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Seed Biopriming Improved Growth and Morpho-physiological Traits in Early Vegetative Phase of Compact Cotton

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ABSTRACT: Cotton is commonly known as "White gold" and "King of natural fibre" owing to its higher economic value amid the cash crops. The use of plant growth-promoting rhizobacteria (PGPR) is a promising alternative tool to improve plant efficiency for effective utilization of fertilizers by cotton. PGPR as biofertilizer favours increase in crop productivity in terms of yield and quality. Plant bacterial interactions in the rhizosphere are the determinants of plant health and soil fertility. However, more studies into the impact of seed biopriming on cotton plant growth and development is needed. This study aims to evaluate the potential of seed priming with PGPR strains to promote growth in cotton plants. Shoot length, root length, shoot dry matter, root dry matter, total dry matter, plant growth traits, chlorophyll content and index, soluble protein and normalized difference vegetation index were analysed. The highest shoot length and root length was 47.0 and 19.6 cm and the highest shoot and root dry matter was 3.80 g and 0.37 g respectively in the plants treated with strain *Azospirillum* sp7. Similarly, highest chlorophyll content (1.48 mg g^{-1}) and chlorophyll index (38.3) were recorded in the *Azospirillum* sp7 strain treated plants, indicating the possible positive effect on yield and quality of cotton.

Keywords: Cotton, *Azospirillum, Pink pigmented facultative methylotrophs,* phosphobacteria and potash bacteria and growth traits.

INTRODUCTION

Cotton is one of the most important cash crop, which possess unique natural fiber producing ability and it plays a major role in sustainable economy and the livelihood of Indian cotton farming community. It is true that yield increment over the years has achieved by the excessive use of chemical fertilizer. However, the marginal productivity of soil is declining due to the excessive use of chemical fertilizers. In addition to decline in soil fertility, ever increasing population also made sure to use excessive fertilizers and pesticides to meet global food demand, which ultimately results in unfavourable effects on human health. In that case, biofertilizers can be an interesting option in the countenance of degraded agricultural areas and to increase productivity in a sustained manner. The usage of biofertilizers, plant growth promoting rhizobacteria (PGPR) in agriculture substantially reduce the amount of minerals with no losses in crop productivity in a more ecological friendly way. Some species of bacteria, micro algae, yeast, fungi and other organic sources are used as biofertilizers.

Biofertilizer refers to products consisting of selected and beneficial living microbes, which are added to soil as microbial inoculants. These inoculants are effective in enhancing the plant's ability to promote crop health and are widely recognized as plant growth-promoting rhizobacteria (PGPR) (Nascente *et al*., 2019). The application of biofertilizer triggers signalling molecules in primary and secondary metabolism to enrich the plant growth and productivity (Calvo *et al*., 2014). PGPR produces phytohormones such as auxin, cytokinin and gibberellic acid as secondary metabolites promotes plant growth (Katiyar *et al*., 2017) and performs specific functions, notably nitrogen fixation, siderophore production, phosphate solubilization and potassium mobilizing ability that leads to plant growth promotion. Applying microorganisms to seed is an attractive option because of the combination of specific effect and limited environmental impact. Biopriming of seed is an emerging technique to enhance the seed quality by soaking the seeds in the bacterial suspension for precalculated period of time to allow the bacterial imbibition into the seed (Abuamsha *et al*., 2011). Seed treatment has the potential to deliver agents "in the right amount, at the right place, and at the right time" (Chandra and Greep, 2010).

Liquid bacterial cultures *viz*., *Azospirillum, Pseudomonas, Azotobacter* (Shaukat *et al*., 2006) and *Methylobacterium* (Nkpwatt *et al.,* 2006) are found to promote seed germination and seedling growth. Phosphate solubilizing bacteria (PSB) and potash bacteria improves overall soil health by reducing phosphate and potassium deficiency in soil. Thus, seed treatment not only plays a dominant role in improving the seed quality, it also aids in the better crop establishment during the early vegetative phase, which contributes to the higher productivity. Single and co-inoculation of PGPR have been proven to increase the seed metabolism and hastens germination and increased the seedling establishment. The present study focuses on evaluation of different biofertilizer strains on the morpho-physiological traits and early vegetative growth in compact cotton.

MATERIALS AND METHODS

Seed source and general experimental details

Seed of a newly released compact cotton (CO 17 variety) was obtained from the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore. The experiment was conducted at Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, located in the Western Agro-climatic zone of Tamil Nadu (11° 02′N latitude, 76°93′E longitude and at an altitude of 428.5 metres above mean sea level). The experiment was laid out in the completely randomized block design in August 2021 with six treatments and four replicates. Experimental soil was sandy clay loam with pH 7.91 and the crop was supplied with fertilizers and other cultivation operations as per recommended package of practices of Tamil Nadu Agricultural University, Coimbatore were followed.

Seed biopriming. Four PGPR strains *i.e., Azospirillum* sp7*,* Phosphobacteria PS1, Potash releasing bacteria KRB9 and *Pink pigmented facultative methylotrophs* TNAU1 containing *Azospirillum brasilense* sp7 strain*, Bacillus megaterium* PS1 strain*, Bacillus mucilaginosus* KRB9 strain and *PPFM* TNAU1 strain were collected from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. The treatments include T1 - Control, T2 - *Azospirillum* sp7 (50 ml/acre of seeds), T3 - *Pink Pigmented Facultative Methylotrophs (PPFM)* TNAU 1 (50 ml/acre of seeds), T4 – *Azospirillum* sp7 + Phosphobacteria PS1 + Potash releasing bacteria KRB9 (50 ml/acre of seeds), T5 – *PPFM* TNAU1+ Phosphobacteria PS1 + Potash releasing bacteria KRB9 (50 ml/acre of seeds), T6 – *PPFM* TNAU1+ Phosphobacteria PS1 + *Azospirillum* sp7 + Potash releasing bacteria KRB9 (50 ml/acre of seeds). Cotton seeds were bioprimed using 2% CMC (Carboxymethyl Cellulose) solution and then shade dried prior to sowing.

Data collection. The effects of bio-stimulants on morphological, physiological and biochemical characteristics were observed during early vegetative stage (squaring stage).

Shoot length was measured from the ground soil level to the tip of the terminal leaf and denoted in centimeters (cm).

Root length was calculated by measuring distance from bottom of the shoot to the longest end of the root and expressed in cm.

The main stem nodes and internodes were counted from labelled plants. The average number of main stem nodes and internodes per plant was worked out. The reproductive sympodia arising from extra axillary buds were expressed in numbers per plant.

Four plants at random were removed for the estimation of dry matter production (DMP). Plants were collected, and shoots and roots were separated, washed in running water and placed into paper bags. The samples were initially air dried and subsequently oven dried at 65°C for 72 hours until a constant weight was reached. Dry matter was then determined using an analytical balance and expressed as g $plant^{-1}$.

The number of leaves was recorded and expressed as number per plant.

Leaf area per plant was measured using a Leaf area meter (LICOR, Model LI 3000) and expressed as cm^2 $plant^{-1}$.

The leaf area index of the plant was computed as per the procedure suggested by Williams, (1946) by using the following formula

$$\text{LAI} = \frac{\text{Total leaf area of the plant}}{\text{Ground area occupied by the plant (spacing)}}$$

Specific leaf weight of the samples were computed according to Nelson and Schweitzer, (1988) by using the formula,

$$\text{SLW} = \frac{\text{leaf dry weight}}{\text{leaf area}}$$

A portable chlorophyll meter (Minolta SPAD, Model 502) was used to record chlorophyll index under field condition. Four readings were taken from each replication and the average values were computed using the method described by Monje and Bugbee, (1992), Green Seeker®, a crop sensor with an active light source optical sensor was used to assess plant biomass, plant canopy measurement and displayed as Normalized Difference Vegetation Index (NDVI, Trimble).

The photosynthetic pigments were determined using DMSO method. Spectrophotometer readings at 645 and 663 nm were performed according to Hiscox and Israelstam (1979). Three plants were randomly chosen from each treatment for the analysis. Samples were collected from the third leaf at two-thirds the height of the plant, as measured from the base and at 1 cm from the leaf margin and values were expressed as mg g^{-1} of fresh weight.

Soluble protein content in the leaf was estimated by following Lowry *et al.,* (1951) with folin ciocalteau reagent and expressed as mg g^{-1} fresh weight.

Data were analysed by using the software SPSS Statistics (version 16.0) and a comparison of means were done at 5 % significance level with Duncan's multiple range test.

RESULTS AND DISCUSSION

Shoot and root length is an important morphological trait, where nodes and internodes arise to determine the morphological frame work of the plant. Experimental result from Fig. 1 disclosed that plants treated with PGPR strains showed significant improvement in shoot and root length. The data clearly indicated that, the plants receiving strain of *Azospirillum* sp7 (T2) showed maximum shoot length followed by *PPFM* TNAU1 (T3) inoculated plants *i.e*., 47.0 cm and 46.4 cm respectively and maximum root length was seen in *Azospirillum* sp7 (T2) and *PPFM* TNAU1 (T3) strain inoculated plants (19.6 cm). However, shoot and root length was higher in the combined inoculation of the strains T4 – *Azospirillum* sp7 + Phosphobacteria PS1 + Potash releasing bacteria KRB9*,* T5 – *PPFM* TNAU1+ Phosphobacteria PS1 + Potash releasing bacteria KRB9 and T6 – *PPFM* TNAU1+ Phosphobacteria PS1 + *Azospirillum* sp7 + Potash releasing bacteria KRB9 over the uninoculated control plants (T1 - 36.3 cm and 13.5 cm). The shoot length was increased by 29.5% and 28.0% in *Azospirillum* sp7 (T2) and *PPFM* TNAU1 (T3) strains inoculated plants respectively over the control. The inoculation of PGPR strains increased the production of phytohormones like auxin, gibberellins and cytokinin and nutrient availability which in turn involved in cell elongation and caused growth of the crop, which resulted in higher shoot length (Singh *et al*., 2015). *Azospirillum* sp7 inoculated plants, known to synthesis hormone Indole-3 Acetic Acid (IAA) abundantly, which is mainly involved in cell elongation that contributed for higher shoot length compared to other strains. The plants treated with individual strains of *Azospirillum* sp7 (T2) and *PPFM* TNAU1 (T3) had recorded about 29.5 and 28.0% increment in shoot length and 45.5 and 45.1% increment in root length respectively. Similiar findings were reported by Efthimiadou *et al.,* (2020); Hamed *et al*., (2019) in maize. Dhale *et al.,* (2010) reported increased shoot length with the application of bioinoculants (*Azospirillum, Azotobacter*, PSB and PGPB) in cotton. Our results are in accordance with Taha *et al.,* (2020); El-Gamal *et al.,* (2020). The increase in shoot length resulted in increasing plant height by influencing cell metabolism and improved nutrient uptake. Our results were in accordance with Dhale *et al*., (2010); Pindi *et al.,* (2014) where plant growth promoting bacterial (PGPB) inoculation stimulates the production of plant growth hormones that favours root growth and alters root morphology.

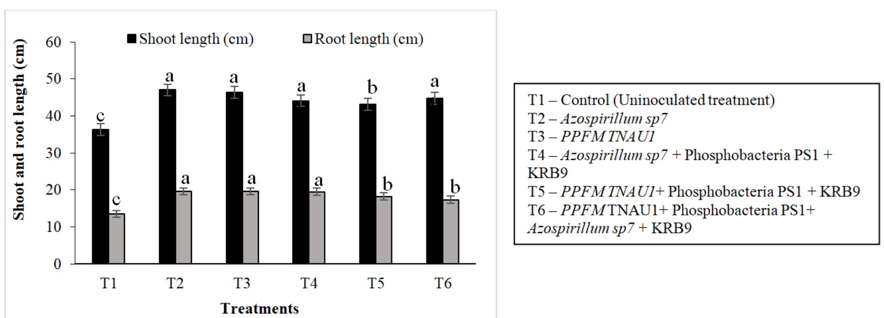

Fig. 1. Effect of individual and combined inoculation of PGPR strains on shoot length and root length of compact cotton. Values are mean of replicates; values followed by the same letter in each column are not significantly different from each other as determined by DMRT ($p \leq 0.05$).

The number of nodes and internodes of main stem was high in PGPR strains inoculated plants than control plants (Fig. 2). The *Azospirillum* sp7 (T2) inoculation caused significantly greater number of nodes (9.3) and internodes (5.50) followed by the treatment T3, *PPFM* TNAU1 strain inoculated plants (9.0 and 5.25 respectively). The treatment T4, combined inoculants of *Azospirillum* sp7 + Phosphobacteria PS1+ Potash bacteria KRB9 was on par with the treatment T3, *PPFM* TNAU1 with respect of number of nodes (9.0) in main stem. However, there was no significant difference among the treatments in number of internodes in main stem. The inoculation of *Azospirillum* sp7 and *PPFM* TNAU1 strain resulted in an increment of 37.0% and 33.3% in the number of nodes, and 29.41% and 23.53% in the number of internodes respectively.

Leaf number and leaf area have reflective outcome on the production of crop canopy. Leaf area is an important component that is closely related to the physiological process controlling dry matter production and yield. Experimental results revealed that, plants treated with PGPR showed substantial improvement in number of leaves and leaf area (Table 1).

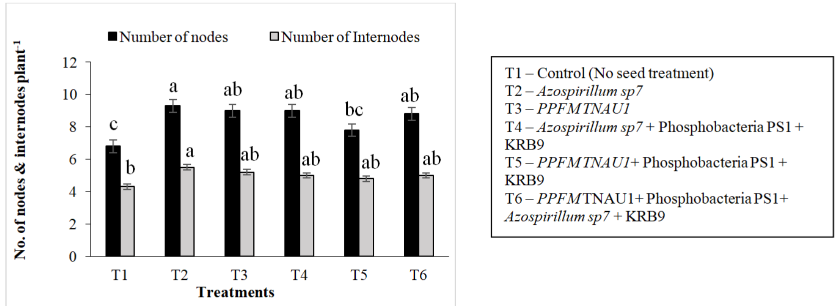

Fig. 2. Effect of individual and combined inoculation of PGPR strains on number of nodes and internodes in main stem of compact cotton. Values are mean of replicates; values followed by the same letter in each column are not significantly different from each other as determined by DMRT ($p \leq 0.05$).

The treatment T3, *PPFM* TNAU1 strains inoculated plants exhibited a greater number of leaves (20.5) and hence recorded higher foliage size (432.92 cm^2) followed by the treatment T2, *Azospirillum* sp7 strain inoculated plants (19.8 and 416.60 cm^2). There was 28.13 and 21.35% increase in number of leaves and leaf area in inoculated plants over the control plants.

The increase in leaf number and leaf area resulted in increased growth traits such as Leaf Area Index (LAI) and Specific Leaf Weight (SLW). LAI is the ratio of green leaf laminae to ground area, often used to characterize a crop's ability to capture photosynthetic active radiation (PAR). SLW signifies the thickness of leaf which consequently had positive correlation with photosynthetic rate (Ashwathama *et al.*, 2003). Similarly, data furnished in the Table 1 showed that the plants treated with *PPFM* TNAU1 strain (T3) recorded more LAI (0.32) and SLW (4.71 mg cm^{-1}) followed by the *Azospirillum* sp7 strain (T2) inoculated plants (0.31 and 4.32 mg cm^{-1}). Sufficient quantity of nutrients and phytohormones facilitated the plants to produce more leaves through higher cell division of leaf primordia in the PGPR inoculated plants (Reddy *et al.,* 1990). The percent increment in LAI and SLW was 21.35, 16.55 and 26.98, 16.49% in *PPFM* TNAU1 and *Azospirillum* sp7 strain treated plants respectively over the control. Increased nutrient availability mainly nitrogen and phosphorus and increased hormone activity *i.e.,* auxin, gibberellins and cytokinins in the PGPR inoculated plants increases the foliage size by regulating the cell division and differentiation. PGPR strains are reported to increase the allocation of photosynthetic assimilate to foliage development and hence improved the plant nutritional status causing higher leaf weight or leaf area. The observations in the present study are in corroboration with the findings of El-Gamal *et al.*, (2020); Chakma *et al.,* (2021); Porter, (1989).

Table 1: Effect of individual and combined inoculation of PGPR strains on plant growth and physiological traits of compact cotton. Values are mean of replicates; values followed by the same letter in each column are not significantly different from each other as determined by DMRT ($p \leq 0.05$).

Treatments	No. of leaves plant^{-1}	Leaf Area (cm^2)	Leaf Area Index	Specific leaf weight (mg cm^{-2})
T1 – Control	16.0^{c}	356.76^{c}	0.26^{c}	3.71^{b}
T2 – *Azospirillum*	19.8^{ab}	416.60^{ab}	0.31^{ab}	4.32^{ab}
T3 – PPFM	20.5^{a}	432.92^{a}	0.32^{a}	4.71^{a}
T4 – *Azospirillum* + PSB + KRB	18.8^{ab}	404.04^{ab}	0.30^{ab}	4.12^{ab}
T5 – PPFM + PSB + KRB	18.3^{b}	384.32^{bc}	0.28^{bc}	3.85^{b}
T6 – PPFM + PSB+ *Azospirillum* + KRB	17.8^{bc}	387.61^{bc}	0.29^{bc}	3.74^{b}
Mean	18.53	397.04	0.29	4.08
SEd	0.90**	15.16**	1.12**	0.32**
CD (*P*=0.05)	1.85	31.86	2.36	0.67

The total biomass production is a function of photosynthesis and respiration rate. The shoot and root dry weight of cotton plant was significantly improved with the inoculation of PGPR strains. From Fig. 3, we could infer that these individual factors contributed for the increase in the total dry matter content in the *Azospirillum* sp7 strain treated plants. Among the treatments, T3 *PPFM* TNAU1 and T2 *Azospirillum* sp7 strain brought significantly maximum shoot (3.794 and 3.550 g per plant) and root biomass (0.374 and 0.369 g per plant) compared to other co-inoculated treatments and control (Table 2). The plants treated with individual strains of *Azospirillum* sp7 (T2) and *PPFM* TNAU1 (T3) had recorded about 46.5 and 37.2% increment in shoot biomass and 44.1 and 42.4% increment in root biomass respectively. This increase in shoot and root biomass is due to higher shoot length, number of leaves and alteration in root morphology.

Table 2: Effect of individual and combined PGPR strains in plant growth and physiological traits of compact cotton. Values are mean of replicates; values followed by the same letter in each column are not significantly different from each other as determined by DMRT ($p \leq 0.05$).

Treatments	TDMP (g plant^{-1})		Soluble Protein (mg g^{1})	NDVI	No. of fruiting branches plant^{-1}
	Shoot weight	Root weight			
T1 – Control	2.59^{c}	0.26^{c}	12.7^{b}	0.75^{b}	4.3^{b}
T2 – *Azospirillum*	3.55^{a}	0.37^{ab}	15.4^{a}	0.78^{ab}	5.5^{a}
T3 – PPFM	3.79^{a}	0.37^{a}	15.3^{a}	0.80^{a}	5.0^{ab}
T4 – *Azospirillum* + PSB + KRB	3.11^{b}	0.35^{ab}	13.7^{a}	0.79^{a}	4.8^{ab}
T5 – PPFM + PSB + KRB	2.89^{bc}	0.29^{bc}	14.8^{ab}	0.79^{a}	4.8^{ab}
T6 – PPFM + PSB+ *Azospirillum* + KRB	2.85^{bc}	0.29^{abc}	13.6^{ab}	0.78^{ab}	5.0^{ab}
Mean	3.13	0.32	30.9	0.78	4.9
SEd	0.16**	3.31*	0.95	1.47	0.51
CD (*P*=0.05)	0.34	7.06	1.99	3.09	1.06

T1 - Control, T2 - *Azospirillum* sp7, T3 - *Pink Pigmented Facultative Methylotrophs (PPFM)* TNAU 1, T4 – *Azospirillum* sp7 + Phosphobacteria PS1 + Potash releasing bacteria KRB9, T5 – *PPFM* TNAU1+ Phosphobacteria PS1 + Potash releasing bacteria KRB9, T6 – *PPFM* TNAU1+ Phosphobacteria PS1 + *Azospirillum* sp7 + Potash releasing bacteria KRB9.

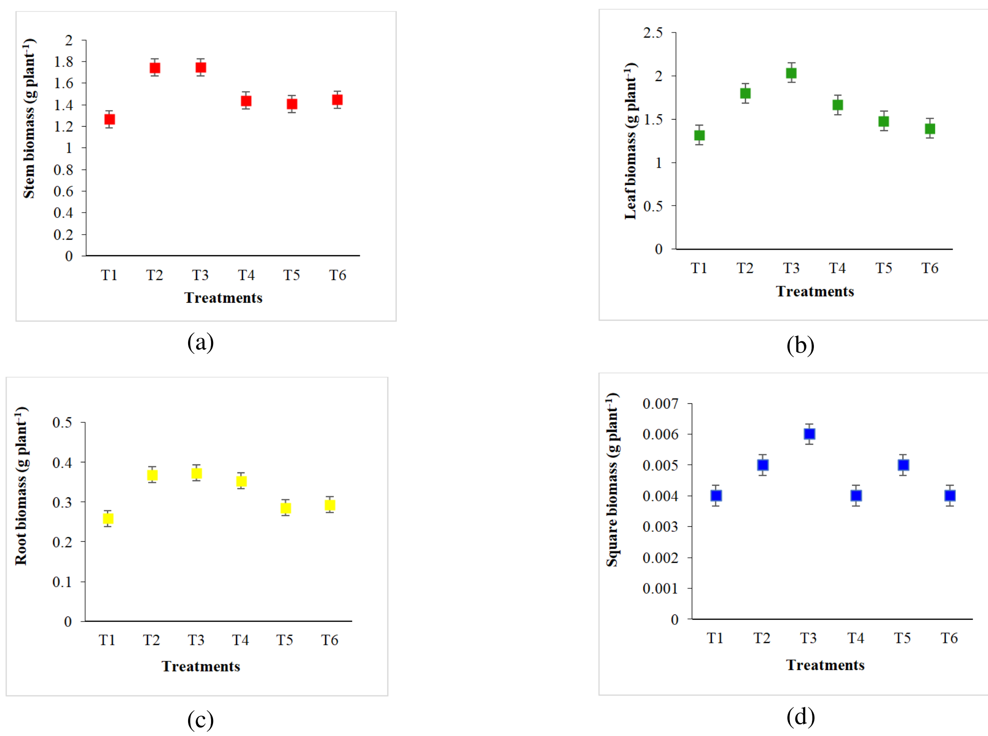

Fig. 3. Effect of individual and combined inoculation of PGPR strains on total dry matter content of compact cotton. (a) Stem biomass (g plant^{-1}) (b) Leaf biomass (g plant^{-1}) (c) Root biomass (g plant^{-1}) (d) Square biomass (g plant^{-1}). Values are mean of replicates; values followed by the same letter in each column are not significantly different from each other as determined by DMRT ($p \leq 0.05$).

The present study is in concordance with (Dhale *et al*., 2010; Pindi *et al.,* 2014; Calvo *et al*., 2017) where significant improvement of shoot and root biomass of cotton seedlings was observed in PGPR inoculated plants over uninoculated plants. Chlorophyll index is one of the important parameters for understanding the nitrogen and photosynthetic pigment status of plants. Estimation of total chlorophyll content is necessary to understand the photosynthetic process and assimilate production. Our results revealed that plants inoculated with PGPR strain significantly improved the accumulation of photosynthetic pigments, chlorophyll content and chlorophyll index over untreated control plants (Fig. 4). Total chlorophyll index and chlorophyll content were recorded higher in *Azospirillum* sp7 strain (T2) inoculated plants (38.30 and 1.48 mg g^{-1}) followed by *PPFM* TNAU1 strain (T3) inoculated plants (38.25 and 1.46 mg g^{-1}). The plants treated with individual strain of *Azospirillum* sp7 (T2) and *PPFM* TNAU1 (T3) had recorded about 11.0% and 10.9% increment in chlorophyll index and 35.4 and 33.5% increment in total chlorophyll content respectively.

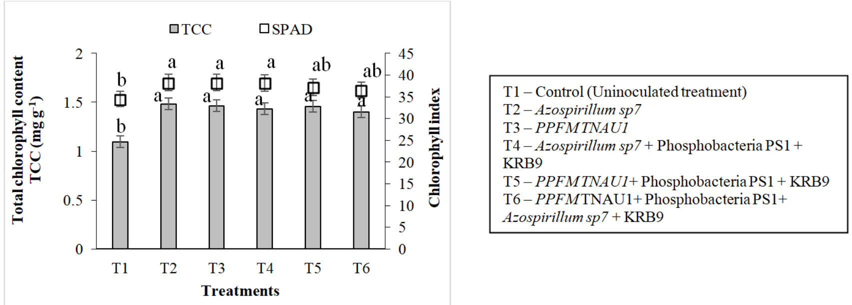

Fig. 4. Effect of individual and combined inoculation of PGPR strains on total chlorophyll content (mg g^{-1}) and chlorophyll index of compact cotton. Values are mean of replicates; values followed by the same letter in each column are not significantly different from each other as determined by DMRT ($p \leq 0.05$).

These findings are in conformity with the outcomes of Hamed *et al*., (2019); Compant *et al.,* (2005). Our results of increased chlorophyll index and total chlorophyll content in plants with single and combined inoculation of PGPR were supported by the above findings. Soluble protein serves as an indicator of photosynthetic efficiency in terms of the RuBP (Ribulose-1,5 bisphosphate) carboxylase activity (Evans, 1975). The plants treated with individual strain of *Azospirillum* sp7 (T2) and *PPFM* TNAU1 (T3) recorded greater protein content than the co-inoculated (T4, T5 and T6) plants (Table 2). The treatment *Azospirillum* sp7 strain recorded higher soluble protein content followed by *PPFM* TNAU1 (T3) treated plants (15.4 and 15.2 mg g^{-1}). The inoculation of *Azospirillum* sp7 (T2) and *PPFM* TNAU1 strain (T3) had recorded about 21.5 and 20.1% increase in soluble protein content compared to control plants. The findings of Lotfi *et al*., 2015 and Al-Arjani *et al*., 2020 are in accordance with our present study.

Normalized difference vegetation index (NDVI) is a measure of the status of plant health-based on the capacity of the plants to reflect light at certain frequencies. It indicates the presence of chlorophyll and photosynthetic capacity, which correlates with plant health (Wu *et al*., 2016). The treatment T3, plants inoculated with individual strain of *PPFM* TNAU1 recorded maximum NDVI values (0.80) followed by the treatment T4, co-inoculation of *Azospirillum* + Phosphobacteria + Potash bacteria strains (0.79), which indirectly reveals the presence of more chlorophyll a and hence improvement in plant health (Table 2). Single and co-inoculation of the PGPR strains had recorded higher NDVI values than non-inoculated control plants. The present study is in line with the findings of Hou *et al.,* (2019) and it is understood that the overall plant health could be assessed using NDVI.

Generally, in cotton, increase in plant height is accompanied by increase in sympodial branches (fruiting branches). The individual and combined inoculation of these PGPR strains resulted in the production of higher number of fruiting branches per plant (Table 1). The treatment T2, *Azospirillum* sp7 strain inoculated plants recorded higher number of sympodial branches (5.50) than other individual and combined inoculants of PGPR strain and control. There was about 29.5% increase in the number of fruiting branches per plant in the *Azospirillum* sp7 strain (T2) inoculated plants. Individual treatments of *Azospirillum* sp7 and *PPFM* TNAU1 strains also increased the number of fruiting branches. Similar finding is reported by Hamed *et al*., 2019, where two bacterial strains significantly improved the number of fruiting branches over uninoculated control. The increase in plant height, root length, dry matter, chlorophyll index and photosynthetic pigments helped in improving the number of sympodial branches (fruiting branches), which ultimately results in higher number of squares.

CONCLUSION

The results obtained in the present study revealed that, plants bioprimed with PGPR strains significantly enhanced the growth of cotton in terms of morphological, physiological and biochemical parameters. Among the different inoculants, seeds treated with individual PGPR strains improved shoot length, root length, shoot and root dry mass, total chlorophyll content, leaf soluble protein, chlorophyll index, NDVI and number of fruiting branches during the early vegetative period of crop growth. The increase in fruiting branches directly correlated with the increase in boll number and yield. Hence, the tested PGPR strains could be used as biostimulants/biofertilizers to improve cotton yield and quality. It is concluded that the beneficial microbes tested as biopriming agents in the current investigation bears physiological capacity to improve plant growth and development in cotton.

FUTURE SCOPE

Plant growth enhancement due to PGPRs suggest that they are employed to improve the overall plant growth, physiological potential and to increase the yield in cotton. In order to provide practical recommendations to farmers supporting the agricultural crops, the PGPR strains need to be investigated in different dimensions of types, quantity, timing and the number of applications, with reference to crop specificity.

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Conflict of interest. None.

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