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Soaking seeds in biostimulants restores germination, growth, and physiology of *Glycine max* seedlings affected by salt stress

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ABSTRACT

The main approach to control stressed crops is using plant biostimulants, such as diluted lemon fruit juice (DLFJ) and bee honey (DBH), which promote plant growth and physiology. This study investigated the potential promotive effects of soaking *Glycine max* seeds in DLFJ or DBH under normal conditions (NCs) or salt stress conditions (SSCs) on seed germination percentage (GP), seedling growth, and physiology. The result indicated that the ideal requirement for seed soaking was to soak the seeds for 8 h in 4% DLFJ or 6% DBH to obtain optimal results, with 6% DBH outperforming 4% DLFJ. The SSCs (irrigation with saline water; EC = 8.60 dS m^{-1}) markedly reduced seed PG, seedling fresh and dry weights, root activity, photosynthetic efficiency (total chlorophyll content, PSII F_v/F_m, PSII PI_{ABS}), relative water content, and membrane stability index compared with the NCs (irrigation with normal water; EC = 1.60 dS m^{-1}). When soybean seedlings were irrigated with normal water or saline water, soaking seeds in 4% DLFJ or 6% DBH significantly increased the above parameters compared to controls (soaking seeds in distilled water). The responses of the above parameters were generally more pronounced under SSCs than under NCs and were typically more pronounced with 6% DBH treatment than with 4% DLFJ treatment. In conclusion, 6% DBH is a natural multi-biostimulator that is significantly better than DLFJ in reducing the effects of salt stress on *G. max* seedlings.

Keywords: Biostimulators, Soybeans, Salinity, Growth, Physiology

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1. INTRODUCTION

Soil salinity is a global problem, affecting about 7% of the planet's surface (**Balasubramaniam *et al.*, 2023**). Intense solar radiation and high temperatures often combine with salt stress, reducing water availability (**Regni *et al.*, 2021**), and negatively impacting plant growth and physiology. Salinity causes metabolic changes, nutrient imbalances, and cell membrane disruption, resulting in negative morpho-physio-biochemical responses in many crop plants (**Kaya *et al.*, 2023; Alsamadany *et al.*, 2023**). Primarily, salinity causes osmotic fluctuations in the rhizosphere, which negatively affects the ability of plant roots to absorb water and nutrients from the soil, leading to water and nutritional imbalances in plant cells. Salinity causes excessive Na^+ and Cl^- accumulation, limiting nutrient uptake. Accumulation of Na^+ ions in plants leads to K^+ deficiency and enzyme inactivity, and enzyme metabolic activities are inhibited due to the high Na^+/K^+ ratio (**Zaki and Rady, 2015; Taha *et al.*, 2020**). Salinity, a major abiotic stress, inactivates the photosynthetic system of plants due to chlorophyll breakdown by activating the chlorophyllase-degrading enzyme and inhibiting chlorophyll biosynthesis (**Abou-Sreea *et al.*, 2021; AbdEl-Azeem *et al.*, 2023a, 2023b; Hassan, 2024**).

Applications of biostimulants to plants enable them to resist salt stress (**Semida *et al.*, 2019; Abou-Sreea *et al.*, 2021; Abdelkhalik *et al.*, 2023; Tarfayah *et al.*, 2023**). The biostimulant is defined as any microorganism or substance, depending on its composition of biologically active substances, which is used to improve the quality characteristics of crops, resistance to abiotic stress, and nutritional efficiency (**du Jardin, 2015**). Among efficient biostimulants, diluted solutions from bee honey (DBH) and lemon fruit juice (DLFJ), which are applied efficiently for stressed plants (**Abou-Sreea *et al.*, 2021; Abdelkhalik *et al.*, 2023; Rady *et al.*, 2023a; Tarfayah *et al.*, 2023**). It was observed in these papers that DBH and DLFJ applications enhanced seed germination percentage (GP), seedling growth, and physiological functions, including photosynthesis efficiency and water relations. Since biostimulants contain different bioactive components and have high antioxidant activity, the enhancing mechanisms of these components for stressed plants remain incompletely understood. The enhancing effects of a biostimulant often arise from the different synergistic activities of two or more components. To restore seed vigor, seedling growth, and physiological functions under stress, biostimulants enhance plant stress tolerance. DBH and DLFJ (DBH is superior to DLFJ) have strong antioxidant activity, various antioxidants, osmoregulatory substances, and various nutrients, and have been used repeatedly to enable stressed plants to perform well under stress (**Abou-Sreea *et al.*, 2021; Abdelkhalik *et al.*, 2023; Rady *et al.*, 2023a; Tarfayah *et al.*, 2023**).

DBH and DLFJ are unique biostimulants that are rarely used. In this study, DBH and DLFJ were successfully applied to *G. max* seeds and seedlings exposed to salt stress. It has been reported that the salinity threshold for soybean is at 5.0 dS m^{-1}, however, soybean grain yield decreased by 20% at a salinity of 4.0 dS m^{-1} and 56% at 6.7 dS m^{-1} salinity (**Aini *et al.*, 2014**). We suggested that to improve seedling immunity against the harmful effects of salt stress, seed priming with 6%

DBH or 4% DLFJ could improve seed GP, seedling growth (fresh and dry weight), and physiological functions. This study aimed to determine how *G. max* seed priming in 6% DBH or 4% DLFJ compared to priming in distilled water affects the evaluation of seed GP, seedling fresh and dry weights, and physiological functions, including photosynthetic efficiency and tissue water content in seedlings exposed to salt stress.

2. MATERIALS AND METHODS

2.1. Seed germination tests (preliminary trials)

Glycine max seeds were disinfected using 70% (v/v) ethanol and NaClO (0.25% Cl) for 0.5 and 3 min, respectively, and washed several times with sterile deionized water (SD.H_2O). Six groups of 40 seeds each were soaked for 2, 4, 6, 8, 10, and 12 h in SD.H2O. After soaking seeds with the tested durations, the seeds of each group were placed on seven layers of Whatman (No. 1) filter paper in each Petri dish with a diameter of 15 cm. This trial aimed to select the ideal soaking duration (8 h) for soybean seeds through the best seed GP. After 2 weeks, seedling fresh and dry weights, and total chlorophyll (TChls) content were evaluated.

The same procedures were applied with 4 groups of 40 seeds each to test the diluted lemon fruit juice (DLFJ) at the concentrations of 2, 4, 6, and 8% (of total soluble solids; TSS) and the diluted bee honey (DBH) at the concentrations of 3, 6, 9, and 12% (of TSS). In all treatments, the soaking duration was 8 h. This trial aimed to select the ideal concentration of DLFJ and DBH (4 and 6%, respectively) for soaking soybean seeds through the best seed GP. After 2 weeks, seedling fresh and dry weights, and total chlorophyll (TChls) content were also evaluated.

In the main study, 6 sets of 40 seeds were soaked in SD.H_2O, 4% DLFJ, or 6% DBH (optimal concentrations in previous trials) for 8 h (optimal seed soaking duration in previous trials). The seeds were placed on seven layers of Whatman filter paper (No. 1) in each 15 cm diameter Petri dish. The seeds soaked in SD.H_2O, 4% DLFJ, or 6% DBH were then irrigated with normal water (EC = 1.60 dS m^{-1}) or saline water (EC = 8.60 dS m^{-1}), which was added at a rate of 20 ml per Petri dish daily until full germination, and then increased gradually increased to reach 40 ml per Petr dish in the last 3 days of the experiment. Each of the 6 treatments was replicated 5 times (5 Petri dishes each containing 8 seeds). The order of treatments was completely randomized. At a specific time, each day, seed germination was recorded. When the length of a seed radical reached approximately 2 mm, it was considered germinated (**Canavar *et al.*, 2023**).

2.2. Growing conditions of the main study

During the same period (1–15 May 2023), three pot experiments were conducted simultaneously in three adjacent appropriate places in the Faculty of Agriculture, Fayoum University, Egypt. The natural growing conditions were natural sunlight intensity, 26 ± 3/14 ± 2 °C mean day/night temperatures, 62 ± 4% humidity, and 12/12 h mean day/night photoperiods. Uniform and healthy *Glycine max* (L.) seeds (cultivar Giza-111) were secured from the Egyptian Agricultural Research Center (Egy-ARC). After disinfection with 70% ethanol for 0.5 min and NaClO (0.25% Cl) for 3 min, the disinfected seeds were rinsed with SD.H_2O several times. In each of the three experiments, seeds were sown in 60 plastic pots (25 cm diameter, 22 cm depth), with three seeds in each pot. Before sowing, each

pot was filled with 6 kg of growth medium (GM) as suggested by **Rady and Rehman (2016)** with modification in **Hassan *et al.* (2023)**. The GM contained 16.5, 33.4, 50.0, and 0.1% of acid-treated pure sand, vermiculite, peat moss, and humic acid, respectively. The GM was disinfected with Moncut SC (a fungicide, Central Glass Co., Ltd., Tokyo, Japan) at a rate of 0.125 g and then fertilized with 0.42 g NH_4NO_3 + 0.5 g $CaH_4P_2O_8$ + 0.33 g K_2SO_4 + 0.83 g $MgSO_4$ L^{-1}. To adjust pH and increase GM fertility, 1.25 g $CaCO_3$ L^{-1} and 2% acidified compost (**Rady *et al.*, 2023b**) were added, respectively.

2.3. Treatments and trial setup

In each of the three experiments, the 60 pots were divided into two equal sections (each containing 30 pots) for the normal treatments (NW; 1.60 dS m^{-1}) and saline treatments (SW; 8.60 dS m^{-1}), which were applied through irrigation water. Each section was divided into three equal subsections (each containing 10 pots) to soak the seed in SD.H_2O, 4% DLFJ (lemon fruit juice diluted to 4.0% from 8.82%, which was the TSS in juice), and 6% DBH (bee honey diluted to 6.0% from 83.8%, which was the TSS of honey). The treatments were as follows: (1) seed soaking in SD.H_2O + irrigation with normal water (NW) having EC = 1.60 dS m^{-1} (normal control; NCt), (2) seed soaking in 4% DLFJ + irrigation with NW, (3) seed soaking in 6% DBH + irrigation with NW, (4) seed soaking in SD.H_2O + irrigation with saline water (SW) having EC = 8.60 dS m^{-1} (saline control; SCt), (5) seed soaking in 4% DLFJ + irrigation with SW, and (6) seed soaking in 6% DBH + irrigation with SW.

The analysis for major bioactive components in lemon fruit juice (LFJ) and bee honey (BH) is present in **Table 1**, and the chemical composition of normal irrigation water is present in **Table 2**.

Table 1. Major bioactive components of lemon fruit juice (LFJ) and bee honey (BH):

Components	Units	LFJ	BH
Osmo-regulatory compounds			
Total free amino acids	%, based on fresh matter	4.13±0.22	0.35±0.02
Total soluble sugars		1.18±0.05	81.3±4.02
Free proline		0.02±0.00	0.01±0.00
Antioxidant compounds			
Vitamin C (Ascorbate)	%, based on fresh matter	0.03±0.00	0.05±0.00
Total anthocyanins		0.01±0.00	0.05±0.00
Total phenols		0.12±0.01	0.13±0.00
Antioxidant activity	mM Trolox eq. L^{-1}	17.2±0.78	19.8±0.92
Nutrient elements			
Calcium	%, based on fresh matter	0.8±0.00	0.9±0.00
Magnesium		0.7±0.00	0.9±0.00
Phosphorus		0.7±0.00	0.8±0.00
Potassium		0.7±0.03	0.8±0.00
Iodine		0.2±0.00	0.4±0.00

eq.; equivalent

Table 2. Chemical composition of irrigation water:

Concentration of ions (meq L^{-1})								EC (dS m^{-1})	pH	SAR
CO_3^{2-}	HCO_3^-	SO_4^{2-}	Cl^-	Mg^{2+}	Ca^{2+}	K^+	Na^+			
0.00	2.10	3.14	8.24	1.78	3.60	3.52	3.44	1.60	7.48	2.42

EC; electrical conductivity, and SAR; Sodium adsorption ratio.

The soaking duration was 8 h at 25±1 °C for all treatments. The soaking time and concentrations of DLFJ (4.0% TSS) and DBH (6.0% TSS) were selected based on three preliminary experiments conducted from May 1 to 15, 2022 (**Tables 3–5**).

Table 3. Effect of soaking duration for soybean (*Glycine max* L., cv. Giza-111) seeds in distilled water on germination percentage (GP), seedling fresh and dry weights, and total chlorophyll (TChls) content:

Soaking duration	GP (%)	Seedling FW (g)	Seedling DW	TChls content (mg g^{-1} FW)
2 h	73.2±2.34c	2.14±0.14d	0.21±0.01d	1.68±0.06c
4 h	77.6±٢.٩0b	2.56±0.17b	0.28±0.02b	1.84±0.11b
6 h	78.2±3.00b	2.58±0.19b	0.29±0.02b	1.90±0.٠٩b
8 h	82.8±3.22a	2.98±0.21a	0.36±0.02a	2.20±0.11a
10 h	82.4±3.20a	2.94±0.20a	0.34±0.02a	2.15±0.11a
12 h	72.6±2.91c	2.33±0.16c	0.25±0.02c	1.76±0.06c

Based on the LSD test, mean values (± standard errors) followed with similar letters in the same column not differed significantly at $p \leq 0.05$ level of probability.

Table 4. Effect of concentration of diluted lemon fruit juice (DLFJ) for soaking soybean (*Glycine max* L., cv. Giza-111) seeds on germination percentage (GP), seedling fresh and dry weights, and total chlorophyll (TChls) content:

DLFJ levels	GP (%)	Seedling FW (g)	Seedling DW	TChls content (mg g^{-1} FW)
2 %	72.9±2.32b	2.10±0.13b	0.20±0.01b	1.72±0.06b
4 %	86.4±3.50a	3.05±0.22a	0.38±0.02a	2.10±0.10a
6 %	85.0±3.44a	3.02±0.21a	0.37±0.02a	2.08±0.10a
8 %	70.8±2.22b	1.98±0.12b	0.20±0.01b	1.60±0.05c

Based on the LSD test, mean values (± standard errors) followed with similar letters in the same column not differed significantly at $p \leq 0.05$ level of probability. GP; germination potential, GR; germination rate, and TChls; total chlorophylls.

Table 5. Effect of concentration of diluted bee honey (DBH) for soaking soybean (*Glycine max* L., cv. Giza-111) seeds on germination percentage (GP), seedling fresh and dry weights, and total chlorophyll (TChls) content:

DBH levels	GP (%)	Seedling FW (g)	Seedling DW	TChls content (mg g^{-1} FW)
3 %	62.2±2.14c	2.10±0.12c	0.20±0.01c	1.68±0.06c
6 %	89.4±3.74a	3.48±0.31a	0.44±0.02a	2.66±0.12a
9 %	89.3±3.80a	3.46±0.29a	0.43±0.02a	2.60±0.11a

12 %	80.5±3.00b	2.80±0.18b	0.32±0.02b	2.24±0.09b

Based on the LSD test, mean values (± standard errors) followed with similar letters in the same column not differed significantly at $p \leq 0.05$ level of probability. GP; germination percentage,and TChls; total chlorophylls.

All trials were arranged in a completely randomized design and ended 15 days after sowing. In salt treatments, salt concentration was kept in the GM at EC = 8.60 dS m^{-1} and controlled by periodic evaluation with ICP-AES (inductively coupled plasma atomic emission spectrometry; IRIS-Advan type, Thermo, USA). The pots were irrigated based on the weighing method, which was done once every 48 h. In this method, each pot was irrigated to the capacity of the soil, after weighing, by compensating for the water lost through evapotranspiration. In conjunction with watering the pots, throughout the experimental period (15 days), the pots were rotated to avoid the impacts of fluctuating local environmental conditions. Taking into account the full implementation of the recommendations of the Egy-ARC, weeds and pathogens were controlled by following standard plant protection measures.

The trials were terminated 15 days after sowing and seedlings were collected from the six treatments for the following observations: fresh and dry weights of seedlings, photosynthetic efficiency indices, leaf relative water content (RWC), and leaf membrane stability index. All samples were collected in the early morning and quickly transported to laboratories for analysis.

2.4. Assessment of growth, root activity, photosynthetic efficiency, and leaf integrity

G. max seedlings were weighed to reach the average fresh weight (g) per seedling. Then, the seedlings were exposed to 70 ± 2°C until the dry weights were constant to reach the average dry weight (g) per seedling.

To evaluate *G. max* root activity, the procedures of **Rehman *et al.* (2018)** were followed. All seedling roots received 5 mL of 100 mM Na-P buffer (pH 7.0) in a 25-mL test tube and the mixture was shaken for 30 min. In another test tube, the roots of another seedling were shaken for 3 h after the addition of 5 mL α-naphthylamine (α-NA). ρ-Aminobenzene sulfonic acid and $NaNO_2$ (0.01%) were added at a rate of 1 mL of each to 1 µL of both solutions and then incubated at 30 °C for 10 min. Optical density was measured at 510 nm against the control without roots. In this method, the root contents of auto-oxidized α-NA were measured. α-NA contents were shaken for 30 min and 3 h, and were evaluated by comparison with α-NA standard solutions. The oxidized α-NA was computed as a reduction in the α-NA amount from 30 min to 3 h. The root activity was expressed as a subtraction of the oxidized α-NA in the control from the oxidized α-NA (µg g^{-1} fresh root h^{-1}).

Leaf TChls content (mg g^{-1} FW) was estimated by applying the procedure of **Wellburn (1994)**. The pigment was extracted by homogenizing the leaf sample in acetone solution (80%). Spectrophotometrically, the extract absorbance was recorded at 470, 653, and 666 nm. The photosynthesis efficiency was assessed as F_v/F_m (**Maxwell and Johnson, 2000**) and PSII performance index (PI_{ABS}) (**Clark *et al.*, 2000**).

Respectively, the protocols of **Osman and Rady (2014)** and **Rady (2011)** were harnessed to measure relative water content (RWC, %), and membrane stability (MSI,

%). The equations below were utilized to calculate RWC, MSI, and EL percentages:

$$RWC\ (\%) = \left[\frac{(fresh\ mass - dry\ mass)}{(turgid\ mass - dry\ mass)}\right] \times 100$$

$$MSI\ (\%) = \left[1 - (\frac{EC1}{EC2})\right] \times 100$$

2.5. Statistical analysis

The (one-way) ANOVA technique was practiced to analyze all data obtained after testing the homogeneity of error variance (**Gomez and Gomez, 1984**). The differences among means were tested by applying LSD at $p \leq 0.05$ by the CoHort Software (Costat 6.29 computer program) (Snedecor and Cochran, 1989).

3. RESULTS AND DISCUSSION

3.1. Bioactive components of lemon fruit juice (LFJ) and bee honey (BH)

The major bioactive components obtained from LFJ were osmoregulatory compounds; ORCs (total free amino acids; TFAAs, total soluble sugars; TSsug, and free proline; FPro), antioxidant compounds (ascorbate; AsA, total anthocyanins, and total phenolics), and nutrients (Ca, Mg, P, K, and iodine), all of which were present in significant amounts. The antioxidant content of the LFJ conferred an antioxidant activity (TAA) equivalent to 17.2 mM Trolox (**Table 1**). All of the above-mentioned bioactive components were detected in BH in higher amounts than in LFJ, thus giving a TAA equivalent to 19.8 mM of Trolox (**Table 1**). The results of LFJ analysis in this study confirm those of **Klimek-Szczykutowicz *et al.* (2020)** and **Rady *et al.* (2023a)** who reported that LFJ is a promising plant growth organic biostimulator for plant growth under stress. **Rady *et al.* (2023a)** reported that the high DPPH radical-scavenging activity of LFJ (86.8%) was utilized to screen the antioxidant activity to prevent lipid peroxidation (**Rady *et al.*, 2021; Semida *et al.*, 2019**), giving antioxidative properties of LJS. In addition, the results of BH analysis in this study confirm those of **Semida *et al.* (2019), Rady *et al.* (2021), Alghamdi *et al.* (2023), Belal *et al.* (2023),** and **Tarfayah *et al.* (2023)**. In these papers, it was reported that the high DPPH radical-scavenging activity of BH (86.60-89.84%) was used to screen the antioxidant activity to prevent lipid peroxidation, thus improving plant growth and productivity under stress. The significant contents of TSsug, AsA, FPro, anthocyanins, and phenolics acquired the FLJ and BH high TAA, making them promising organic biostimulators for plant growth and productivity under stress.

Khalid et al. (2024) reported that plant biostimulants enhance plant resilience to abiotic stresses, including salinity, through mechanisms such as enhancing different nutrient uptake, especially the ionic K^+, regulating stress-responsive genes (e.g., improving gene expressions of enzymes, particularly genes related to the ascorbate-glutathione cycle and others). In addition, biostimulants encourage to improve the biosynthesis of plant hormones, improve cell osmotic adjustment by increasing the contents of soluble sugars, glycine betaine, proline, and total free amino acids. and reinforce enzymatic and non-enzymatic antioxidant activity to scavenge the increased reactive oxygen species (ROS).

3.2. Effect of seed soaking duration on germination percentage (GP), seedling growth, and total chlorophyll (TChls) content

Soaking soybean seeds in distilled water for 2, 4, 6, and 8 h gradually increased GP, seedling fresh weight (SdFW), seedling dry weight (SdDW), and TChls content (**Table**

3). During the soaking period of 2 to 8 hours, the recorded values of GP were 73.2–82.8%, SdFW 2.14–2.98g, SdDW 0.21–0.36g, and TChls content 1.68–2.20 mg g^{-1} FW. After that, these values started to decrease insignificantly with the soaking duration of 10 h, while they significantly reduced with the soaking duration of 12 h compared to the soaking duration of 8 h.

The physio-biochemical changes that precede the morphological changes during germination are closely related to the early establishment, survival rate of seedlings, and growth. These changes begin with soaking seeds in water, which activates several metabolic processes, helping to shorten the germination time and improve overall performance. Each crop seed requires a critical soaking time, which should be below the safe limit (**Nithyadevi *et al.*, 2022**). In this study, the highest performance for all studied parameters was recorded with the 8-h soaked seeds. Therefore, soaking soybean seeds for more than 8 h as excessive soaking duration started to generate adverse impacts. The highest performance of 8-h soaked seeds indicates that the seeds have absorbed the optimum moisture level required for saturation to activate the embryo to initiate several processes, including cell division, differentiation, and proliferation to differentiate into vigor seedlings (**Sabongari and Aliero, 2004**), and hence vigorous growth due to the high TChls content. Water absorption in seeds follows a three-stage pattern. Due to the seed's low water potential, water absorption occurs rapidly for biochemical activities, which leads to seed germination (**Bewley, 1997**). **Nithyadevi *et al.* (2022)** stated that the enhanced performance of soaked seeds could be attributed to the completion of pre-germination processes, including DNA replication, increased RNA and proteins biosynthesis, higher ATP levels and embryo growth, repair of degraded seed parts, reduced metabolite leakage and lipid peroxidation, and increased antioxidant activities (**Issam *et al.*, 2012**). Finally, the optimum moisture level in the seeds due to the ideal soaking duration improves seed germination and growth. This is probably due to the breakdown of complex sugars into simple sugars easily used in auxin and protein biosynthesis. The biosynthesized auxins help soften cell walls to facilitate growth and proteins are easily used to produce new tissues.

3.3. Effect of seed soaking in diluted lemon fruit juice (DLFJ) or diluted bee honey (DBH) concentration on GP, seedling growth, and TChls content

Soaking soybean seeds for 8 h in DLFJ at concentrations of 2 and 4% (of total soluble solids; TSS of juice) gradually increased GP, seedling fresh weight (SdFW), seedling dry weight (SdDW), and TChls content (**Table 4**). As a result of soaking seeds in 2 to 4% DLFJ, the recorded values of GP were 72.9–86.4%, SdFW 2.10–3.05g, SdDW 0.20–0.38g, and TChls content 1.72–2.10 mg g^{-1} FW. After that, these values started to decrease insignificantly with the soaking in 6% DLFJ, while they significantly reduced with the soaking in 8% DLFJ compared to the soaking in 4% DLFJ. The highest performance for all studied parameters was recorded with soaking seeds in 4% DLFJ. Therefore, soaking soybean seeds in DLFJ solution with a concentration of more than 4% where excessive DLFJ started to generate adverse impacts.

Soaking soybean seeds for 8 h in DBH at concentrations of 3 and 6% (of total soluble solids; TSS of honey) gradually increased GP, seedling fresh weight (SdFW), seedling

dry weight (SdDW), and TChls content (**Table 5**). As a result of soaking seeds in 3 to 6% DBH, the recorded values of GP were 62.2–89.4%, SdFW 2.10–3.48g, SdDW 0.20–0.44g, and TChls content 1.68–2.66 mg g^{-1} FW. After that, these values started to decrease insignificantly with the soaking in 9% DBH, while they significantly reduced with the soaking in 12% DBH compared to the soaking in 6% DBH. The highest performance for all studied parameters was recorded with soaking seeds in 6% DBH. Therefore, soaking soybean seeds in DBH solution with a concentration of more than 6% where excessive DBH started to generate adverse impacts.

The highest performance of soaking seeds in 4% DLFJ or 6% DBH solution indicates that the seeds have absorbed the optimum bioactive components of DLFJ or DBH, including ORCs (TFAAs, TSsug, and FPro), antioxidant compounds (AsA, total anthocyanins, and total phenolics), and nutrients (Ca, Mg, P, K, and iodine) (**Table 1**). These bioactive components are required to nourish and activate the embryo to develop several processes, including cell division, differentiation, and proliferation to differentiate into strong seedlings and hence vigorous growth due to the high TChls content. The plant life cycle includes seed germination as the first stage. Plant growth generally depends on strong germination and a high germination percentage (**Rady *et al.*, 2023a**). In this study, the bioactive components of 4% DLFJ or 6% DBH solution penetrated the seed for embryo development by activating starch mobilization in the endosperm and transporting simple sugars to the embryonic axis, contributing to the nourishment of the embryonic axis (**Rady *et al.*, 2023a**). This leads to strong germination with a high percentage and thus increased seedling growth due to increased TChls content (**Tables 4** and **5**). The significant improvements in GP, seedling growth, and TChls content may be attributed to the increased ORCs, nutrients, and antioxidative machinery in 4% DLFJ-soaked seeds or 6% DBH-soaked seeds and then in produced seedlings to by established in integrity state with strong root system (**Rucińska-Sobkowiak, 2016**). This is probably attributed to higher accumulations of FPro and TSsug that contributed to the maintenance of suitable water content in seedling tissues, which supports the continuity of the physiological and metabolic activities through the osmo-regulation process (**Abdelkhalik *et al.*, 2023; Sitohy *et al.*, 2020**). The seedling integrity in this study in response to soaking soybean seeds in 4% DLFJ or 6% DBH solution initiates a suitable medium for TChls biosynthesis and inhibits chlorophyll degrading enzyme, increases PSII activity and activates the photosynthetic machinery (**Rady *et al.*, 2023a**).

3.4. Effect of seed soaking in 4% DLFJ or 6% DBH on GP, seedling growth, and physiology of soybean seedlings grown under salt stress

As displayed in **Table 6**, irrigation of soybean seedlings with saline water; SW (EC = 8.60 dS m^{-1}) markedly reduced seed GP, seedling FW, seedling DW, root activity, TChls content, PSII F_v/F_m, PSII PI_{ABS}, relative water content (RWC), and membrane stability index (MSI). The decreases were 61.6, 47.4, 50.0, 37.9, 56.8, 41.8, 45.6, 42.2, and 49.4%, respectively, compared with irrigation with normal water; NW (EC = 1.60 dS m^{-1}).

Table 6. Response of germination, growth, and physiology of soybean (*Glycine max* L., cv. Giza-111) seeds and seedling fresh and dry weights under irrigation with normal water (NW; EC = 1.60 dS m^{-1}) or saline water (SW; EC = 8.60 dS m^{-1}) to soak the seeds in diluted lemon fruit juice (DLFJ) or diluted bee honey (DBH) at 4.0 or 6.0%, respectively:

Treatments (IrrW + Bio-Ss)	Parameters			
	GP %	Seedling	Seedling DW g	Root activity (µg α-NA g^{-1} FW
NW (NCt)	83.2±3.30c	3.04±0.24c	0.38±0.02c	84.4±5.24c
NW + DLFJ$_{4.0}$	87.0±3.60b	3.26±0.27b	0.41±0.02b	89.6±5.65b
NW + DBH$_{6.0}$	89.8±3.76a	3.58±0.30a	0.46±0.02a	95.2±6.76a
SW (SCt)	48.6±2.12e	1.60±0.15e	0.19±0.01e	52.4±3.32e
SW + DLFJ$_{4.0}$	73.4±2.90d	2.62±0.19d	0.34±0.01d	76.8±4.67d
SW + DBH$_{6.0}$	81.4±3.21c	3.01±0.26c	0.39±0.02c	86.2±5.21c

	Parameters				
	TChls content mg g^{-1} FW	F_v/F_m	PI_{ABS}	RWC	MSI
				%	
NW (NCt)	2.13±0.09c	0.79±0.03c	15.1±0.31c	86.2±3.84b	62.4±3.2c
NW + DLFJ$_{4.0}$	2.44±0.11b	0.84±0.04b	16.4±0.33b	89.9±4.20a	65.4±3.3b
NW + DBH$_{6.0}$	2.70±0.12a	0.88±0.04a	17.9±0.35a	91.1±4.45a	67.8±3.6a
SW (SCt)	0.92±0.05e	0.46±0.02e	8.22±0.18e	49.8±2.22d	31.6±1.8e
SW + DLFJ$_{4.0}$	1.48±0.07d	0.68±0.03d	12.5±0.24d	70.6±3.20c	51.8±2.3d
SW + DBH$_{6.0}$	2.06±0.08c	0.77±0.03c	14.8±0.30c	84.8±3.88b	61.2±3.1c

Based on the LSD test, mean values (± standard errors) followed with similar letters in the same column not differed significantly at $p \leq 0.05$ level of probability. IrrW; irrigation water, Bio-Ss; biostimulators, NW; normal water (EC = 1.60 dS m^{-1}), SW; saline water (EC = 8.60 dS m^{-1}), NCt; normal control, SCt, saline control, DLFJ$_{4.0}$; soaking the seeds in 4.0% DLFJ, DBH$_{6.0}$; soaking the seeds in 6.0% DBH, FW; fresh weight, GP; germination percentage, α-NA; α-naphthylamine, F_v/F_m; chlorophyll "a" fluorescence, and PI_{ABS}; photosynthetic performance index.

When soybean seedlings were irrigated with SW or NW, soaking seeds in 4% DLFJ or 6% DBH significantly increased the above parameters compared to controls (soaking seeds in distilled water). The responses of the above parameters were generally more pronounced under salt stress conditions (SSCs) than under normal conditions (NCs) and were typically more pronounced with 6% DBH treatment than with 4% DLFJ treatment. Therefore, soaking seeds in 6% DBH increased seed GP by 7.9 and 67.5%, seedling FW by 17.8 and 88.1%, seedling DW by 21.1 and 105.3%, root activity by 12.8 and 64.5%, TChls content by 26.8 and 123.9%, PSII F_v/F_m by 11.4 and 67.4%, PSII PI_{ABS} by 18.5 and 80.0%, RWC by 5.7 and 70.3%, and MSI by 8.7 and 93.7% under NCs and SSCs, respectively, compared to corresponding control (**Table 6**).

Salinity stress is one of the most important abiotic variables affecting over 32 Mha of cultivated soils worldwide, particularly in arid and semiarid regions (**Shrivastava and Kumar, 2015; Desoky *et al.*, 2020**). Short-term osmotic stress and long-term accumulation of phytotoxic ions are the earliest signs of salinity's harmful effects on

plants (**Ullah *et al.*, 2021**). Under salty conditions, the gradual absorption of salts and the resulting decrease in the water potential surrounding the root zone reduce water conductivity in plant cells and largely cause plant growth to stop, the first stage of salt exposure causes salt-induced osmotic stress. Extended exposure to high salt levels results in the buildup of harmful ions, such as Na^+ and $Cl^–$, which worsens the damage to plant cells and tissues by causing ion toxicity and reducing nutrient uptake. According to **Isayenkov and Maathuis (2019)** and **Balasubramaniam *et al.* (2023)**, the unfavorable effects of salt stress on plants can be seen in adversely affected seed germination, morphology (stunted growth and chlorosis), and physiology (inhibition of photosynthesis, and reduced RWC and MSI). In this study, the adversely affected seed germination, seedling growth, and physiology of *G. max* seedlings were restored under irrigation with saline water (8.60 dS $m^{–1}$) by soaking seeds in 4% DLFJ or 6% DBH.

Due to their abundance in ORCs (TFAAs, TSsug, and FPro), antioxidant compounds (AsA, total anthocyanins, and total phenolics), and nutrients (Ca, Mg, P, K, and iodine) (**Table 1**), DLFJ and DBH play effective roles in reducing the adverse effects of salt stress on *Glycine max* seedlings (**Table 6**). According to **Rady *et al.* (2023a)**, 4% DLFJ is a strong biostimulant of growth, which enabled *Phaseolus vulgaris* plants to grow well under cadmium stress conditions. Additionally, **Semida *et al.* (2019)** and **Abou-Sreea *et al.* (2021)** reported that 6% DBH is a strong biostimulant of growth, which enabled onion and chili pepper plants to grow well under SSCs. In this study, soaking seeds in 6% DBH or 4% DLFJ enabled *Glycine max* seedlings to grow well under SSCs. However, 6% DBH treatment outperformed 4% DLFJ treatment in increasing seed GP, seedling FW, seedling DW, root activity, TChls content, PSII F_v/F_m, PSII PI_{ABS}, RWC, and MSI under NCs or SSCs (**Table 6**). These results can be attributed to the increased bioactive components in BH compared to those in LFJ. In addition, total antioxidant activity (TAA) in BH is equivalent to 19.8 mM of Trolox compared to 17.2 mM Trolox equivalent in LFJ (**Table 1**). TAA is frequently used to screen for antioxidant activity to stop lipid peroxidation (**Semida *et al.*, 2019; Rady *et al.*, 2021, 2023a**). This affords a higher DBH ability to overcome salt stress effects than DLFJ. In this study, the GP of *G. max* seeds was found to be lowered when irrigated with saline water. Even the seeds that did germinate saw a decrease in growth, as evidenced by a reduction in the seedlings' fresh and dry weights as well as root activity. While salt stress impacts all stages of plant growth, the seed germination stage is particularly vulnerable. High quantities of salt and chloride ions restrict seed germination by primarily decreasing the osmotic potential of the surrounding environment—which, in turn, hampers seed imbibition and embryo growth. Furthermore, ion toxicity not only destroys macromolecular components but also impacts energy use and metabolism during the germination process (**Rady *et al.*, 2023a**).

Multiple studies have demonstrated that exposure to high salt levels can considerably diminish the strength and impede the seed germination process and early seedling development in various species (**Chen *et al.*, 2021**), also observed in the current investigation. The decreased germination of

G. max seeds caused by salinity (**Table 6**) may be due to reducing the water availability for seed embryo growth, inhibiting the movement of starch into the endosperm, and limiting the transportation of soluble sugars to the embryonic axis of the seed. These factors contribute to the starvation of the embryonic axis (**Haider *et al.*, 2023; Rady *et al.*, 2023a**). However, soaking G. max seeds in DLFJ or DBH markedly increased seed germination up to 51%–68%. This suggests that both treatments, with outperforming DBH, effectively counteracted the inhibitory effect of salts. Treatment with DLFJ significantly increased plant growth, as measured by seedling fresh and dry weights. DBH treatment outperformed DLFJ in this effect in salt-free and salt-laden environments, by enhancing root activity. These data suggest that increased root growth aided in plant tolerance to stressful environmental conditions. These beneficial effects are mediated by multiple signaling pathways that influence plants' ability to adapt to environmental challenges. Biostimulants enhance shoot development and promote root activity (**Table 6**), thus improving photosynthetic efficiency (e.g., TChls content, PSII F_v/F_m, and PSII PI_{ABS}) and water absorption and ultimately boosting stress tolerance. The use of biostimulators in plants can activate metabolic pathways and enhance the synthesis of phenylpropanoids, which might reduce the negative effects of stress on plants (**Ma *et al.*, 2022**). The improved growth characteristics observed in our findings with DLFJ or DBH are attributable to their composition of ORCs (TFAAs, TSsug, and FPro), antioxidant compounds (AsA, total anthocyanins, and total phenolics), and nutrients (Ca, Mg, P, K, and iodine) (**Table 1**). These bioactive components can stimulate seed germination and seedling growth, as well as facilitate rapid cell enlargement, division, and multiplication (**Abou-Sreea *et al.*, 2021; Rady *et al.*, 2023a, 2023b, 2023c**).

According to **Rady *et al.* (2023b),** salinity adversely impacts photosynthesis. In this study, the exposure to high levels of salt stress (8.60 dS m^{-1}) significantly decreased TChls content, PSII F_v/F_m, and PSII PI_{ABS} in *G. max* plants, as indicated in **Table 6**. Salinity reduces pigment synthesis by inhibiting the activities 5-aminolevulinic acid dehydratase, porphobilinogen deaminase, coproporphyrinogen III oxidase, porphyrinogen IX oxidase, Mg chelatase, and protochlorophyllide oxidoreductase (**Alsamadany *et al.*, 2023**), activating the catalytic activity of chlorophyllase (an enzyme that degrades chlorophyll), and inhibiting chlorophyll biosynthesis due to increased production of ethylene caused by higher levels of Na^+ and Cl^- or unstable pigment–protein complexes. Hence, TChls content serves as a fundamental physiological measure that indicates the effectiveness of photosynthesis in plants (**Alsamadany *et al.*, 2023**). The decline in TChls content was linked to a reduction in PSII activity; F_v/F_m and PI_{ABS}. This led to the deterioration of photosynthetic machinery and the inhibition of photosynthesis due to the disruption of the essential nutritional balance caused by salt stress (**Azzam *et al.*, 2022; Rady *et al.*, 2022**). **Rady *et al.* (2021)** reported that the reduction in photosynthesis under salt stress is caused by oxidative and osmotic stress as well as nutritional imbalance. The findings of our study indicated that the use of DLFJ or DBH (with DBH outperforming DLFJ) improved the photosynthetic efficacy in the *G. max* seedlings under salinity stress.

In this study, seed priming in DLFJ or DBH (with DBH outperforming DLFJ) provided a water balance (RWC) through seed absorption (during soaking) of TSsug, FPro, and K^+ as ORCs, which led to increased MSI and thus the seedlings tolerated the adverse effects of salt stress. The results of our study support recent research that validated the involvement of DBH in preserving the structural integrity of photosynthetic membranes and aiding in the restoration of TChls content in *G. max* (**Rady *et al.*, 2021**). The DBH treatment maximized ORCs levels in *G. max* seedlings, allowing for the appropriate preservation of RWC according to cellular needs. This led to greater water conservation in plants experiencing salt stress. **Table 6** demonstrates a notable correlation between the increase in the RWC of *G. max* seedlings and the enhancement of plant hydration status resulting from DLFJ or DBH treatment (with DBH outperforming DLFJ). Under salt stress, the use of DLFJ or DBH enhanced RWC and MSI and maintained the proper functioning of metabolic processes. Biostimulants have been found to preserve the structural integrity of cell membranes and to enable plants to thrive in both normal and high-salt stress circumstances. This can explain the positive effects observed in leaf tissue membranes and RWC (**Rady *et al.*, 2023b**).

In conclusion, 6% DBH was superior to 4% DLFJ, however, 6% DBH or 4% DLFJ acts as a powerful natural biostimulant for *G. max* plants exposed to salt stress. Applying 6% DBH markedly enhanced the ability of *G. max* seedlings to tolerate a high salt level (EC = 8.60 dS m^{-1}) by noticeably promoting seed germination, seedling growth, and physiological functions, including photosynthetic efficiency and leaf integrity. Thus, 6% DBH can be used as a cost-effective biostimulant for plants in normal and stressed situations, offering a more affordable alternative to costly synthetic chemicals. Further research is necessary to investigate the exact mechanisms of this intriguing DBH as a multi-stimulator in signaling pathways and physiological responses to abiotic stressors.

4. CONCLUSIONS

In conclusion, 6% DBH is a natural multi-biostimulator that is significantly better than DLFJ in reducing the effects of salt stress on *G. max* seedlings.

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