

## Article

# Seed Priming Enhances Seed Germination and Morphological Traits of *Lactuca sativa* L. under Salt Stress

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**Abstract:** Seed germination is the stage in which plants are most sensitive to abiotic stress, including salt stress (SS). SS affects plant growth and performance through ion toxicity, decreasing seed germination percentage and increasing the germination time. Several priming treatments were used to enhance germination under SS. The objectives of this study were (1) to identify priming treatments to shorten the emergence period, (2) to evaluate priming treatments against the SS, and (3) to induce synchronized seed germination. Salt-sensitive ‘Burpee Bibb’ lettuce seeds were treated with 0.05% potassium nitrate, 3 mM gibberellic acid, and distilled water. All the primed and non-primed seeds were subjected to 100 mM sodium chloride (NaCl) or 0 mM NaCl (control). The seven-day experiment, arranged in a complete randomized block design with four replications, was conducted in a growth chamber maintained with 16/8 h photoperiod (light/dark), 60% relative humidity, and a day/night temperature of 22/18 °C. The result indicated that hydro-primed (HP) seeds were better synchronized under SS. Similarly, fresh mass (FM) and dry mass (DM) of cotyledon, hypocotyl, and radicle were the highest in HP lettuce regardless of SS. Electrolyte leakage was the lowest in the HP lettuce, while other priming methods under SS increased membrane permeability, leading to osmotic stress and tissue damage. Overall, hydro-priming can be a good priming method for synchronizing germination and increasing FM and DM by creating the least osmotic stress and ion toxicity in lettuce under SS.

**Keywords:** hydro-priming; lettuce; sodium chloride; synchronization; electrolyte leakage



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## 1. Introduction

Salt stress (SS) is destructive abiotic stress that affects crop production in arid and semiarid areas [1]. For this reason, it poses a severe threat to food security. At least 20% of crop cultivation land has been damaged by salt accumulation worldwide [2]. It is estimated that around 4000 hectares of land are affected daily and 50% of the arable land is affected by the salt in the soil [3,4]. It is speculated that more than 50% of the cultivable land will turn into non-arable land by the mid-21st century [5]. SS, especially under sodium chloride (NaCl), affects plant growth and performance through ion toxicity and osmotic stress in leaves and roots. Several reports on the relation between SS and seed germination state that SS also suppresses seed germination percentage and increases the germination time [6–8]. Most crops are reported to be sensitive to SS even as low as 3 dSm<sup>-1</sup> electrical conductivity [9]. The suppression is due to the adverse physiological and biochemical changes in germinating seeds exposed to SS [1]. Specifically, Na<sup>+</sup> and Cl<sup>-</sup> impair the seed growth and development through osmotic and ion-specific toxic effects [5,10,11]. Na<sup>+</sup>, on the one hand, can replace the K<sup>+</sup> cation and promote ion toxicity, while Cl<sup>-</sup> controls the vegetative growth [12].

Seeds are supposed to turn into healthy and vigorous plants with proper metabolisms and physiological performances. To achieve that outcome, seeds maintain low moisture content during their physiological maturity [13]. Lettuce seeds with moisture levels less

than 5% can be stored for a long period [14]. However, the extended seed storage period can deteriorate the germination and viability of the seed [15]. Seeds stored for an extended period when subjected to SS can result in even poorer germination and seedling growth. Seed germination and seedling growth are critical stages for crop establishment and are considered the stages in which plants are the most sensitive to abiotic stresses [16,17]. The 'two-phase growth response to SS' concept by Lauchi and Grattan [18] stated that the germination rate is directly correlated with the level of salt treatments applied.

Several seed priming treatments, including hydro- (HP), potassium nitrate- ( $\text{KNO}_3$ ), and gibberellic acid-priming ( $\text{GA}_3$ ), were used to enhance germination and performance under SS [19,20]. The purpose of these priming treatments was to shorten the emergence period and protect the seeds from biotic and abiotic factors during the crucial phase of seedling establishment, synchronizing emergence and leading to a uniform stand and improved yields. Priming is a rehydrating approach that uses different biochemical agents to allow regulated seed rehydration, stimulating metabolic processes that are ordinarily active during the early stages of germination, while preventing the seed from progressing to complete germination [21]. Plant species/genotype and physiology, environmental conditions exposed to the seed lot and vigor, and the priming method used all play roles in the success of seed priming [22]. Priming treatments have some beneficial effects, such as germination enhancement, emergence synchronization, early seedling growth, and minimizing the deleterious effect of abiotic and biotic stress. This has been demonstrated in many crops, such as wheat (*Triticum aestivum*) [23], chickpea (*Cicer arietinum*) [24], sunflower (*Helianthus* pp.) [25], and cotton (*Gossypium* spp.) [26]. Additionally, several other reports on maize (*Zea mays*), broccoli (*Brassica oleracea* var. *italica*), cauliflower (*Brassica oleracea* var. *botrytis*), and other leafy vegetables stated that priming reduced the mean germination time (MGT) and increased the mean germination rate (MGR) and synchronization index (Z) [27,28]. MGT is a measure of the time spread of the germination while MGR is the reciprocal of MGT [29,30]. Similarly, Z is a degree of homogeneity of germination over time [31], which indicates the highest synchronized germination when the Z value is the lowest [32]. These parameters are the measures of germination, seed vigor, and response to abiotic and biotic stress, which help to improve understanding of the roles of different priming methods used under different environmental conditions and on different crops [33,34].

Several studies have been conducted to investigate the influence of different priming methods on different  $\text{C}_3$  plants. However, there has been very little information on the interactive effect of various priming methods and salt levels on the germination synchronization, germination parameters, or morphological and biochemical traits in lettuce. Hence, the objectives of this study are (1) to distinguish the priming treatments that can effectively shorten the emergence period, (2) to evaluate the responses of priming treatments under SS, and (3) to induce synchronized seed germination in lettuce. Overall, the goal of this study is to examine priming approaches to overcoming the negative effects of SS on lettuce germination.

## 2. Materials and Methods

### 2.1. Planting Materials and Priming Treatment

The salt-susceptible *Lactuca sativa* (cv. 'Burpee Bibb') (BB) seeds were purchased from Burpee (Warminster, PA, USA). The susceptibility of BB was determined from a study by Adhikari et al. [35]. Before sowing, 200 lettuce seeds were treated with three different priming treatments: (1) 0.5%  $\text{KNO}_3$ , (2) 3 mM  $\text{GA}_3$ , and (3) hydro-priming. These priming agents were prepared using distilled water. A set of non-primed (NP) seeds were also included for comparison with primed seeds. Seeds were immersed in different priming treatments for different numbers of hours ( $\text{KNO}_3$  = 2 h 30 min;  $\text{GA}_3$  = 12 h 45 min; and hydro-priming = 2 h 30 min), as described by Mahmoudi et al. [36], with few modifications. Soon after the priming, seeds were rinsed four times with distilled water and were left to

dry at room temperature until they regained their original moisture content, which took approximately 2 days (maximum).

## 2.2. Salt Treatment and Germination

All the primed and non-primed seeds were subjected to either 0 mM NaCl (control) or 100 mM NaCl concentration. The NaCl solution was prepared using distilled water. Before sowing, seeds were soaked in 200 mL of either control or 100 mM NaCl for around 2 h and left to dry for 2 more hours to regain their original moisture content at room temperature. The germination was carried out by placing 25 seeds per treatment per replication in a 10 cm Petri dish with a double-layer filter paper in the growth chamber. The 0 mM NaCl treatment was considered as a control. The growth chamber was maintained with 16/8 h photoperiod (light/dark), 330  $\mu\text{mol}/\text{m}^2/\text{s}$  of photosynthetic photon flux density (PPFD), 50–60% relative humidity, and a day/night temperature of 22/18 °C. Treatments were arranged in a complete randomized block design with four replications each.

## 2.3. Germination Parameters and Morphological Traits

### 2.3.1. Germination Parameters

Seed germination was recorded every 24 h for 5 days. Seeds were considered germinated when the radicle protruded through the seed coat and had a length of at least 2 mm. The mean germination time (MGT), mean germination rate (MGR), germination index (GI), and synchronization index (Z) were measured using the following equations:

$$\text{MGT} = \frac{\sum_{i=1}^k n_i \times t_i}{\sum_{i=1}^k n_i} \quad [37]$$

where

$n_i t_i$  = seeds germinated at  $i$ th interval with the corresponding time interval

$n_i$  = number of seeds germinated in the  $i$ th time

$t_i$  = time taken for seeds to germinate at  $i$ th count

$$\text{MGR} = \frac{1}{\text{MGT}} \quad [29]$$

$$\text{GI} = \sum_{i=1}^k \frac{n_i}{t_i} \quad [38]$$

$$Z = \frac{\sum_{i=1}^k C_{ni,2}}{C_{\sum ni,2}} \quad [31]$$

where

$$C_{ni,2} = n_i (n_i - 1) / 2$$

### 2.3.2. Seeds' Morphological Traits

Five days after sowing, seeds were divided into cotyledon, hypocotyl, and radicle for the determination of growth parameters. Fresh mass (FM), dry mass (DM), and length of all parts of the seeds were recorded.

## 2.4. Electrolyte Leakage

Electrolyte leakage (EL) was determined using the method described by Quartacci et al. [39]. The cotyledon and the hypocotyl parts of the lettuce plant were cut into 3–4 mm pieces and placed in a 50 mL tube containing 23–30 mL of double-distilled water. The initial electrical conductivity ( $EC_1$ ) was recorded after two hours using the digital EC meter (Fisherbrand™ accumet AP85 portable waterproof pH/Conductivity meter, Thermo Fisher Scientific, Waltham, MA, USA). The samples were then immersed in liquid nitrogen for 2–3 min and placed back in the same 50 mL tube for an additional 2 h, with continuous shaking. The final electrical conductivity ( $EC_2$ ) was then recorded. EL was calculated using the following equation:

$$EL (\%) = \frac{EC_1}{EC_2}$$

### 2.5. Data Analysis

Statistical analysis of the data was performed using SAS (version 9.4; SAS Institute, Cary, NC, USA). Data were analyzed using PROC GLM analysis of variance (ANOVA), followed by mean separation. The standard errors were based on the pooled error term from the ANOVA table. Tukey's test ( $p \leq 0.05$ ) was used to differentiate between genotype classifications and treatment.

## 3. Results

The effects of different priming methods on lettuce seeds subjected to salt stress (SS) were studied through various germination indicators, such as mean germination time (MGT), mean germination rate (MGR), germination index (GI), and synchronization index (Z). These parameters help to achieve a better understanding of seed germination and growth in primed lettuce seeds subjected to SS. Additionally, the electrolyte leakage in germinated seed parts and the morphological traits of cotyledon, hypocotyl, and radicle, including fresh mass (FM), dry mass (DM), and length, were recorded.

### 3.1. Germination Parameters

None of the priming methods affected MGT, MGR, and GI values in lettuce subjected to SS (Table 1). There was no interaction effect observed between different priming and salt treatments, except for Z ( $p < 0.05$ ). The Z value was recorded as the lowest in HP lettuce under both salt treatment levels. The Z values of other priming methods (GA<sub>3</sub> and KNO<sub>3</sub>) were recorded highest; however, they were not significantly different when compared with the NP control.

**Table 1.** Mean responses of various germination parameters of lettuce seeds for evaluation of the effects of different priming methods (GA<sub>3</sub>, hydro-priming, KNO<sub>3</sub>, and NP) and two salt treatment levels (control and 100 mM NaCl).

Priming	Treatment	MGT <sup>3</sup>	MGR	Z	GI
GA <sub>3</sub>	Control	2.16 ± 0.03a <sup>1,2</sup>	0.5 ± 0.01a	0.72 ± 0.05a	11.8 ± 0.14a
	NaCl	2.23 ± 0.11a	0.4 ± 0.02a	0.65 ± 0.11ab	11.5 ± 0.12a
Hydro	Control	2.01 ± 0.02a	0.5 ± 0.03a	0.32 ± 0.08c	12.5 ± 0.01a
	NaCl	2.04 ± 0.01a	0.4 ± 0.08a	0.42 ± 0.03c	12.3 ± 0.02a
KNO <sub>3</sub>	Control	2.17 ± 0.04a	0.5 ± 0.01a	0.71 ± 0.09a	11.8 ± 0.16a
	NaCl	2.37 ± 0.18a	0.4 ± 0.03a	0.55 ± 0.13b	11.2 ± 0.18a
Non-priming	Control	2.17 ± 0.02a	0.5 ± 0.01a	0.71 ± 0.03a	11.8 ± 0.08a
	NaCl	2.32 ± 0.11a	0.4 ± 0.02a	0.56 ± 0.18ab	11.2 ± 0.43a
Priming		***	***	***	***
Salt		**	**	***	***
Priming × Salt treatment		NS	NS	**	NS

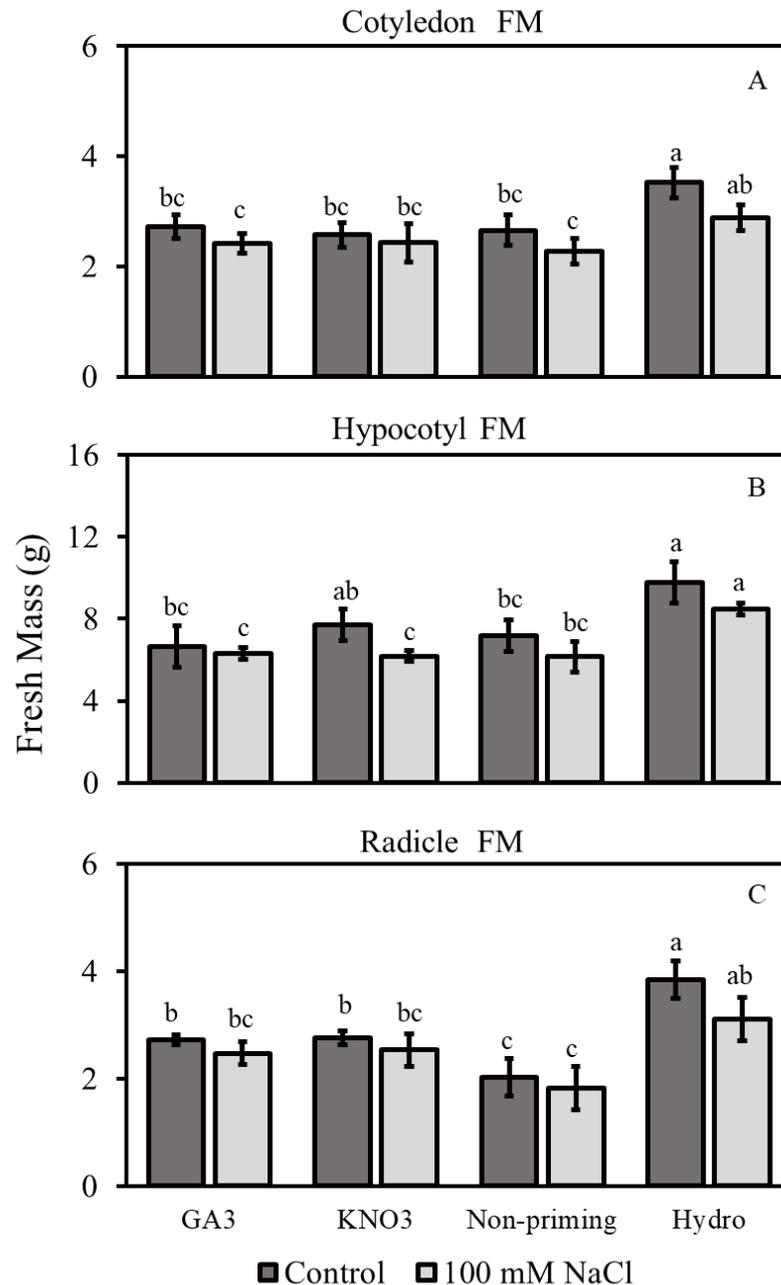
<sup>1</sup> Values represent the mean ± SE,  $n = 25$ . <sup>2</sup> Different lowercase letters indicate significant differences in parameters ( $p < 0.05$ ) as assessed by Tukey's test. NS represents nonsignificant  $p > 0.05$ ; \*\*, \*\*\* represent significance levels at  $p \leq 0.01$  and  $p \leq 0.001$ , respectively. <sup>3</sup> MGT = mean germination time (day); MGR = mean germination rate ( $\text{day}^{-1}$ ); GI = germination index (unitless); and Z = synchronization index (unitless).

### 3.2. Morphological Traits

#### 3.2.1. Fresh Mass

The FM data (Figure 1) indicated a significant (priming × salt treatment) interaction effect on FM of all morphological parameters (hypocotyl, cotyledon, and radicle). Figure 1A demonstrates that hydro-priming resulted in higher cotyledon FM compared to other priming methods (GA<sub>3</sub> and KNO<sub>3</sub>). Although there was no significant difference in the hypocotyl FM compared to its control, the hypocotyl FM was recorded highest (Figure 1B) compared to other priming methods, irrespective of the salt treatments. There was no significant effect of GA<sub>3</sub>- and KNO<sub>3</sub>-priming on hypocotyl FM when treated with salt, compared to the control. Additionally, the cotyledon and radicle FMs of GA<sub>3</sub>- and KNO<sub>3</sub>-

primed lettuce were not significantly different from non-primed (NP) lettuce under both salt treatment levels. Similarly, there was no significant difference in radicle FM of HP salt-treated lettuce compared to the control (Figure 1C). However, the highest radicle FM was observed for HP lettuce.

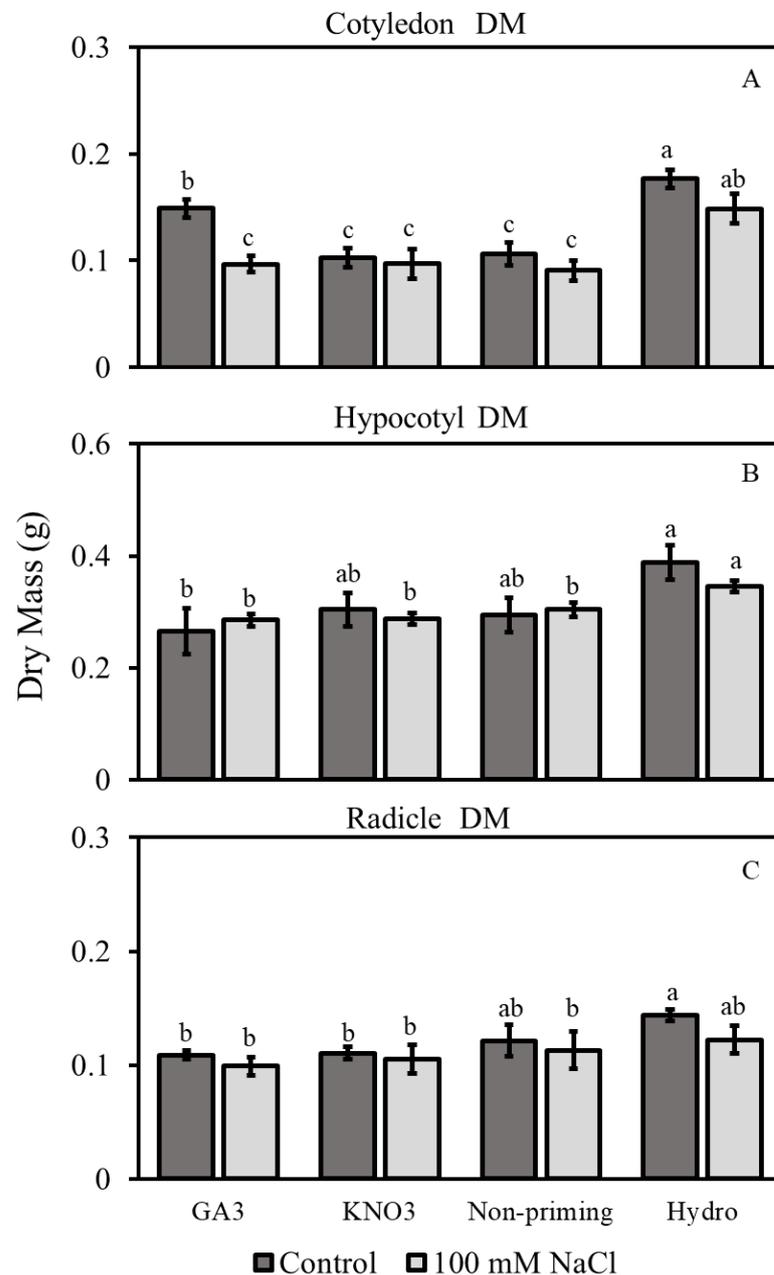


**Figure 1.** Fresh Mass (FM) of the: (A) cotyledon; (B) hypocotyl; and (C) radicle, (mean  $\pm$  SE,  $n = 4$ ), of BB lettuce, non-primed and seed-primed with GA<sub>3</sub>, KNO<sub>3</sub>, and hydro-priming, and further subjected to 100 mM of NaCl salt stress. 0 mM NaCl was considered as a control. Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly difference test at  $\alpha = 0.05$ .

### 3.2.2. Dry Mass

The dry mass (DM) of lettuce responded differently to different priming methods when subjected to two salt treatments, as presented in Figure 2. Priming under different salt treatment levels had a significant interaction effect on cotyledon, hypocotyl, and radicle DMs. Cotyledon DM in HP lettuce was significantly higher compared to other priming

methods ( $GA_3$  and  $KNO_3$ ). Interestingly, there was no significant difference in the cotyledon DM of HP lettuce subjected to SS compared to the control (Figure 2A). It is also worth noting that DM due to hydro-priming increased by almost 100% compared to  $GA_3$ -,  $KNO_3$ - and NP in both salt treatment levels.



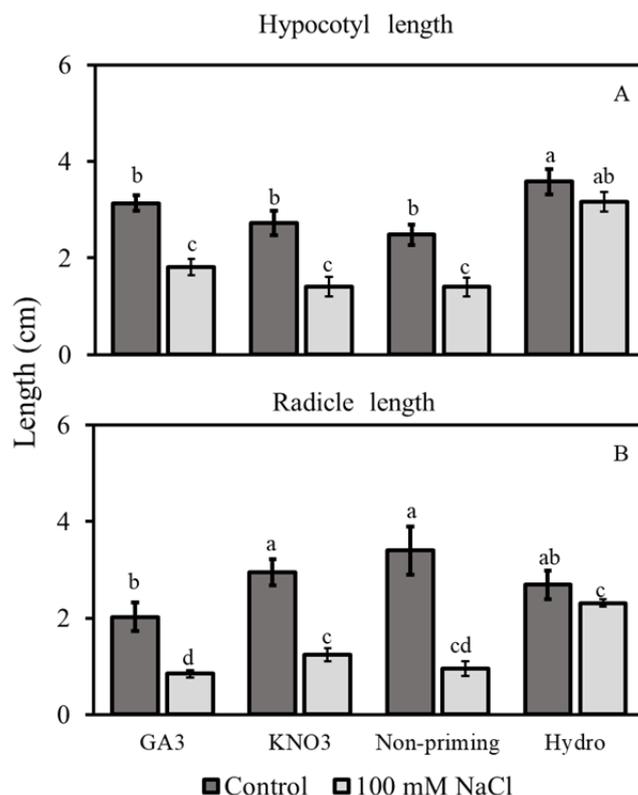
**Figure 2.** Dry Mass (DM) of the: (A) cotyledon; (B) hypocotyl; and (C) radicle (mean  $\pm$  SE,  $n = 4$ ) of BB lettuce, non-primed and seed-primed with  $GA_3$ ,  $KNO_3$ , and hydro-priming, and further subjected to 100 mM of NaCl salt stress. 0 mM NaCl was considered as a control. Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly difference test at  $\alpha = 0.05$ .

The DM of hypocotyl was found to be significantly different ( $p < 0.05$ ) in different priming methods studied under different salt treatment levels (Figure 2B). Although no salt treatment effect was observed within the priming methods, a significant interaction effect showed that hypocotyl DM was measured the highest in HP lettuce and the lowest in  $GA_3$ -primed lettuce. As in cotyledon DM, priming and salt treatment were significantly

affected in the radicle DM. DM of the radicle was recorded highest in the HP lettuce, followed by the NP lettuce (Figure 2C).

### 3.2.3. Hypocotyl and Radicle Lengths

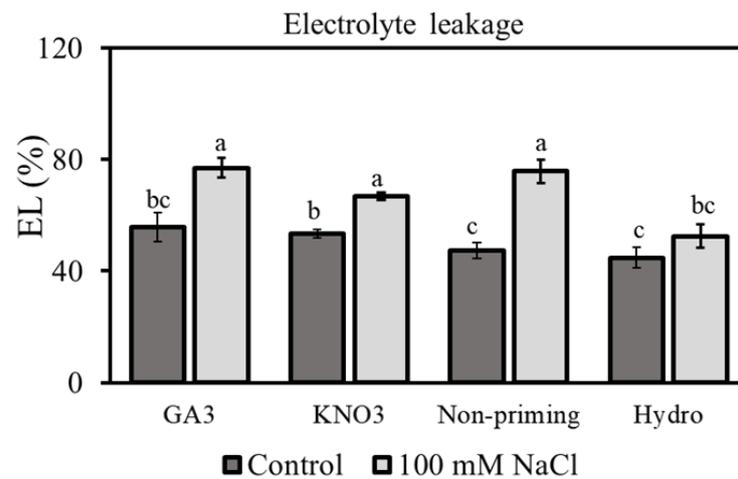
The priming methods and salt treatment interacted to significantly affect the hypocotyl and radicle lengths (Figure 3). There was a significant decrease ( $p < 0.05$ ) in hypocotyl length by 42%, 49%, and 44% in GA<sub>3</sub>-primed, KNO<sub>3</sub>-primed, and NP lettuce, respectively, when subjected to SS, as compared to the control (Figure 3A). However, the HP lettuce remained unaffected by the salt treatment compared to the control. The hypocotyl length was recorded highest in the HP lettuce. As in hypocotyl length, a significant decrease in radicle length by 58%, 59%, and 72% was observed in GA<sub>3</sub>-primed, KNO<sub>3</sub>-primed, and NP lettuce, respectively, under SS as compared to the control (Figure 3B). However, there was no significant decrease (14%) in radicle length recorded in HP and salt-treated lettuce compared to the control. Overall, the length parameters revealed the non-significant suppression in the hypocotyl and radicle length of HP lettuce subjected to 100 mM NaCl.



**Figure 3.** Length of: (A) hypocotyl; and (B) radicle, (mean  $\pm$  SE,  $n = 4$ ) of BB lettuce, non-primed and seed-primed with GA<sub>3</sub>, KNO<sub>3</sub>, and hydro-priming, and further subjected to 100 mM of NaCl salt stress. 0 mM NaCl was considered as a control. Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly difference test at  $\alpha = 0.05$ .

### 3.3. Electrolyte Leakage

The membrane injury (EL) was noticeably increased in lettuce primed with GA<sub>3</sub>, KNO<sub>3</sub>, and NP subjected to 100 mM NaCl compared to the control, except for HP lettuce (Figure 4). The increase in EL by 38%, 25%, and 60% was recorded in GA<sub>3</sub>, KNO<sub>3</sub>, and NP lettuce, respectively, under SS compared to the control. However, there was no significant membrane damage caused in HP lettuce. Salt-treated HP lettuce reflected the lowest EL compared to other salt-treated primed lettuce.



**Figure 4.** Electrolyte leakage (mean  $\pm$  SE,  $n = 4$ ) in BB lettuce, non-primed and seed-primed with GA<sub>3</sub>, KNO<sub>3</sub>, and hydro-priming, and further subjected to 100 mM of NaCl salt stress. 0 mM NaCl was considered as a control. Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly difference test at  $\alpha = 0.05$ .

#### 4. Discussion

Seed germination and seedling growth are two important stages for crop establishment [17]. These stages are critically sensitive to any abiotic stress, including SS, resulting in poor or delayed germination [7,16]. Lettuce is a moderately salt-tolerant crop (electrical conductivity threshold of 1.3–2.0 dS/m), and can be affected by the osmotic effect of salt in the first phase of salt absorption (osmotic stress phase), followed by the second phase of inhibition of potassium (K<sup>+</sup>) ions, the third phase (salt-specific effect phase) of inability to prevent salt ions from accumulating to toxic levels, and the final phase of oxidative stress and cell death [40–42]. During the first phase, the water potential is affected, leading to decreased water uptake by plants, while the second and third phases disturb the ion homeostasis of the cell, leading to membrane disorganization and inhibition of photosynthesis in plants [42]. This four-phase effect of SS was reported in several crops, such as tomato (*Solanum lycopersicum*) [43], wheat (*Triticum aestivum*) [44], sugar beet (*Beta vulgaris*) [45], beans (*Phaseolus vulgaris*) [46], and several other C3 crops [42]. Under normal conditions, the cytosol in plants contains 100 mM of K<sup>+</sup> and less than 10 mM Na<sup>+</sup> [47]. However, under SS, the cytosolic Na<sup>+</sup> and Cl<sup>-</sup> can increase up to 100 mM, becoming cytotoxic [47]. A cytotoxic situation leads to protein denaturation, membrane damage, and destabilization, ultimately causing electrolyte leakage and cell death [47]. The current study demonstrated that 100 mM NaCl treatment increases the mean germination time (MGT) and reduces the mean germination rate (MGR). Supporting these results, several reports on SS stated that concentration beyond the crop threshold could decrease the germination rate and increase germination time [6,7,16]. This situation could be caused by the plant's quick response to the high osmotic pressure created by SS, leading to the inhibition of cellular and biosynthetic processes [47]. While the SS caused seed growth inhibition in crops and leafy vegetables with moderate to low salt tolerance, seed priming, in contrast, was reported to improve the overall physiological and morphological performance of those crops [21]. For example, calcium chloride, used on wheat (*Triticum aestivum*) and sorghum (*Sorghum bicolor*); potassium chloride, used on wheat; deionized or distilled water, used on maize (*Zea mays*), barley (*Hordeum vulgare*), and Chinese cabbage (*Brassica rapa* subsp. *Pekinensis*); Gibberellic acid, used on pea (*Pisum sativum*), maize (*Zea mays*), rice (*Oryza sativa*), and alfalfa (*Medicago sativa*); glycine betaine, used on safflower (*Carthamus tinctorius*); potassium nitrate, used on sunflower (*Helianthus* spp.); and potassium chloride, used on broccoli (*Brassica oleracea* var. *italica*), were reported effective in SS amelioration through improved seed germination rate and improved yield [23,48–56]. Priming methods are crop-specific,

and a recent study was conducted to demonstrate the difference in the efficacy levels of different priming methods based on several reports in C<sub>3</sub> and C<sub>4</sub> plants.

Previously, it has been reported that synchronized seed growth is also enhanced by the crop-specific seed priming method [11]. In our study, HP seeds had more synchronized seed growth, with the lowest synchronization index (Z), which aligned with the earlier report by Khajeh-Hosseini et al. [11,32]. The current study also demonstrated that hydro-priming inhibited the negative effects of SS on seed germination, while there was a minimal response to KNO<sub>3</sub> and GA<sub>3</sub> of lettuce seeds under SS. Previous research by Mahmoudi et al. [36] found that HP lettuce seedlings could alleviate the NaCl effects on lettuce seedlings better than KNO<sub>3</sub> and GA<sub>3</sub>. The NaCl-inhibiting ability of hydro-priming could be due to the ambient or high-water potential ( $\Psi = 0$  MPa) environment created by hydro-priming during the early germination period [47]. Moreover, the decline in synchronization in KNO<sub>3</sub>- and GA<sub>3</sub>-primed lettuce could be due to a reduction in water potential and cell dehydration caused by SS, leading to a decrease in metabolic activities and protein destabilization during the germination period [47]. Thus, it is worth noting that HP lettuce maintained seed germination and growth in a synchronized pattern even under SS better than the rest of the priming treatments under SS, which agrees with the results reported by Mahmoudi et al. [36].

The salt treatment severely impacted the overall morphological parameters. The use of different priming methods reflected the significantly different growth responses compared to the SS created by 100 mM NaCl. Under SS, FM and DM in KNO<sub>3</sub>- and GA<sub>3</sub>-treated lettuce were found to be indifferent to those in non-primed (NP) lettuce, and were also reduced significantly, as reported earlier in melon (*Cucumis melo*) [57] and chickpea (*Cicer arietinum*) [58]. The germination and seedling improvement with KNO<sub>3</sub> and GA<sub>3</sub> have previously been documented in 'Vista' lettuce [59] and cabbage (*Brassica oleracea* var. *capitata*) [60]. However, the effectiveness of KNO<sub>3</sub> and GA<sub>3</sub> on salt-stressed BB lettuce was limited in the current study. The previous report documented that poor performance of priming agents can be due to amorphous tissue in the seed coat, which controls the permeability of seeds, disrupting the endogenous osmotic equilibrium [21,61]. The disparity can result in nutritional imbalance and poor seedling growth [62]. Although KNO<sub>3</sub> was reported to be effective in improving the seedling growth and establishment in sunflower (*Helianthus* spp.) and GA<sub>3</sub> was reported to be effective in onion (*Allium cepa*) and sesame (*Sesamum indicum*), the poor performances of KNO<sub>3</sub> and GA<sub>3</sub> suggested that the effects of priming under SS can be crop-specific [63]. On the contrary, the FM and DM of lettuce primed with distilled water (HP) increased significantly, irrespective of the salt treatments. The positive response of hydro-priming even under SS can be due to the stimulatory effect of priming on the mediation of cell division in germinating seeds in the early germination stage [64]. The better performance of HP lettuce in terms of water absorption can also be due to the early commencement of metabolic activity in the seeds before the start of radicle emergence [65]. Additionally, improved water-use efficiency has been documented with hydro-priming [66]. The improved water-use efficiency could be due to the efficient breakdown of stored food reserves in the seed during the second phase (lag phase of seed germination), which initiates the onset of cell wall loosening and expansion for the third phase (post-germination phase) [47]. Hydro-priming was reported to improve seed germination, seedling emergence, and productivity in many field crops [8,24,26,63]. The current study also aligned with a study conducted in romaine lettuce by Mahmoudi et al. [36], which demonstrated that HP seeds exhibited higher adaptive potential under SS compared to KNO<sub>3</sub>- and GA<sub>3</sub>-primed lettuce seeds. Thus, the use of hydro-priming for lettuce under SS could be an effective priming method to improve the FMs and DMs of different morphological traits in germinated seeds.

Abiotic stress is the major source of reactive oxygen species production (ROS), which deteriorates the growth and performance of plants. Since seed degradation is linked to a loss of structural and metabolic integrity and biochemical aberrations, assessing changes in oxidative stress biomarkers, such as electrolyte leakage (EL), is a good method for figuring

out which factors promote seed synchronization [67,68]. EL is prevalent in a range of species, tissues, and cell types, and can be caused by a variety of stresses, including salt stress [69,70]. In the current study, an increase in EL leakage was significantly different in salt-treated lettuce, causing membrane injury, unlike in the control. The most prominent EL was observed in the  $\text{KNO}_3^-$ ,  $\text{GA}_3^-$ , and non-primed lettuce subjected to SS. Chiu et al. [71] reported that improvement in germination by any priming might be due to enhanced repair of the membrane. However,  $\text{KNO}_3^-$  and  $\text{GA}_3^-$  priming failed to suppress the ROS induced by SS, leading to significant membrane injury. On the contrary, HP lettuce maintained the suppression of ROS even under SS, thus inhibiting the EL and maintaining the seed germination, as reported by Chiu et al. [71] and Mahmoudi et al. [36]. The outcome of the current study establishes that the positive effect of hydro-priming on EL in lettuce could be related to the higher FM and DM reduction, as well as the lowest synchronization index in the presence of SS, thus sparing few electrons for the production of reactive oxygen species [72]. In this study, the FMs and DMs of seed parts were statistically similar in  $\text{KNO}_3^-$  and  $\text{GA}_3^-$  primed seeds, while HP lettuce was significantly different and highest compared to the NP control. This suggests that BB lettuce can ensure better seed growth and germination with HP seeds even under SS, but not with  $\text{KNO}_3^-$  or  $\text{GA}_3^-$  primed seeds. In addition, the lowest seed synchronization index and electrolyte damage due to hydro-priming provides enough evidence to validate the positive impact of hydro-priming in seed germination and synchronization with minimal cell damage. Overall, this research suggests that hydro-priming has a mitigating impact on membrane injury in plants exposed to SS.

## 5. Conclusions

This study investigated the effects of different priming methods on salt-sensitive BB lettuce cultivars subjected to SS. Seed priming has the potential to improve seed germination and establishment by initiating germination metabolism without radicle protrusion. Thus, the current report shows the benefits of seed priming in the improvement of productivity in several field crops [73]. In addition, seed priming is cost- and resource-effective [73]. To validate the response of several priming methods in lettuce, this research was conducted to demonstrate that HP seeds showed better synchronization under both salt treatment levels than any other primed seeds. Similarly, the FMs and DMs of cotyledons, hypocotyls, and radicles were recorded the highest in HP lettuce, irrespective of salt treatments. It is important to note that low electrolyte leakage could be attributed to the better seed synchronization and higher FM and DM in HP lettuce under SS. After analyzing the overall morphological, biochemical, and germination traits, it is concluded that HP lettuce performed better under SS compared to lettuce primed with  $\text{KNO}_3$  and  $\text{GA}_3$ . Based on these results, using hydro-priming for lettuce subjected to SS can be a suitable priming method to ameliorate the deleterious effect of SS and enhance the synchronized seed germination. In addition, hydro-priming can also be beneficial in stimulating various signaling cascades during the early growth phase, producing faster and more efficient defense responses in lettuce. Thus, suggesting hydro-priming as an effective priming technique in lettuce tends to benefit a grower in terms of better growth synchronization and higher FM and DM.

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