Chemical seed priming alleviates salinity stress and improves *Sulla carnosa* germination in the saline depression of Tunisia

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**Abstract**
In the saline depressions (Sebkhas) of Tunisia, *Sulla carnosa* expresses anarchic distribution, sometimes in association with halophytes sometimes individually. In order to explain this distribution, we investigated the effects of salinity on seed germination, the osmotic and toxic limiting factors, and the importance of some stimulating agents (priming agents) in the improvement of the tolerance to salinity at the germinating stage. A study was conducted on seeds harvested from the natural biotope Sebkha d’El Kelbia (35°50’34”N, 10°16’18”E), and an increasing concentration of NaCl (0, 5, 10, 15, and 20 g L⁻¹) was applied. Some priming agents were used to propose efficient, rapid, and low-cost tools to improve the seed germination and tolerance of *Sulla carnosa* (Desf.) in saline depression. Salinity stress significantly decreased germination capacity and rate and delayed its initiation and maximum. Until 15-g L⁻¹ NaCl, the most limiting factor of seed germination is the osmotic effect. At 20-g L⁻¹ NaCl, the toxic effect dominates, and germination is irreversibly inhibited. Some priming agents have shown their efficiency in improving the germination capacity at 10-g L⁻¹ NaCl and conferring a salt tolerance of up to 15-g L⁻¹ NaCl.

**KEYWORDS**
germination, osmotic effect, priming, salinity, *Sulla carnosa*, toxic effect

**1 | INTRODUCTION**

Soil salinity, whether natural or anthropogenic, affects more than 20% of the world’s arable land surface. A soil is considered saline if its NaCl concentration is over 40 mM, roughly equivalent to 7% of seawater salinity. These soils, commonly regarded as marginal, are progressively expanding due to land clearing, unsustainable irrigation, and bringing marginal lands into production (Kopittke et al., 2019). Munns and Tester (2008) announced that salinity deprives 1.5 million hectares of land from production each year. Thus, 50% of cultivable lands will be lost by the middle of the 21st century (Wang et al., 2009). It has long been believed that the salt-affected areas are unavailable for cultivation. However, extensive research revealed that it is possible to cultivate these lands using salt-tolerant crops. Despite the principal plant sensitivity to salinity, some species can survive and thrive under saline conditions. Flowers and Colmer (2008) confirmed that 1% of the world species are halophytes and can complete their lifecycles under saline conditions (over 200 mM of NaCl in the rhizosphere). The introduction of such tolerant plants in the salinized area would help valorize these zones and use salt water for irrigation.

**Abbreviations:** FGC, final germination capacity; GC, germination capacity; GR, germination rate; MDG, mean daily germination; MTG, meantime germination; RGC, recovery of germination capacity; RRGC, rate of recovery germination capacity; SG, speed of germination; Vc, velocity coefficient.

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Germination is the first stage in the plant’s life cycle, known to be the most sensitive (Patade et al., 2011). Hubbard et al. (2012) demonstrated that seed germination and seedling growth are the two critical stages for establishing crops. Various abiotic stresses are known to delay or prevent seed germination (Fazlali et al., 2013). Multiple authors stated that increasing salt concentration delays germination and decreases its percentage (Ansari & Husain, 2012). Increasing salt concentrations prevents seed germination and extends the germination time by delaying its initiation (Thiam et al., 2013). At the cellular level, the toxicity of Na$^+$ would result from its ability to compete with potassium in binding processes on key proteins. More than 50 enzymes require K$^+$ to be active and Na$^+$ would not perform the same function (Bhandal & Malik, 1988). Therefore, a high concentration of Na$^+$ in the cytoplasm would inhibit the activity of many enzymes and proteins leading to cellular dysfunctions. Regarding all these data, we suggest that low salt concentration induces a state of dormancy and decreases the germination rate, whereas high salt concentration inhibits seed germination and decreases its percentage. However, seed priming is a process that can regulate germination by the rearrangement of its metabolism. It is considered cost-effective, low risk, and efficient to improve seed germination, seedling emergence, and plant growth in salt-affected soils (Subramanyam et al., 2019). Saddiq et al. (2019) demonstrated that seed priming induced metabolic activities, structural and genetic repair, RNA and protein synthesis, and antioxidant activity that accompanies the induced metabolic activities, structural and genetic repair, RNA and protein synthesis, and antioxidant activity that accompanies the proper germination and seedling development. This treatment allows seeds to overcome environmental stresses during germination (Farooq et al., 2006). Several compounds such as silicon, ascorbic acid, salicylic acid, polyamines, KNO$_3$, KCl, CuSO$_4$, and ZnSO$_4$ have been proposed as seed priming agents. They may significantly enhance plant growth and development (Abu El-Soud et al., 2013; Khaing et al., 2020). Similarly, Hua-long et al. (2014) demonstrated that proline enhanced the germination rate and the relative germination energy of rice seeds under salinity. Li et al. (2014) announced the positive effect of spermidine on germination, germination vigor, root viability, and length while shortening the mean germination time under different water stress conditions. Sheteiwy et al. (2020) demonstrated that seed priming or foliar application of jasmonic acid and/or their combination significantly improved water potential, osmotic potential, water use efficiency, relative water content, net photosynthesis, and chlorophyll content of soybean seedlings grown under salinity stress. They observed that the transcriptional levels of the FeSOD, POD, CAT, and APX genes increased significantly in the NaCl-stressed seedlings irrespective of jasmonic acid treatments. Li, Xu, et al. (2017) reported that priming with salicylic acid, H$_2$O$_2$, or their combination reduced seed germination time and enhanced seed vigor and seedling growth as compared with nonpriming treatments under low temperature. Comparable results were obtained in rice upon seed priming with spermidine and 5-aminolevulinic acid (Sheteiwy et al., 2017) or with 2.5-mM methyl jasmonate (Sheteiwy et al., 2018). The combination of salicylic acid and H$_2$O$_2$ for seed priming increased the antioxidant enzymes activities and their corresponding ZmPAL, ZmSOD4, ZmAPX2, ZmCAT2, and ZmGR gene expression (Li, Xu, et al., 2017). The abscisic acid catabolism gene and the expressions of genes encoding response receptors in ABA signaling pathway were all upregulated (Li, Xu, et al., 2017). In *Oryza sativa* L. subjected to nano-ZnO stress, plant growth parameters were significantly increased in seeds primed with 30% polyethylene glycol (Sheteiwy et al., 2015). The superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and malondialdehyde (MDA) contents were significantly reduced. The evidence that melatonin seed priming could improve the tolerance of safflower to drought through the antioxidative mechanism was also reported by Heshmati et al. (2021). García et al. (2021) demonstrated that hydroelectrostatic hybrid priming confers fast germination of tomato seeds via hormone regulation and the reprogramming of gene expression. This technique enhances the germination index and vigor, increases gibberellin and decreases abscisic acid levels. Significant changes in the transcription levels of SINCED2 and SIDELLA (genes playing important roles during seed germination) were also observed during the priming procedures.

The Sebkha d’El Kelbia is an intermittent lake in Tunisia (Kairouan Governorate) that covers 8000 ha, in addition to 7000 ha of surrounding swamps, at 35°50′34″N, 10°16′18″E south of Kondar. It is classified in the lower semi-arid bioclimatic stage (Korked et al., 2017).

In a previous study, the Fabaceae *Sulla carnosa* (Desf.) species was found to express a particular distribution. It occupies the sebkha border mainly. Its distribution decreases going towards the center of the sebkha, both individually and in association with other halophytes (*Salsola vermiculata, Atriplex halimus, …*). In the rhizosphere of *S. carnosa*, the EC was about 13 dS m$^{-2}$ while reaching 64 dS m$^{-2}$ in the areas without vegetation. We hypothesized that the most sensitive stage limiting *S. carnosa* distribution and development in saline depression is germination. The high salinity in the nude areas inhibits the germination of seeds, normally dispersed randomly in the sebkha. Regarding the effect of salinity stress on *S. carnosa* seed germination, the importance of seed priming in alleviating salt stress effects and germination improvement remains very limited. Therefore, this study’s primary aim was to evaluate the germination response of *S. carnosa* seeds to salinity. We aimed also to assess the extent of factors controlling germination, osmotic, or toxic effects. Different methods are now used to improve seed germination under stress and non-stress conditions. For this, we used different seed priming agents aiming to propose efficient, rapid, and low-cost tools to improve the seed germination and tolerance of *S. carnosa* in saline areas.

### 2 | MATERIALS AND METHODS

All experiments were carried out at the Research Unit Valorization and Optimization of the Resources of the Faculty of Sciences and Techniques of Sidi Bouzid. Seeds of *S. carnosa* were collected from the Sebkha Kelbia. The seeds were surface sterilized in a 1% sodium hypochlorite solution for 10 min and then rinsed with distilled water before being used in the germination tests.
The experiments were implemented in two consecutive phases. The first phase aimed to evaluate the germination capacity (GC) and germination rate (GR) of the seeds and determine the osmotic and toxic effects. This procedure was intended to collect some preliminary data regarding germination response to different concentrations of NaCl. For this purpose, seeds were germinated in Petri dishes containing an ascendant concentration of NaCl (0, 5, 10, 15, and 20 g L\(^{-1}\)) for 9 days. NaCl concentrations were chosen regarding previous results on S. carnosa rhizosphere salinity (soil salinity = 9 g L\(^{-1}\), Korked et al., 2017). In order to determine the extent of the osmotic/toxic effect on seed germination, we used an extended range of concentration on either sides of 9 g L\(^{-1}\). Fifty seeds were used per treatment, arranged in 5 Petri dishes of 10 seeds each, and the experiment was repeated twice. Presented results are the mean of 100 seeds. The seed germination recovery (GRec) allows us to discriminate between the toxic effect and the osmotic ones. For this purpose, the non-germinating seeds were transferred to deionized water for another 9 days. Beyond the objectives mentioned above, these experiments allowed us to select the best salt concentration to assess various seed priming agents in the subsequent phase.

The second phase of the experiments consisted of the priming tests. We soaked the seeds into solutions containing one of the following salts each, NaCl (5 g L\(^{-1}\)), KNO\(_3\) (500 mg L\(^{-1}\)), KCl (500 mg L\(^{-1}\)), CuSO\(_4\) (500 mg L\(^{-1}\)), ZnCl\(_2\) (500 mg L\(^{-1}\)), or MnCl\(_2\) (500 mg L\(^{-1}\)) for 6 h, then transferred to new Petri dishes containing NaCl solution at 10 g L\(^{-1}\). Fifty seeds by Petri dish were used for each treatment, and the experiment was replicated twice. Presented results are the mean of 100 seeds.

The various experiments produced estimates of the following traits:

- **GC (%):** This parameter represents the best tool for identifying the saline concentration that constitutes the physiological limit of S. carnosa seed germination, expressed as the ratio of germinated seeds to the total number of seeds.
- **GR (day\(^{-1}\): This parameter constitutes an important tool for monitoring germination as a function of time, expressed as the daily ratio of germinated seeds to the total number of seeds.
- **GRec (%):** expressed as the capacity to recover the GC lost on NaCl treatment, calculated using the equation \[ \left( \frac{a - b}{c - b} \right) \times 100 \], where \( a \) = total number of seeds germinated after recovery, \( b \) = total number of seeds germinated in NaCl treatments, and \( c \) = total number of seeds (Gulzar & Khan, 2001).
- **Rate of germination recovery (RGRRec, day\(^{-1}\):** Expressed as the daily number of germinated seeds after the transfer to deionized water to the number of nongerminated seeds.
- **Final germination capacity (FGC, %):** This parameter allows us to calculate the maximum GC considering the recovery potentiality, expressed as the sum of GC and GRec at each treatment.
- **Speed of germination (SG, days):** This parameter allows expressing the germination energy responsible for depressing seed reserves. The SG was estimated by the average time (T\(_{SG}\)) corresponding to 50% of seeds germination.

\[ V_c = \frac{(N_1 + N_2 + N_3 + \ldots + N_n) \times 100}{(N_1 T_1 + N_2 T_2 + N_3 T_3 + \ldots + N_n T_n)} \]

\( N_n \) is the number of germinated seeds between time \( T_{n-1} \) and \( T_n \).

### 2.1 Statistical analysis

To analyze the data obtained from these experiments, we perform analysis of variance (ANOVA) and check whether the different experimental factors (NaCl concentrations & priming agents) affect germination; we used Statplus Pro software. Results are presented as mean ± standard error. The significance of differences among treatments was determined by Fisher’s least significant difference test (LSD) (\( P < .05 \)). Treatment means were declared significant when the difference between any two treatments was greater than the LSD value generated from the ANOVA. They are marked by different letters in the figures.

### 3 RESULTS

Depending on the concentration, sodium chloride inhibited the GC of S. carnosa seeds. This inhibition was 48% at 5-g L\(^{-1}\) NaCl and reached 72% when adding 10-g L\(^{-1}\) NaCl. Although germination decreased, it was not negligible until 20-g L\(^{-1}\) NaCl in the medium (Figure 1a). Similarly, when transferring nongerminated seeds in deionized water, the GRec was significant and was affected only beyond 15-g L\(^{-1}\) NaCl (Figure 1b). This result indicated that the most limiting factor of seed germination until 15-g L\(^{-1}\) NaCl is the osmotic effect. The toxic effect became significant only at 20-g L\(^{-1}\) NaCl (the toxic effect become higher, 68% than the osmotic effect, 32%).

By adding the GRec to the initial germination, that is, eliminating the osmotic effect, the FGC becomes very important until 15-g L\(^{-1}\) NaCl (64%, Figure 2, instead of 18% without recovery, Figure 1a). In fact, Figure 3, which represents the evolution of the osmotic and toxic effects connected with NaCl concentration in the medium, shows a gradual decline of the osmotic effect in favor of toxic ones. This last factor overcame the first ones only at the highest salt concentration (20-g L\(^{-1}\) NaCl).
The calculated GR as a function to time revealed that seed germination was initiated after 3 days at 0 and 5-g L⁻¹ NaCl, 5 days at 10-g L⁻¹ NaCl, and 6 days at 15-g L⁻¹ NaCl (Figure 4a). The maximum seed germination was reached after 6 days in the control treatment (0 NaCl), 7 days at 5-g L⁻¹ NaCl and 8 days at 10-g L⁻¹ NaCl and over concentrations. The GRec rate represented as previously shown a rapid recovery starting after 1 day of stay in deionized water, reaching the maximum after 4 days for seeds nongerminated at 5-g L⁻¹ NaCl and 5 days for seeds nongerminated at 10-g L⁻¹ NaCl and further concentrations (Figure 4b).

The calculation of the Vc, germination mean time (GMT), and MDG shows an inverse relationship with NaCl concentration for the first parameter and linear relationship for the second and third ones (Table 1). Increasing NaCl concentration decreased Vc and increased GMT and MDG, meaning that sodium chloride delays the seed germination and inhibits the GC. The salt stress also disrupts the SG estimated by the T₅₀. This parameter increased significantly by increasing NaCl concentration.

In order to improve S. carnosa germination in salt-affected soils, we investigated the importance of seed pretreatment with some chemical agents before their transfer on 10-g L⁻¹ NaCl (concentration inhibiting 72% of seed germination). Figure 5 shows that germination of pretreated seeds was initiated 1 day before that of nontreated ones. Otherwise, seeds pretreated with NaCl, KNO₃ and CuSO₄ reached their maximum GC after 6 days (7 days for seeds pretreated with KCl, ZnCl₂, and MnCl₂). When examining the GC (Figure 6), we conclude that NaCl, KNO₃, and CuSO₄ are interesting agents by improving GC more than two times (compared with salinity stress). The other agents also increased GC but did not reach an interesting level. The Vc increased, and the GMT decreased significantly, particularly when the seeds were pretreated with NaCl or KNO₃ (Table 2). The MDG grew from 3.3 when seeds were germinated in 10 g NaCl...
without pretreatment to 8.3 and 7.8, respectively, when seeds were pretreated with either NaCl or KNO3. The pretreatment of seeds before applying salt stress also resolved, to some extent, the disruption of germination speed estimated by $T_{50}$ (Table 2).

### DISCUSSION

Salinity stress significantly decreased the FGC in *S. carnosa* at 10-g L$^{-1}$ NaCl and above. The GR demonstrated that salinity also delays the initiation of germination and the maximum reach. It is well established that salinity stress increases osmotic pressure, disturbs ion uptake, and induces oxidative stress due to toxic ions (Tahjib-Ul-Arif et al., 2018). Patade et al. (2011) showed that germination is one of the most sensitive stages of the plant cycle to salinity. Hubbard et al. (2012) demonstrated that seed germination and seedling growth are the two most critical stages for establishing crops. In accordance with our results, multiple authors stated that increasing salt concentration decreases the germination percentage and increases germination time by delaying its initiation (Ansari & Husain, 2012; Patade et al., 2011; Thiam et al., 2013). This is confirmed by the gradual decrease of $V_c$ and MDG in a saline environment, conversely with the gradual increase of GS (or $T_{50}$) and MTG. Sima et al. (2013) suggested that the high sensitivity of germination to salinity is attributed to the damages and delayed activation of enzymes. Thus, we suggest that low salt concentration in the medium induces a dormancy state and decreases the GR without acting as a toxic component. In contrast,

**TABLE 1** Velocity coefficient ($V_c$), meantime germination (MTG, days), mean daily germination (MDG, %), and germination speed (GS, days) at different sodium chloride concentrations (NaCl, g L$^{-1}$)

| NaCl | $V_c$       | MTG       | MDG        | GS ($T_{50}$) |
|------|-------------|-----------|------------|---------------|
| 0 NaCl | 22.75 ± 2.31a | 4.40 ± .49d | 1.67 ± 1.22a | 3.68 ± .41c   |
| 5 NaCl | 19.70 ± 2.12b | 5.08 ± .52c  | 5.78 ± .61b   | 3.86 ± .40c   |
| 10 NaCl | 15.22 ± 1.83c | 6.57 ± .58b   | 3.11 ± .29c   | 6.25 ± .56b   |
| 15 NaCl | 14.52 ± 1.62cd | 6.89 ± .59ab  | 2.00 ± .18d   | 6.50 ± .53ab  |
| 20 NaCl | 13.64 ± 1.45d | 7.33 ± .61a   | .67 ± 0.11c   | 6.75 ± .61a   |

Note: Within cells, means with the same letter are not significantly different at $\alpha = .05$ according to Fisher’s least significant difference. Standard error of the mean ($n = 100$, two replicates of 50 seeds).
the high salt concentration inhibits seed germination and decreases its percentage due to sodium and chloride’s toxic effect. When representing the osmotic effect and the toxic one in function to salinity concentration (Figure 3), we observed the osmotic effect’s dominance until 15-g L⁻¹ NaCl. Beyond this concentration, the toxic effect takes over and dominates the situation. So normally, if we alleviate the osmotic effect, we can improve the germination percentage and rate. In this study, when transferring nongerminated seeds in deionized water (recovery of germination), we improved the GC from 28% and 18%, respectively at 10- and 15-g L⁻¹ NaCl, to a FGC of 72% and 64%, respectively at 10- NaCl and 15 g L⁻¹ NaCl. If we consider that a GC below 50% state of sensitivity, between 50% and 75% state of relative tolerance and above 75% state of tolerance, we can see clearly that we have gone from a clear sensitivity at 10-g L⁻¹ NaCl and over to a relative tolerance of up to 15-g L⁻¹ NaCl. The GRec rate (Figure 4b) also demonstrated that 2 days are gained in germination initiation and time to reach the maximum until 15-g L⁻¹ NaCl (the 20-g L⁻¹ NaCl is considered a state of sensitivity, GC remain < 50%). Thus, we can conclude that until 15-g L⁻¹ NaCl the S. carnosa seeds suffer from an osmotic limitation for their germination that can be overcome by water supply. The irreversible toxic effect of sodium chloride is only expressed at 20-g L⁻¹ NaCl where the GRec rate remains very low. This also indicates that, in low and moderate saline environments, the osmotic component is the main driver of germination of S. carnosa seeds rather than the toxic ones. In soils with high salinity, this relationship reflects. Our first conclusion is that S. carnosa suffer from difficulties of seed germination due to osmotic stress until 15-g L⁻¹ C0 NaCl which can be overcame by dilution. Beyond this concentration, the toxic effect dominates and inhibits irreversibly the germination. We suggest, as previously discussed, that high accumulation of Na⁺ inhibits the activity of many enzymes and proteins leading to cellular dysfunctions.

It is well documented that rapid seed germination and plant establishment are critical factors affecting crop production under stress conditions. Due to the new achievements in the germination sciences and the considerable extent of our understanding of this process, multiple germination improvement methods are identified in favor of agricultural applications. The most used method is known as “seed priming”
(Paparella et al., 2015). The priming process involves the exposure of seeds to a stimulating factor (chemical or other agent) that makes plants more tolerant to further stress exposure. It is used to improve seed germination under optimal and adverse conditions (Jisha et al., 2013). In this study, several pretreatment agents were used on S. carnosa seeds before their transfer on 10-g L⁻¹ NaCl (concentration at which GC did not exceed 28%). Three priming agents demonstrated their capacity to improve germination more than two times (2.5 times for NaCl, 2.3 times for KNO₃, and 2 times for CuSO₄), bringing us to the relative tolerance zone (GC exceeded 50%), whereas the other agents (KCl, ZnCl₂, and MnCl₂), although they increased GC, remained in the sensitive zone (GC less than 50%). The positive effects of seed priming under salinity conditions have been reported in many crops, such as lettuce (Nasri & Khalatbari, 2011), maize (Tabatabaei Mirakabadi et al., 2014), pea (Naz et al., 2014), pepper (Aloui et al., 2014), soybean (Miladinov et al., 2015), and chickpea (Aziz & Peksen, 2020).

Several other studies demonstrated that seed priming is one of the most efficient physiological approaches that could adapt glycophyte species to saline conditions (Gholami et al., 2015). The positive effects of priming under unfavorable conditions were also confirmed (Ashraf & Foolad, 2005; Chen et al., 2011). This approach is an easy, low-cost, and low-risk technique to overcome the salinity problem in agricultural systems. Earlier studies demonstrated that seed priming with diverse plant regulators (salicylic acid, asbiscic acid, ascorbic acid, melatonin, spermidine, & jasmonic acid) and several other priming agents improved germination, seedling vigor, and the overall metabolic functions (plant growth, ion regulation, water and nutrient uptake, photosynthesis, chlorophyll pigments, ...) and yield in wheat (Bajwa et al., 2018; Mir et al., 2018; Tabassum et al., 2017).

Khan et al. (2020) demonstrated that seed priming with silver nanoparticles (AgNPs) at different levels (0, 10, 20, and 30 mM) mitigated the adverse impacts of salt stress and improved plant growth and defense system. In accordance with our results, Sarkar et al. (2020) demonstrated that bamboo seed priming with 1% KNO₃ gave the highest rise in germination (39.1%). Saed-Mooheshi et al. (2014) showed that priming maize seeds with KNO₃ and urea lead to high antioxidant defensive enzymes and increase the tolerance level to abiotic stresses such as salt and drought. On the other hand, priming of oat grains with CuSO₄ and ZnSO₄ showed an improved growth as compared with nonprimed seeds. It was concluded that the seed priming technique could partly overcome the effects of salinity (Iqbal et al., 2020). In the same way, Khan et al. (2020) revealed that wheat seed priming with ZnSO₄ improved the growth and antioxidant enzyme activities more than CuSO₄ under different salinity levels. Mahadi et al. (2020) observed that 1-mM KCl is the adequate concentration that enhances the germination of drought-stressed Malaysian indica rice seeds. Saddiq et al. (2019) demonstrated that with KCl treatment, we could overcome the harmful effects of salt stress in wheat. Khang et al. (2020) confirmed that seed priming with 2.5% KCl and 1% K₃PO₄ triggered specific changes in the amino acids (AA) and fatty acids (FA) compositions in grain. Bruce et al. (2007) stated that seed priming improves the role of phytohormones and regulatory substances (auxins, gibberellins, asbiscic acid, ethylene, jasmonic acids, etc.), which enable plants to perform adequately under stress condition. As in our study (pretreatment with low NaCl concentration), Basra et al. (2017) supported the significant role of presewing seeds with various salt concentrations to induce salt tolerance. Recent literature shows that priming with KCl and CaCl₂ alleviates salt stress by upregulating seedling growth, photosynthetic activity, proline, and phenolic contents (Islam et al., 2015). Priming also improved antioxidant enzyme activities in plants grown under salt stress (Paparella et al., 2015).

Sheteyi et al. (2020) reported that the transcriptional levels of the FeSOD, POD, CAT, and APX genes increased significantly in the NaCl-stressed seedlings irrespective of jasmonic acid treatments. The combination of salicylic acid and H₂O₂ for seed priming increased the antioxidant enzymes activities and their corresponding genes expression (Li, Xu, et al., 2017). The abscisic acid catabolism gene and the expressions of genes encoding response receptors in ABA signaling pathway were all upregulated (Li, Yu, et al., 2017). Garcia et al. (2021) demonstrated that hydroelectrostatic hybrid priming confers fast germination of tomato seeds via hormone regulation and the reprogramming of gene expression. Finally, we suggest that seed priming results from multiple mechanisms that can act individually or synergistically.

## 5 Conclusion

Taken together, our results demonstrated that increasing salt stress decreases GC and rate and disturbs the associated parameters (Vc, MDG, GMT, & Ts0). Soil containing 10-g L⁻¹ NaCl represents an inhibitory environment of seed germination in S. carnosa. Nevertheless, the inhibition’s main source was the osmotic effect. This latter can be surpassed by water supply or other stimulating agents because the level of toxicity that damages the embryo has not been reached. The pretreatment with some priming agents like low concentrations of NaCl, KNO₃, or CuSO₄ draws particular importance. This approach enhances GC at 10-g L⁻¹ NaCl and confers a salt tolerance of up to 15-g L⁻¹ NaCl. Finally, the most sensitive stage limiting S. carnosa distribution and development in the saline depression of Tunisia is the germination. This stage is highly sensitive to soil salinity, and any program of rehabilitation of these areas should necessarily find solutions to overcome this obstacle. The use of some stimulating agents as priming factors like KNO₃ or CuSO₄ might be a good candidate. Another alternative is the short-time water supply that can decrease the soil salinity by dilution effect. This short-time low salinity also represents a considerable priming agent.

## Conflict of Interest

The authors declare no conflict of interest associated with the work described in this manuscript.

## Author Contributions

Abdelmajid Krouma and Mohamed Chaieb set up the experimental protocol, followed its realization, and formatted the last version of the article. Amal Bouzidi conducted the experiments, carried out analyzes, and wrote the first version of the paper.
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