

Original Research

Enhancement of Root System Architecture, Seedling Growth, and Germination in Lentil under Salinity Stress by Seed Priming with Silicon and Salicylic Acid

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Abstract

To deal with increasing salinization, plants show an array of responses such as root system architecture remodelling, thereby enhancing stress tolerance. Although various chemical, molecular and genetic techniques such that generally expensive and difficult applications are used to enhance stress tolerance, out of them, seed priming with suitable substrates is an easy-applicable and cost-effective treatment. The experiment aimed to evaluate the effects of salicylic acid (SA), silicon (Si), and sodium chloride (NaCl) priming on lentil seed germination parameters, seedling development, and root system architecture. In 2022, the experiment was carried out in the Field Crops Department laboratory at Siirt University in Turkey. Petri and pot investigations were part of the two stages of the experiment. Three concentrations of NaCl and priming treatments were utilized. Germination characteristics were impeded by rising saline levels. But seed priming, which boosted uniformity of germination by 50% as compared to unprimed seeds under saline stress, enhanced germination characteristics. A pot experiment revealed that the dry matter accumulation in roots was reduced by 24.6% due to salt stress. Si priming increased number of lateral roots by 32.6% compared to control seeds, whereas salt stress lowered it by 22.8%. The total length of lateral roots (TLLR) and mean length of lateral roots (MLLR) were inhibited by salinity stress by 75% and 63, respectively. Total root area was reduced by salinity stress by almost 70%, although seed priming increased it by as much as 29.3%. The SA and Si priming treatments improved germination stage and induced seedling growth by reducing salinity stress via more effective shoot

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development and root system architecture. In conclusion, seed priming with SA and Si is an affordable and sustainable method for reducing salt stress in lentil farming.

Keywords: *Lens culinaris*, root phenotyping, salt stress, stress management, sustainability

Introduction

Lentil (*Lens culinaris* Medik.) is one of the most produced and eaten agricultural foods in the world due to its high nutritional value, which includes protein, amino acids, carbs, energy, fibre, vitamins, phenolic compounds, and antioxidants. Lentil is a good source of digestible dietary protein, and radically contributes to the soil by fixing environmental N_2 [1]. This is especially true in poor nations. Turkey is a significant producer of lentils, ranking third globally behind Canada, India, and Australia with an annual production of 370.000 tons on a 250.000 ha area [2]. Despite the fact that lentils are very tolerant to several adverse environmental conditions like extreme cold temperatures and drought, salinity stress (SS) plays an inhibitory role on lentils from germination to the productive stage [3].

Forecasts indicate that excessive salinity affects 33% of irrigated cultivated fields and 20% of all arable land. Furthermore, according to experts, salinized lands have been growing at a pace of 10% each year for a variety of causes, including excessive surface evaporation, a lack of precipitation, saline irrigation water, the weathering of natural rocks, and improper cultural practices. Basic soil salinity affects plants in two ways: It restricts water intake through osmotic pressure and causes an ion imbalance in cells owing to an excess of Na^+ and Cl^- ions [4]. Similar to how SS limits nutrient uptake by roots and their movement through the vascular system, hinders enzymatic activity, causes osmotic stress and disturbs ion homeostasis, reduces photosynthesis efficiency, causes the release of reactive oxygen species (ROS) and stress hormones, and ultimately inhibits plant growth and development throughout its lifespan [5]. The germination and early seedling phases are the most crucial times for healthy development, high yield, and quality in field crops due to soil salinity and the high sensitivity of plants. Due to an excess of Na^+ and Cl^- in plant tissues, soil salinity boosted toxicity while lowering osmotic potential and water absorption by seeds.

Additionally, SS during the early seedling stage prevents root elongation and shoot development, which limits the improvement of root system architecture (RSA), a crucial marker of plant growth, yield, and quality because the RSA directly affects water and nutrient uptake, and raises the plants' stress tolerance [6]. Ondrasek et al. [5] summarized the basic solutions to SS under five titles, including usage of agro-technical practices, treatment of agrochemicals, genetic and breeding approaches, screening and modelling technologies, and improving soil beneficial microbiomes, however, these management strategies generally need

to spend many times or require high treatment cost. Out of them, seed priming is an easy-applicable, low-cost, and sustainable approach for stress management in plant increase antioxidant enzyme activities and osmoprotectants, scave ROS, lower lipid peroxidation and malondialdehyde (MDA) content, therefore, it enhances stress tolerance and induces plant growth under saline conditions [7]. There is, however, a dearth of knowledge about seed priming-based SS management in lentils, and in particular, its effects on the RSA in both stress- and non-stress-free environments. In this experiment, the effects of salt stress (SS) on lentil germination and seedling development, the role of seed priming with salicylic acid (SA), silicon (Si), and sodium chloride (NaCl) in stress management at petri and cylindrical container experiments, and the impact of seed priming on lentil RSA under saline and non-saline conditions are all investigated. In particular, using of cylindrical container system provide a controlled conditions to observe the RSA depending on seed priming treatments under saline and optimal conditions, therefore, it leads to take natural root growth and obtaining susceptible observations [8]. Moreover, when the scientific literature is examined, there is a lack of knowledge about the effects of the priming materials used in the experiment on both the germination process and the RSA depending on the seedling development under salinity stress.

Material and Methods

Experimental Site

The experiments were laid out under laboratory and growth chamber conditions from March 16th, 2022, to April 20th, 2022 at the Siirt University, Turkey. The experiment area is located at an altitude of 37°58'N, 41°50'E, 580 m above sea level and under terrestrial climate.

Experimental Materials

The experiment employed the early maturing crop Tigris (*Lens culinaris* M.), which is highly adapted to the climatic conditions of the Southern Anatolia area. A unique, certified seed material was used in the experiment. The endosperm impact was avoided by using homogeneous seeds. Artificial SS was created using sodium chloride (NaCl). To prime the seeds, SA, Si, and NaCl were used. Sodium metasilicate (Na_2SiO_3) was used as a source of Si.

Experimental Design and Treatments

The experiment was set up in two stages, with the first stage consisting of a Petri study to investigate the inhibitory effect of SS, and its mitigation by seed priming treatments on the germination characteristics of lentil seeds, and the second stage consisting of a cylindrical container experiment to observe the effects of treatments on seedling growth and the RSA. In the incubator, a petri experiment was carried out for seven days in complete darkness with the temperature set at 24°C. The growth chamber in which the pot experiment was conducted had a 14:10-hour light/dark cycle, a temperature range of 18 to 25°C, and a relative humidity range of 60 to 70%. The petri experiment employed three salt concentrations (S0: control, S1: 100 mM, and S2: 200 mM), and four priming treatments (control, SA, Si, and NaCl). To the control seedlings, distilled water was used for hydro-priming. The experiment was set up with a factorial layout and six replications using a completely randomized design (CRD). For SA, Si, and NaCl, the priming suspension concentrations were set at 0.2 mM, 4 mM, and 1 mM, respectively. Prior to the experiment, the test materials were autoclave sterilized at 12°C for 20 minutes. For surface sterilization, seeds were exposed to 10% sodium hypochlorite for 5 minutes. The seeds were sterilized, then rinsed three times in distilled water and dried for 24 hours using filter paper. The incubator temperature was adjusted to 24°C, and it was maintained in the dark for 20 hours with the petri dishes inside. Seeds were placed in filter paper after priming to finish drying to their original moisture content. The 25 seeds were eventually planted in Petri dishes between 2 layers of filter paper. Each Petri dish received 4 ml of the saline solutions, with distilled water serving as the control. Throughout the experiment, the Petri dishes were maintained at 24°C. Until the completion of the trial, 2 ml of suspension were applied to each Petri plate every 48 hours. On the seventh day, the study was finished. Under the regulated growth chamber circumstances described above, the second stage of research was carried out as a cylindrical container study. The concentrations, priming ingredients, and salinity levels were identical to those used in the Petri experiment.

The second experiment was carried out using a factorial design with five replications in accordance with CRD. Prior to planting, seedlings underwent the same procedure of surface cleaning and were primed with the aforementioned suspensions. Five homogeneous seeds were inserted on each filter paper (40x40 cm) following the priming and re-drying of the seeds. Each replication consisted of six filter sheets, meaning that observations for each treatment were gathered from 30 seeds. Filter sheets were stacked one over the other after being wet. Rolling the filter papers and placing them in cylindrical containers with the same amounts of solution [8].

Germination Percent, Seedling Growth and Root Phenotyping

During a petri experiment lasting seven days, regular germination of seeds was counted every day. At the conclusion of the seventh day, it was calculated the germination percentage (GP), mean germination time (MGT), germination index (GI), uniformity of germination (UG), germination rate (GR), and germination energy (GE). Under saline and non-saline circumstances, the parameters that were utilized to determine germination characteristics were estimated using the equations below:

$$GP (\%) = \frac{G7}{N} \times 100$$

$$MGT (day) = \sum \frac{G \cdot t}{N} \times 100$$

$$GI = \sum \frac{G_t}{t}$$

$$UG = \frac{GP}{MGT}$$

$$GR = \frac{\sum_x^y N}{\sum_x^y T}$$

$$GE = \frac{G1}{N} \times 100$$

G stands for the number of seeds that germinated, G7 for the total number of seeds that germinated on the seventh day, N for the total number of seeds sown, and t for the amount of time between sowing and counting day. T is the number of days in the trial, where x is the first day to be counted and y is the last day to be counted.

Rolls of filter paper were carefully opened at the conclusion of the cylindrical container experiment, and roots were scanned at 600 dpi resolution using a handheld scanner called ISCAN. Samples' roots and branches were divided after scanning. Samples were put in a 68 °C oven after being immediately measured for the root fresh weight (RFW) and shoot fresh weight (SFW). The root dry weight (RDW) and shoot dry weight (SDW) were calculated using a precision scale after the samples were dried until there was no difference between the final two weights. A meter was used to manually measure the shoot length (SL) using Image J analysis software, and root pictures were examined to look into variations in the RSA of samples [9]. Using ImageJ software, the features that make up the RSA-taproot length (TL), number of lateral roots (NLR), total length of lateral roots (TLLR), mean length of lateral roots (MLLR), and total root area (TRL) were manually quantified.

Statistical Analysis

The Shapiro-Wilks test was used to determine if the data were normal. ANOVA was used in both trials to evaluate data with a normal distribution at significance levels of 0.05 and 0.01. Statistical analysis software R (ver. 3.5.2) was used to compare and categorize the data using a TUKEY multivariate test.

Results

Influence of Seed Priming Treatments on Germination under Different Salinity Levels

To examine the impact of various seed priming treatments on key growth metrics under SS, a two-stage experiment was designed. According to treatments and salt levels, different germination characteristics were seen in the initial phase of the experiment. Different salinity levels, priming treatments, and their combinations, as determined by ANOVA, resulted in statistically significant changes (0.01) in every aspect of germination characteristics (Table 1).

The ranges for the GP, MGT, GI, UG, GR, and GE were 85.3-100.0%, 1.1-2.9 days, 329.0-946.6, 322.7-908.7, 82.3-236.7, and 1.0-89.3, respectively. Salinity levels rose, which caused a degradation in germination properties. While the lowest values were achieved under 200 mM NaCl circumstances, the maximum GP, GI, UG, GR, and GE were seen under non-stress settings. SS enhanced the MGT relative to control, unlike before (Table 2). Under stressful and non-stressful situations, seed priming treatments virtually enhanced the germination qualities. SA-primed seeds showed the greatest GP, GI, UG, GR, and GE, and in a similar vein, SA treatment led to the shortest germination time. Due to SA-low priming's SS, there was no GP loss; however, at high SS, there was a 2.7% loss. Under high salinity circumstances, the SA, Si, and NaCl priming, respectively, the MGT climbed to 2.9 days from

1.9 days in control seeds whereas it was 2.2, 2.1, and 2.3 under those conditions. Under non-stressful conditions, SA-primed seeds had the highest GI, a crucial germination characteristic indicator, while control seeds had the lowest GI under very salty conditions. For the GI, all priming techniques outperformed the control group. Under pot or field circumstances, the UG signifies the creation of a homogenous stand, giving plants an advantage. SA and NaCl priming improved the UG above control seeds by around 50%. When it came to SA-primed seeds, the GR was the greatest. It was superior to the other priming strategies, and increased the GR by 53% as compared to control seeds. On the first day of the experiment, the GE displayed the sprouted seeds. Seedlings primed with SA or NaCl showed the effects that were greater than those of untreated or Si-primed seeds (Table 2).

Influence of Seed Priming Treatments on Seedling Growth and Root System Architecture under Different Salinity Levels

The second stage of the experiment examined the effects of seed priming with SA, Si, and NaCl on seedling development and RSA in salinity and typical circumstances. Salinity levels affected the SFW, SL, NRL, and TLLR at a significance level (0.05) in the second trial, but only at a highly significance level (0.01) for the RFW, SDW, RDW, TL, MLLR, and TRA. Seed priming treatments had a 5% (0.05) significance level effect on the RFW, NRL, and TLLR, but only a 1% (0.01) significance level effect on TL, MLLR, and TRA. Additionally, seed priming had no discernible impact on the SFW, SDW, RDW, and SL. The SFW was unaffected by the SS and priming treatments' interaction.

The SFW and RFW altered between 97.0 and 124.4 g, and 64.2 and 165, respectively. Although SA-primed seeds produced the greatest SFW, there was no discernible difference across treatments. Compared to non-stress circumstances, SS reduced the SFW and RFW by 9.9 and 46.7%, respectively; as a result, SS

Table 1. Analysis of variance belonging to germination characteristics depending on salinity stress, seed priming treatments and their interactions.

| Source of variation | DF | GP | | MGT | | GI | |
|---------------------|----|-----------|---------|----------|---------|----------|---------|
| | | MS | F prob. | MS | F prob. | MS | F prob. |
| Salinity (S) | 2 | 312.00 | ** | 579.90 | ** | 907.50 | ** |
| Priming (P) | 3 | 135.10 | ** | 148.50 | ** | 216.30 | ** |
| SxP | 6 | 31.10 | ** | 9.20 | ** | 21.00 | ** |
| Source of variation | DF | UG | | GR | | GE | |
| | | MS | F prob. | MS | F prob. | MS | F prob. |
| Salinity (S) | 2 | 726371.50 | ** | 56640.90 | ** | 21313.50 | ** |
| Priming (P) | 3 | 168622.60 | ** | 13539.70 | ** | 5478.30 | ** |
| SxP | 6 | 27595.20 | ** | 1317.30 | ** | 1673.70 | ** |

Table 2. Differences in germination characteristics depending on seed priming treatments under different salinity stresses.

| Germination Percentage (%) | | | | | Mean Germination Time (Day) | | | | |
|----------------------------|---------|---------|---------|--------|-----------------------------|---------|----------|---------|--------|
| | S0 | S1 | S2 | Mean | | S0 | S1 | S2 | Mean |
| Control | 98.7ab | 97.3a-c | 92.0c | 96.0B | Control | 1.9de | 2.0cd | 2.9a | 2.3A |
| SA | 100.0a | 100.0a | 97.3a-c | 99.1A | SA | 1.2h | 1.6fg | 2.2bc | 1.6C |
| Si | 98.7ab | 93.3bc | 85.3d | 92.4C | Si | 1.5g | 1.7ef | 2.1bc | 1.8B |
| NaCl | 98.7ab | 97.3a-c | 93.3bc | 96.4B | NaCl | 1.1h | 1.6fg | 2.3b | 1.7C |
| Mean | 99.0A | 97.0B | 92.0C | | Mean | 1.4C | 1.7B | 2.4A | |
| Germination Index | | | | | Uniformity of Germination | | | | |
| | S0 | S1 | S2 | Mean | | S0 | S1 | S2 | Mean |
| Control | 568.7de | 480.0ef | 329.0g | 459.2C | Control | 530.0cd | 477.0d-f | 322.7g | 443.2C |
| SA | 946.6a | 697.7bc | 462.3f | 702.2A | SA | 908.7a | 622.3bc | 444.3ef | 658.4A |
| Si | 767.7b | 613.3cd | 416.3fg | 599.1B | Si | 676.3b | 557.7cd | 399.3fg | 544.4B |
| NaCl | 906.7a | 700.0bc | 429.0f | 678.6A | NaCl | 852.0a | 628.0bc | 409.0fg | 629.7A |
| Mean | 797.4A | 622.8B | 409.2C | | Mean | 741.8A | 571.3B | 393.8C | |
| Germination Rate | | | | | Germination Energy | | | | |
| | S0 | S1 | S2 | Mean | | S0 | S1 | S2 | Mean |
| Control | 142.0de | 120.0ef | 82.3g | 114.8C | Control | 16.0de | 1.0e | 1.0e | 6.0C |
| SA | 236.7a | 174.3bc | 115.7f | 175.6A | SA | 89.3a | 40.0bc | 2.0e | 43.8A |
| Si | 192.0b | 153.3cd | 104.0fg | 149.8B | Si | 56.0b | 29.3cd | 2.0e | 29.1B |
| NaCl | 226.7a | 175.0bc | 107.3f | 169.7B | NaCl | 82.7a | 42.7bc | 1.0e | 42.1A |
| Mean | 199.3A | 155.7B | 102.3C | | Mean | 61.0A | 28.3B | 1.5C | |

(S0: No salt application, S1: 100 mM NaCl, and S2: 200 mM NaCl, SA: Salicylic acid, Si: Silicon)

had a greater impact on the root growth than the shoot growth. Si- and NaCl priming outperformed the control, whereas SA priming generated the best RFW outcomes. The ranges of SDW and RDW were 1.22-1.61 g and 0.81-1.20 g respectively. While SS significantly reduced the amount of dry matter that accumulated in aboveground organs, seed priming and its combination with SS had no discernible impact on the SDW. Similar to this, SS prevented the buildup of dry materials in root organs. Depending on salinity, the accumulation of dry matter in roots were reduced by 24.6%. SS had a considerable impact on the SL, which varied between 1.22 and 1.61 cm, but seed priming treatments had little effect. As much as 24% less SL was achieved by SS compared to control. Salinity and its interactions with seed priming treatment had an impact on the TL. The SA-primed seeds under control circumstances had the maximum TL (20.1 cm), whereas the NaCl-primed seeds under high salinity conditions had the lowest value (6.6 cm). The individual effect of SS reduced the TL by 44.8%. Depending on treatments and salinity, the NLR fluctuated between 8.2 and 19.3. Si priming increased the NLR above control seeds by 32.6%, whilst SS lowered it by 22.8%. The TLLR and MLLR were, respectively, 5.7-44.1 cm and 0.52-2.66 cm. The TLLR

and MLLR were both reduced by SS by 75% and 63%, respectively. While SA- and Si priming were more efficient for TLLR, NaCl priming was more efficient for MLLR. Depending on the SS and seed priming treatments, the TRA changed from 8.8 to 50.9 cm². About 70% of the TRA was reduced by SS, but up to 29.3% was boosted by seed priming. The results clearly show that seed priming applications (especially SA and Si) not only increased cellular expansion and dry matter accumulation in the plant, but also contributed to the development of the lateral root system, which enables plants to utilize water and nutrients more effectively. In addition, it is understood that tolerance develops in plants growing under stress by spreading more root amount per unit area, thus plant growth shows a positive changes (Table 4).

Discussion

Germination Characteristics

In the trial, SS considerably reduced the GP, GI, UG, GR, and GE, while raising the MGT. To the best of our knowledge, even though salt stress has a detrimental

Table 3. Analysis of variance belonging to root and shoot development depending on salinity stress, seed priming treatments and their interactions.

| Source of variation | Shoot Fresh Weight | | | Root Fresh Weight | | Shoot Dry Weight | |
|---------------------|-------------------------|--------|---------|-------------------------------|---------|------------------------------|---------|
| | DF | MS | F prob. | MS | F prob. | MS | F prob. |
| Salinity (S) | 2 | 823.9 | * | 21850 | ** | 447399 | ** |
| Priming (P) | 3 | 260.2 | ns | 956.6 | * | 36657 | ns |
| SxP | 6 | 135.4 | ns | 1145.9 | ** | 9213 | ns |
| Source of variation | Root Dry Weight | | | Shoot Length | | Taproot Length | |
| | DF | MS | F prob. | MS | F prob. | MS | F prob. |
| Salinity (S) | 2 | 284320 | ** | 4412.8 | * | 2444630 | ** |
| Priming (P) | 3 | 12667 | ns | 267.6 | ns | 1047269 | ** |
| SxP | 6 | 29446 | * | 220.9 | ns | 741226 | ** |
| Source of variation | Number of Lateral Roots | | | Total Length of Lateral Roots | | Mean Length of Lateral Roots | |
| | DF | MS | F prob. | MS | F prob. | MS | F prob. |
| Salinity (S) | 2 | 15257 | * | 21933036 | * | 10390662 | ** |
| Priming (P) | 3 | 52577 | * | 2477439 | * | 474447 | ** |
| SxP | 6 | 118161 | ns | 1064447 | ns | 629228 | ** |
| Source of variation | Total Root Area | | | | | | |
| | DF | MS | F prob. | | | | |
| Salinity (S) | 2 | 4766.8 | ** | | | | |
| Priming (P) | 3 | 299.5 | ** | | | | |
| SxP | 6 | 79.2 | ** | | | | |

effect on plants at all phases of their development, the germination stage is the most vulnerable since it is the plant's most vulnerable time [3]. The buildup of harmful ions (Na^+ and Cl^-), and the restriction of water uptake by seeds are the primary causes of salinity's inhibiting influence on germination. Salinity thus prevents good germination and stand development by blocking enzymatic processes that convert endosperm reserves into sugar [10]. According to Liu et al. [11], salinity during the germination stage reduces seed germination by lowering α -amylase enzyme activity and restricting the amount of bioactive gibberellin. In addition, K^+ ions, which are essential for osmoregulation, cell growth, membrane polarization, enzyme activity, and the neutralization of negative ions, compete with Na^+ buildup. Additionally, Na^+ and Cl^- can enter cells and harm cell membranes and cytosolic metabolic processes [12]. So, much like other crops, salt has a negative impact on the germination process in lentil [13]. Osmo-, halo-, nano-, solid matrix-, hormonal-, and bio-priming, among other approaches, control pre-germination phases, boost water absorption, foster enzyme activities, and hasten seed germination under SS and non-stress circumstances [14]. Additionally, several studies have demonstrated that seed priming boosts antioxidant enzyme activity during SS, enhancing germination properties [15]. SA-

priming was the most effective in promoting favorable germination traits both in stress- and non-stress-filled environments. According to one definition, the SA is a signal molecule that affects a number of physiological procedures during germination. As a result, it promotes and quickens seed germination [16]. Furthermore, Janda et al. [17] suggested that induced genes expressing resistance that appear to effect on germination may be the reason why reducing SS during seed germination requires on SA-priming. The rate of water intake affects the pace of seed germination. However, an osmotic barrier caused by salt prevents the intake of water. SA is essential for the promotion of cytokinin and indoleacetic acid. Thus, it stimulates cell division and improves seed germination when exposed to SS [18]. According to Afzal et al. [19], seed priming with 50 ppm SA reduced electrolyte leakage, encouraged seedling development, enhanced germination traits, and sped up germination time.

Therefore, the results are consistent with the earlier experiments. Si is considered as a crucial element for plants since, in the event of a deficit, different anomalies may be seen in growth processes. Si is the second most prevalent element in soil after oxygen. Due to its mitigating effects on plants under stress, Si is often referred to as a plant booster and protective substance

Table 4. Differences in shoot and root growth, and root system architecture depending on seed priming treatments under different salinity stresses.

| Shoot Fresh Weight (g) | | | | | Root Fresh Weight (g) | | | | |
|-----------------------------------|--------|---------|--------|--------|------------------------------------|---------|---------|---------|---------|
| | S0 | S1 | S2 | Mean | | S0 | S1 | S2 | Mean |
| Control | 111.8 | 115.0 | 97.0 | 107.9 | Control | 130.2bc | 75.2de | 81.2de | 95.5B |
| SA | 117.0 | 124.4 | 109.4 | 116.9 | SA | 165.8a | 100.8cd | 72.6de | 113.1A |
| Si | 118.4 | 104.2 | 103.8 | 108.8 | Si | 134.2ab | 98.4cd | 64.2e | 98.9AB |
| NaCl | 115.8 | 116.6 | 107.0 | 113.1 | NaCl | 127.8bc | 115.4cb | 79.2de | 107.5AB |
| Mean | 115.8A | 115.1AB | 104.3B | | Mean | 139.5A | 97.5B | 74.3C | |
| Shoot Dry Weight (g) | | | | | Root Dry Weight (g) | | | | |
| | S0 | S1 | S2 | Mean | | S0 | S1 | S2 | Mean |
| Control | 1.41 | 1.26 | 1.22 | 1.30 | Control | 1.06a-d | 1.01a-d | 0.81d | 0.96 |
| SA | 1.61 | 1.48 | 1.28 | 1.46 | SA | 1.20a | 1.13ab | 0.87cd | 1.07 |
| Si | 1.56 | 1.45 | 1.23 | 1.41 | Si | 1.13ab | 1.10a-c | 0.81d | 1.01 |
| NaCl | 1.55 | 1.41 | 1.25 | 1.40 | NaCl | 1.18a | 1.10a-c | 0.94b-d | 1.07 |
| Mean | 1.53A | 1.40B | 1.25C | | Mean | 1.14A | 1.09A | 0.86B | |
| Shoot Length (cm) | | | | | Taproot Length (cm) | | | | |
| | S0 | S1 | S2 | Mean | | S0 | S1 | S2 | Mean |
| Control | 11.6 | 11.6 | 8.0 | 10.3 | Control | 16.9a | 13.4ab | 6.9b | 12.4B |
| SA | 12.2 | 11.4 | 10.3 | 11.3 | SA | 20.1a | 18.4a | 14.2ab | 17.6A |
| Si | 12.6 | 11.0 | 9.2 | 10.9 | Si | 19.7a | 19.7a | 14.6ab | 18.0A |
| NaCl | 12.3 | 10.7 | 9.1 | 10.7 | NaCl | 20.2a | 16.8a | 6.6b | 14.5AB |
| Mean | 12.1A | 11.9A | 9.2B | | Mean | 19.2A | 17.1B | 10.6C | |
| Number of Lateral Roots | | | | | Total Length of Lateral Roots (cm) | | | | |
| | S0 | S1 | S2 | Mean | | S0 | S1 | S2 | Mean |
| Control | 16.0 | 12.8 | 8.2 | 12.4B | Control | 29.1 | 19.7 | 5.7 | 18.2B |
| SA | 18.1 | 19.1 | 12.0 | 16.4AB | SA | 36.6 | 25.6 | 12.3 | 24.8A |
| Si | 16.8 | 19.3 | 19.2 | 18.4A | Si | 44.1 | 19.1 | 8.8 | 24.0A |
| NaCl | 13.9 | 14.3 | 10.4 | 12.9B | NaCl | 37.3 | 21.6 | 9.8 | 22.9AB |
| Mean | 16.2A | 16.3A | 12.5B | | Mean | 36.8A | 21.5B | 9.2C | |
| Mean Length of Lateral Roots (cm) | | | | | Total Root Area (cm ²) | | | | |
| | S0 | S1 | S2 | Mean | | S0 | S1 | S2 | Mean |
| Control | 1.78c | 1.64cd | 0.74e | 1.38B | Control | 39.3b | 21.5d | 8.8f | 23.2B |
| SA | 2.00bc | 1.39cd | 1.01de | 1.47AB | SA | 40.4b | 36.0bc | 15.7d-f | 30.7A |
| Si | 2.63ab | 0.99de | 0.52e | 1.38B | Si | 50.9a | 34.9bc | 12.6ef | 32.8A |
| NaCl | 2.66a | 1.51cd | 1.09de | 1.76A | NaCl | 47.9a | 31.3c | 18.1de | 32.4A |
| Mean | 2.27A | 1.39B | 0.84C | | Mean | 44.6A | 30.9B | 13.8C | |

(S0: No salt application, S1: 100 mM NaCl, and S2: 200 mM NaCl, SA: Salicylic acid, Si: Silicon)

[20]. Under stress and non-stress settings, Si-priming reduced the MGT and boosted GI, UG, GR, and GE compared to control seeds. First of all, Si-priming stimulates the germination process and increases water

intake. As a result, prepared seeds have the potential for quick germination. Secondly, Si reduced the negative effects of SS on germination traits. Additionally, when a cell is active, it secretes ROS, which is crucial for

breaking dormancy and initiating the germination stage [21]. Additionally, even while hydration promotes the restoration of mitochondrial function, there may be circumstances in which the electron transport chain directly transfers electrons to O_2 , making decreased O_2 the unavoidable main source of ROS in mitochondria [22]. Thus, ROS buildup and neutralisation mechanisms are necessary for proper germination [23]. During the activation phase of germination, Si-priming controls ROS buildup and enzyme activity. On the other hand, whereas Si-priming improves seed germination in SS, its precise function is unknown. Additionally, it is widely known that Si interacts in certain unresolved ways with phytohormones, which play a crucial role in seed germination and development [24]. Under both normal and saline circumstances, NaCl priming reduced the MGT, and improved germination parameters such as the GI, UG, GR, and GE compared to control seeds. The NaCl priming strategy boosted germination qualities because, according to the earlier SS management experiment, it offers a chance to promote adaptation to SS. Additionally, the capacity of salt-resistant plants to adapt SS is predicated on the avoidance of dehydration that results from osmoregulation. By actively absorbing inorganic ions or synthesizing organic substances like amino acids, organic acids, sugars, etc., plants may regulate their internal osmotic pressure [25]. NaCl priming enhances proline and total sugar accumulation in melon seedlings, which reduces SS, [26]. Additionally, NaCl priming has been shown by Nakaune et al. [27] to promote gibberellic acid (GA) accumulation by activating genes for GA production, improve seed germination by activating genes for endosperm cap weakening, and influence the amount of abscisic acid (ABA).

Seedling Growth and Root System Characteristics

Both aboveground and subsurface organs were injured by the SS. Because to the buildup of harmful ions, osmotic stress, and the production of reactive oxygen species (ROS), SS limits the growth of seedlings and the development of their roots. Increasing salinity caused a drop in the SFW, SDW, RFW, and RDW. Additionally, SS weakened the RSA, and seed priming made it easier for the plants to fight back under SS. Numerous studies have shown that SS negatively impacts plant growth and hinders physiological, morphological, and molecular development [13, 28], which is in line with the findings of our study. The most common changes in plant metabolism under SS are related to the accumulation of metabolites such as proline soluble carbohydrates, γ -aminobutyric acid and glycine betaine [29], that carry on the osmotic balance in the cell, reduce the entropy, and provide the maintenance of proteins [30]. Besides, the accumulation of ascorbate and glutathione contributes to the mitigation of the adverse influence of ROS secreted by SS and is a major indicator of stress [31].

SS, therefore, inhibits the accumulation of dry matter, new biomass, root development, and plant growth. Despite the fact that many experiments revealed that root biomass and fine root formations were restricted by SS in various crops, Munns and Tester [32] noted that underground organs, i.e., similar to how the NLR was lowered up to 22.8% under SS compared to ideal circumstances, the TL declined by 44.8% as saline levels rose. However, other studies claimed that in cultivars of *Cicer arietinum*, the NLR rose as saline levels increased [33].

Consequently, Noreen and Ashraf [34] noted that by increasing the net photosynthetic rate under SS, SA-which is regarded as a significant plant growth regulator promotes seedling development and stress tolerance. However, the efficiency of SA and its effective concentration may vary amongst plant species. While some investigations failed to find any promotional effects of SA therapy under SS, others showed superior outcomes to control treatment [35, 36]. The secretion of ROS is crucially influenced by SA's suppression of catalase. Additionally, SA induces the antioxidant defense mechanism to become active in plant tissues when hydrogen peroxide (H_2O_2) levels rise [35]. The initial sign of stress under salinity is thought to be the buildup of different compatible solutes such as glycine betaine and proline, which are brought on by SS. Treatment with SA raises the content of proline in SS. Additionally, SS decreases cell turgor, which limits the production of new biomass and dry matter in plants. SA therapy enhances the storage of water and dry matter, and modulates turgor via increasing proline accumulation [37]. These conclusions concur with our findings. Additionally, in suitable and salty circumstances, SA priming dramatically promoted RSA, which resulted in an increase in TL, NLR, TLLR, and TRA compared to control. According to Miao et al. [38], the modulation of the transcriptional level of the RSA genes in cucumber seedlings is the reason why exogenous SA enhances root growth and the creation of primary and secondary lateral roots under SS. Similar to this, Pasternak et al. [39] found that SA treatment influences RSA and root meristem shape by enhancing the concentration and dispersion of produced auxin. The positive effects of Si in plants under abiotic stress conditions including salt, drought, severe temperature, etc. were thoroughly covered by Liang et al. [40]. An increase in exchangeable Al^{3+} in acid soils, a decrease in citrate and malate exudation, an upregulation of phosphorus transporter genes, and an increase in internal phosphorus consumption by lowering Fe and Mn uptake are all effects of Si application, according to research from earlier experiments [41]. Additionally, Si treatment increases nitrogen absorption, boosts chlorophyll index, and enhances shoot and root development in wheat and maize crops, according to Galindo et al. [42]. Additionally, Si applications promote nutrition control and ROS detoxification [43]. Additionally, according to Abdelaal et al. [44], foliar Si treatment enhances the levels of mineral nutrients, chlorophyll a and b, and hydration status in leaves. Si administration during SS

also lowers superoxide levels, lipid peroxidation, and electrolyte leakage. According to Shamshiripour et al. [45], an ideal Si concentration increases nodulation, soil biological properties, and shoot and root development in greenhouse settings. With the exception of the MLLR, Si priming offered the greatest results for root system attributes. As a result, it enhanced the RSA in both ideal and stressful circumstances (Table 4). According to Guo et al. [46], the buildup of ABA in the roots caused by Si administration encourages the growth of lateral roots. The transpiration stream pulls Si from the soil, and moves it to the shoot region. Within the whole body, Si is deposited [47]. Organ Si buildup boosts secondary metabolites, including a variety of plant hormones that control several growths and developmental processes [48].

According to several researchers, halo-priming (seed priming with salts), which was used in our work, can reduce SS in a variety of crops. Halo-priming regulates osmotic adjustment and prevents ionic toxicity, assisting plants in adjusting to the fatal effects of SS. Additionally, halo-priming causes treated plants to accumulate more osmolytes, while decreasing the Na⁺ content [49]. In comparison to untreated plants, Alzahrani et al. [50] noted that NaCl priming increased the number of photosynthetic pigments and salt tolerance, decreased the content of osmolytes and malondialdehyde (MDA), and promoted the expression of salt-responsive genes (TaSOS1, TaNHX1, TaHKT1, TaSOS4, TaAKT1, and TaHKT2).

Conclusions

Lentil germination and seedling development were severely hindered by SS. Salt stress were reduced during seed germination and seedling development by seed priming with SA, Si, and NaCl. All priming ingredients had a positive effect on SS and developmental processes, however, in the experiment, the 0.2 mM SA and 4 mM Si treatments performed better in terms of germination traits, shoot growth, and root system development. In summary, since this research was conducted under laboratory conditions, it is only a starting point and an easily applicable solution. In order to fully observe the effects, it should be examined with repeated field trials in areas with salinity problems. However, this research carried out under artificial and controlled conditions has shown positive results in terms of improving salinity stress, improving germination process, and measuring the response of seedling and root development to applications. In conclusion, seed priming may be a simple, affordable, and practical strategy for SS mitigation in lentil farming.

Conflict of Interest

The authors declare no conflict of interest

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