SCREENING THE EFFECTS OF PHOSPHATE SOLUBILIZING ACTERIA ON THE GERMINATION AND GROWTH RATE OF BLACK GRAM

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Abstract

Phosphorus is an important macronutrient in plant metabolism, ultimately reflected on crop yields. Phosphate solubilizing bacteria (PSB) play essential role in inorganic and organic soil P solubilization and mineralization for plant growth. This experiment was conducted to study the effect of phosphate solubilizing bacteria (PSB) on the growth of Black gram. Among 10 isolated phosphate solubilizing bacteria from the rhizosphere of *Vigna catjang* Walp (Cow pea) and *Phaseolus mungo* Lin (Black gram), six strains, PSBCPR-1, PSBCPR-2, PSBBGR-2, PSBBGR-6, PSBBGS-1 and PSBBGS-2 were selected and used as inoculums. Laboratory experiment with six treatments and one control (only diluted peptone water) with five replicates was carried out in Microbiology Laboratory, Zoology Department, Pathein University during June to December 2019. In this study, root length, shoot length and total seed germination rate of treated plants significantly increased ($p<0.05$) over control. The maximum root length on treatment 2 (PSBCPR.2) and maximum shoot length on treatment 3 (PSBBGR-2) and treatment 6 (PSBGBS-2) were observed. Fresh weight and dry weight of treated seedling were also significantly increased over control. The isolates obtained in this study showed a significant *in vitro* plant growth promoting activity onto black gram. The use of these bacteria as bioinoculants could be a sustainable practice to facilitate the nutrient supply to black gram plants and preventing negative side-effects.

Keywords: Phosphate solubilizing Bacteria, Rhizosphere, Black gram

Introduction

Phosphorus has been reported as one of the key elements in crop production which is associated with several vital functions and is responsible for many characteristics of plant growth such as nodule formation, cell division and organization, fat formation and transfer of heredity. Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition (Qureshi *et al.,* 2012 and Saxena and Sharma, 2003).

Bacteria are more effective in phosphorus solubilization than fungi (Alam *et al.,* 2002). Phosphorus (P) is one of the essential macronutrients for plant growth and development. Plants acquire this element from soil solution as phosphate anions (Bhattacharyya and Jha, 2012).

Black gram (*Vigna mungo* L.) is one of the most important pulse crops next to chickpea, lentil and mungbean both in area and production (AIS, 2017). Black gram is important legume crop characterized by a relative high content of protein (25.67%), carbohydrates (5.4%), fat (1-3%), fibers (3.5-4.5%) and ash (4.5-5.5%), while calcium and phosphorus are 132 and 367 mg per 100 g of seed, respectively (Ahmad *et al*., 2008). Productivity of black gram is low in general due to poor management and low soil fertility. Chemical fertilizers are frequently used to achieve maximum crop production legume. These cost effective chemicals, however, when used roughly, have resulted in loss of soil fertility and consequently, the crop production. Due to these reasons, focus in recent times has been shifted towards the use of cost competitive biological resources such as Plant Growth Promoting Bacteria (Tiwari *et al.,* 2017).

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Plant growth promoting bacteria (PGPB) are a group of bacteria that actively colonize plant roots and increase plant growth and yield (Shaukat *et al.,* 2006). *Azospirillum, Pseudomonas* and *Azotobacter* strains could affect seed germination and seedling growth. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in canola as well as wheat and potato (Glick *et al*., 1997).

Phosphate Solubilizing Bacteria (PSB) or Phosphobacteria (Muleta *et al.,* 2013), being an important member of PGPB family and are a viable substitute to phosphate fertilizers. Many mechanisms like lowering the pH by acid production, ion chelation, exchange reactions and polymeric substances formation in the growth environment have been reported to play a role in phosphate simultaneously P uptake by the plant and crop yields (Hameeda *et al*., 2008 and Shahab *et al.,* 2009).

Crop establishment depends on an interaction between seedbed environment and seed quality. Among the stages of the plant life cycle seed germination, seedling emergence and seed establishment are the key processes in the survival and growth of any plant species (Hadas, 2004).

Keeping in view the importance of low cost production of black gram using environmentally safe management approach, this research was carried out to investigate the effects of isolated bacteria on the germination rate and to compare the effects of isolated bacteria on the growth of Black gram seedlings as starter culture for biofertilizer.

Materials and Methods

Experimental site and study period

The experiment was conducted at Microbiology Laboratory, Department of Zoology, Pathein University during June to December 2019.

Inoculum preparation

Bacteria were grown in Pikovskaya's (PVK) agar medium. Selected isolates were grown in 10mL peptone water for 24hours at 37 ºC. Final concentrations of inoculums were made to 10^8CFU mL^{-1}. PSBCPR-1, PSBCPR-2, PSBBGR-2, PSBBGR-6, PSBBGS-1 and PSBBGS -2 were used as treatments and control (only diluted peptone water).

Sterilization of seeds

Seeds of Black gram were surface-sterilized with 0.02% sodium hypochlorite for 2 minutes and rinsed thoroughly in distilled water about five times.

Inoculation of seeds

Surface sterilized seeds (50 black gram seeds per selected isolate) were dipped into each suspension of bacteria (10^8 $CFUmL^{-1}$) for 5 hours. 0.5% of insoluble phosphate solution (gmL^{-1}) was also added in each treatment. Control seeds (50 black gram seeds) were immersed in sterile diluted peptone water for 5 hours.

Seed germination

Ten seeds per plate of inoculated and control Black gram were placed in Petri dishes with sterilized filter paper and five replicates were carried out. Petri dishes with treatments and control seeds were incubated at 30 ºC for 7 days. After 3 days the number of germinated seeds was counted. Then, root and shoot length of individual seedlings (50 seedlings from each treatments and control) were measured after 7 days.

Germination rate parameters

The seed germination was calculated by using the following formula (Gholami *et al.*, 2009).

$$\text{Germination rate (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Statistical analysis

Data of experiment was subjected to statistical analysis using IBM- SPSS software (version 25). The differences between the treatment and control means were determined by using One-way ANOVA with LSD, post-hoc test.

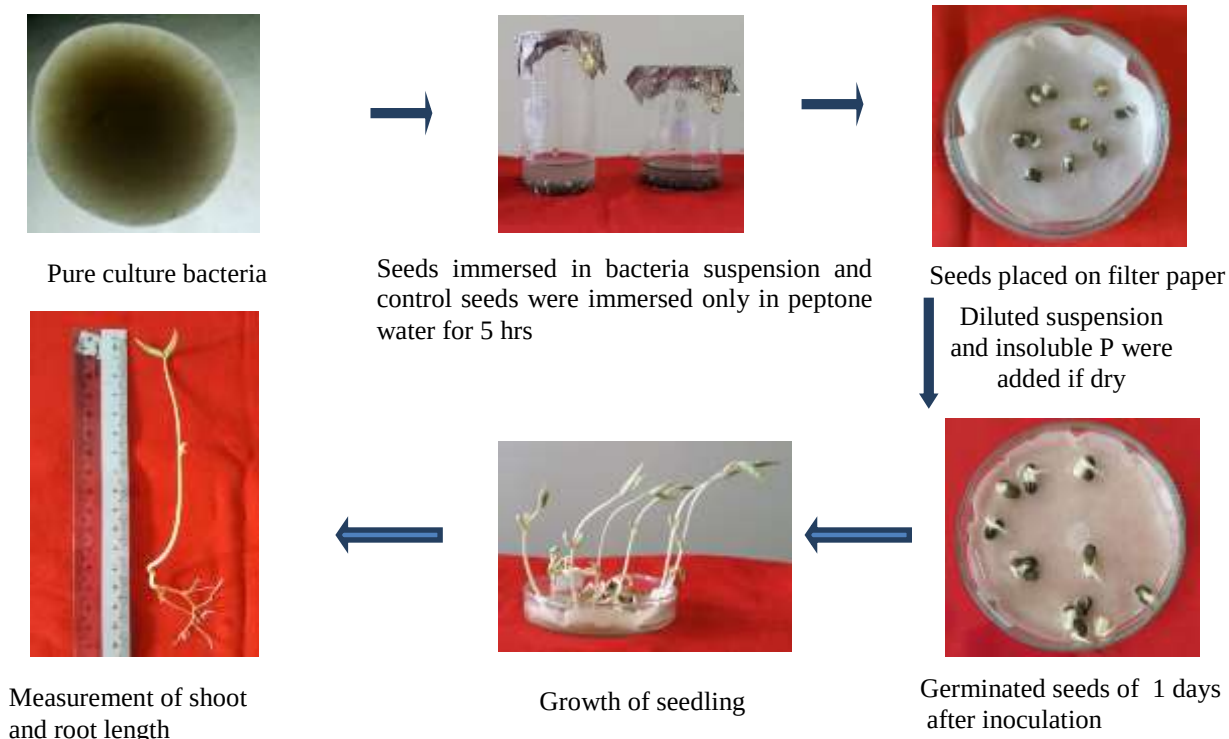

Figure Steps of the present study

Results

The seed inoculation significantly enhanced seed germination of Black gram. To evaluate the effects of selected PSB on the growth of Black gram, Laboratory experiment with six strains and Control (only diluted peptone water) with five replicates were carried out in Microbiology Laboratory, Zoology Department, Pathein University. The highest germination percentage (100%) was observed in T-4 (PSBGBR.6) and T-6 (PSBGBS.2) inoculated seedlings (Table 1, 2 and Fig 2). The maximum root length (5.0 ± 2.0 cm) was observed in T-2 (PSBCPR.2) and maximum shoot length (13.0 ± 3.1cm) was observed in T-3 (PSBBGR.2) inoculated seedlings (Table3-4 and Fig. 3). The maximum fresh root weight (0.186 ± 0.028 g) was observed in T-2 (PSBCPR.2) and maximum fresh shoot weight (0.379 ± 0.030 g) was observed in T-3 (PSBBGR.2). The maximum dry root weight (0.004 ± 0.001 g) was observed in T-2 (PSBCPR.2) and maximum fresh shoot weight (0.028 ± 0.006 g) was observed in T-3 (PSBBGR.2). In this study, total seed germination rate, root length and shoot length of treated seedlings significantly increased ($p<0.05$) over control (Table 1 to 4). Fresh shoot weight, dry shoot weight, fresh root weight and dry root weight of all treatment were also significantly increased ($p<0.05$) over control (Table 5 to 8 and Fig 4, 5).

Table 1 Effect of Phosphate solubilizing bacteria inoculation on the germination rate of Black gram

Treatment	Germination rate (Mean ± SD) (3Days)	Germinated seed (%)
Control	9.1 ± 1.0[a]	91.33
PSBCPR-1 (T-1)	9.6 ± 0.6[b]	97.33
PSBCPR-2 (T-2)	9.8 ± 0.4[b]	98.00
PSBBGR-2 (T-3)	9.9 ± 0.3[b]	98.67
PSBBGR-6 (T-4)	10.0 ± 0.0[b]	100.00
PSBBGs-1 (T-5)	9.9 ± 0.3[b]	98.67
PSBBGs-2 (T-6)	10.0 ± 0.0[b]	100.00

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

Table 2 ANOVA result for the effect of isolated bacteria on germination rate of Black gram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.981	6	1.330	5.313	.000
Within Groups	24.533	98	.250		
Total	**32.514**	**104**			

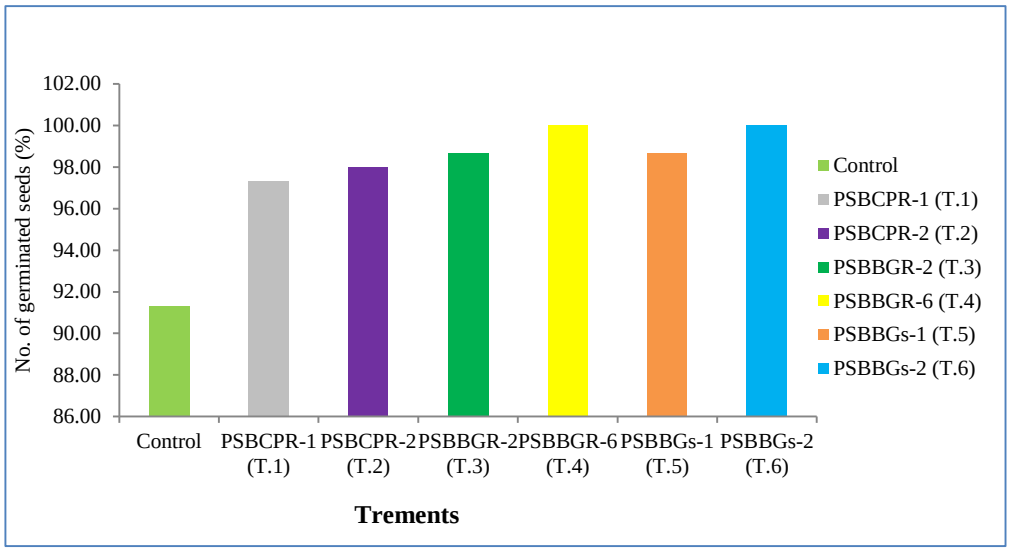

Figure 2 Effect of Phosphate solubilizing bacteria inoculation on the germination rate of Black gram

Table 3 Mean Root and Shoot length of Black gram seedlings

Treatment	Root Length (cm)(7Days)	Shoot Length (cm)(7Days)
Control	2.7 ± 1.0[a]	9.4 ± 2.8 [a]
PSBCPR-1 (T-1)	4.8 ± 2. 0 [c]	11.7 ± 2.7 [b]
PSBCPR-2 (T-2)	5.0 ± 2.0 [c]	12.3 ± 3.5 [c]
PSBBGR-2 (T-3)	4.6 ± 1.7 [b]	13.0 ± 3.1 [c]
PSBBGR-6 (T-4)	4.1 ± 1.5 [b]	12.0 ± 3.0 [b]
PSBBGs-1 (T-5)	4.0 ± 1.5 [b]	11.6 ± 2.3 [b]
PSBBGs-2 (T-6)	4.4 ± 1.8 [b]	12.9 ± 3.1 [c]

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

Table 4 ANOVA result for the effect of isolated bacteria on root and shoot length of Black gram

		Sum of Squares	df	Mean Square	F	Sig.
Root Length	Between Groups	151.418	6	25.236	8.909	.000
	Within Groups	971.590	343	2.833		
	Total	1123.009	349			
Shoot Length	Between Groups	487.833	6	81.305	9.192	.000
	Within Groups	3033.959	343	8.845		
	Total	3521.792	349			

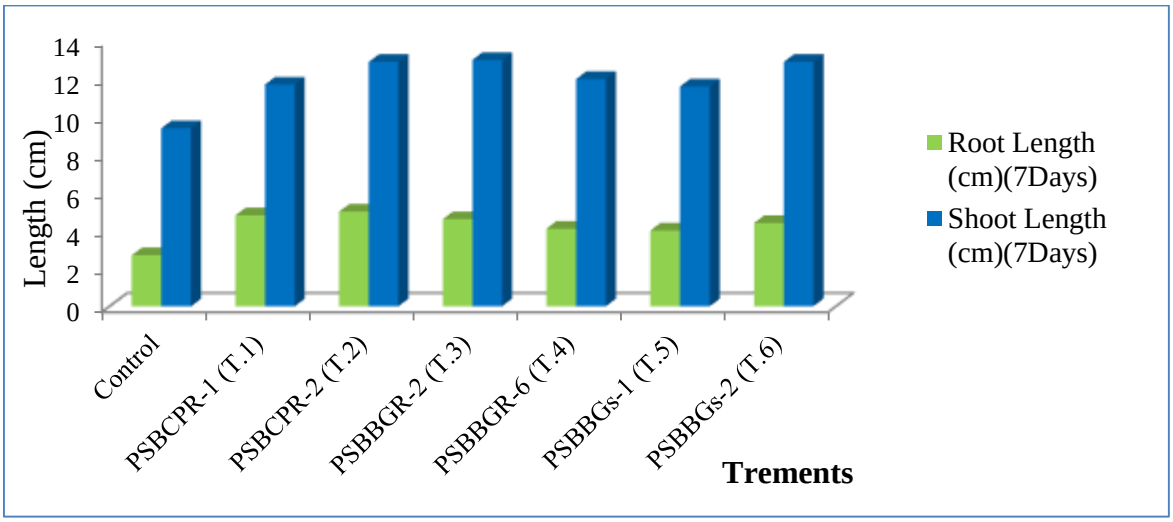

Figure 3 Effect of Phosphate solubilizing bacteria inoculation on the root length and shoot length of Black gram seedlings

Table 5 Mean fresh and dry weight of root of Black gram seedlings

Treatment	Fresh Root Weight (g)	Dry Root Weight (g)
Control	0.084 ± 0.013[a]	0.001 ± 0.001[a]
PSBCPR-1 (T-1)	0.132 ± 0.033[b]	0.003 ± 0.001[b]
PSBCPR-2 (T-2)	0.186 ± 0.028[c]	0.004 ± 0.001 [c]
PSBBGR-2 (T-3)	0.142 ± 0.043[b]	0.003 ± 0.001[b]
PSBBGR-6 (T-4)	0.134 ±0.036[b]	0.002 ±0.001[b]
PSBBGs-1 (T-5)	0.132 ± 0.030[b]	0.002 ± 0.001[b]
PSBBGs-2 (T-6)	0.136 ± 0.034[c]	0.003± 0.001[b]

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

Table 6 ANOVA result for the effect of isolated bacteria on fresh root weight and dry root weight of Black gram

ANOVA (Root and Shoot Lengths)						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Root Length	Between Groups	151.418	6	25.236	8.909	.000
	Within Groups	971.590	343	2.833		
	Total	1123.009	349			
Shoot Length	Between Groups	487.833	6	81.305	9.192	.000
	Within Groups	3033.959	343	8.845		
	Total	3521.792	349			

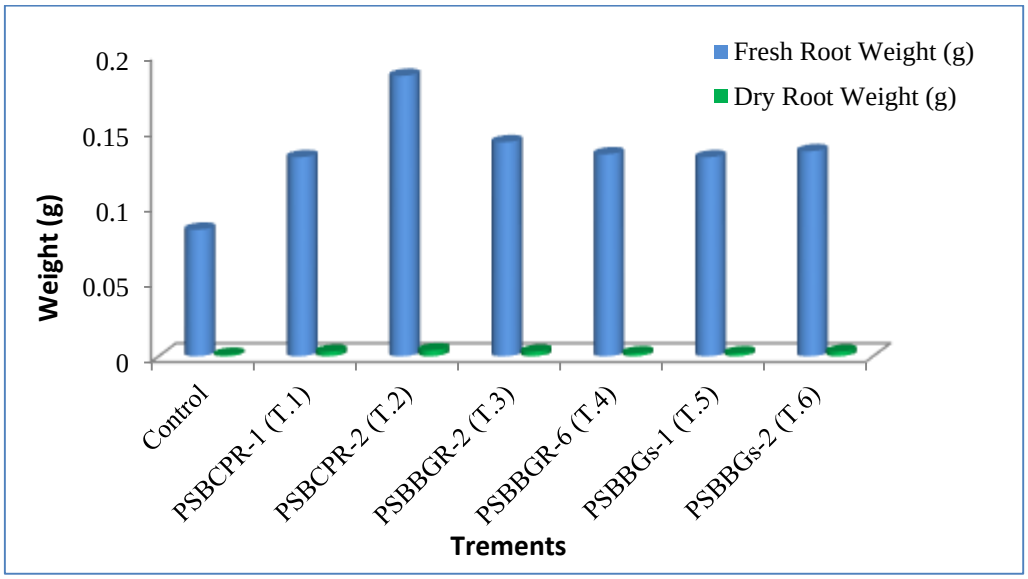

Figure 4 Fresh and dry root weight of Black gram seedlings

Table 7 Fresh and dry shoot weights of Black gram seedlings

Treatment	Fresh Shoot weight (g)	Dry Shoot weight (g)
Control	0.226 ± 0.027[a]	0.013 ± 0.004[a]
PSBCPR-1 (T-1)	0.327 ± 0.042[b]	0.019 ± 0.002[b]
PSBCPR-2 (T-2)	0.331 ± 0.035[b]	0.023 ± 0.004[b]
PSBBGR-2 (T-3)	0.379 ± 0.030[c]	0.028 ± 0.006[c]
PSBBGR-6 (T-4)	0.322 ±0.034[b]	0.021 ±0.004[b]
PSBBGs-1 (T-5)	0.310 ± 0.043[b]	0.019 ± 0.002[b]
PSBBGs-2 (T-6)	0.351 ± 0.037[c]	0.022± 0.005[c]

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

Table 8 ANOVA result for the effect of isolated bacteria on fresh shoot weight and dry shoot weight of Black gram

ANOVA for Shoot Weight

		Sum of Squares	df	Mean Square	F	Sig.
Fresh Shoot Weight	Between Groups	.685	6	.114	89.396	.000
	Within Groups	.438	343	.001		
	Total	1.123	349			
Dry Shoot Weight	Between Groups	.007	6	.001	69.192	.000
	Within Groups	.005	343	.000		
	Total	.012	349			

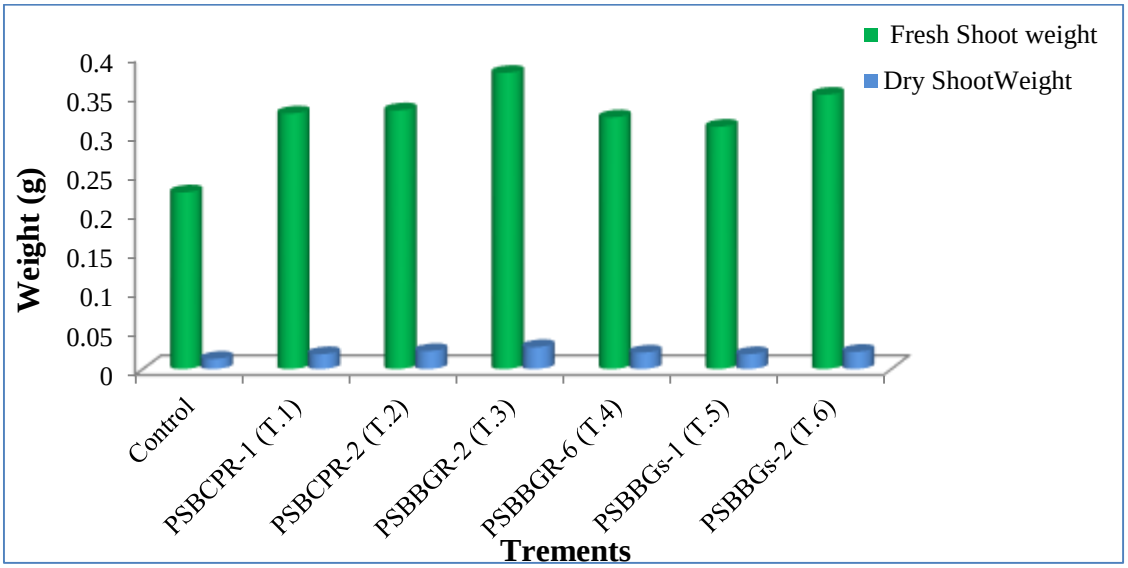

Figure 5 Fresh and Dry shoot weight of Black gram seedlings

A. Inoculated seeds (1 day) B. Inoculated seeds (2 day)

Figure 6. Observation of seed germination

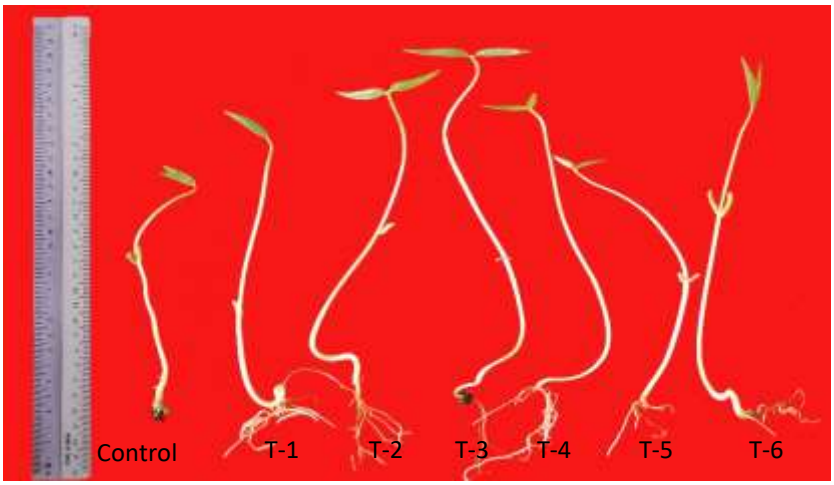

Figure 7 Root and Shoot Length of Control and Treatment after 7days inoculation

Discussion

In this research, the effect of isolated phosphate solubilizing bacteria on Black gram was observed in Laboratory. Gholami *et al.* (2009) used 0.02% sodium hypochlorite for 2 minutes to sterilize seeds of maize and rinsed thoroughly in sterile distilled water. Nwanyanwu *et al.* (2015) also made the sterilization of maize, beans and groundnut seeds by using 0.02% sodium hypochlorite for 2 minutes and rinsed thoroughly in sterile distilled water. In this study, seeds of Black gram were surface-sterilized with 0.02% sodium hypochlorite for 2 minutes and rinsed thoroughly in sterile distilled water.

Nonmavo *et al.* (2013) inoculated the seeds of maize with bacterial suspension of 10^8 CFU/ml^{-1}. Gholami *et al.* (2009) stated that maize seeds were dipped into the suspension of bacteria (10^8 CFU mL^{-1}). Demissie *et al.* (2013) also inoculated the seeds of Fava bean by dipping into the bacteria suspension of 10^8 CFU mL^{-1} for 5 hours. In this experiment, surface sterilized seeds were dipped into the suspension of bacteria 10^8 CFUmL^{-1} for 5 hours.

Gholami *et al.* (2009) observed the percent of geminated maize seeds for 1-3 days, root and shoot length of individual seedling was measured up to 7 days. Demissie *et al.* (2013) studied the percent of geminated Fava bean seeds for 1-3 days, root and shoot lengths of individual seedling were measured up to 7 days. In this observation, the percent of geminated seeds were observed at 1-3 days, root and shoot length of germinated seeds were taken up to 7 days.

Demissie *et al.* (2013) reported that Fava bean of seeds germination percentage, root and shoot length showed significant ($p<0.05$) increased over control. Nonmavo *et al.* (2013) described that the seeds of maize germination percentage, root and shoot length showed significant ($p<0.05$) increased over control. In this study, germination percentage, root and shoot length showed significantly ($p < 0.05$) increased over control.

Demissie *et al.* (2013) conducted the Fava bean plant dry weight after air dried and further oven dried at 72ºC. In the present study, Black gram plant dry weights were observed in the same manner of previous work.

Gholami *et al.* (2009) stated that fresh weight and dry weight of maize plant root and shoot increased over their respective controls. Demissie *et al.* (2013) observed fresh weight and dry weight of Fava bean plant root and shoot length and these weights increased over their respective controls. Shazia *et al.* (2016) stated that fresh weight and dry weight of mung bean root and shoot significantly increased over their respective controls. In this observation, fresh weight and dry weight of Black gram root and shoot increased over their respective controls.

Conclusion

In conclusion, microbial inoculation (biotechnological approach) could be a sustainable practice to facilitate germination rate and plant growth. In order to fulfill this biotechnological approach, different phosphate solubilizing bacteria from legume rhizosphere were isolated. Some strains showed a significant plant growth promoting activity onto black gram. As a final point, current investigation is in progress to evaluate the performance of these native strains and their relationship with native soil micro-organisms under field conditions.

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