi et al. (2020), salinity decrease the germination rate due to an increase in the osmotic pressure of the soil solution, resulting in a slowdown in the uptake and a decrease in the absorption of water required for the movement of various metabolic processes. The study conducted by Wang et al. (2020) showed that salt stress leads to a reduction in germination rate and this is mainly related

to seed inability to take in the required amount of water, and also because of the poisoning of the foetus by high concentrations of some ions such as chlorine (Hakim et al., 2010).

The used technique is 'seed priming', it's a commonly used approach that involves submerging the seeds in water and/or a chemical solution (Pawar & Laware, 2018). Seed priming technique has been discovered as a successful approach to enhance stress tolerance in several plant species. Several physiological treatments have been extensively studied to enhance seed germination and emergence of seedlings under various stress conditions (Ghoohestani et al., 2012; Moaaz et al., 2020; Ikram et al., 2023; Mangal et al., 2023). In addition, the combination of growth regulators of plant and different pre-sowing seed treatments could improve seed performance in a number of vegetable crops. Favorable results of KNO_3 priming have been obtained for several plant species, including soybean, tomato and wheat (Ghassemi-Golezani et al., 2011; Feghhenabi et al., 2020; Moaaz et al., 2020; Hadia et al., 2023). Salles et al., 2019 demonstrated that calcium nitrate priming increased the germination rate of eggplant seeds. Previously, Ghoohestani et al., 2012 demonstrated the effect of ASA, and salicylic acid on tomato seed germination improvement.

Hence, the aim of this research is to examine the effect of chemical priming (ASA, KNO_3 and $CaNO_3$) on germination performance and seedling growth of tomato in salt conditions within different priming periods (24 and 48 hours).

MATERIALS AND METHODS

Tomato seeds (GRObite Desi Tomato Vegetable Seeds) were sterilized with 0.25% sodium hypochlorite solution for 10 minutes. Seeds were firstly divided into 7 groups: (No pre-treated seed group, 3 groups were pre-treated with different priming solutions (KNO_3 2%, ASA 150 mg L and $CaNO_3$ 0.2%) for 24 hours, and 3 groups were pre-treated with the same priming solutions for 48 hours, with gentle shaking (Ghoohestani et al., 2012; Salles et al., 2019; Moaaz Ali et al., 2020). After that, the priming seeds were placed for germination in Petri dishes soaked with distilled water or NaCl solution (100 mM). Finally, each priming agent resulted in 4 treatments (2 time periods of priming and 2 irrigation solutions). Two controls are designed: Negative control means no stressed and no primed seeds; positive control means salt stressed and no primed seeds. For each treatment, 3 petri dishes were used with twenty seeds in each. The treatments were replicated three times.

Seeds were considered germinated when the radicle and epicotyl emerged. Samples were sorted by epicotyl and radicle for determination of fresh weight, photosynthetic pigments, malondialdehyde (MDA) and proline content.

According Wu et al., 2019, we determined Final germination percentage (FGP).

$$1. FGP = NGS/NTS \times 100$$

Also mean germination time (MGT)

$$2. MGT = \sum (N_i T_i) / \sum (N_1 + N_2 \dots + N_i)$$

We analyzed Germination rate index (GRI) and germination index (GI) using the formula proposed by Shah et al. (2021).

$$3. GRI = N_1/T_1 + N_2/T_2 + \dots + N_i/T_i$$

$$4. GI = (10 \times N_1) + (9 \times N_2) + \dots + (1 \times N_i)$$

Abbreviations used in above equations were:

NGS: number of final germinated seeds (in the end of experiment: 6 days)

NTS: number of total tested seeds

Ni: number of seeds germinated in the i^{th} time

Ti: time taken for seed germination at i^{th}

The methodology utilized to determine chlorophyll was that of Arnon (1949). At 663 nm and 645 nm, the absorbance of each sample was measured. Using the formula provided by MacKinney (1941), the chlorophyll content was computed and expressed in mg per g FW.

$$\text{Total Chlorophylls (mg L)} = 20.2 \times A_{645} + 8.02 \times A_{663}$$

A represents the extract's absorbance at the specified wavelength.

According to the ninhydrin method, we estimated proline content (Pro) in tomato seedlings (Bates et al., 1972).

In epicotyl, the total amount of malondialdehyde (MDA) was determined using the approach outlined by Heath & Packer in 1968. An extraction procedure was conducted on leaf samples utilizing a solution comprising 10% trichloroacetic acid (TCA) and 0.65% 2-thiobarbituric acid. The MDA concentration in epicotyl sample was expressed as nmol g^{-1} FW.

ANOVA analysis and Tukey's HSD tests were used to ascertain significant variations between the means of different treatments at probability level ≤ 0.05 .

RESULTS AND DISCUSSION

Germinations parameters

Globally, tomatoes are ingested due to their rich nutrient and bioactive compound content (Li et al., 2021; Ali et al., 2021). Recent studies declared that salt is one of major factors that affect plant yield and fruit quality (Zhang et al., 2022). Although salinization of soil and water is a natural process, it is exacerbated by anthropogenic activities such as land clearance and improper irrigation techniques.

In the absence of seed priming, salt treatment inhibited seed germination (Fig. 1, A). Various chemical priming methods were shown to enhance seed germination under salt stress, with the most effective treatment being seed priming for 48 hours (as depicted in Fig. 1, A, Fig. 1, B, and Fig. 1, C). In normal conditions, KNO_3 and CaNO_3 priming had no effect on final germination percentage (FGP) (Fig. 1, D). However, ASA agent induced a slight decrease of FGP (10%). In absence of seed priming, salt reduced FGP by 25% (Fig. 1, D). With 24-h chemical priming, salt reduced too FGP by 28%, 13 and 10% in ASA, KNO_3 and CaNO_3 , respectively. In 48-h pre-treated seeds, ASA and KNO_3 agents decreased FGP by about 20% and 11% respectively, however CaNO_3 priming gave 100% FGP (Fig. 1, D). Found result is supported by several studies which reported the effect of seed priming in improving germination rates, increase grain production, and improve seedling growth (Goiba et al., 2018; Nouri & Haddioui, 2021). Different methods were used to mitigate harmful effect of salt: as seed priming (Ben Youssef et al., 2023), salt co-treatment in media (Moghaddam et al., 2023) or foliar spray (El-Hawary et al., 2023).

Recently Ben Youssef et al., 2023 revealed the effectiveness of seed priming with calcium chloride on germination and seedling growth in barley species. Exogenous KNO_3 application alleviates salt effect on glasswort growth (Moghaddam et al., 2023)

and foliar application of ASA improved growth of wheat under salt treatment (El-Hawary et al., 2023). The current study found that different chemicals used in seed priming improved germination under salt stress; the most effective treatment for alleviating salt stress seems to be the CaNO_3 -48-hour priming treatment (Fig. 1, D). For ASA agent, more research should be done to identify an effective ASA concentration that improve salt stress mitigation in tomato.

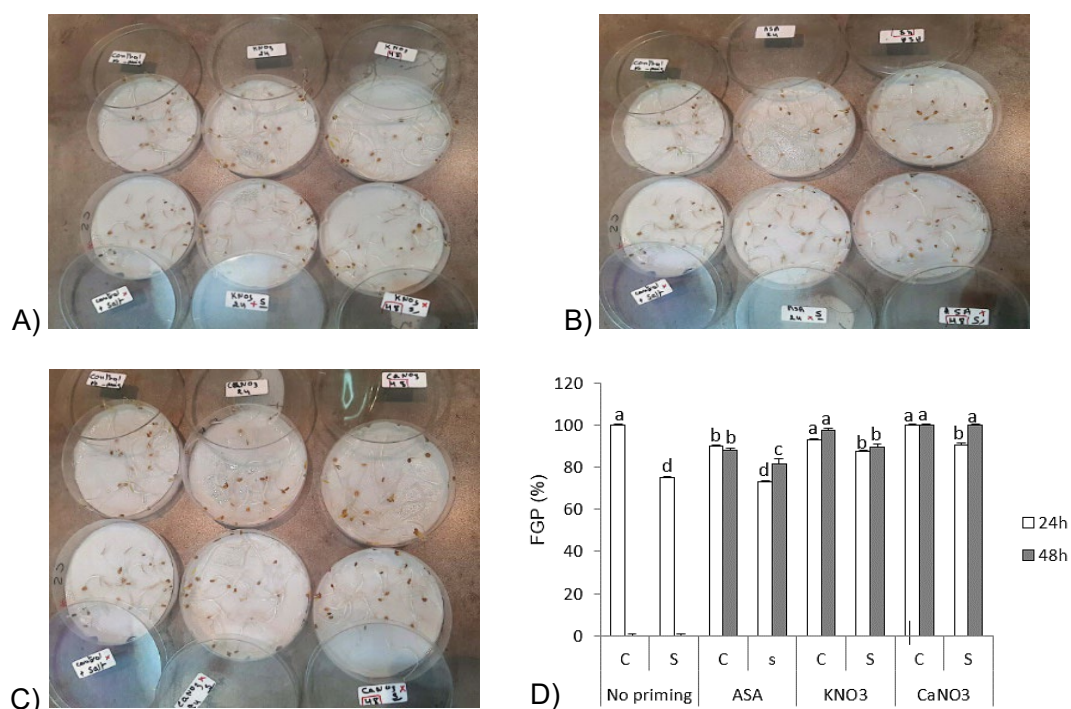


Figure 1. Germination test of tomato seeds in Petri dishes with different chemical priming: Ascorbic Acid priming (ASA) (A), potassium nitrate (KNO_3) (B) and calcium nitrate priming (CaNO_3) (C) within 24 h and 48 h. Effect of different chemical priming on Final germination percentage (FGP (D) within 24 and 48 hours after 6 days of germination under salt conditions. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.

In normal conditions, chemical priming had minor effect on mean germination time (MGT) (Fig. 2, A). Under salt treatment, MGT increased in all chemical priming agents by about 28% that which was close to MGT in positive control both in 24-h and 48-h treated seeds (Fig. 2, A). So, different priming agents had no significant effect on germination time in normal conditions (Fig. 2, A). However, under salinity, priming agents decreased MGT in comparison with positive control, particularly with a 48-h CaNO_3 treatment (11%).

In normal conditions, KNO_3 and CaNO_3 increased germination rate index (GRI) in both 24-h primed seeds and 48-h primed seeds. GRI increase was most significant in KNO_3 and CaNO_3 -48-h primed seeds by 13% and 29%, respectively (Fig. 2, B). In no primed seeds, salt reduced enormously GRI by 75%. In primed seeds, salt reduced less GRI especially in 48-h treated seeds. In ASA and KNO_3 pretreated seeds, salt reduced

GRI by more than 60% referring to positive control. In 48-h- CaNO_3 primed seeds, salt treatment reduced GRI only by 54% referring to positive control (Fig. 2, B).

In normal conditions, different chemical priming had no significant effect on germination index (GI) (Fig. 2, C). In no primed seeds, salt reduced GI by 47% referring to negative control. In ASA primed seeds, salt reduced GI by 37% and 27% after 24 h and 48 h of seed pretreatment, respectively. In KNO_3 primed seeds, salt reduced GI by about 25% in both 24 and 48 h of seed priming. In CaNO_3 primed seeds, salt reduced GI respectively by 26% and 16% in 24-h and 48-h seed priming. So, CaNO_3 priming restored more GI after 48 h of treatment in comparison with others priming agents (Fig. 2, C).

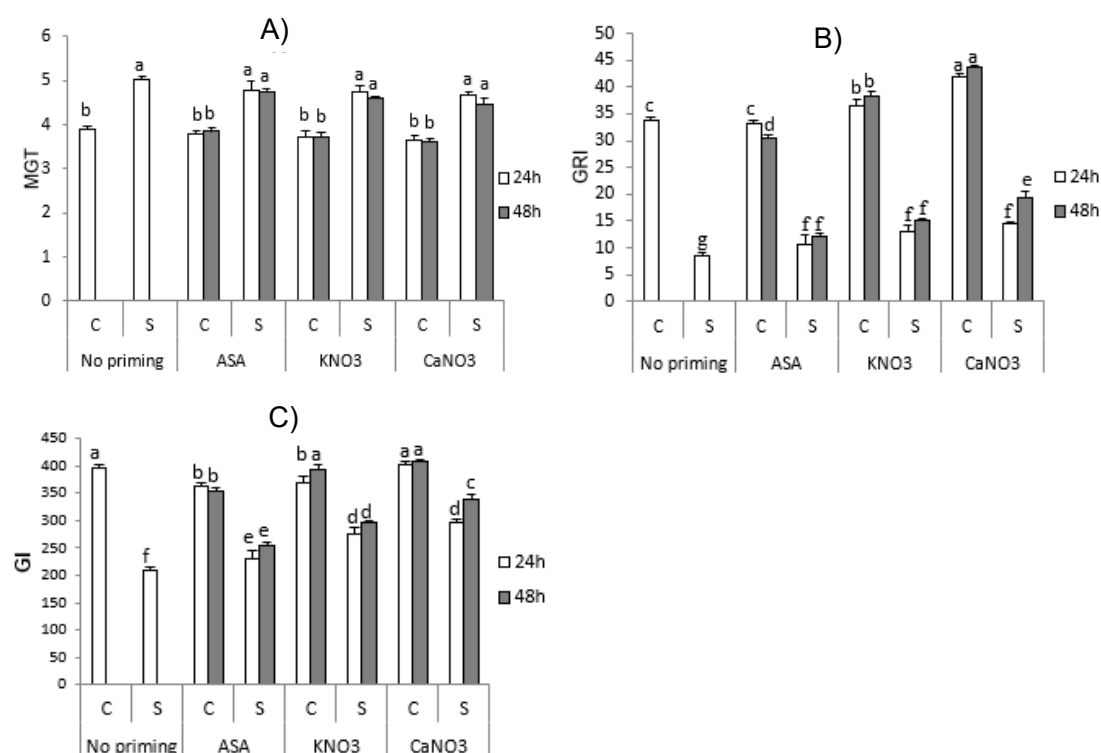


Figure 2. Effect of different chemical priming (ASA/ KNO_3 / CaNO_3) and time (24 h/48 h) on mean germination time (MGT) (A), Germination rate index (GRI) (B) and germination index (GI) (C) in normal and salt conditions. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.

The current study revealed that KNO_3 and CaNO_3 seed priming increased GRI in normal conditions. Khoshvaghti et al., 2013 reported same results in *Anethum graveolens*. In addition, KNO_3 seed priming has been reported to enhance the germination rate of pepper seeds (Tu et al., 2022).

Chemical priming had no significant effect on MGT in normal conditions (Fig. 2, A). However, in several studies, seed priming reduced MGT, indicating that primed seeds are capable of germination in a shorter period of time (Nazari et al., 2017; Arun et al., 2022).

Found result revealed that without priming, salt treatment restrained germination seed (Fig. 1, A). Salt reduced FGP and GRI by 25% and 75%, respectively (Fig. 1, C; Fig. 2, B). This result is supported by Chakma et al. (2019) which demonstrated that salt stress decreased the rate of germination in tomato plant.

In current study, different chemical priming agents restored germination under salt treatments, especially 48-h seed priming. In CaNO_3 -48-h pretreated seeds, FGP remain equal to normal conditions (100%) as shown in Fig. 1, C. Results showed that GRI increase was most significant in 48-h primed seeds by CaNO_3 (29%) referring to positive control (Fig. 2, B). CaNO_3 priming restored more GI after 48 hours of treatment in comparison with other chemical priming agents (Fig. 2, C).

Growth parameters

The chemical priming agents induced growth enhancement of tomato seedlings under both normal and salt conditions, as demonstrated in Fig. 3, C, Fig. 3, D, and Fig. 3, E. Notably, the 48-h CaNO_3 priming agent exhibited particularly significant effects.

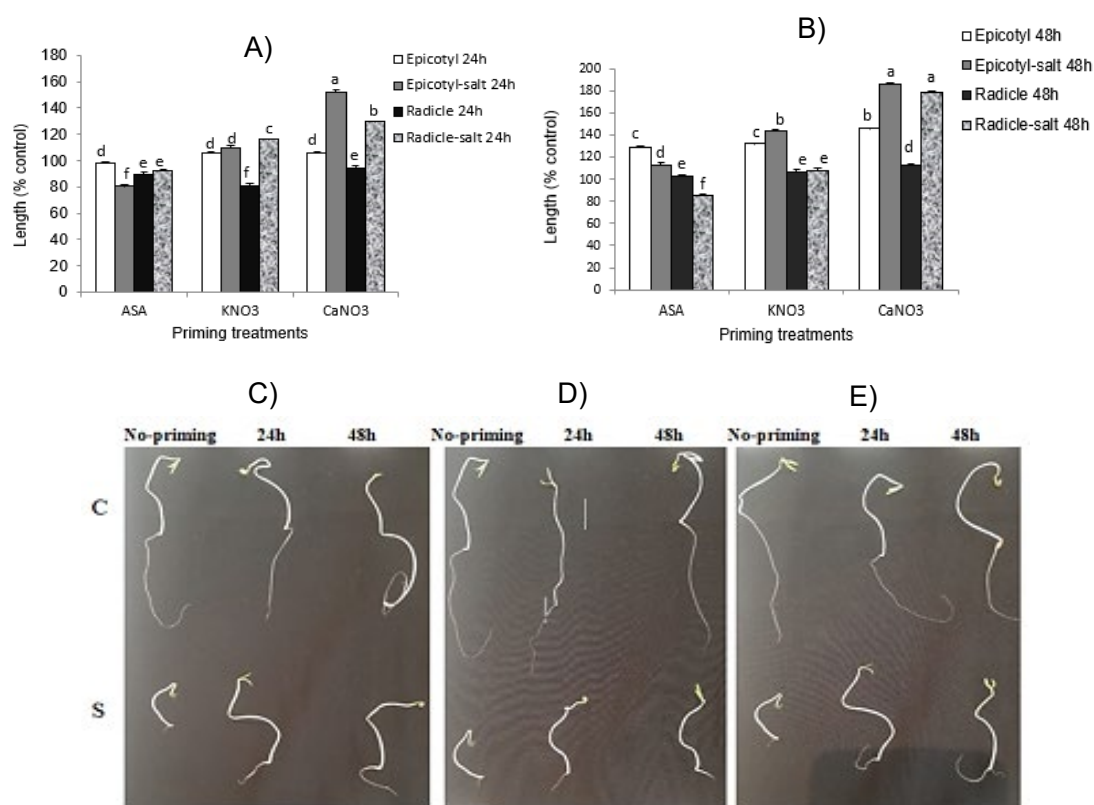


Figure 3. (A–B). Effect of different chemical priming (ASA/ KNO_3 / CaNO_3) on epicotyl and root length (% controls) after 24 h (A) and 48 h (B). Negative control means no stressed and no primed seeds; positive control means salt stressed and no primed seeds. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability. **Figure 4. (C–E):** Effect of different chemical priming ASA, KNO_3 and CaNO_3 , respectively, on seedling growth within 24 and 48 hours after 6 days of germination.

Under normal conditions, the 24-h seed priming with different chemicals had no significant effect on epicotyl length (Fig. 3, A). While in roots, KNO_3 and CaNO_3 treatments induced length by about 5% referring to negative control. Under salt treatment, 24-h chemical priming, especially CaNO_3 , increased epicotyl and root length by 50% and 29%, respectively, referring to positive control.

Under normal conditions, 48-h priming with ASA, KNO_3 and CaNO_3 treatments induced epicotyl length by 28%, 32% and 45%, respectively, refer to negative control (Fig. 3, B). In roots, the effect was less than those in epicotyl. Under salt treatment, ASA induced epicotyl length, while radicle length was decreased. Especially, CaNO_3 priming induced both epicotyl and radicle length by 85% and 78%, respectively, referring to positive control (Fig. 3, B).

In normal conditions, the 24-h seed priming with different chemicals increased mainly epicotyl fresh weight (FW) and especially with CaNO_3 priming (21%) refer to negative control (Fig. 4, A). Under salt treatment, chemical priming, especially CaNO_3 , increased both epicotyl and root FW by 46% and 24%, respectively, referring to positive control.

In normal conditions, 48-h priming with ASA had no effect on epicotyl and root FW (Fig. 4, B). KNO_3 and CaNO_3 treatments induced epicotyl FW by respectively 16%, 34% refer to negative control (Fig. 4, B). Under salt treatment, KNO_3 and CaNO_3 priming increased both epicotyl and radicle FW (Fig. 4, B). Especially, CaNO_3 priming increased both epicotyl and radicle FW by 85% and 80%, respectively, referring to positive control (Fig. 4, B).

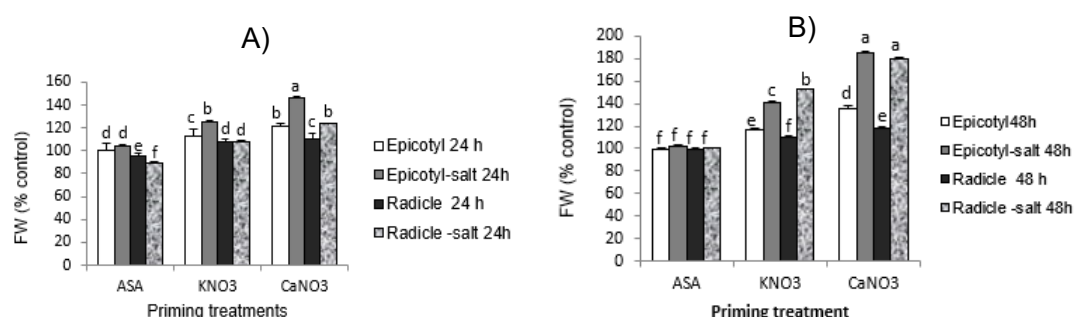


Figure 4. Effect of different chemical priming (ASA/ KNO_3 / CaNO_3) on epicotyl and root fresh weight (% controls) after 24 h (A) and 48 h (B). Negative control means no stressed and no primed seeds; positive control means salt stressed and no primed seeds. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.

Although used chemicals have no significant effect on seed germination in normal conditions, ASA, KNO_3 and CaNO_3 treatments induced an increase in both epicotyl and root length (Fig. 3, B). Seed priming with different chemicals increased epicotyl fresh weight (FW); especially with CaNO_3 priming within 24 h and 48 h (Fig. 4, B).

The current results suggested that under salinity, the decrease in plant growth, specifically the length and fresh weight of the epicotyl and root, (Fig. 3, A, Fig. 3, B, Fig. 4, A and Fig. 4, B), could be attributed to the osmotic effect caused by salt stress. This effect leads to a decrease in growth promoters, an increase in growth inhibitors and

a disruption in the water balance (Rady, 2012; Rady et al., 2019a; Semida & Rady, 2014). Under salt treatment, both time of priming and all chemical agents, especially 48-h CaNO_3 , increased epicotyl and root length and FW (Fig. 3, B; Fig. 4, B).

Previously, Oliveira et al. 2019, demonstrated that KNO_3 has the potential to effectively mitigate the detrimental impacts of salt treatment during the first stages of melon seed germination and plantlet growth. As in Papaya, results demonstrated that calcium ions had a more significant salt-alleviation effect on seedling growth in comparison to potassium ions (Maneesha et al., 2019). While ASA has demonstrated efficacy as a compound in wheat cultivation amidst saline conditions, its impact on tomato plants remains relatively insignificant when compared to KNO_3 and CaNO_3 El-Hawary et al., (2023).

The Fig. 5 illustrated the effect of 48-h chemical priming on chlorophyll content. In normal conditions, there was no significant effect on chlorophyll content (Fig. 5). Under salt treatment, chlorophyll content decreased by 6.7% refers to positive control.

Different chemical priming restored chlorophyll content referring to positive control. The decrease of chlorophyll level was more alleviated by CaNO_3 priming: the chlorophyll content decreased by about only 1% refers to control CaNO_3 primed seedlings.

In general, salt decreased the chlorophyll content of an extensive variety of plant species as in canola (Iqbal et al., 2022), alfalfa (Wang et al., 2020) and tomato (Shin et al., 2020). In several studies, priming technique mitigates harmful salt effect and restored chlorophyll content as in cowpea with CaCl_2 priming (Farooq et al., 2020) and in wheat with ASA priming (Baig et al., 2021).

In normal conditions, the 48-h seed priming with different chemicals had no significant effect on leaf MDA content (Fig. 6, A). With no priming, salt increased the MDA content in leaves by 77%. All chemical priming reduced the salt induced-MDA increase. Under salt treatment, leaf MDA content in ASA, KNO_3 and CaNO_3 pretreated seedlings was respectively 54%, 41% and 14% referring to control chemical primed seedlings (Fig. 6, A). Recently, several studies demonstrated that CaCl_2 and KNO_3 seed priming reduced effectively MDA accumulation caused by salt stress (Abdelhamid et al., 2019; Ben Youssef et al., 2021).

In normal conditions, ASA priming increased proline content in leaves while KNO_3 and CaNO_3 priming had no significant effect on proline content (Fig. 6, B). In no primed seedlings, salt increased proline content more than 8-fold refers to control. The chemical

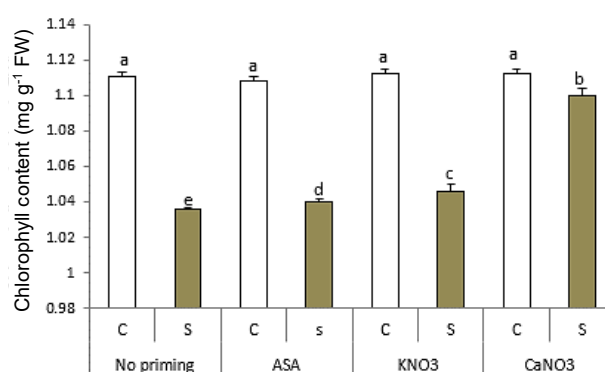


Figure 5. Effect of 48-h chemical priming type (ASA/ KNO_3 / CaNO_3) on chlorophyll content (mg g^{-1} FW) in leaves of tomato seedlings. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.

priming alleviated salt effect especially CaNO_3 agent. In stressed CaNO_3 primed seedlings, salt increased only 3-fold proline content refers to control CaNO_3 primed seedlings.

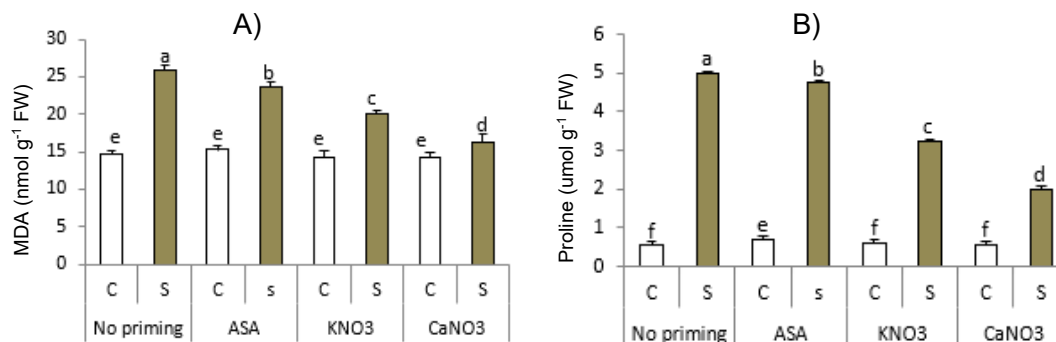


Figure 6. Effect of 48-h chemical priming type (ASA/ KNO_3 / CaNO_3) on malondialdehyde (MDA) content (A) and Proline content (B) in leaves of tomato seedlings. Data are means of six replicates. Comparative lowercase letters (a, b, c, etc.) denote treated and control samples. The Tukey test reveals no significant difference between bars denoted by identical letters with a 5% probability.

Generally, salt treatment induced proline accumulation in plant tissues. Plants accrue compatible solutes, such as proline, to withstand salt stress. By reducing the cytoplasmic osmotic potential, this process promotes water absorption and removes reactive oxygen species (ROS) molecules (Rady et al. 2019b). Recently, Abdelhamid et al revealed that seed priming alleviates detrimental salt effect by inducing proline accumulation (Abdelhamid et al., 2019).

However, in current study, the application of ASA, KNO_3 and CaNO_3 under salinity decreased the undesirable effect of salinity on seed germination and plant growth while decreasing proline accumulation. These results are supported by other studies where several chemical seed priming reduced proline content under salinity stress as in wheat (Salama et al., 2015) in tomato (Mimouni et al., 2016), sweet peppers (Abdelaal et al., 2020) and soybeans (Hasanuzzaman et al., 2022). Chemical used in seed priming could mitigate salt effect by the restriction of sodium and/or chloride absorption. For example, in salt-stressed wheat, calcium improve plant growth via its apoplastic effects on the transport of Na and K across the root plasma membrane (Reid & Smith, 2000).

CONCLUSIONS

In tomato, salt treatment reduced seed germination parameters and seedling growth. In normal conditions, the germination parameters (FGP, GI, GRI, and MGT) and seedling growth were not significantly affected by the applications of used chemical priming. The duration of seed priming and used chemical agent are crucial in determining chemical efficacy in salt stress alleviation. ASA, KNO_3 and CaNO_3 demonstrated efficiency in alleviation of salt effect on FGP and GI, GRI and MGT, especially 48-h as priming duration. Under salinity, chemical priming improved more germination parameters, growth of issued seedlings and leaf chlorophyll content, particularly after 48 hours of CaNO_3 priming.

The effectiveness of the mitigation strategy is traduced by low level of MDA indicating less significant oxidative stress. The improvement in seedling growth was concurrent with a decrease in the level of proline, which is an indicator of salt-induced osmotic stress, which suggests that used priming agents possibly reduced the absorption of salt ions and consequently the osmotic stress. It is determined that these priming agents could be categorized into three ranges according to their effectiveness on the various studied parameters. Among these, CaNO_3 is the most effective, closely followed by KNO_3 . Whereas ASA agent needs more studies to determine an effective concentration that mitigate effectively salt stress on tomato seeds.

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