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*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 most sensitive stage to abiotic stress, including salt stress (SS). SS affects plant growth and performance through ion toxicity, decreases seed germination percentage, and increases the germination time. Several priming treatments were used to enhance germination under SS. The objectives of this study are to 1) identify priming treatments to shorten the emergence period; 2) evaluate priming treatments against the SS; 3) induce synchronized seed germination. Salt-sensitive "Burpee bibb' lettuce seeds were treated with 0.05% Potassium nitrate, 3 mM Gibberellic acid, and distilled water (HP). All the primed and non-primed seeds were subjected to 100 mM NaCl or 0 mM NaCl. The 7-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 day/night temperature of 22/18 °C. The result indicated that HP seeds were better synchronized under SS. Similarly, FM and DM of cotyledon, hypocotyl, and radicle were 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, the HP can be a good priming method to synchronize germination and increase 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

## 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. Thirty-four million hectares of irrigated land are affected by salt accumulation worldwide [2]. Around 1.5 million hectares of land are affected by high salt accumulation each year [3,4]. It is speculated that 50% of the cultivable land will turn into non-arable land by the mid-21st century [5]. SS, especially 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

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seed germination percentage and increases the germination time [6–8]. Most crops are reported sensitive to SS even as low as 3 $dSm^{-1}$ electrical conductivity [9]. The suppression is due to the adverse physiological and biochemical changes in germinating seeds exposed to SS [1]. Specifically, $Na^+$ and $Cl^-$ impair the seed growth and development through osmotic and ion-specific toxic effects [5,10,11]. $Na^+$ on the one hand, can replace the $K^+$ cation and promote ion toxicity and $Cl^-$ controls the vegetative growth [12].

Seeds are meant for turning into a healthy and vigorous plant with proper metabolism and physiological performance. To attain that, 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 poor germination and seedling growth. Seed germination and seedling growth are critical stages for crop establishment and are considered the most sensitive stages 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.

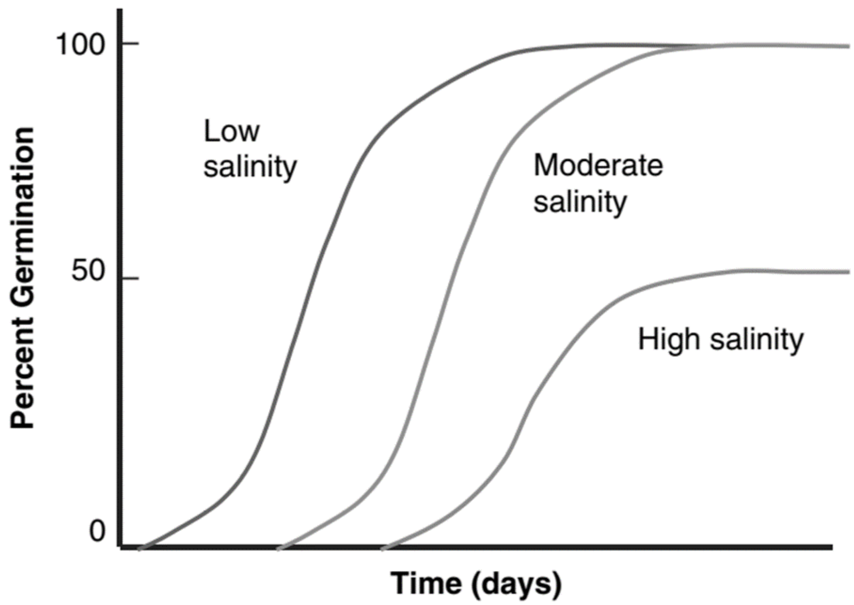


Figure 1. Relationship between the germination % rate and time after sowing at different salt stress levels [18]

Several seed priming treatments (hydro- (HP), potassium nitrate- ($KNO_3$), and gibberellic acid-priming ($GA_3$)) were used to enhance germination and performance under SS to enhance seed germination [19,20]. The purpose of these priming treatments is to shorten the emergence period and protect the seeds from biotic and abiotic factors during the crucial phase of seedling establishment, synchronize emergence, and lead to a uniform stand and improved yields. Priming is a water-based approach that allows regulated seed rehydration to stimulate metabolic processes that are ordinarily active during the early stages of germination but prevents the seed from progressing to complete germination [21]. Plant species/genotype and physiology, environmental conditions exposed to the seed lot and vigor, as well as the priming method used, all play a role in the success of seed priming [22]. Priming treatments has some beneficial effects such as germination enhancement, emergence synchronization, early seedling growth, and minimizing the deleterious effect of abiotic and biotic stress as demonstrated in many crops wheat [23], chickpea [24], sunflower [25] and cotton [26]. Also, several other reports on maize, broccoli, cauliflower, and other leafy vegetables stated that priming reduces the mean germination time (MGT) and increase 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 degree of homogeneity of germination over time [31] which indicates that highest synchronized germination when the Z value is the lowest [32]. These parameters are the measure of germination, seed vigor and response to abiotic and biotic stress which helps in better understanding the role of different priming methods used under different environmental conditions and crops [33,34].

Several studies have been conducted to investigate the influence of different priming methods in different $C_3$ plants. However, there have been very little information on the interactive effect of varying priming methods and salt levels on the germination synchronization, germination parameters, morphological and biochemical traits in lettuce. Hence, the objectives of this study are 1) to identify priming treatments to shorten the emergence period; 2) to evaluate priming treatments against the SS; 3) to induce synchronization in seed germination. Overall, the present study aims to evaluate priming techniques to overcome the adverse effects of SS during the germination period in lettuce.

## 2. Materials and Methods

### *2.1. Planting materials and priming treatment:*

The salt susceptible ‘Burpee Bibb’ lettuce seeds were purchased from Burpee (Warminster, PA). The susceptibility of Burpee Bibb was determined from a study by Adhikari et al. [35]. Before sowing, 200 lettuce seeds were treated with three different priming treatments: 1) 0.05% Potassium nitrate ($KNO_3$); 2) 3 mM Gibberellic acid ($GA_3$), and 3) distilled water (hydro-primed (HP)). A set of non-primed (NP) seeds were also included for comparison with primed seeds. Seeds were immersed in different priming treatments for the different numbers of hours ($KNO_3$= 2 hours 30 min; $GA_3$= 12 hours and 45 min; and HP= 2 hours 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 it regained their original moisture content, approximately 2 days (maximum).

### *2.2. Salt treatment and germination:*

All the primed and non-primed seeds were subjected to either 0 mM or 100 mM Sodium chloride (NaCl) concentration, and 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 unprimed seeds treated with distilled water (0 mM NaCl) were considered as the control. The growth chamber was maintained with 16/8 h photoperiod (light/dark), 330 μmol/m$^2$/s of photosynthetic photon flux density (PPFD), 50-60% relative

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humidity, and 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 hours 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

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

$ti$ = time taken for seeds to germinate at $i^{th}$ count

- $\text{MGR} = \frac{1}{MGT}$ [29]
- $\text{GI} = \sum_{i=1}^{k} \frac{n_i}{t_i}$ [38]
- $Z = \frac{\sum_{i=1}^{k} C_{ni,2}}{C_{\sum ni,2}}$ [31]

Where;

$Cn_{i,2} = n_i\,(n_i - 1)/2$

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. Biochemical Analysis:*

Electrolyte leakage (EL) was determined using the method described by Quartacci et al. [39]. Cotyledon and the hypocotyl part of the lettuce plant were cut into 3-4 mm pieces and placed in a 50 ml tube containing 23-30 ml 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 let immersed into liquid nitrogen for 2-3 minutes and placed back in the same 50 ml tube for additional 2 hours 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). 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.

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## 3. Results

The effect of different priming methods on lettuce seeds subjected to salt stress (SS) was 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 in the better understanding of response of seed germination and growth of primed lettuce seeds subjected to SS. Also, the morphological traits (fresh mass (FM), dry mass (DM), and length) of cotyledon, hypocotyl, and radicle, and electrolyte leakage in germinated seed parts were recorded.

### *3.1. Germination parameters*

All the priming methods did not affect MGT, MGR, and GI values in lettuce when subjected to SS (Table 1). There was no interaction effect observed between different priming and salt treatment, except for Z ($P < 0.05$). The Z value was recorded the lowest in HP lettuce under both salt treatment levels. The Z values of other priming methods ($GA_3$ and $KNO_3$) were recorded the highest; however, were not significantly different when compared with NP control.

Table 1. Mean responses of various germination parameters of lettuce seeds for evaluation of effect of different priming methods ($GA_3$, Hydro, $KNO_3$, and Non-priming) and two salt treatment levels (Control and 100 mM NaCl).

| Priming | Treatment | MGT[3] | MGR | Z | GI |
|---|---|---|---|---|---|
| $GA_3$ | Control | 2.16 ± 0.03a[1,2] | 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 |
| $KNO_3$ | 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 |

[1] Values represent the mean ± SE, n=25. [2] Different lowercase letters indicate significant differences with parameters ($p < 0.05$) as assessed by Tukey's test. [3] MGT=mean germination time (day); MGR= mean germination rate ($day^{-1}$); GI=germination index (unitless); and Z=synchronization index (unitless).

### *3.2. Morphological traits*

#### 3.2.1. Fresh mass

The Fresh mass (FM) data (Figure 2) indicated a significant (priming × salt treatment) interaction effect in FM of all morphological parameters (hypocotyl, cotyledon, and radicle). Figure 2A demonstrated that seeds primed

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with distilled water (hydro-primed (HP)) resulted in higher cotyledon FM compared to other priming methods (Gibberellic acid ($GA_3$) and Potassium nitrate ($KNO_3$). Although there was no significant difference in the hypocotyl FM compared to its control, the hypocotyl FM was recorded the highest (Figure 2B) compared to other priming methods irrespective of the salt treatments. There was no significant effect of $GA_3$- and $KNO_3$- priming in hypocotyl FM when treated with salt, compared to control. Also, the cotyledon and radicle FM of $GA_3$- and $KNO_3$- 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-primed salt-treated lettuce compared to control (Figure 2C). However, the highest radicle FM was observed for HP lettuce.

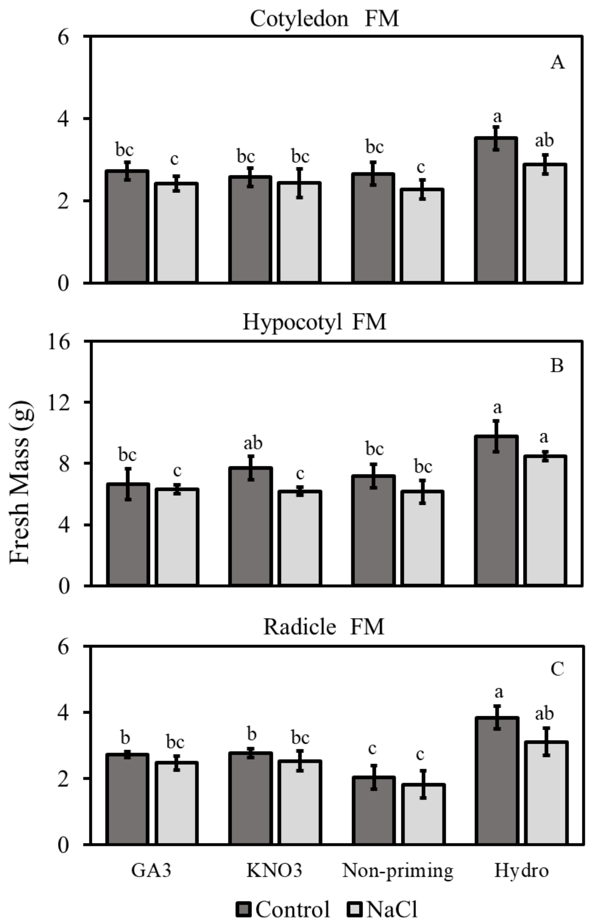


Fig 2. Fresh Mass (FM) of cotyledon (A), hypocotyl (B), and radicle (C) (mean ± standard deviation, n=4) of 'Burpee bibb' lettuce, non-primed (NP), and seed-primed with Gibberellic Acid ($GA_3$), Potassium Nitrate ($KNO_3$), and distilled water and further subjected under 100 mM of NaCl salt stress including 0 mM NaCl as 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

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The dry mass (DM) of lettuce responded differently to different priming methods subjected to two salt treatments, as presented in Figure 3. Priming under different salt treatment levels had a significant interaction effect on cotyledon, hypocotyl, and radicle DM. 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 cotyledon DM of HP lettuce subjected to SS compared to control (Figure 3A). It is also worth noting that DM due to HP increased by almost 100% compared to $GA_3$-, $KNO_3$- and NP in both salt treatment levels.

The DM of hypocotyl was found significantly different ($p<0.05$) in different priming methods studied under different salt treatment levels (Figure 3B). Although no salt treatment effect was observed within the priming methods, a significant interaction effect showed that hypocotyl DM was measured highest in HP and the lowest in $GA_3$-primed lettuce. Like cotyledon DM, priming and salt treatment were significantly affected in the radicle DM. DM of the radicle was recorded highest in the HP followed by NP lettuce (Figure 3C).

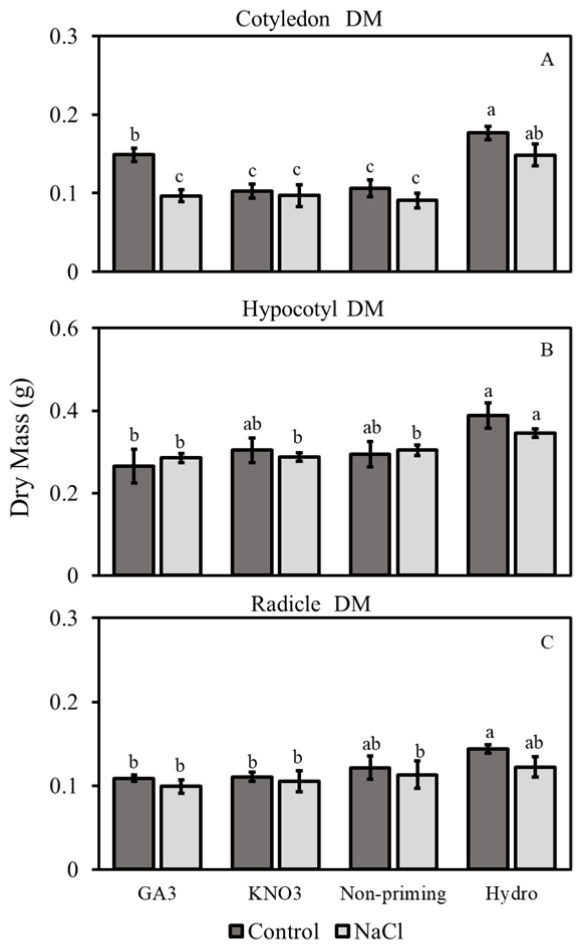


Fig 3. Dry Mass (DM) of cotyledon (A), hypocotyl (B), and radicle (C) (mean ± standard deviation, n=4) of 'Burpee bibb' lettuce, non-primed (NP) and seed-primed with Gibberellic Acid ($GA_3$), Potassium Nitrate ($KNO_3$), and distilled water and further subjected under 100 mM of NaCl salt stress

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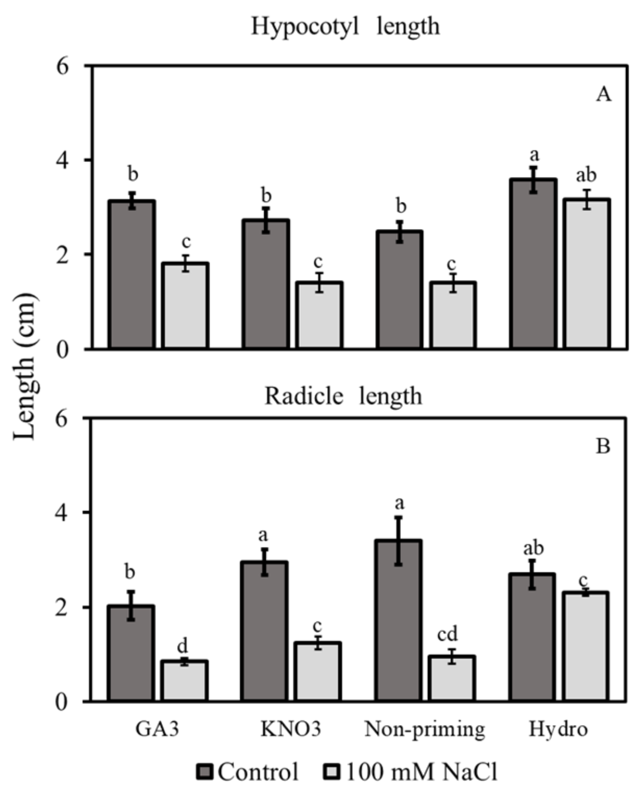
including 0 mM NaCl as control. Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly difference test at $\alpha = 0.05$.

3.2.3. Hypocotyl and radicle length

The priming methods and salt treatment interact to significantly affect the hypocotyl and radicle length (Figure 4). There was a significant decrease ($p < 0.05$) in hypocotyl length by 42%, 49%, and 44% in $GA_3$-primed, $KNO_3$-primed, and NP lettuce, respectively, when subjected to SS compared to control (Figure 4A). However, the HP lettuce remained unaffected by the salt treatment compared to the control. The hypocotyl length was recorded as the highest in the HP lettuce. Like hypocotyl length, a significant decrease in radicle length was observed in $GA_3$-primed, $KNO_3$-primed, and NP lettuce by 58%, 59%, and 72%, respectively, under SS as compared to control (Figure 4B). However, there was no significant decrease (14%) in radicle length recorded in HP + salt-treated lettuce compared to control. Overall, the length parameters revealed the non-significant suppression in the hypocotyl and radicle length of lettuce subjected to 100 mM NaCl salt treatment when primed with distilled water (HP).



1
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Fig 4. Length of hypocotyl (A) and radicle (B) (mean ± standard deviation, n=4) of 'Burpee bibb' lettuce, non-primed (NP) and seed-primed with Gibberellic Acid ($GA_3$), Potassium Nitrate ($KNO_3$), and distilled water and further subjected under 100 mM of NaCl salt stress including 0 mM NaCl as 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 $GA_3$, $KNO_3$, and NP subjected to 100 mM NaCl compared to control, except for HP lettuce (Figure 5). The increase in EL was recorded in $GA_3$, $KNO_3$, and NP lettuce by 38%, 25%, and 60%, 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 priming lettuce.

section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

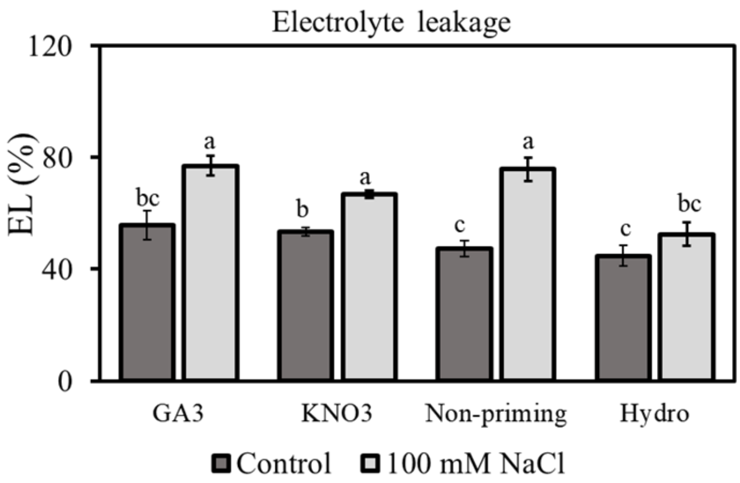


Fig 5. Electrolyte leakage (mean ± standard deviation, n=4) in 'Burpee bibb' lettuce, non-primed (NP) and seed-primed with Gibberellic Acid ($GA_3$), Potassium Nitrate ($KNO_3$), and distilled water and further subjected under 100 mM of NaCl salt stress including 0 mM NaCl as control. Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly difference test at $\alpha$ = 0.05.

## 4. Discussion

Authors 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 being 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 ($K^+$) ion, third phase (salt-specific effect phase) of inability to prevent salt ions from accumulating to its toxic levels and 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 second and third phase disturb the ion homeostasis of cell leading to membrane disorganization and inhibition of photosynthesis by plants [42]. These four-phase effect of SS were reported in several crops like tomato [43], wheat [44], sugar beet [45], beans [46], and several other C3 crops [42]. Under normal condition, the cytosol in higher plants l contain 100 mM of $K^+$ and less than 10 mM $Na^+$ [47]. However, under SS, the cytosolic $Na^+$ an d $Cl^-$ can increase up to 100 mM, becoming cytotoxic [47]. A cytotoxic situation leads to protein denaturation, membrane damage and destabilization, ultimately leading to 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). In support of the 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 due to the quick response of the plant 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 moderately to low salt-tolerant crops and leafy vegetables, seed priming, on the other hand, was reported to improve the overall physiological and morphological performance of those crops [21]. For example, calcium chloride (wheat, sorghum), and potassium chloride (wheat), deionized or distilled water (maize, barley, Chinese cabbage), Gibberellic acid (Pea, maize, rice, alfalfa), glycine betaine (safflower), potassium nitrate (sunflower) and potassium chloride (broccoli), were reported effective in SS amelioration through improved seed germination rate and improved yield [23,48–56]. Priming methods are crop-specific, and the recent study was conducted to demonstrate the difference in efficacy levels of different priming methods based on the several reports in C3 and C4 plants.

Previously it is reported that the synchronized seed growth is also enhanced by crop-specific seed priming method [11]. In our study, HP seeds have more synchronized seed growth with the lowest synchronization index (Z), which aligned with the report earlier by Khajeh-Hosseini et al. [11,32]. The current study also demonstrated that HP inhibits the negative effect of SS on seed germination while there is a minimal response of $KNO_3$ and $GA_3$ for SS lettuce seeds. Previous research by Mahmoudi et al. [36] found that lettuce seedlings treated with distilled water (HP) could alleviate the NaCl effect on lettuce seedlings better than $KNO_3$ and $GA_3$. The NaCl inhibiting ability of HP could be due to the ambient or high water potential ( $\Psi = 0$ MPa) environment created by HP during the early germination period [47]. Moreover, the decline in synchronization in $KNO_3$ and $GA_3$-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 maintained the seed germination and growth in a synchronized pattern even under SS 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 against the 100 mM NaCl salt treatments. Under SS, the fresh mass (FM) and dry mass (DM) of $KNO_3$ and $GA_3$ were found indifferent to that of non-primed (NP) lettuce and were also reduced significantly, as reported earlier in melon [57] and chickpea [58]. The germination and seedling improvement with $KNO_3$ and $GA_3$ has been documented earlier for 'Vista' lettuce [59] and cabbage [60]. However, the effectiveness of $KNO_3$ and $GA_3$ under salt-stressed 'Burpee bibb' 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, thus disrupting the endogenous osmotic equilibrium [21,61]. The disparity can result in nutritional imbalance and poor seedling growth [62]. Although $KNO_3$ was reported effective in improving the seedling growth and establishment in sunflower and $GA_3$ was reported effective in onion and sesame, the poor performance of $KNO_3$ and $GA_3$ suggested that the effect of priming under SS can be crop-specific [63]. On the contrary, the FM and DM primed with distilled water (HP) were increased significantly irrespective of the salt treatments. The positive response of HP even under the SS can be due to the stimulatory effect of priming in the mediation of cell division in germinating seed at the early germination stage [64]. The better performance of HP in terms of water absorption can also be due to the early commencement of metabolic activity in the seed before the start of radicle emergence [65].

Also, the improved water use efficiency has been documented with HP [66]. The improved water use efficiency could be due to efficient breakdown of stored food reserve 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]. HP was reported to improve seed germination, seedling emergence and productivity in many field crops [8,24,26,63]. The recent 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 $KNO_3$ and $GA_3$ primed lettuce seeds. Thus, use of HP for lettuce under SS could be an effective priming method to improve the FM and DM of different morphological traits of germinated seeds.

Abiotic stress is the major source for 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 like electrolyte leakage (EL) is a good method to figure out what 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 stress, including salt stress [69,70]. In the recent study, an increase in EL leakage was significantly different in salt-treated lettuce to cause the membrane injury compared to control. The most prominent EL was observed in the $KNO_3$-, $GA_3$-, 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, $KNO_3$- and $GA_3$- 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 to maintain the seed germination, as reported by Chiu et al. [71] and Mahmoudi et al. [36]. The outcome of the recent study established that the positive effect of HP on EL in lettuce could be related to the higher FM and DM reduction as well as lowest synchronization index in the presence of SS and thus sparing few electrons for the production of reactive oxygen species [72]. The outcomes of this research established that FM and DM of seed parts are statistically similar in $KNO_3$ and $GA_3$ primed seeds while HP was significantly different and highest compared to NP control. Thus, suggesting that burpee bibb lettuce can ensure the better seed growth and germination with HP seeds even under SS, but not with $KNO_3$ and $GA_3$ primed seeds. In addition, the lowest seed synchronization index and electrolyte damage due to HP provided enough evidence to validate the positive impact of HP in seed germination and synchronization with minimal cell damage. Overall, this research suggests that HP had a mitigating impact against the membrane injury in the plants exposed to SS.

## 5. Conclusions

This study investigated the effect of different priming methods in salt-sensitive lettuce cultivar 'Burpee bibb' subjected to salt stress (SS). Seed priming has a potential to improve the seed germination and establishment by initiating germination metabolism without radicle protrusion. Thus, the beneficiaries of seed priming has been held true to improve productivity in several field crops in the previous report [73]. In addition, seed priming are cost and resource-effective too [73]. To validate the response of several priming methods in lettuce, this research was conducted which demonstrated that HP primed seeds showed better synchronization under both salt treatment levels than any other priming seeds. Similarly, FM, and DM of cotyledon, hypocotyl, and radicle 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 $KNO_3$ and $GA_3$. Based on the result, using HP 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, HP can also be beneficial to stimulate various signaling cascade during early growth phase for faster and efficient defense responses in lettuce. Thus, suggesting HP as an effective priming technique in lettuce tend to benefit a grower in terms of better growth synchronization and higher FM and DM.

## 6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

**Author Contributions:** B.A.: conceptualization, methodology, validation, formal analysis, investigation, writing—original draft, writing—review and editing, visualization, methodology, validation, investigation. O.J.O.: methodology, validation, formal analysis, investigation. J.C.W.: methodology, validation, formal analysis, investigation, writing review and editing. T.C.B.: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing review and editing, visualization, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

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**Conflicts of Interest:** The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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