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Synergetic effect of PGPR on growth, yield and nutrients of black soybean

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ABSTRACT

A pot experiment was conducted at College of Basic Science Humanities, Govind Ballabah Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India.Numerous rhizobacteria fix the atmospheric nitrogen and solubilize the insoluble phosphorus present in the soil and make available to the plants and also contribute towards better growth, yield and nutrient uptake of plants through other direct and indirect plant growth-promoting activities. This communication deals with the individual or synergetic effect of Azospirillum lipoferum, Bacillus megaterium and Rhizobium radiobacter on plant health and productivity of Glycine soja. Compatible PGPR inoculation either single or mixed (A. lipoferum + B. megaterium + R. radiobacter) had positive effect on overall performance of plants. All the treatments significantly ($p<0.05$) enhanced the vegetative growth, nodulation and yield attributing parameters as compared to control. The chlorophyll content of leaves and nutrient concentration (N, P, K and Fe) in different plant parts were reported maximum in mixed followed by individual treatments over control. The mixed PGPR inoculants also reduced the crop maturity time by 25 days. Moreover, treatment of soil and seeds with PGPR improved the quality of soil in terms of physicochemical characteristics and nutrient content. Therefore, the results indicated the potential of consortium (mixed) in stimulation of plant growth and yield in black soybean for sustainable agriculture and environment.

Keywords:*Azospirillumli poferum*, *Bacillus megaterium,* black soybean,consortium,PGPR, *Rhizobium radiobacter*

INTRODUCTION

Black soybean (*Glycine soja* Sieb. and Zucc.), a wild species, is an annual herbaceous plant of the Leguminosae family and sub-family Papilionaceae. It is a close relative of cultivated worldwide soybean. *Glycine max* and *Glycine soja* both include 20 chromosomes (2n=40), hybridize simply, reveal normal meiotic chromosomes pairing and create viable productive hybrids. Black soybean is also characterized by its high nutrient content, cold toughness, salt tolerance and disease resistance among other soybean varieties. Traditionally, it is grown in different hilly states of India like Himachal Pradesh, Uttarakhand, East Bengal, Khasi hills, and central hilly regions. Black soybean extract is an anticancerous agent with several secondary metabolites like flavonoids and alkaloids (Hidayat and Dwira, 2018). Black soybean ethanolic extract had a cytotoxic effect on HCT-116 colorectal cancer cell growth. Plant growth-promoting rhizobacteria (PGPR) are soil bacteria inhabiting in or around the root surface and directly or indirectly involved in plant growth promotion through production and secretion of assorted regulatory chemicals in the rhizosphere region. There are several PGPR genera like *Agrobacterium*, *Azospirillum, Azotobacter, Arthrobacter,Alcaligenes, Acinetobacter, Burkholderia, Bacillus, Bradyrhizobium, Enterobacter, Flavobacterium, Frankia, Klebsiella, Pseudomonas*, *Rhizobium, Serratia* and *Thiobacillus* have a bio-fertilizer supplement of nitrogen, phosphorous, and potassium for improved crop production (Goswami *et al.* 2016). All these microbial inoculants can enhance plant growth through atmospheric nitrogen fixation, solubilization of mineral nutrients like phosphorus, potassium, and iron, phytohormone production, and improving plant confrontation towards pests and diseases (Gouda *et al.* 2018). Beneficial interactions of black soybean with the rhizosphere microbial community might lessen the consumption of synthetic fertilizers and defend plants from many destructive abiotic and biotic stresses (Sugiyama *et al.* 2017). Inoculation of seed or soil with PGPR can be an eco-friendly approach to reduce the consumption of chemical fertilizers, enhanced crop production and promote soil fertility by their biological activity in the rhizosphere. Hence, the present

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experiment was performed to assess the effect of individual or combined inoculation of *Azospirillum lipoferum*, *Rhizobium radiobacter*, and *Bacillus megaterium* on the growth promotion, yield and nutrient uptake of *Glycine soja*.

MATERIALS AND METHODS

Bacterial strains, media and inoculum preparation: Three bacterial strains *Azospirillum lipoferum* (MTCC 2306), *Rhizobium radiobacter* (MTCC 9756), and *Bacillus megaterium* (MTCC 8755) were used for inoculation procured from Microbial Technical Collection Centre (MTCC), Chandigarh, India. For inoculation, *Azospirillum lipoferum* was cultured in Modified Peptone Succinate Salt broth medium (MPSSB), *Rhizobium radiobacter* in Nitrogen-Free broth medium (NFB), and *Bacillus megaterium* in Nutrient broth medium (NB). Bacterial strain was separately incubated in a 500 ml flask filled with 100 ml respective broth at 28 ± 30^0C for 24 h in an orbital shaking incubator at 150 rpm. Optical cell density was calculated at 600 nm to accomplish a uniform population of bacteria (10^7-10^8 cfu/ml) in the broth prior to inoculation. The bacterial suspension was used to adjust at A_{600nm}= 0.6.

Bio-assay for plant growth-promoting characters: Various plant growth promoting features were tested for *Azospirillum lipoferum*, *Rhizobium radiobacter*, and *Bacillus megaterium* strains. The nitrogen fixation test was done using Jensen medium (Jensen, 1954), and phosphate solubilization test was done on Pikovaskaya medium (Pikovaskaya, 1948). The production of siderophore and Indole Acetic Acid (IAA) were tested using CAS agar medium (Schwyn and Neilands, 1987) and Salkowasky's reagent in tryptophan amended medium (Gordon and Weber, 1951). The origin of each bacterial strain can be found by using MTCC Accession numbers details at https://mtccindia.res.in/catalog.

Experimental procedure: A pot experiment was conducted at Rhizosphere Lab, College of Basic Science Humanities, Govind Ballabah Pant University of Agriculture &Technology, Pantnagar, Uttarakhand, India during 2017. The soil used in the pot trial was a mixture of soil and farmyard manure (cow dung) (2:1). The experimental soil had: pH (6.98); EC (1.39 dSm^{-1}); OC (1.56%); TKN (0.12%), P_2O_5 (0.011%); and K_2O (0.14%). Five treatments were: seeds treated with sterile broth (T-1: control), seeds treated with *A. liopferum* (T-2); seeds treated with *R. radiobacter* (T-3); seeds treated with *B. megaterium* (T-4), and seeds treated with mixture of bacterial strains (*A. lipoferum* + *R. radiobacter* + *B. megaterium*) (T-5). The pot experimentation was conceded out in a completely randomized design (CRD) with four replication set for each treatment.

Plant material, bacterization, growth condition, experimental design: Black soybean variety VL-65 used in the present study was obtained from VPKAS, Almora, Uttarakhand, India. For pot experiments, surface-sterilization of seeds were performed by immersion in 3% (v/v) solution of sodium hypochlorite and 70% ethanol for 3 min and 1 min, respectively and then thoroughly washed 3-4 times with sterile distilled water. Pots with an inner diameter of 20 cm and a length of 35 cm contained 7 kg mixture of unsterile soil and farmyard manure (cow dung) (2:1). Sterilized seeds of uniform size were soaked for 3 hrs in their respective inoculums-broth as per individual treatment, for consortium all broth suspension were mixed in 1:1:1 (v/v/v) and for control treatment sterile broth was used. Inoculated black soybean seed was sown at 10 spots in each pot and 1 ml of respective inoculum was poured around seed whereas, the sterile broth was used for the control treatment. Throughout, the experimental period seeds were grown with the temperature range between 32^0C-37^0C (maximum) and 24^0C-29^0C (minimum). None chemical fertilizers and pesticides were applied during the test period. After ten days of sowing, thinning was done, and only 8 seedlings per pot were kept.

Measurement of growth parameters and physico-chemical characteristics: Data on vegetative characteristic (shoot and root length; fresh and dry root weight; number of leaves and primary branches, number of nodules; nodules fresh and dry weight) were recorded at 30 and 120 DAS. Yield attributes (length of pod; number of pods per plant; number of seed per pod, seed weight per plant and 1000-seed weight) were recorded at harvest. The pH and EC of the soil samples was determined using pH meter and conductivity meter, respectively. Organic content was determined by adopting modified Walkey and Black method (Jackson, 1973). Nitrogen in plant root, stem, seeds, and soil was estimated by Kjeldahl method. Phosphorus was determined using Vandomolybdophosphoric acid method; potassium and iron on atomic absorption spectrometer in diacid extract by flame photometer. The concentration of photosynthetic pigments such as chlorophyll a, chlorophyll b and total chlorophyll content were analyzed following the Arnon method (Arnon, 1949).Data were subjected to an analysis of variance (ANOVA) using the STPR software. Following the analysis of variance (ANOVA), differences among treatment (wherever applicable) was determined by the Duncan's New Multiple Range Test (DMRT) method at 5% level of significance.

RESULTS AND DISCUSSION

Plant growth promoting potential

The three bacterial strains *Azospirillum lipoferum* (MTCC 2306), *Bacillus megaterium* (MTCC 8755), and *Rhizobium radiobacter* (MTCC 9756) were screened for plant growth-promoting characters. Growth on N-free medium indicated the capability of the PGPR to fix atmospheric nitrogen. All strains showed a positive result for nitrogen fixation on Jensen medium plate. Development of a halo zone around the streaked colony on media supplemented with an insoluble form of tricalcium phosphate indicated the presence of mineral phosphate solubilizing feature. *A. lipoferum* and *B. megaterium* had the capacity to solubilize phosphate on Pikovaskaya plate, whereas, *R. radiobacter* did not form halo zone around the colony. Among the phytohormone, the three bacterial strains gave positive results for IAA in succinate broth. The appearance of the orange-halo zone on the CAS-agar plate was considered as positive for siderophore production and all cultures resulted in positive for siderophore production.

Morphological character

The consortium inoculation performed better than individual treatments as compared to control.The highest shoot length (97.30 cm) was recorded with the mixed culture (T-5) followed by T-3, T-4, and T-2 treatment. Root length also followed the same trend (Table 1). Individual and mixed inoculation positively influenced the number of leaves and primary branches; their number was significantly higher than those in the control (Table 1).

Table 1: Effect of PGPR on the growth characteristics of black soybean

Treatments	Shoot length (cm)		Root length (cm)		Number of leaves		Number of primary branches	
	30 days	120 days	30 days	120 days	30 days	120 days	30 days	120 days
T_1	39.7 ± 0.58^{a}	56.2 ± 1.31^{a}	6.7 ± 0.1^{a}	9.2 ± 0.1^{a}	19.2 ± 0.72^{a}	27.1 ± 0.62^{a}	5.4 ± 0.16^{a}	10.2 ± 0.24^{a}
T_2	53.3 ± 0.76^{b}	68.2 ± 0.65^{b}	7.5 ± 0.3^{b}	12.2 ± 0.1^{b}	21.7 ± 1.44^{a}	32.2 ± 0.90^{b}	7.0 ± 0.10^{b}	15.3 ± 0.14^{b}
T_3	71.8 ± 0.53^{d}	86.7 ± 1.29^{c}	8.2 ± 0.3^{b}	16.4 ± 0.4^{c}	26.3 ± 0.74^{b}	37.4 ± 0.70^{c}	7.6 ± 0.07^{c}	17.1 ± 0.16^{c}
T_4	60.5 ± 0.74^{c}	71.5 ± 0.93^{b}	7.9 ± 0.2^{b}	13.5 ± 0.5^{b}	23.7 ± 0.80^{b}	35.1 ± 0.89^{c}	7.3 ± 0.14^{b}	14.9 ± 0.13^{b}
T_{5*}	87.5 ± 1.04^{e}	97.4 ± 0.45^{d}	10.2 ± 0.4^{c}	20.3 ± 0.7^{d}	34.0 ± 1.44^{c}	40.1 ± 1.04^{d}	9.4 ± 0.06^{d}	21.4 ± 0.24^{d}
Mean	62.5	76.0	8.1	14.3	25.0	34.4	7.3	15.8
SEm±	1.57	1.69	.24	.46	1.13	0.88	0.15	0.25
LSD ($p \leq 0.05$)	4.85	5.19	.75	1.4	3.49	2.72	0.46	0.77
CV%	5.03	4.44	5.97	6.4	9.07	5.13	4.03	3.16

Among the treatments, T-5 exhibited the largest effect on leaf (40.1) and primary branches (21.4) followed by T-3, T-4, T-2 treatment. The number of root nodules (11.8) per plant significantly increased with the mixed cultures of *A. lipoferum*, *B. megaterium* and *R. radiobacter* (T-5) over control. For fresh nodule weight, the trend was T-5>T-3>T-2>T-4, however dry weight of nodule was observed maximum in T-5 (0.35 g) and minimum in T-2 (0.16 g) (Table 2).

Table 2: Effect of PGPR on nodulation of black soybean

Treatments	Nodule Number	Nodule fresh weight (g)	Nodule dry weight (g)
T_1	5.13±0.16^a	0.46±0.0002^a	0.11±0.0002^a
T_2	7.38±0.13^b	0.68±0.0002^b	0.16±0.0003^b
T^3	9.38±0.22^c	0.87±0.0002^d	0.25±0.0002^d
T^4	7.31±0.12^b	0.79±0.0002^c	0.18±0.0003^c
T^5	11.75±0.27^d	0.97±0.0004^e	0.35±0.0002^e
Mean	8.19	0.75	0.21
SEm±	0.19	0.00006	0.0003
LSD (p ≤0.05)	0.58	0.0002	0.0008
CV%	4.60	0.02	0.25

The different treatments increased the fresh and dry weight of root and shoot were significantly increased over the control (Table 3) and maximum values were achieved in the T-5 treatment and minimum in T-2 treatment. PGPR and leguminous plants in holobiant relationships through bio-mineralization and synergistic effect have great potential for improving soil quality and fertility (Agler *et al.* 2016; Gouda *et al.* 2018). Application of PGPR either single or consortium enhanced the growth, biomass, nodulation, yield and nutrient uptake of the black soybean and quality of soil in terms of nutrients and physical properties. IAA secretion involvement as root initiation increases roots surface area, cell partition, cell growth and development of root system architecture resulted in the uptake of minerals and water and thus, increases the growth and biomass of plant. Combined inoculation of bacterial strains (*B. megaterium,P. polymyxa* and *Rhizobium*) promoted the root and shoot dry weight as compared to individual inoculation of *Rhizobium* in common bean (Korir *et al.* 2017). Our results showed that triple PGPR inoculants *A. lipoferum*, *B. megaterium* and *R. radiobacter* positively affected all the growth parameters of black soybean, as two to four- fold increase was recorded and T-5 was the best treatment.

Table 3: Effect of PGPR on biomass of black soybean

Treatments	Fresh shoot weight(g)		Fresh root weight (g)		Dry shoot weight (g)		Dry root weight (g)	
	30 days	120 days	30 days	120 days	30 days	120 days	30 days	120 days
T_1	1.22±0.07^a	4.03±0.17^a	0.05±0.00^a	0.67±0.04^a	0.32±0.02^a	1.28±0.17^a	0.015±0.001^a	0.23±0.14^a
T_2	1.54±0.10^b	8.07±0.41^b	0.07±0.00^{ab}	1.02±0.28^b	0.37±0.01^{ba}	2.13±0.09^b	0.019±0.001^b	0.60±0.03^b
T^3	1.64±0.32^b	8.26±0.11^b	0.09±0.00^b	1.62±0.13^c	0.42±0.04^b	2.15±0.04^b	0.024±0.002^c	0.83±0.02^c
T^4	1.79±0.07^c	8.53±0.39^b	0.09±0.01^b	0.90±0.35^a	0.41±0.02^b	2.07±0.23^b	0.020±0.001^b	0.69±0.02^b
T^5	2.64±0.24^d	10.53±0.49	0.28±0.11^c	1.98±0.07^d	0.63±0.07^c	3.42±0.23^c	0.031±0.003^d	0.93±0.02^c
Mean	1.77	10.02	0.12	2.56	0.43	2.21	0.02	0.66
SEm±	0.08	0.36	0.01	0.11	0.02	0.06	0.0008	0.07
LSD (p ≤0.05)	0.24	1.12	0.02	0.34	0.05	0.20	0.003	0.21
CV%	8.70	7.27	13.28	8.63	8.20	5.86	7.47	20.32

Seed characteristics and Yield component

Maximum values for all traits were observed in T-5, whereas the least values were recorded in T-2 (Table 4). The results for PGPR treatments showed that there was a significant change (p ≤ 0.05) in days required for first flowering (Table 4).

The minimum number of days required after sowing for first flowering was 39 in T-5, 60 in T-3, 69 in T-4, and 70 in T-2. The treatments also reduced the duration of the first pod ripening. The number of days required for the first pod ripening was 75 for co-inoculation (T-5), 84 for T-3, 87 for T-4, 95 for T-2 and 100 for T-1 (Table 4). Pod length, seeds per pod, number of pod per plant, seed yield per plant, and 1000-seed weight were determined at the time of harvest (120 DAS). All features were significantly improved in consortium treatment (T-5), followedby individual treatment as compared to control. The maximum amount of seeds per pod was produced following inoculation of seeds with mixed cultures (T-5), followed by treatments T-3,

Table 4: Effects of PGPR on yield and yield characteristics of black soybean

Treatments	1000-seed weight (g)	Number of pod/plant	Number of seed per pod	Seed weight per plant (g)	Pod per Length (cm)	1st flowering	1st pod maturity
T_1	118.4±0.01^a	3.56±0.06^a	3.19±0.19^a	3.57±0.002^a	4.00±0.18^a	80±0.42^e	100±0.20^e
T_2	139.7±0.01^b	5.19±0.28^b	5.44±0.12^b	5.88±0.002^b	5.31±0.06^b	70±0.12^d	95±0.19^d
T^3	215.2±0.55^d	5.69±0.19^c	6.44±0.12^c	8.86±0.001^d	5.94±0.12^c	60±0.32^b	84±0.18^b
T^4	205.0±0.22^c	4.69±0.36^b	5.81±0.16^b	7.87±0.003^c	5.56±0.12^b	69±0.20^c	87±0.28^c
T^5	257.0±0.30^e	8.63±0.16^d	8.56±0.06^d	11.87±0.001^e	7.56±0.12^d	39±0.14^a	75±0.10^a
Mean	1.87	5.55	5.89	7.61	5.68	63.57	88.12
SEm±	0.07	0.22	0.16	0.20	0.13	0.21	0.22
LSD (p≤0.05)	0.23	0.68	0.48	0.63	0.39	0.64	0.67
CV%	7.88	7.93	5.31	5.33	4.50	0.65	0.50

T-4, and T-2. Seed yield increased by 69.96% (T-5), 59.78% (T-3), 54.69% (T-4) and 39.42% (T-2) over the control. All treatments showed a positive effect on test weight (p ≤ 0.05) and maximum value was observed in T-5 (256.97 g) treatment whereas least in T-2 (139.73 g). Our results also supported the findings of Reneta *et al.* (2017) and Marinkovic *et al.* (2018). The mixing of organic manure (cow dung) with soil, and individual or combined inoculation of seeds or soil with PGPR resulted in multiplication of beneficial microorganisms and participate in nutrient cycling and consecutively enhanced growth and yield of the black soybean. Similar results were reported by Adeyeye *et al.* (2017).

Table 5: Physicochemical properties of the soil collected from the rhizosphere of different treatments

Treatments	SOIL (120 DAS)					
	pH	EC (dSm^{-1})	OC(%)	TKN(%)	P_2O_5(%)	K_2O(%)
T_1	7.67±0.01^a	1.42±0.002^e	0.65±0.001^a	0.18±0.001^a	0.17±0.004^a	0.19±0.016^a
T_2	8.09±0.01^c	0.61±0.0001^b	0.87±0.002^c	0.35±0.002^c	0.47±0.004^d	0.53±0.003^d
T^3	7.93±0.01^b	0.71±0.0002^c	0.77±0.002^b	0.47±0.002^d	0.34±0.001^c	0.43±0.004^c
T^4	8.11±0.01^c	0.92±0.0003^d	0.97±0.001^d	0.25±0.003^b	0.27±0.001^b	0.35±0.002^b
T^5	8.42±0.01^d	0.53±0.0003^a	1.04±0.001^e	0.78±0.002^e	0.87±0.002^e	0.97±0.002^e
Mean	8.04	0.84	0.86	0.41	0.42	0.49
SEm±	0.01	0.0008	0.002	0.002	0.003	0.01
LSD (p ≤0.05)	0.03	0.003	0.005	0.01	0.01	0.02
CV%	0.20	0.19	0.35	0.94	1.39	3.20

Soil properties and nutrient content

The electrical conductivity decreased with all treatments over control and was noticed minimum in T-5 (Table 6). The pH (8.42), organic carbon (1.04%), nitrogen (0.78%), phosphorus (0.87%), and potassium (0.97%) contents of the rhizosphere soil samples were significantly improved in all treatments over the control. The values for the mentioned physicochemical characteristics were reported maximum in T-5, followed by individual T-3, T-4, and T-2 treatments (Table 6). The N, P, K and Fe content of roots, stems and seeds were significantly higher in all treatments than those in the control and maximum values were recorded in T-5 treatment, while minimum in T-2 (Table 5). The consortium *Azospirillum*, *Bacillus* and*Rhizobium* boost the growth of test crop by supplying nitrogen through symbiotic and asymbiotic association. It is supposed that PGPR can improve plant nutrition through associative nitrogen fixation, phosphate solubilization and siderophore production. PGPR inocula do not only solubilize soil P unavailable to plants but they also mobilized its organic form through mineralization and facilitate the translocation of phosphate (Owen *et al.* 2015). The major processes involved in mobilization of insoluble forms of potassium are acidolysis and chelation of cation with potassium minerals through the production of various organic acid by microbes resulting an increased K availability in the rhizosphere. In the present study, the enhanced uptake of iron by the plants can be correlated with the siderophore production by

PGPR in the rhizosphere soil and plants with an accessible form of iron. Moreover, siderophore producing isolates can be very effective for enriching the soil fertility and yield of agricultural crops.

Table 6: Effect of PGPR on nutrient contents (%) of root, shoot, and seed and chlorophyll content of black soybean

Nutrient %	T1	T2	T3	T4	T5	Mean	SEm±	LSD (≤0.05)	CV%
Nitrogen (TKN %)									
Root	0.48±0.007^{a}	1.63±0.018^{b}	1.77±0.016^{c}	1.85±0.024^{c}	2.04±0.044^{d}	1.55	0.03	0.08	3.48
Shoot	1.68±0.016^{a}	2.39±0.023^{c}	2.54±0.008^{d}	1.99±0.005^{b}	2.99±0.018^{e}	2.32	0.02	0.05	1.41
Seed	3.05±0.004^{a}	4.48±0.018^{c}	4.89±0.065^{d}	4.37±0.001^{b}	5.85±0.006^{e}	4.53	0.03	0.10	1.39
Total Phosphorus (P_2O_5%)									
Root	0.42±0.005^{a}	0.50±0.006^{b}	0.54±0.014^{c}	0.59±0.002^{d}	0.69±0.004^{e}	0.55	0.01	0.02	2.39
Shoot	0.38±0.003^{a}	0.40±0.001^{b}	0.43±0.002^{c}	0.59±0.003^{d}	0.67±0.001^{e}	0.49	0.002	0.01	0.97
Seed	0.31±0.001^{a}	0.95±0.0002^{d}	0.85±0.0004^{c}	0.48±0.0003^{b}	1.17±0.004^{e}	0.55	0.01	0.02	2.39
Total Potassium (K_2O%)									
Root	1.73±0.066^{a}	2.38±0.028^{b}	2.67±0.034^{c}	2.89±0.023^{d}	3.78±0.045^{e}	2.69	0.04	0.14	3.29
Shoot	3.39±0.008^{a}	3.92±0.006^{b}	4.06±0.003^{c}	4.19±0.024^{d}	4.72±0.030^{e}	4.06	0.02	0.05	0.85
Seed	0.97±0.002^{a}	1.17±0.003^{b}	1.26±0.001^{b}	1.34±0.002^{c}	1.70±0.005^{d}	2.69	0.04	0.14	3.29
Iron(Fe mg/100g)									
Root	1.40±0.027^{a}	1.74±0.025^{c}	1.74±0.022^{c}	1.55±0.023^{cb}	1.94±0.014^{d}	1.67	0.02	0.07	2.62
Shoot	3.01±0.006^{a}	3.86±0.034^{b}	4.70±0.014^{d}	4.45±0.012^{c}	4.87±0.025^{e}	4.18	0.02	0.06	0.97
Seed	2.96±0.004^{a}	3.34±0.009^{c}	3.44±0.009^{d}	3.23±0.002^{b}	3.65±0.002^{e}	1.67	0.02	0.07	2.62
Chlorophyll content (mg/g FW)									
Chl a	1.88±0.04^{a}	2.32±0.07^{b}	2.36±0.08^{b}	2.35±0.02^{b}	2.51±0.09^{b}	2.29	0.07	0.21	0.60
Chl b	1.12±0.07^{a}	1.34±0.10^{b}	1.71±0.22^{b}	1.41±0.03^{c}	2.19±0.34^{d}	1.55	0.05	0.17	6.97
TotalChl(a+b)	3.00±0.11^{a}	3.65±0.10^{b}	4.07±0.29^{c}	3.76±0.05^{b}	4.70±0.30^{d}	3.84	0.11	0.35	5.96

Photosynthetic content

Total chlorophyll content, chlorophyll a, and chlorophyll b were highest in T-5 (consortium), followed by individual treatment T-3, T-4 and lowest in T-2 (Table 6). An interaction between drought stress and beneficial rhizobacteria had a remarkable effect on all physiological features and positively improved the water stress, membrane stability index, proline, chlorophyll, and nitrogen content (Mutumba *et al.* 2018). Numerous PGPR directly controls plant physiology i.e. chlorophyll content, by mimicking synthesis of plant hormones, whereas other increases mineral and nitrogen availability in the soil as a way to augment the crop growth. Rhizobacteria inoculation showed improvement in total chlorophyll and both chlorophyll a and b contents which may positively affect the photosynthesis (Rotaru *et al.* 2015).

It can be concluded from the present study that *Azospirillum lipoferum*, *Bacillus megaterium* and *Rhizobium radiobacter* can enhance the production of leguminous crop when used separately or in combination. However, these strains were more effective when used in combination rather than individual treatment. They were compatible with each other and showed positive synergism towards growth, yield and nutrient uptake of black soybean. Application of mixed strains of bio-inoculants in rainfed leguminous crop of hilly and tarai region of Uttarakhand may seek the attention of agriculturists for better yield and soil fertility through increasing the biotic component and nutrient profile to reduce the risk of chemical fertilizer toxicity.

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