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Influence of Microbial Priming on Germination and Seedling Growth Traits of Compact Cotton CO17

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cotton, known as "the King of fibers", is the predominant fiber 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

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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.,* frequent 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 and promotes toxicity to the soil. Lately, efforts have been focused on minimizing the usage of chemical fertilizers in order to optimize production cost 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 prompted researchers to take a second look at the range of microorganisms which provide benefits to agricultural production by stimulating plant growth and producing a higher yield [1,2].

Plant growth promoting rhizobacteria (PGPR) are bacteria living in the rhizosphere region which interacts with plant metabolism and advances 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 by the activation of germination related enzymes, increasing metabolism that helps in the rapid growth of radicle and plumules. These PGPRs helps in better seed germination and colonizes plant roots to improve seedling vigour, modifies root morphology to facilitate better acquisition of nutrients which ultimately enhances the yield and quality of crop [3,4].

Higher production and productivity of the crop is achieved through the use of good quality seeds and following proper management practices in any cultivar [5]. 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. Therefore, this present study was formulated with an objective to evaluate different PGPR strains on cotton seed germination, seedling emergence and growth traits.

2. MATERIALS 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 laid out in the 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 (CO 17 variety) seeds were collected from Department of Cotton, Tamil Nadu Agricultural University, Coimbatore. Sowing was done by dibbing at row to row 90 cm and plant to plant 15 cm spacing by placing the seeds at each hill. Irrigation was given as per requirement of crop. 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 then shade dried before sowing.

2.3 Data Collection

The efficiency of different PGPR strains as bioinoculants 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 % [6].

$$\text{Germination percentage} = \left[\frac{TNG}{TNP}\right] \times 100$$

where TNG is total number of germinated seeds and TNP is total number of planted seeds

2.3.2 Mean Germination Time - MGT (day)

Mean germination time (MGT) was given by [7].

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

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 [8].

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

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 [9].

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

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 [10].

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

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.

2.3.6 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 [11].

2.3.7 Root length and shoot length (cm)

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

2.3.8 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.

2.3.9 Aerial and underground weight (g seedling^{-1})

Fresh aerial and underground weight of 10 days 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 by 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) [12] 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 [13]. The mean germination time was found to be 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 co-inoculated with *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 had recorded about 16.2 and 14.4% increase respectively, over the control. The present study is in corroboration with the findings of Prathibha and Siddalingeshwara. [14] and Sirohi et al. [15] 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. [16], El-Sheekh et al. [17], Gowtham et al. [18] and Roman-Ponce et al. [19].

Inoculation of PGPR strains exhibited 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 [10]. 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 [8]. 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 [9]. The inoculation of *PPFM* TNAU1 and *Azospirillum* sp7 strain had 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 gibberellin, the hormone which stimulates the activity of the germination-specific enzymes, alpha-amylase, protease and nuclease which is mainly involved in starch breakdown and assimilation [20] and mitochondrial enzyme activities [21].The present study is in corroboration with the results of Xiao et al. [22] and Hossain et al. [23] in which PGPR inoculation significantly improved germination percentage and germination rate index with reference to the control in rice. Similar findings were reported by Makhaye et al. [24] and Hamidi et al. [25] where seed inoculation of PGPRs significantly improved all 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 [26]. and Meena et al. [27].

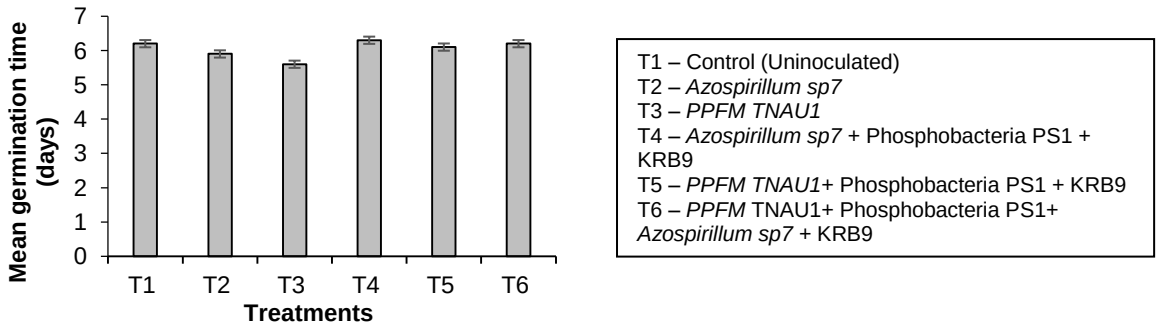

Fig.1. Effect of PGPR strains on mean germination time (MGT) (d) on cotton

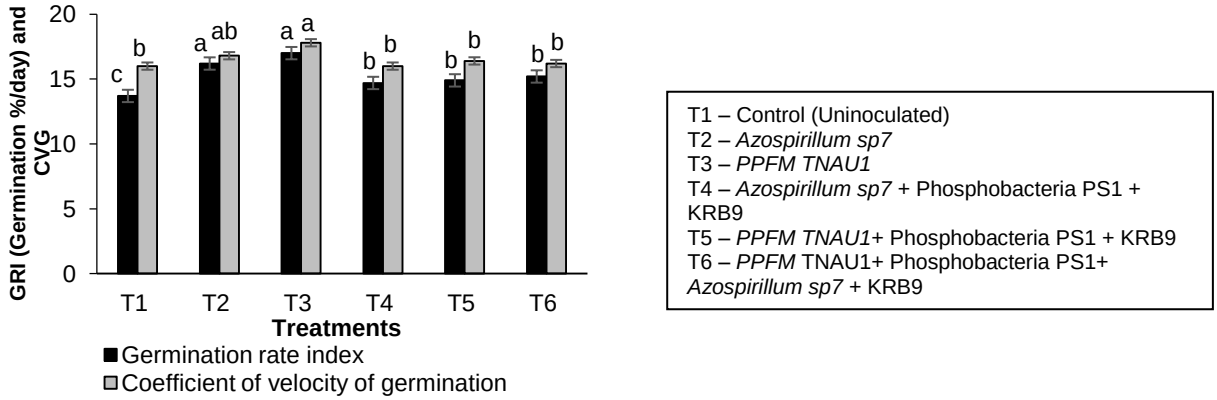

Fig. 2. Effect of PGPR strains on germination rate index GRI (Germination %day^{-1}) and coefficient of velocity of germination of cotton seeds

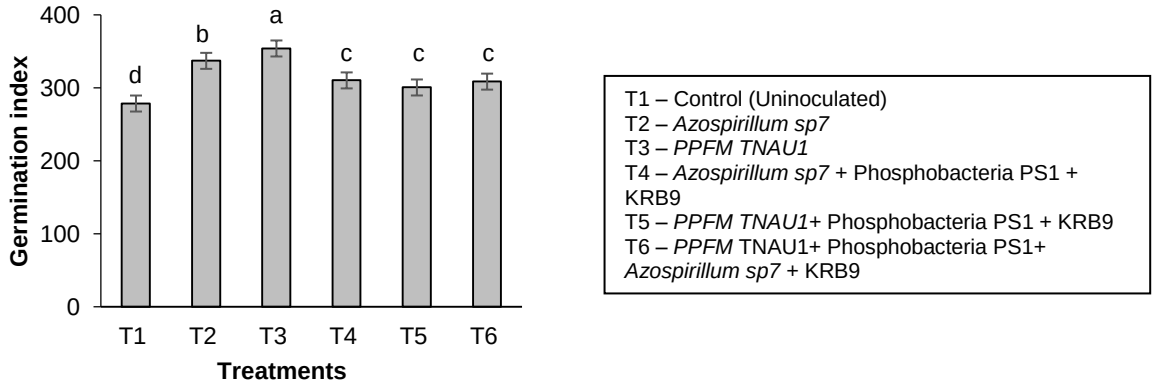

Fig. 3. Effect of PGPR strains on germination index GI of cotton seeds

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). The experimental results indicated that shoot and root length of PGPR inoculated seedlings were significantly higher compared to the uninoculated control (Fig. 4). The treatment, *Azospirillum* sp7 strain (T2) inoculated seeds resulted in maximum shoot length and root length (12.0 and 6.0 cm respectively) followed by the treatment, T3,

PPFM TNAU1 (T3) (11.9 and 5.8 cm respectively). The combined inoculation of PGPR strains (T4, T5 and T6) recorded higher shoot length and root length compared to the non-inoculated 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 phytohormone, IAA (Indole-3 Acetic Acid) and acquires more nutrients such as nitrogen and phosphorus, which in turn increases root length and shoot length [28]. In addition 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*.* [29], Taha et al*.* [30] and El-Gamal et al*.* [31]. Similar findings were reported by Dhale et al. [32], Zamioudis et al. [33] and Pindi et al*.* [34] 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 documented higher seedling vigour index (1714.4) which has higher germination percentage followed by the treatment T2, *Azospirillum* sp7 strain (1709.6) as it showed higher values in shoot and root length compared to other treatments and control. The findings by Noumavo et al. [35] are in accordance with the 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 [36,37].

3.3 Effect of PGPR Strains on Growth Traits

Experimental results obtained showed that cotton seeds treated with PGPR strains had substantial improvement in leaf area, aerial fresh and dry weight and underground fresh and dry weight (Table.1). The treatment T3, *PPFM* TNAU1 and the treatment T2, *Azospirillum* sp7 strains inoculated plants exhibited a higher foliage size compared to the combined inoculation of strains and control plants. *PPFM* TNAU1 strain (T3) inoculated plants recorded maximum leaf area (17.4 cm^2) followed by the treatment T2, *Azospirillum* sp7 strain inoculated plants (16.1 cm^2). The rate of cell division is higher in the *PPFM* TNAU1 inoculated plants 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 increment of 57.3 and 45.6% respectively, over the control. Our results are in corroboration with Wang et al. [38] and Namwongsa et al*.* [39].

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 the 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 the 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 $seedling^{-1}$) strain treated seedlings. The present findings are in line with Wang et al. [38] that increased transcriptional levels of auxin biosynthesis genes enhanced the seedling growth thus improved the biomass.

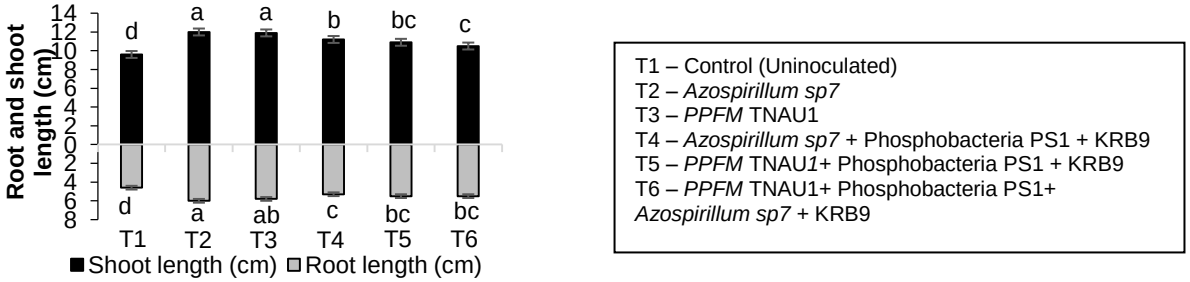

Fig. 4. Effect of PGPR strains on shoot and root length (cm) on cotton

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^{d}	1149.5^{c}	11.0^{b}	2.33^{c}	0.22^{b}	50.3^{c}	5.78^{b}
T2 – *Azospirillum*	95.3^{a}	1709.6^{a}	16.1^{a}	3.19^{a}	0.40^{a}	70.5^{a}	6.85^{a}
T3 – *PPFM*	96.8^{a}	1714.4^{a}	17.4^{a}	3.25^{a}	0.38^{a}	72.8^{a}	6.83^{a}
T4 – *Azospirillum* + PSB + KRB	88.5^{c}	1459.4^{b}	11.7^{b}	2.75^{b}	0.30^{ab}	55.5^{b}	6.60^{a}
T5 – *PPFM* + PSB + KRB	88.8^{c}	1453.5^{b}	11.6^{b}	2.65^{b}	0.35^{ab}	55.0^{b}	6.63^{a}
T6 – *PPFM* + PSB+ *Azospirillum* + KRB	91.3^{b}	1453.6^{b}	11.3^{b}	2.61^{b}	0.32^{ab}	54.0^{bc}	6.60^{a}
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)

Our results of increased seedling fresh and dry weight were in harmony with Etesami and Alikhani. [40], Turan et al. [41], Asari et al. [42] and Egamberdieva et al. [28]. This considerable improvement in seedling growth parameters suggests that selective inoculation of PGPRs could be considered as an effective alternative biofertilizer for promoting cotton seed germination, biomass, and yield.

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 revealed 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. On the contrary, variables of treatment, control (T1) 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.

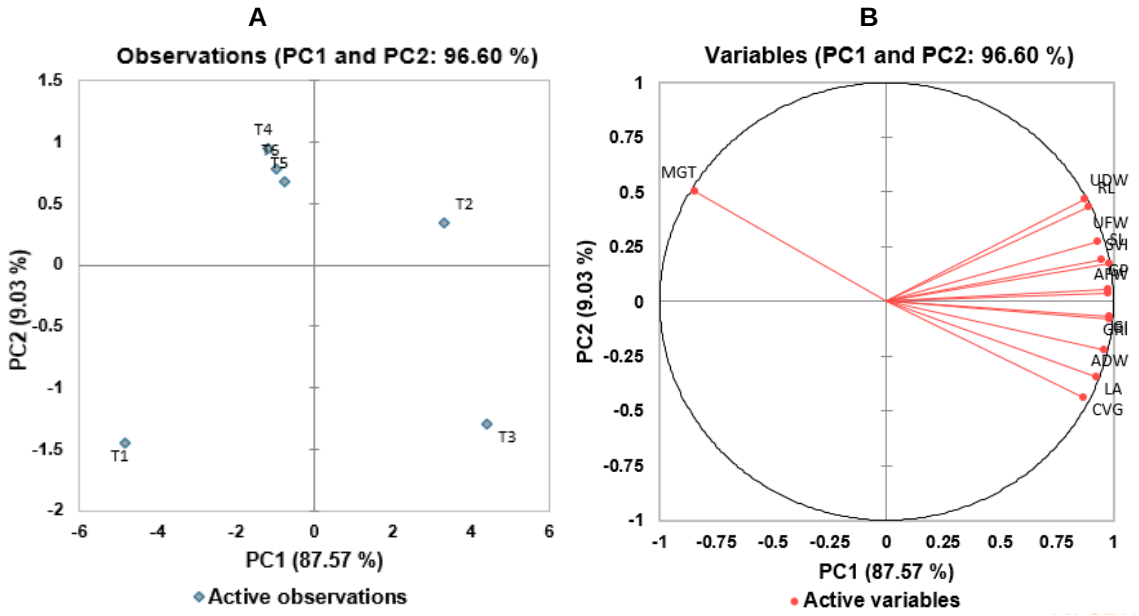

Fig. 5. Principal component analysis (PCA) of cotton as influenced by different PGPR strains (A) Scoring plot of treatments and (B) Loading plot of variables. Variables are GP, Germination percentage; MGT, mean germination time; GI, Germination index; GRI, Germination rate index; SVI, Seedling vigour 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 achieve higher yield.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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