Influence of microbial priming on germination and seedling growth traits of compact cotton CO17

ABSTRACT

Cotton, known as "the King of fibers", is the predominant fibre in the Indian textile industry. Plant growth-promoting rhizobacteria (PGPR) represent a potential sustainable alternative for the enhancement and protection of crops. The germination and seedling growth of cotton can be optimized by inoculating with PGPR. An experiment was conducted to evaluate the effect of different PGPR strains on seed germination and seedling establishment characters on cotton. The highest germination percentage, maximum vigour index and leaf area was obtained with the *PPFM* TNAU1 strain inoculation. The maximum shoot and root length were observed with seeds treated with *Azospirillum* strain sp7 with an increase of 24.4 and 42.8 % over the control. Underground fresh and dry matter was higher in seedlings treated with *Azospirillum* sp7 strain compared to control, while the *PPFM* TNAU1 strain treatment increased the aerial fresh and dry matter content because of its larger leaf area. Seeds inoculated with individual strain of *Azospirillum* sp7 and *PPFM* TNAU1 outperformed the combined inoculation of PGPR strains. The increase in germination traits and seedling characters by PGPR strains indicates the positive influence on improving cotton seedling establishment traits associated to higher yield.

Keywords: Cotton, plant growth promoting rhizobacteria, microbial priming, germination and seedling emergence traits.

1. INTRODUCTION

Cotton is one of the predominant commercial crops with global significance, playing an important role in foreign exchange and industrial economy. Current production of cotton fiber is not sufficient to meet the increasing demand of world population which arises due to many limiting factors, *i.e.,* droughts, soil degradation, salinity, and alkalinity. To date, many farming practices were employed to increase cotton production, as a consequence, toxic impacts were implied on water and soil resources. In addition, a major part of the chemical fertilizers applied to the crops remain in the soil as insoluble inorganic compounds which promote soil toxicity. Lately, efforts have been focused on minimizing the use of chemical fertilizers in order to optimize cost of production and protect the environment against pollution without compromising the seed cotton yield. The use of biological stimulators in Indian agriculture has many economic and ecological advantages. The search for alternative solutions has stimulated research to take a second look at the range of microorganisms which provide benefits to agricultural production by stimulating plant growth and producing higher yield [1,2].

Plant growth promoting rhizobacteria (PGPR) are bacteria living in the rhizosphere which interacts with plant metabolism and improving their growth PGPR inoculants are considered as a part of integrated nutrient management system to improve plant growth and development. The use of beneficial bacteria such as *Azotobacter, Azospirillum, Acetobacter, Pseudomonas, Methylotrophs, Bacillus, Phosphobacteria etc.* colonize plant root and promotes growth through nitrogen fixation, phosphorus and potassium solubilization. Seed priming by the microbial inoculants favours increased germination activating germination related enzymes, increasing metabolism that helps in the rapid growth of radicle and plumules. These PGPRs help in improve seed germination and colonizes plant roots improving seedling vigour, and modifies root morphology to facilitate better acquisition of nutrients increasing crop yield and quality.

Higher production and productivity of the crop is achieved through the use of good quality seed and proper management practices in any cultivar. Good quality seed implies vigour, uniformity and structure, in addition to genetic and physical purity. Seed priming, identified as an effective seed invigoration method has become a common seed treatment to increase the rate and uniformity of germination and crop establishment. The present study was conducted with the objective to evaluate different PGPR strains on cotton seed germination, seedling emergence and growth traits.

2. MATERIAL AND METHODS

2.1. Experimental site and soil characteristics

The field experiment was conducted at Eastern block farm, Tamil Nadu Agricultural University, Coimbatore, located in the Western Agro-climatic zone of Tamil Nadu (11° 02' N latitude, 76° 93' E longitude, and an altitude of 428.5 masl). The experiment was planted following a randomized block design with four replications. Experimental soil was sandy clay loam with pH 8.49 and organic matter content 0.47%. Newly released compact cotton seeds (CO 17 cultivar) were obtained from the Cotton Department, Tamil Nadu Agricultural University, Coimbatore. Planting was done in rows 90 cm apart and plant to plant 15 cm spacing by placing the seeds at each hill. Irrigation was given as required. Standard plant protection schedule was followed to protect the crop from diseases and pests as per recommended package of practices of Tamil Nadu Agricultural University, Coimbatore.

2.2. Seed treatment details

Four PGPR strains included *Azospirillum* sp7, Phosphobacteria PS1, Potash releasing bacteria KRB9 and *Pink pigmented facultative methylotrophs* TNAU 1 were obtained 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). *Azospirillum, PPFM, Phosphobacteria* and Potash releasing bacteria are the commercial formulations of biofertilizers which contains *Azospirillum brasilense* sp7 strain, *PPFM* TNAU1 strain, *Bacillus megaterium* PS1 strain and *Bacillus mucilaginosus* KRB9 strain, respectively. Cotton seeds were bioprimed using 2% CMC (Carboxymethyl Cellulose) solution and shade dried before planting.

2.3. Data collection

The efficiency of different PGPR strains as bio-inoculants as an individual and interactive effect on seed germination efficiency, seedling emergence and growth in cotton were recorded.

2.3.1. Germination percentage - GP (%)

Final germination percentage was calculated by dividing the number of seeds germinated by the total number of seeds and expressed in % [3].

2.3.2. Mean germination time - MGT (day)

Mean germination time (MGT) was given by [4].

$$\text{MGT} = \sum\left(\frac{ni*ti}{ni}\right)$$

where ni is the number of germinated seeds on germination days, ti is the number of days during the germination period (between 0 and 10 days)

2.3.3. Germination rate index - GRI (Germination % day^{-1})

The germination rate index was computed by the formula given by [5]

$$\text{GRI} = \frac{G1}{1} + \frac{G2}{2} + \frac{GX}{X}$$

where G1=Germination percentage at the first day after sowing, G2= Germination percentage at the second day after sowing.

2.3.4. Coefficient of velocity of germination - CVG

The coefficient of velocity of germination was calculated by the formula given by [6]

$$\text{CVG} = \frac{\sum ni}{\sum ni*ti} * 100$$

where ni is the number of germinated seeds on day ti; ti = the number of days during the germination period (between 0 and 10 days)

2.3.5. Germination index - GI

The germination index (GI) was calculated based on the formula of [7]

$$\text{GI} = (10 * n1) + (9 * n2) + \cdots + (1 * n10)$$

where n1 = number of seedlings emerging on first day after planting; n2 = number of seedlings emerging on second day; n10 = number of seedlings emerging on tenth day.

2.3.7. Seedling vigour index - SVI

The vigour index value was obtained by multiplying germination of seeds in percentage and total seedling length in centimeter and expressed in whole number as described by [8].

2.3.8. Root length and shoot length (cm)

Root and shoot length were measured at 10 d old seedlings and expressed in centimeters (cm)

2.3.9. Leaf Area (cm^2)

Leaf samples collected from each replication were cleaned and inserted into a leaf area meter (LICOR, Model LI 3000) and leaf area measured was expressed as cm^2 per plant.

2.3.10. Aerial and underground weight (g $seedling^{-1}$)

Fresh aerial and underground weight of 10 d old seedlings were taken and expressed in g $seedlings^{-1}$. Seedlings used for growth measurement were placed in a paper cover and dried in shade for 24 h and then placed in a hot air oven at 65 ˚C for 48 h. The dried seedlings were weighed to estimate the aerial and underground dry matter production and the mean values were expressed in mg $seedlings^{-1}$.

2.4. Statistical analysis

Data were analysed using the software SPSS Statistics (version 16.0) and XLSTAT version 2019.2.1 (XLSTAT, 2019) and comparison of means were done at 5 % significance level with Duncan's multiple range test. The principal component analysis (PCA) [9] was performed with seed germination and growth traits as influenced by different PGPR inoculation in cotton.

3. RESULTS AND DISCUSSION

3.1. Effect of PGPR strains on germination efficiency

Inoculation with different PGPR strains significantly influenced the germination efficiency of cotton seeds. Experimental results in Table 1 revealed that seeds treated with PGPR strains showed significant improvement in germination percentage. Cotton seeds inoculated with strain of *PPFM* TNAU 1 (T3) showed maximum germination percentage followed by seeds inoculated with *Azospirillum* sp7 strain (T2) with 96.8 and 95.3 %, respectively. Likewise, cotton seeds treated with different PGPR strains positively influenced the mean germination time (Fig. 1). Mean germination time determines how faster the seed germinates in shorter period of time [10]. The mean germination time was lower in the strains treated with *PPFM TNAU1* (T3) (5.6 d) followed by *Azospirillum* sp7 (T2) strain (5.9 d) treated seeds whereas seeds inoculated with both *Azospirillum sp7* + Phosphobacteria PS1 + Potash bacteria KRB9 (T4) strains resulted in higher mean germination value of 6.3 d. Seeds treated with individual strains of *PPFM TNAU1* and *Azospirillum* sp7 strain recorded about 16.2 and 14.4 % increase, respectively, over the control. Results obtained coincide with those obtained by Prathibha and Siddalingeshwara [11]n and Sirohi *et al*. [12] where seed inoculation of *Bacillus sp.* and *Pseudomonas* significantly improved germination percentage and rate of germination in sorghum and wheat seeds. Similar findings were reported by Nehra *et al*. [13], El-Sheekh *et al*. [14], Gowtham *et al*. [15] and Roman-Ponce *et al.* [16]

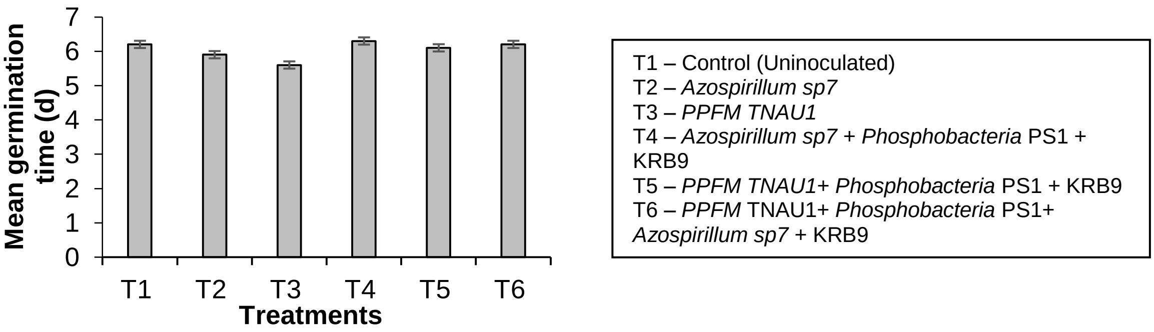

Fig.1. Effect of PGPR strains on mean germination time (MGT) (d) on cotton seeds.

Inoculation of PGPR strains showed significant improvement in germination index (GI), germination rate index (GRI) and coefficient of velocity of germination (CVG) (Fig. 2. and Fig. 3.). Cotton seeds inoculated with *PPFM TNAU* 1 (T3) strain showed maximum germination index (354.3) followed by the treatment T2, *Azospirillum* sp7 strain (337.3). GI accentuates both germination percentage and speed. Higher GI values indicate a higher germination percentage and rate at which germination occurs [7]. Germination rate index was higher in the treatment T3, *PPFM* TNAU1 strain followed by the treatment T2 *Azospirillum* sp7 strain treated seeds *i.e.,* 17 % day^{-1} and 16.2 % day^{-1}, respectively. The GRI measures the percentage of seeds that germinate on each day of the germination period, with higher GRI values indicate faster germination [5]. Similarly, the coefficient of velocity of germination also recorded higher in the (T3) *PPFM TNAU* 1 strain (17.8) inoculated seeds. The CVG indicates the speed at which seeds germinate. It increases as the number of germinated seeds increases while time needed for germination declines [6]. The inoculation of *PPFM* TNAU1 and *Azospirillum* sp7 strain recorded about 27.1 and 21 %, 23.5 and 18.3 %,10.7 and 4.8 % increase in germination index, germination rate index and coefficient of velocity of germination over the uninoculated control, respectively. The increased germination efficiency is due to the synthesis of gibbereline, the hormone which stimulates activity of alpha-amylase, the germination-specific enzyme and protease and nuclease mainly involved in starch breakdown and assimilation [17], and mitochondrial enzyme activities [18].The present study coincides with results of Xiao *et al*. [19], and Hossain *et al*. [20] in which PGPR inoculation significantly improved germination percentage and germination rate index over the control in rice. Similar findings were reported by Makhaye *et al.* [21], and Hamidi *et al.* [22], where seed inoculation of PGPRs significantly improved the germination traits such as germination percentage (GP), germination index (GI), germination rate index (GRI), mean germination time (MGT) and coefficient of velocity of germination (CVG) in maize. However, the increased germination traits were also observed in combined inoculants of PGPR strains. The results of the present study are similar to the findings of Anitha [23] and Meena *et al.* [24].

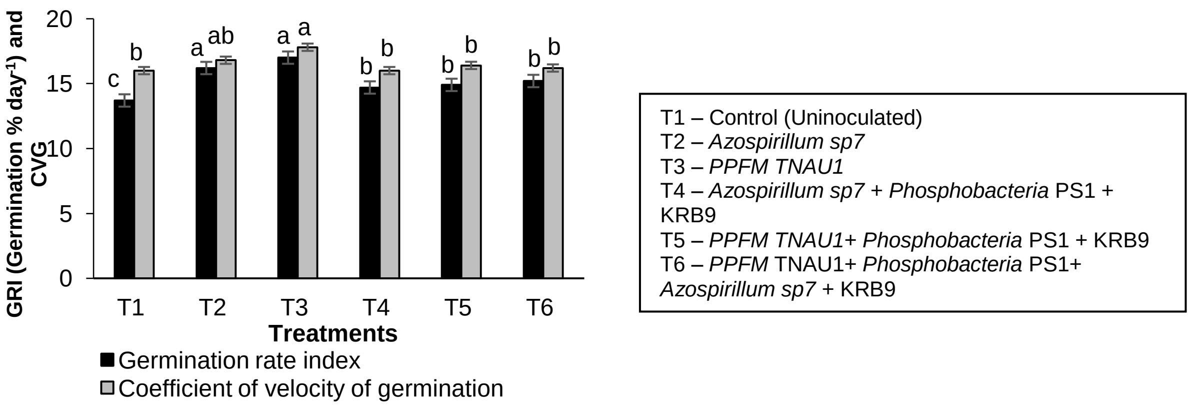

Fig. 2. Effect of PGPR strains on germination rate index GRI (Germination % day^{-1}) and coefficient of velocity of germination of cotton seeds.

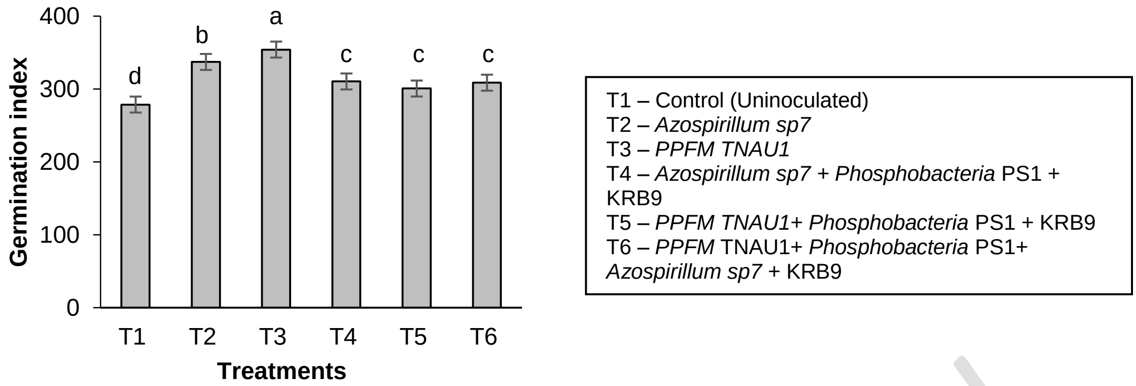

Fig. 3. Effect of PGPR strains on germination index (GI) of cotton seeds.

3.2. Effect of PGPR on seedling emergence traits

Cotton seeds treated with different PGPR strains exhibited significant improvement in the seedling emergence traits such as shoot and root length (Fig. 4.), and seedling vigour index (Table 1). Results obtained indicate that shoot and root length of PGPR inoculated seedlings were significantly higher compared to the uninoculated control (Fig. 4). The treatment of *Azospirillum* sp7 strain (T2) inoculated seeds resulted in maximum shoot and root length (12.0 and 6.0 cm, respectively) followed by the treatment 3 (*PPFM TNAU*1) with 11.9 and 5.8 cm, respectively). The combined inoculation of PGPR strains (T4, T5 and T6) resulted in higher shoot and root length compared to the noninoculated control. The percent increment in shoot and root length was 24.4 and 42.8 % in the *Azospirillum* sp7 (T2) strain inoculated seeds over the uninoculated control. PGPR inoculation stimulates the production of the hormone IAA (Indole-3 Acetic Acid) acquiring more nutrients such as nitrogen and phosphorus, which, in turn, increases root and shoot length [25]. In additions of increasing shoot and root length, IAA is responsible for cell elongation in *Azospirillum* treated plants. The results are similar to those obtained by Efthimiadou *et al.* [26], Taha *et al.* [27], El-Gamal *et al.* [28], Dhale *et al*. [29], Zamioudis *et al*. [30], and Pindi *et al.* [31], where plant growth promoting bacterial (PGPB) inoculation stimulates the production of plant growth hormones that favours root growth and alters root morphology. The plants inoculated with the treatment T3, *PPFM* TNAU1 strain resulted in higher seedling vigour index (1714.4) as it has higher germination percentage, followed by treatment T2, *Azospirillum* sp7 strain (1709.6) which showed higher values in shoot and root length compared to other treatments and control. Results obtained by Noumavo *et al*. [32] are similar with results obtained in the present study. This enhanced seedling vigour index could be associated with greater production and metabolism of hormones, auxin and cytokinin which primarily promotes the cell elongation and cell division induced by PGPR inoculation [33,34].

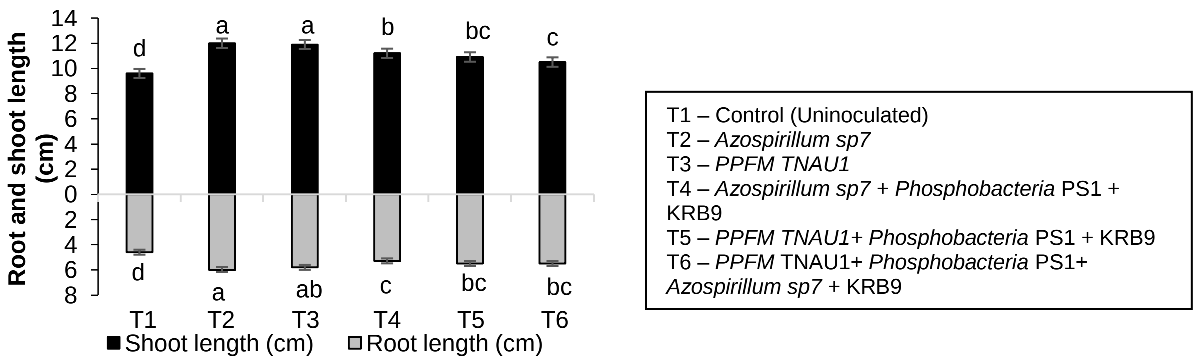

Fig. 4. Effect of PGPR strains on shoot and root length (cm) on cotton seed.

3.3. Effect of PGPR strains on growth traits

Results obtained showed that cotton seeds treated with PGPR strains had improved leaf area, aerial fresh and dry weight and underground fresh and dry weight (Table1). The treatment T3, *PPFM* TNAU1 and the treatment T2, *Azospirillum* sp7 strains inoculated seeds had a higher foliage size compared to the combined inoculation of strains and control plants. *PPFM* TNAU1 strain (T3) inoculated seeds recorded maximum leaf area (17.4 cm^2) followed by the treatment T2, *Azospirillum* sp7 strain inoculated seeds (16.1 cm^2). The rate of cell division is higher in the *PPFM* TNAU1 inoculated seeds due to synthesis of cytokinin, the phytohormone that contributes to the wider leaf surface area. The inoculation of *PPFM* TNAU1 and *Azospirillum* sp7 strain resulted in an increase of 57.3 and 45.6 %, respectively, over the control. Our results are similar to those obtained by Wang *et al*. [35], and Namwongsa *et al.* [36].

Maximum aerial fresh and dry weight were recorded in the treatment T3*, PPFM* TNAU1 strain (3.25 g $seedling^{-1}$ and 72.8 mg $seedling^{-1}$), respectively. Likewise, the maximum underground fresh and dry weight recorded was higher in treatment T2, *Azospirillum* sp7 strain inoculated plants (0.40 g $seedling^{-1}$ and 6.85 mg $seedling^{-1}$). This increase of aerial fresh and dry weight in the treatment T3, *PPFM* TNAU1 strain, is due to the higher leaf biomass. *Azospirillum* sp7 (T2) strain inoculated treatment is found to have maximum underground fresh and dry weight due to increased root length by the synthesis of phytohormones that act on cell division and cell elongation. The maximum leaf area obtained in treatment T3, *PPFM* TNAU1 (T3) inoculated seedlings, contributed to the higher total dry matter production (79.6 mg $seedling^{-1}$) compared to the (T2) *Azospirillum* sp7 (77.3 mg $seedling^{-1}$) strain treated seedlings. The present findings are similar to those obtained by Wang *et al*. [35] where increased transcriptional levels of auxin biosynthesis genes enhanced the seedling growth thus improved biomass. Our results of increased seedling fresh and dry weight were similar to those reported by Etesami and Alikhani. [37], Turan *et al*. [38], Asari *et al*. [39], and Egamberdieva *et al*. [25]. This improvement in seedling growth parameters suggest that selective inoculation of PGPRs could be considered as an effective alternative biofertilizer for promoting cotton seed germination, biomass, and yield.

Table 1. Effect of PGPR strains on germination percentage, seedling emergence and seedling growth traits

Treatments	**Germination percent %**	**Seedling vigour index**	**Leaf Area (cm^2)**	**Fresh weight (g $plant^{-1}$)**		**Dry weight (mg $plant^{-1}$)**	
				Aerial weight	**Underground weight**	**Aerial weight**	**Underground weight**
T1 – Control	83.3	1149.5	11.0	2.33	0.22	50.3	5.78
T2 – *Azospirillum*	95.3	1709.6	16.1	3.19	0.40	70.5	6.85
T3 – *PPFM*	96.8	1714.4	17.4	3.25	0.38	72.8	6.83
T4 – *Azospirillum* + PSB + KRB	88.5	1459.4	11.7	2.75	0.30	55.5	6.60
T5 – *PPFM* + PSB + KRB	88.8	1453.5	11.6	2.65	0.35	55.0	6.63
T6 – *PPFM* + PSB+ *Azospirillum* + KRB	91.3	1453.6	11.3	2.61	0.32	54.0	6.60
Mean	90.7	1490.0	13.2	2.8	0.30	59.7	6.50
SEd	1.06**	34.74**	0.63**	8.28**	5.44	1.74**	0.13**
CD (*P* =0.05)	2.26	74.04	1.33	0.18	0.12	3.72	0.29

Values are mean of replicate. Values followed by the same letter in each column are not significantly different from each other as determined by DMRT (P = 0.05).

3.4. Principal component analysis (PCA) of seed germination and growth traits in cotton influenced by PGPR strains

The PCA analysis was done using the data of seed germination and growth-related variables such as germination percentage (GP), mean germination time (MGT), germination index (GI), germination rate index (GRI), seedling vigour index (SVI), leaf area (LA), aerial and underground fresh (AFW and UFW) and dry weight (ADW and UDW) obtained from six treatments with four replications (Fig. 5.). The PCA showed that the variables were correlated with the principal component value of 96.60 % (PC1 – 87.57 % and PC2 - 9.03 %). The variables obtained from seed treatment of *Azospirillum* sp7 (T2) and *PPFM* TNAU1 (T3) strains were located in the right side of the scoring plot, showing positive correlation between the components. However, variables of the control treatment (T1) were located in the bottom left quarter of the scoring plot in PCs (Fig. 5A). The plant growth variables were positively influenced by PGPR strain inoculation, at the right side of the plot (Fig. 5B). PCA results confirmed that PGPR strain inoculation positively influenced the germination traits and growth characters, while mean germination time was least correlated with the inoculation of different PGPR strains. Results from PCA recorded that the treatment *Azospirillum* sp7 (T2) strain, located in the right side of first quadrant is the best treatment followed by the treatment *PPFM* TNAU1 (T3), located in the right end of the second quadrant, which detailed that PGPR inoculation improved germination efficiency and seedling growth traits.

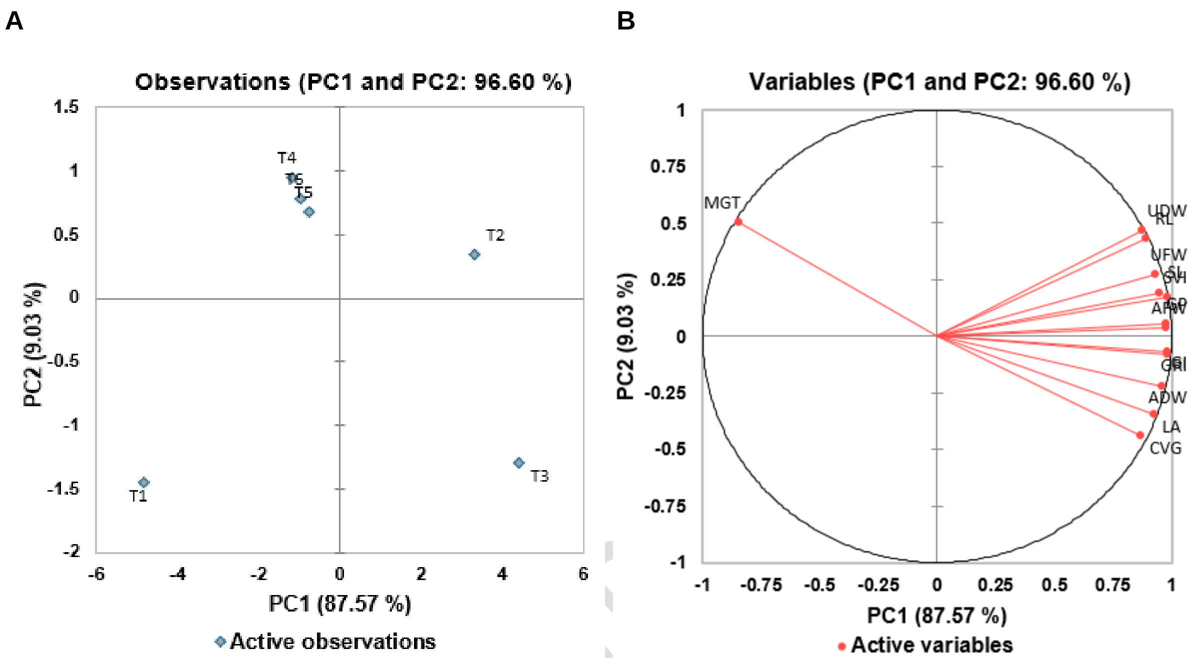

Fig. 5. Principal component analysis (PCA) of cotton as influenced by different PGPR strains (A) Scoring plot of treatments and (B) Loading plot of variables. Variables are GP, Germination percentage; MGT, mean germination time; GI, Germination index; GRI, Germination rate index; SVI, Seedling vigour index; SL, Shoot length; RL, Root length; LA, Leaf area; AFW, Aerial fresh weight; UFW, Underground fresh weight; ADW, Aerial dry weight and UDW, Underground dry weight.

4. CONCLUSION

Single and combined inoculation of PGPR strain in cotton seeds considerably improved the germination efficiency and plant growth during the early growth stage. However, seeds inoculated with individual strain of *Azospirillum* sp7 and *PPFM* TNAU1 outperforms the combined inoculation of PGPR strains. These results indicate that PGPR strain inoculation has a positive stimulant effect on germination efficiency and plant growth promotion in the cotton crop. It is concluded that the investigated beneficial microbes can be employed to increase the physiological potential of cotton to obtain higher yield.

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