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Research Article

Does Seed Inoculation with PGPRs Affect Germination and Final Biomass of Flax Under Drought Stress Conditions?

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Abstract

Seed germination as a primary aspect of growth is sensitive to water deficit. The current experiments were carried out to test the effects of drought stress and plant growth promoting rhizobacteria (PGPR) inoculation on seed germination, seedling growth, and biomass production of flax. Initially, the efficacy of PGPR (control, *Bacillus amyloliquefaciens*, *Bacillus* sp. strain1, *Bacillus* sp. Strain2, *Azotobacter chroococcum*, *Pseudomonas putida*, and *Azospirillum lipoferum*) and drought stress (0, -0.75, -1.5, -2 and -2.5 bar of PEG-6000) were estimated on flax germination under laboratory conditions. Then, bacterial treatments for the pot experiment were selected based on the laboratory experiment results (individually and in combination). Drought stress levels in the pot experiment were included 50%, 75%, and 100% crop water requirement. Results showed that the seeds inoculated with PGPRs under drought conditions positively affected seed germination and seedling growth under laboratory conditions. On the other hand, in the pot, emergence properties, dry biomass production, and root-related traits of bacterial inoculated plants were also improved compared with controls. B. *amyloliquefaciens*, *Bacillus* sp. strain1, and A. *chroococcum* in laboratory and co-application of mentioned rhizobacteria in the pot recorded pronounced impact on most of the traits. Moreover, bacterial inoculation is proved to be an effective technique to increase the performance, growth and final biomass production of plants under unfavorable conditions like drought stress.

Keywords: Azotobacter; Bacillus; Drought; Germination; Oilseed

Introduction

The seeds and oil of oilseeds, a rich source of bioactive compounds, have positive effects on disease prevention [1]. Flaxseed (*Linum usitatissimum L.*) as an oilseed crop has been used for formulation of healthy functional food [2] and non-food applications from ancient times to the present [3]. The nutritional importance of flax is for its proteins, lipids, and minerals. Its oil is also a significant source of omega-3 fatty acids, lignans, fiber, mucilage gums, and lignin [1]. The early establishment of seedlings is the most important stage in plants life cycle. Success germination guarantees plants survival and production. Biotic and abiotic stresses as limiting factors have destructive effects on growth and developmental process. Drought stress is one of the serious threats which influences germination, growth, and developmental process through non-normal physiological mechanisms [4]. Under laboratory conditions, polyethylene glycol (PEG) usually applies to induce drought stress, which is not likely to infiltrate into plant tissue quickly [5]. Reduced germination and establishment under water deficit conditions have been investigated in plethora of plants including wheat [6], maize [7], coffee [8], sorghum [9], and soybean [10]. Seeds priming as an alternate, unexpansive and practical method through metabolic process regulation can upgrade seed germination [11] under unfavorable conditions like drought stress. Seeds biopriming with microorganisms named Plant Growth Promoting Rhizobacteria (PGPR) via entering or adhering the seeds causes increase in germination rate and uniformity, high crop establishment, quality and quantity improvement [12]. Generally, mechanisms of these microorganisms for growth promotions are included growth substances secretion, antifungal compounds production, and induction of plant systematic resistance [13]. The capacity of Azotobacter and Bacillus strains to phytohormones production has been confirmed [14]. It has been reported that combined inoculation of bacteria improved wheat seedlings germination and vigor index of stress conditions [15]. [16] found that the germination rate of soybean plants improved by 50% under unfavorable environments. It was hypothesized that PGPR application can decrease destructive effects of drought stress

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in plants. Based on these considerations, the current research was planned and carry out to examine the effects of individual and combined applications of PGPRs on the establishment and production of flax under drought stress conditions.

Materials and Methods

Investigation of Bacterial Growth Promoting Properties

An agar medium comprising calcium phosphate was used as inorganic phosphate to determine the bacteria's ability to solubilize phosphate. Bacteria were tested on a plate using the National Research Institute's Phosphate (NBRIP) growth medium. PGPRs were cultured in NBRIP broth for four d and inoculated on NBRIP agar plates. A loop filled with each culture was then placed on the plates at 30°C for seven d. The appearance of halo zones around the colonies after five d indicated phosphate solubilization. The emerged halo zones helped to classify low (diameter = < 1 cm), medium (diameter = 1-2 cm) and high phosphate solubilizer (diameter = < 2 cm). In order to distinguish ammonia production capability, the strains were cultured in peptone water broth at 27°C for five days before 1 ml of the Nessler's reagent was mixed in 0.2 ml of the culture supernatant, and then, its volume was made to 8.5 ml using ammonia-free distilled water. The alteration of solution color from brown to yellow was considered as ammonia production [17]. For Indole acetic acid production [18], bacterial strains were added to 100 ml of the nutrient broth under continuous shaking for two days at 25°C. Accordingly, the cultures moved to 50 ml falcon tubes and centrifuged at 3000g for 10 minutes before 2 ml of the Salkowski's reagent was mixed with 1 ml of the supernatant. The Salkowski reagent was made through dissolving 4.5 g of $FeCl_3$ in 1 L of concentrated (10.8 M) H_2SO_4. Reagent was added to the sterile nutrient broth as the control treatment. Indole acetic acid production was specified through solution color variation from yellow to brown. Chrome Azurol S (CAS) agar medium was used for siderophore production [19]. CAS agar plates were prepared by adding 100 ml CAS reagent to 900 ml Luria Bertani (LB) agar medium. Bacterial strains were then spot-inoculated separately on plates and maintain for six days in an incubator at 28°C. Non-inoculated plate was used as the control treatment. An orange zone round the bacterial showed siderophore production [20].

Bacterial Preparation

Bacterial strains were gained from the Faculty of Agriculture, Shahrekord University. Single colonies of the bacteria were separately cultured in 250 ml Erlenmeyer containing 50 ml Trypticase Soy Broth and incubated at 32 ±4°C under continuous shaking for 48 hours before washing the bacterial cells three times with NaCl 0.85% in order to remove Trypticase Soy Broth residues. The suspensions were then diluted in NaCl 0.85% to 5×10^6 CFU. ml^{-1}Ultimately, surface sterilized seeds were inoculated using 150 ml of each bacterial culture for two hours before planting.

Experimental Factors and Investigated Traits

Laboratory tests

Using a factorial in RCBD design with three replications, laboratory tests were carried out at the Seed Technology Laboratory of Shahrekord University, Shahrekord, Iran. The factors involved bacterial strains (non-bacterial inoculation or control (C), Bacillus amyloliquefaciens (B_1), *Bacillus sp. strain1* (B_2), *Bacillus sp. strain2* (B_3), *Azotobacter chroococcum* (A_1), *Pseudomonas putida* (P), and *Azospirillum lipoferum* (A_2)) and drought stress (0, -0.75, -1.5, -2 and -2.5 bar of PEG6000). Flax seeds was soaked by ethanol 70% for ten seconds and then washed with distilled water. Afterward, surface sterilaztion of seeds was done by using NaCl 0.2 % for 10 minutes and washed with distilled water. Surface sterilized seeds were treated by 150 ml of each strain culture over a two-hour period before sowing. 50 inoculated seeds were placed per petri plates and drought stress treatments exposed to each bacterial plates. Germination tests were accomplished in a dark growth chamber at 25±0.5°C and 75±1% of relative humidity. Seeds were considered germinated when radicles length were at least two mm. The number of germinated seeds was noted every day, and the final germination test was seven day. Germination percentage [21], germination rate [22] and the mean germination time [23] were calculated according to following relations, respectively:

$$GP = \left(\frac{NG}{TN}\right)\times 100 \qquad (1)$$

$$GR = \sum\left(\frac{GT}{DT}\right) \qquad (2)$$

$$MGT = \frac{\sum GT \times Tt}{\sum Gt} \qquad (3)$$

where, GP, NG, and TN represent germination percentage, total number of germinated seeds, and total number of seeds, respectively. GR is germination rate; GT stands number of germinated seeds in t days and DT denotes the days after sowing. MGT represent mean germination time and Tt denotes the time of Gt in days. Total radicle and plumule length were considered as seedlings height. For this purpose, ten plants were selected randomly and measured by the ruler. Radicle and plumule dry weight were measured after drying in oven at 70 ºC for 24 hours.

Pot Experiment

Complementary to the lab measurements, the pot experiments were conducted to investigate the bacterial effects more attentively, particularly when combined under controlled conditions. The experiment was carried out as factorial based on RCBD, with three replications on May 22, 2016, at the open area of research farm (2116 m above sea level; 32°21'N, 50°49'E) of Shahrekord University, Iran. Soil texture was clay loam with pH: 7.8, EC: 0.38 dS m^{-1}, N: 0.11%, K: 470 mg kg^{-1} as K, and P: 17.6 mg kg^{-1} as P_2O_5.

Daily temperature and humidity are presented in (Figure 1). The experimental factors included drought stress levels and bacterial strains. Irrigation contains three levels, 100%, 75%, and 50% crop water requirements designated as NDS (Non- Drought Stress), MDS (Moderate Drought Stress), and SDS (Severe Drought Stress), respectively; and bacterial inoculations include single, doublet, and triplet applications of the bacteria which had better result in the first test (C, B_1, B_2, A_1, B_1+B_2, B_1+A_1, B_2+A_1, and B_1+B_2+A_1). Pots with 20 cm diameter and 25 cm depth were filled with 4 kg soil. 15 seeds were planted in each pot and the seedlings were subsequently decreased to 10 after emergence. All the pots were kept in the open field. Growth period was 120 days (Figure 1).

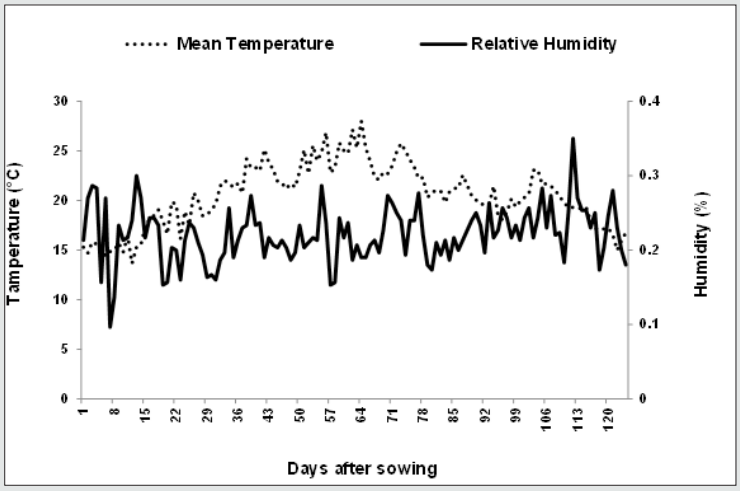

Figure 1: Daily means temperature and relative humidity during the period from planting to harvesting (May 22 to August 22) in 2016 at Shahrekord University, Shahrekord, Iran.

Drought Stress Levels

The seedlings irrigated fully before applying the stress. Designed drought stress levels were initiated at the beginning of the stem elongation. Deficit irrigation was based on maximum allowable water depletion, i.e., MAD (%). To apply drought levels, a moisture meter (Delta-T, SM300, UK) was used to quantity the soil moisture content every second day and the plants were irrigated when the respective MAD threshold was reached. The irrigated water volume was calculated using the following relation:

$$\theta irri = \theta FC - (\theta FC - \theta PWP) \times MAD \quad (4)$$

where, Θ_{irri} (%), Θ_{FC} (%), and Θ_{PWP} (%) represent root depth volumetric water content required by the crop, soil volumetric water content at FC (field capacity), and soil volumetric water content at PWP (permanent wilting point), respectively. The values obtained for Θ_{FC}, Θ_{PWP} (calculated by the volumetric method), and MAD were 34%, 18%, and 50%, respectively. The irrigation depth was determined based on the soil water moisture using following relations (5) and (6) [24]:

$$d = (\theta FC - \theta Soil) \times D \quad (5)$$

$$V = d \times a \quad (6)$$

Where, d is irrigation depth (m), Θ_{soil} denotes soil volumetric water content prior to irrigation (%), D represents pot depth (m), V is the irrigation volume applied (m^3), and A labels pot area (m^2).

The experimental pots were irrigated by means of a graduated cylinder nearly every second day. Emergence percentage, emergence rate and the mean emergence time were calculated like laboratory test (Relation 1, 2, and 3). Samples to measure leaf dry weight and capsulated branches were taken between 11 a.m. and 14 p.m., when the plants in each treatment were at 50% their flowering stage (approximately 50-60 days after the planting date). For this purpose, three plants from each pot were randomly pulled out. Leaves were dried in a hot air oven for one day at 75°C. The number of capsulated branches was obtained from the average of plants. When the plants reached physiological maturity, samples were taken to determine biological yields. Samplings were done for plants that had not been used for prior measurements. Biological yield was obtained from the total dry weight of plants (grain+straw). At the end of the growing season, roots were washed, and all attached soils were removed. Root drying was performed similar to leaf drying. Root volume was measured with the use of graduated cylinder containing water according to Archimedes law [25].

Statistical Analysis

Variance analysis was carried out to compare the effects of different drought stress levels and bacterial treatments on measured traits using the SAS software. Mean comparisons were accomplished using the least significant difference (LSD) test ($P<0.05$).

Results

Bacterial ammonia, indole acetic acid, and siderophore production Growth contributed features of used bacterial strains are displayed in (Table 1). All the strains solubilize phosphate, produce ammonia (except for B_2 and B_3), IAA-like compounds, and siderophore (except B_3 and P) (Table 1).

Table 1: Growth promoting features of bacterial strains (+: positive activity, -: no activity, H: high, M: medium and L; low phosphate solubilization ability).

Traits	B_1	B_2	B_3	A_1	A_2	P
phosphate solubilization	H+	M+	L+	H +	L+	H+
ammonia production	+	-	-	+	+	+
IAA-like compound production	+	+	+	+	+	+
siderophore production	+	+	-	+	+	-

Germination Attributes Traits Under Laboratory Experiment

Percentage, rate, and mean time of germination were negatively affected by drought stress while bacterially inoculated treatments improved the mentioned traits (Figure 2). The highest germination percentage was observed for all inoculated seeds under C conditions. Considering severe stress or -2.5 bar of PEG, similarly, A_1 and B_2 had the highest percentage, with 5.7 and 4.7%%, respectively, in comparison with C treatments under the same stress level (Figure2-A). The highest germination rate (with a rise of 18.6.3%) was recorded in B_1 and B_2 treated seed under non-stress conditions relative to that recorded for C. The B_1, B_2 and A_1 treatments under -2.5 bar PEG presented a significant increase in their rate, compared with C (Figure 2-B). The highest mean germination time was observed in the non-inoculated seeds under severe stress conditions (-2.5 bar PEG). However, significant difference did not observe between control plants with B_1, B_2, B_3, A_1, and P_1 treatments under C conditions (Figure 2C, Figure 2).

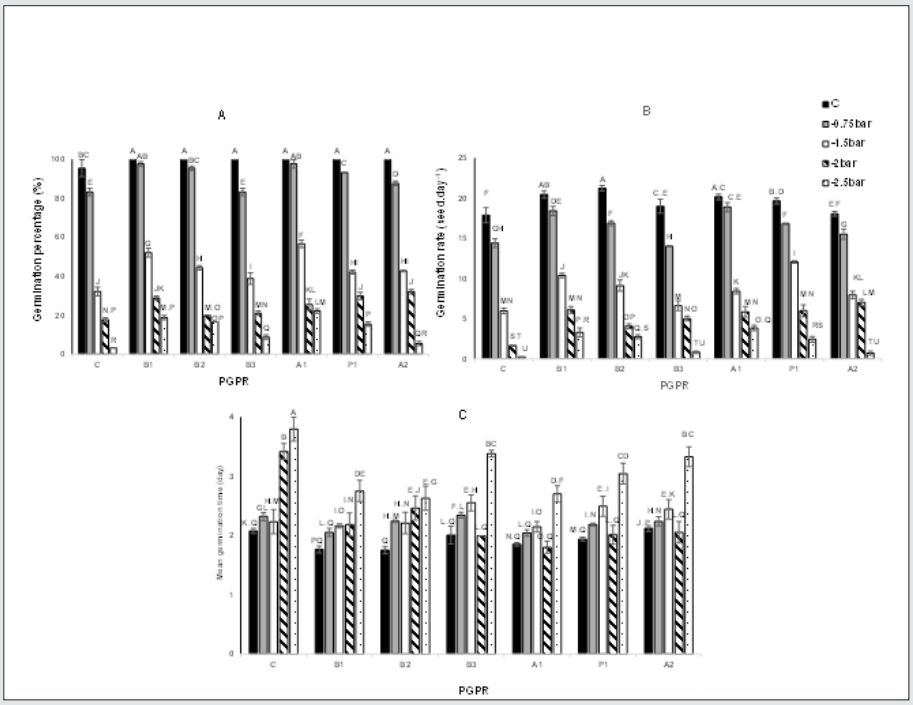

Figure 2: Effects of drought stress and bacterial inoculation on germination percentage (A), germination rate (B), and mean germination time (C) under laboratory experiments. C: control; B_1: B. *amyloliquefaciens*; B_2: *Bacillus* sp. strain1; B_3: *Bacillus* sp. strain2; A_1: A. *chroococcum*; P: P. *putida*; A_2: A. *lipoferum*.

Seedling's Length and Dry Weight Under Laboratory Experiment

Non-stressed and bacterially inoculated treatments had higher length. The B_1 seedlings exposed to normal non-stressed conditions had the highest length, exhibiting an increase of 18.3% relative to the height of the C seedlings. This is while the B_2 treatments subjected to -2.5 bar PEG demonstrated even greater values, which were by 118.2% higher than those of C (Table 3-A). All bacterially

inoculated seedlings had higher dry weight in comparisons with C ones, even though under stress condition the effect of each bacterial strain was different. The highest seedlings dry weight was observed in all bacterial treatments under non-stressed conditions. The B_2 and P treatments subjected to -2. 5 bar PEG recorded the greatest weight, exhibiting an increase of 144.3% relative to the weight of the C seedlings (Table 3-B) (Figure-3).

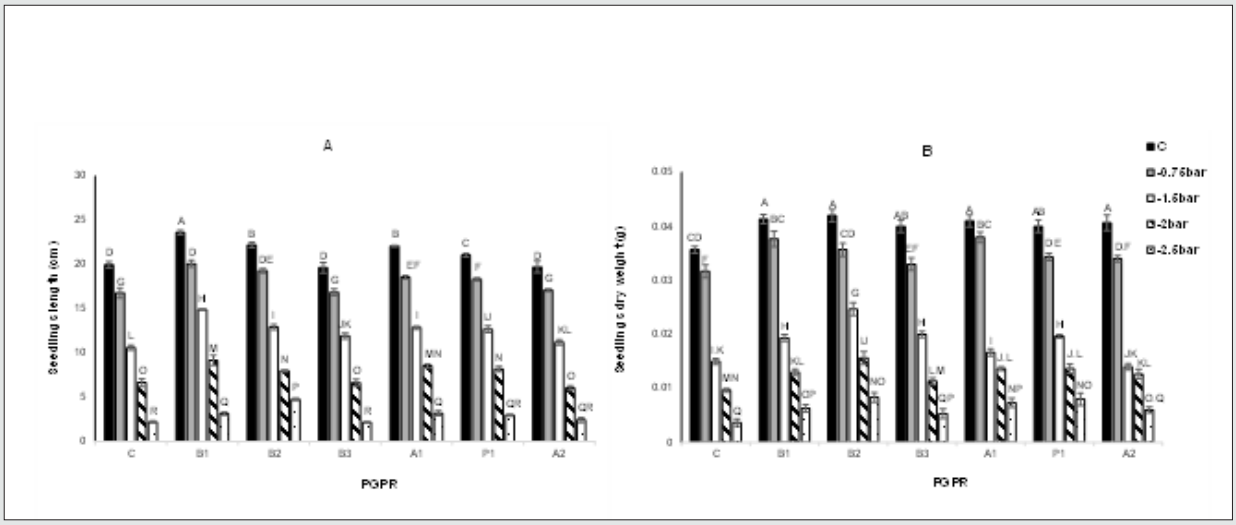

Figure 3: Effects of drought stress and bacterial inoculation on seedlings length (A) and dry weight (B) under laboratory experiments. C: control; B_1: B. *amyloliquefaciens*; B_2: *Bacillus* sp. strain1; B_3: *Bacillus* sp. strain2; A_1: A. *chroococcum*; P: P. *putida*; A_2: A. *lipoferum*.

Emergence Attributes Traits Under Pot Experiment

In pot experiments, the highest emergence percentage was recorded for B_1+B_2+A_1 under NDS, showing an increase of 58%, compared with control NDS plants. Furthermore, B1+B2+A1 under MDS and SDS displayed increased emergence percentage compared to C plants (Figure 4-A). The plants inoculated with B_2+A_1 exposed to NDS, showed the highest germination rate by 78% higher than that of the C plants. B_1 under MDS and B_2 under SDS led to germination rate by 50% and 161.5% respectively, higher than what recorded for C plants in each stress levels (Figure 4-B). Non-inoculated plants under SDS showed mean emergence time by 57.4% higher than that of the C plants subjected to NDS. This is while the B_1+B_2+A_1 plants under NDS conditions showed a reduction of 28.6% in time emergence when compared with those in C plants subjected to NDS (Figure 4-C).

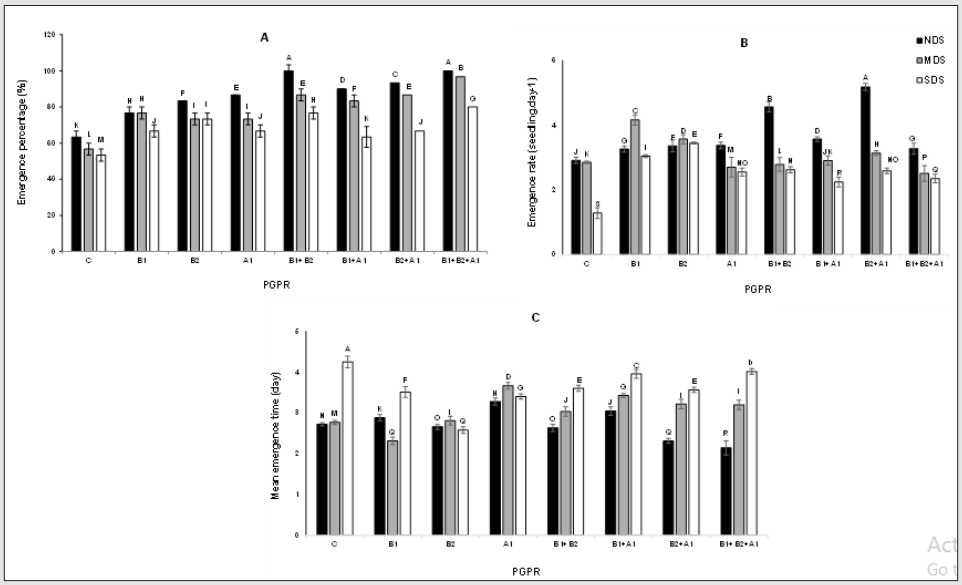

Figure 4: Effects of drought stress and bacterial inoculation on emergence percentage (A), emergence rate (B), and emergence mean time (C) under pot experiments. C: control; B_1: *B. amyloliquefaciens*; B_2: *Bacillus* sp. strain1; A_1: A. *chroococcum*.

Number of Capsulated Branches, Leaves Dry Weight, and Biological Yield

The number of capsulated branches was significantly higher in plants exposed to both non-deficit irrigation and bacterial inoculation. The number of capsulated branches in B_1+B_2+A_1 plants subjected to NDS was 74.9% higher than the number of branches in control NDS plants. A similar result was obtained from MDS conditions. The B_1+B_2+A_1 and B_2+A_1 plants exposed to SDS conditions had the highest capsulated branches number, showing an increase of 90.6% compared with those recorded for control plants under the same irrigation regimes (Figure 5-A). Under the pot experiment, higher leaf dry weight was recorded in bacterial inoculated plants with combined strains in comparison with those inoculated with individual ones. The highest weight was observed for the B_2+A_1 plants under NDS, showing an increase of 80.3% relative to that of C plants. Furthermore, the B_1+B_2+A_1 treatments under MDS and SDS conditions revealed increased dry weight by 60% and 52.9%, respectively higher than the C treatments (Figure 5-B). Water deficit stress was observed to reduce biological yield in all the plants. The bacterially inoculated treatments exhibited increased biomass production, compared with those observed in the C plants. The plants inoculated with B_1+B_2+A_1 showed the highest yield, i.e., 21.6% higher than C ones. This same inoculation treatment under SDS led to a yield that is 22.6% higher than those recorded for control treatments (Figure 5-C).

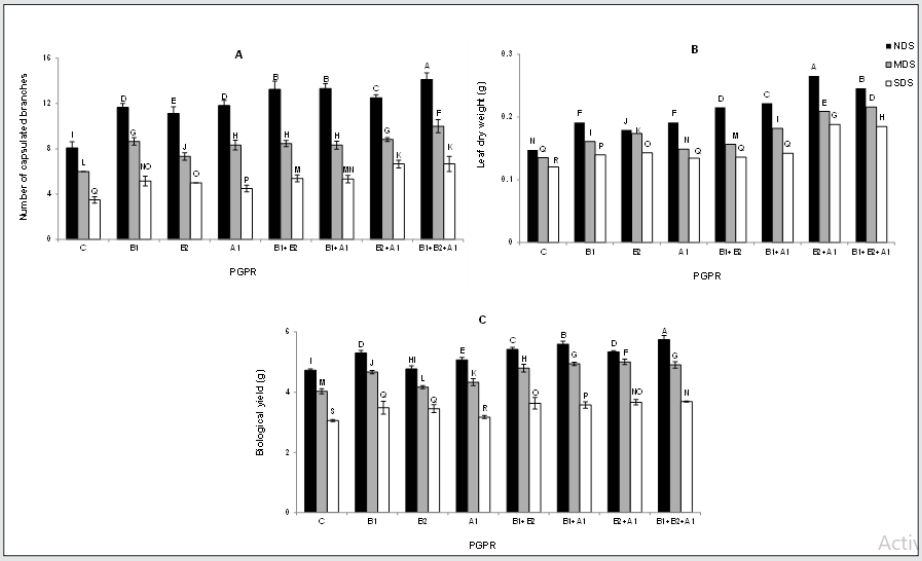

Figure 5: Effects of drought stress and bacterial inoculation on number of capsulated branches (A), leaf dry weight (B), and biological yield (C) under pot experiments. C: control; B_1: B. *amyloliquefaciens*; B_2: *Bacillus* sp. strain1; A_1: A. *chroococcum*.

Roots Volume and Dry Weight

Fully irrigated and bacterially inoculated plants had maximum roots volume and dry weight. B_1+B_2+A_1 treatments exposed to NDS conditions had 40% more volume compared to control NDS ones. Furthermore, B_2+A_1 treatments subjected to SDS had 85.6% more volume than C plants under SDS conditions (Table 2). The highest roots dry weight was observed for B_1+B_2+A_1 ones under NDS conditions, showing an increase of 11.5%, compared to C plants. Similarly, the dry weight in this treatment under SDS recorded 13.3% increase, in comparison with untreated or C treatments under SDS conditions (Table 2).

Discussion

PGPRs use a variety of mechanisms to promote plant growth [26]. In our research, all the strains solubilized phosphate in the in vitro assays nonetheless varied in the degree of solubilization ability. The Bacillus and Pseudomonas genera are the most influential bacteria that typically solubilize phosphate by producing organic acids [27]. All the strains produced ammonia (except B_2 and B_3) which considers as nitrogen source used for the macromolecule synthesis in plants [28]. Nitrogen accessibility through bacteria can have substantial role in providing other nutrients for yield production. All six bacteria confirmed IAA-like compounds production. According to reports, 80% of root-associated bacteria have the ability for IAA secrete [29]. Moreover, all the bacteria (except B_3 and P) created siderophore. Siderophore are low molecular weight were secreted to solubilize iron from the surrounding environment of the plants and to form a ferric-siderophore complexes, which can be absorbed by plants [30]. Germination and emergence are considered as important agronomic performance indices. In time germination and uniform establishment of seedling has key impact on crop's final production. Content of soil water is an important factor affecting seed germination and establishment. In the current examination, PGPR inoculation has positive effects on germination and emergence related traits of flaxseed under drought stress conditions. The improved germination and emergence features of inoculated seeds might be attributed to the bacterial growth promoting properties, especially IAA production (Table 1). IAA is an important phytohormone for plants growth [31]. Bacterial IAA may increase plants endogenous IAA levels by increasing water

uptake, photosynthesis, and IAA-related gene expression [32,33]. Increased seed germination of bacterial inoculated treatments may be related to activity of some enzymes like hydrolytic enzymes [34]. [35] reported that PGPRs through gibberellin production may increase α-amylase activity and starch hydrolysis; therefore, cytoplasmic membrane permeability will increase. At which point minerals transition to embryo and germination will improve [35]. Moreover, in the pot experiment of current research, maximum percentage and rate and minimum time emergence of triply- and doubly inoculated treatments could be due to the synergistic effects of the bacteria in mixed form than their individual application. Application of Bacillus subtilis and *Aospirillium brasilense* and their combination improved seed germination, seedling vigor index, and promptness index of wheat under osmotic stress induced by PEG6000 [36]. In their research with Bromus *Tomentellus Bioss*, [34] found that, compared to the non-inoculated plants, under drought stress, *Azotobacter vinelandii*, and *Azotobacter vinelandii*+ *P.putida+Pantoea agglomerans* at 0.7 FC level showed increased seed germination rate. Studying Brassica napus, [37] reported positive relations between seed bacteria and the mean germination time of seedlings. [38] reported that the shortest germination time of mature pepper seed was recorded in Bacillus strain inoculated treatment while the longest mean germination time was exhibited in the untreated control ones.

According to [39] report, seedlings length is a significant index of crops agronomic performance. The non-inoculated control plants in our study were more affected by drought stress, whereas the bacterially inoculated treatments demonstrated alleviated stress effects. Higher length recorded in inoculated seedlings and plants might be associated with growth promoting properties, particularly IAA production (Table 1). These hormones are responsible for cell division and elongation, consequently, increase plants length. IAA is closely related to growth promotion, not only through embryogenesis, organogenesis, and vascular differentiation but also via root and shoot development [40]. Gibberellins is also causing plant growth and development, seed germination stimulation, flowering inhibition, dormancy breaking, and root formation [41]. [42] reported that in Helianthus annus shoot and root length significantly increased in inoculated PGPR treatments under water stress. In their experiment with mexican fir tree species, [43] found that, compared to the non-inoculated plants, hydroprimed and inoculated treatments with Pseudomonas and Bacillus strains had higher length. Water deficit leads to stomatal closure and reduce the water content of aerial organs that consequently decreases the number of capsulated branches. The increased capsulated branches recorded in the treated flax plants may be as a consequence of mitigated drought conditions through the PGPR possible acting through phytohormones such as IAA which promotes root and shoots development. Alterations in the root morphology of PGPR treated plants could affect the number of stems, shoots and leaves by facilitating the transmission of water and nutrients toward aerial organs [44] It has been reported that Agrobacterium tumefaciens and Rhizobium *leguminosarum in faba* bean acted as PGPR increasing the number of pods and crop yield [45].

Water deficit may through stomata closing or root growth decreeing, lead plants water content reduction and consequently causes dropping leaf growth and final biomass production. The increased leaves dry weight and biological yield detected in inoculated flax plants might be associated with the growth promoting traits of used PGPRs (Table 1) [46]. reported that applying PGPR to maize plants increased dry biomass of whole plant parts. [47,48] attributed the increased in dry biomass of inoculated plants to abilities like phosphate solubilization, production of root promoting hormones namely IAA, cytokinins and gibberellin. In [49] research on onion crops and bulbs, higher dry matter, yield and dry weight were observed in bacterial inoculated treatments. On the other hand, increased final yield in inoculated treatments may be attributed to their on-time and uniform emergence and establishment (Figure 2) which are crucial factors, and essential to final biomass production and involve in complex phenomenon of physiological and biochemical process. Agroecosystems can be contributed with plant growth promoting rhizobacteria mediated root trait changes through improving crop stand, resource use efficiency, stress tolerance, and soil structure. PGPRs by phytohormones, volatile organic complexes, and secondary metabolites production can affect plants root features, subsequent improved nutrient exchange and rhizosphere effects [50]. The effect of PGPRs on root length, biomass and volume have been reported in several experiments [51-53].[54] maintained that *Bradyrhizobium* japonicum and Pseudomonas putida in soybean crops improved root length, surface area, root volume, nodules number mainly due to the bacterially IAA production and phosphorous and nitrogen accumulation. In peanut, the improved root dry weight and root length reportedly belonged to plants receiving inoculation of Bacillus sp., Pseudomonas sp., and co-inoculation with *Bradyrhizobium* sp. by IAA production, antagonistic against root disease, biofilm formation, and lytic enzyme production [55].

Conclusion

Drought was found to have undesirable influences on percentage, rate, and mean time of germination, seedlings dry weight and height in the laboratory experiment and the percentage, rate, and mean time of emergence, number of capsulated branches, leaves dry weight, biological yield, root volume and dry weight in the pot part. However bacterial inoculation was detected to decline the adverse effects of unfavorable conditions. The results indicated that investigated characters varied with different bacterial strains significantly ($P < 0.05$). The B1 strain in the laboratory part and $B_1+B_2+A_1$ treatment in the pot experiment had better results in comparison with other ones. The observed improvement measured traits may be attributed to the phosphate solubilization, ammonia, IAA-like compounds, and siderophore production of the bacteria used and their synergistic effects when used as a combination. Briefly, PGPR application possibly will be a promising technique to increase drought stress tolerance in oilseed crops such as flax.

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