

Significance of plant growth promoting rhizobacteria (PGPR) on the growth of wheat and maize

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ABSTRACT

Worldwide the remarkable enhancement in agricultural and industrial production by using chemical fertilizers, herbicides and pesticides causes severe environmental degradation. To overcome this global hazards, plant growth promotion by plant growth promoting rhizobacteria (PGPR) is an alternative strategy. This experiment was performed to show the significance of plant growth promoting rhizobacteria (PGPR), *Bacillus subtilis* and *Pseudomonas aeruginosa* on growth of wheat and maize. Both the PGPR strains have the ability to produce IAA, solubilise inorganic phosphate and produce ammonia. Higher germination rate, vigour index, chlorophyll content and better morphological characters were observed in when seeds inoculated with *Bacillus subtilis* and *Pseudomonas aeruginosa*. Both the PGPRs are quite efficient to enhance soil fertility as supported by observed values of pH, and NPK. Enhanced seed germination maximum up to 100% was recorded with *B. subtilis* for both the crops whereas *P.aeruginosa* treated seeds were also quite responsive than the controlled seeds. Variations in morphological parameters were also observed.

Keywords: Plant growth promoting rhizobacteria (PGPR), *Bacillus subtilis*, *Pseudomonas aeruginosa*, seed germination, morphological parameters.

INTRODUCTION

Plant growth promoting rhizobacteria are a group of colonizing bacteria that inhabits rhizosphere of soil and increase plant growth and yield by direct and indirect mechanisms. Direct mechanisms include Nitrogen fixation, phosphate solubilisation, phytohormone production, ammonia production and siderophore production. Indirect mechanisms include antibiotic, toxins and lytic enzymes production and induced systemic resistance. Plants colonized by the endophytes grow taller, faster and have higher levels of resistance to stresses. These bacteria are now commonly called Plant Growth Promoting Rhizobacteria (PGPR) and Emergence Promoting Rhizobacteria (EPR) (Kloepper *et al.*, 1986, 1988).

The objective of this research was to access the plant growth promotion traits and significance of *Bacillus subtilis* and *Pseudomonas aeruginosa* on growth of wheat and maize. Significant increase in germination rate and seed vigour index of wheat and maize in response to inoculation with PGPR have been reported. It has been observed that inoculated plant crops resulted in significant changes in various growth parameters and germination rate. *Bacillus subtilis* MTCC NO-121 and *Pseudomonas aeruginosa* MTCC NO- 414 have the properties of IAA production, ammonia production, phosphate solubilisation and increases soil fertility.

MATERIALS AND METHODS

The present experiments were performed in Department of biotechnology, P.G.G.C.G.-42, Chandigarh and are discussed under following headings.

Quantitative determination of IAA produced by PGPR

Lyophilised bacterial species (*Bacillus subtilis* and *Pseudomonas aeruginosa*) were revived according to the instructions given by IMTECH, Chandigarh and growth curve was standardised. Gordon and Weber method of IAA quantification was used with Salkowski's reagent. The bacteria were grown in Yeast Malt Dextrose broth (YMD Broth) for 4 days at 30°C in incubator shaker. Inoculums (2ml) were transferred to each flask containing YMD Broth (50 ml) supplemented with tryptophan (0.5 g/l) and YMD Broth (50 ml) without tryptophan. The broth was incubated at 28°C for 4 days. Broth (2ml) is centrifuged at 10,000 rpm for 30 minutes. After centrifugation, added 2 ml of Salkowski's reagent to 1ml of supernatant and kept in dark for 30 minutes. Pink colour development indicated IAA production and the amount of IAA were measured by colorimetric method at 530 nm.

Phosphate solubilisation test

Phosphate solubilisation test was performed by inoculation of individual bacterial strains on Pikovskaya's agar medium. The plates were incubated for 4-5 days at 25°C. A clear zone around the bacterial colony was considered as a positive indication of phosphate solubilisation.

Ammonia production test

Ammonia production test was performed by inoculation of bacterial strains (0.1ml) in 10 ml peptone water. Inoculated test tubes were incubated at 28°C for 48 to 72 hr. Added 0.5ml of Nessler's reagent to each tube. Development of brown to yellow colour indicated ammonia production.

Seed germination by Paper towel method

Paper towel method was performed to study seed germination under *in vitro* conditions. Seeds were surface sterilized with 0.1% of HgCl₂ for 3 minutes followed by washing with autoclaved distilled water for 2-3 times. The seeds were placed in between the layers of tissue papers wetted with water, *Bacillus subtilis* and *Pseudomonas aeruginosa* labelled as control (C), treatment 1,2 (T1,T2) respectively. *Bacillus subtilis* and *Pseudomonas aeruginosa* were used during their exponential growth phase i.e. 4 hours and 3 hours respectively. The plates were incubated at 25°C for seed germination.

Seed germination by Pot trial method

The soil was air dried, crushed, cleaned and autoclaved. Sterilised, overnight soaked seeds treated with *Bacillus subtilis* and *Pseudomonas aeruginosa* were sown in pots followed by labelling as control (C), treatment 1,2 (T1,T2). The growth rate or germination percentage was recorded every third day till fifteenth day along with morphological parameters. Soil testing was carried out by using soil testing kit to check the content of available Nitrogen, Phosphorus and Potassium in the soil under pot trial method.

Evaluation of per cent germination and Seed vigour index (S.V.I.)

On 3rd, 6th, 9th, 12th and 15th day, number of germinated seeds were counted for control and the inoculated pots. Per cent germination was evaluated by using given formula.

$$\text{Germination Percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

Plant growth promotion of seedling was assessed by using Seed Vigour Index (S.V.I.) formula as follows:

$$\text{Seed Vigour Index} = \text{Percent germination} \times \text{Seedling length (Shoot length + Root length)}$$

Chlorophyll content

Approximately 0.15 gm of fresh leaves of wheat and maize from control and inoculated pots were collected and grinded in mortar pestle. 10 ml of 80% acetone was added to it and transferred to centrifuge tubes after filtration. Centrifuge the filtrate for 15 minutes at 10,000 rpm at 4°C. Supernatant was placed in dark condition. To measure the chlorophyll content, absorbance at A₆₆₃ and A₆₄₅ was taken by using spectrophotometer. The chlorophyll content (mg) was calculated as follows:

$$\text{Chl a} = \left[12.7(A_{663}) - 2.69(A_{645}) \right] \frac{V}{1000} \times W$$

$$\text{Chl b} = \left[22.9(A_{645}) - 4.68(A_{663}) \right] \frac{V}{1000} \times W,$$

Where v = volume of the extract (ml)

W = weight of fresh leaves (g)

(Total chlorophyll = chl a + chl b) and W = weight of fresh leaves (g)

RESULTS AND DISCUSSION**Quantitative determination of IAA**

Both the bacterial strains produced IAA in significant quantities with or without tryptophan. It was observed that *Bacillus subtilis* and *Pseudomonas aeruginosa* produced 5 µg/ml and 3 µg/ml respectively without tryptophan whereas significant increase in IAA concentration i.e. 15 µg/ml and 18 µg/ml IAA for *B. subtilis* and *P. aeruginosa* was recorded when they were cultured with tryptophan treated medium respectively (Table 1). Zahir *et al.*, (2001) also reported IAA production from various rhizobacteria of different Brassica species (ranging from 0.33 to 11.40 µgml⁻¹). Various bacterial isolates of *Bacillus* and *Pseudomonas* species were able to produce IAA in significant quantities with or without L-tryptophan, concentrations ranging from 0 to 500 µg/ml were used. In the presence of 50 µg/ml, some of the isolates produced high concentrations of IAA i.e. 1.4- 2.6 µg/ml.

Phosphate solubilisation test

Both the strains in this study formed clear halo zones with *B. subtilis* diameter (5mm) and *P. aeruginosa* with diameter (4mm) on Pikovskaya's agar plates supplemented with phosphate. This confirms phosphate

solubilisation ability of both the cultures (Table 1, Plate 1a). Application of phosphate-solubilizing bacteria increases soil fertility due to their ability to convert insoluble phosphate to soluble phosphate by releasing organic acids, chelation and ion exchange (Narula *et al.*, 2000; Whitelaw, 2000).

Ammonia production test

Bacillus subtilis (T1) produced high ammonia than *P. aeruginosa* (T2) as it changes brown colour to yellow whereas colour partially changed with *P. aeruginosa*. (Table 1, Plate 1b). Ahemad and Khan, 2011, isolated 62 isolates producing ammonia. Maximum Production of ammonia was shown by *Bacillus cereus* SKR16. The produced amount of ammonia was recorded in the range of 16.9-98.6 $\mu\text{g mL}^{-1}$

Paper towel method

Wheat was not so responsive to C (water) as seed germination percentage was 15% and it gave good germination rate with T1 (*B. subtilis*) i.e. (40%) and T2 (*P. aeruginosa*) i.e. (36%) on 3rd day of sowing. On 15th day, maximum seed germination i.e. 100% was recorded with T1 which is highest if compared with T2 (96%) and C (80%). Maize seeds when treated with C, T1 and T2 showed maximum seed germination of 100% at the 15th day with T1. This germination rate was quite high when compared with T2 (80%) and control (86%). (Table 3, Plate2, fig3). Shahsavani *et al.*, 2005 studied on the effect of plant growth-promoting rhizobacteria (PGPR) on seed germination, seedling growth and yield of field grown maize. Six bacterial strains include *P.putida* strain R-168, *P.fluorescens* strain R-93, *P.fluorescens* DSM 50090, *P.putida* DSM291, *A.lipoferum* DSM 1691, *A.brasilense* DSM 1690 were used.

Pot trial method

The germination rate of wheat was highest with T1 (92%) followed by T2 (85%) and for C it was only 64%. Maize when treated with C, T1 and T2 showed a maximum seed germination of 85% at 15th day with T1 and followed by T2 (68%). This germination rate was quite high when compare to C in which 42%. (Table 3, fig2). Cakmakci *et al.*, 2007 studied that seed inoculation of barley with plant PGPR increased root weight by 17.9%-32.1% as compared to the control, and increased shoot weight by 28.8%-54.2%, depending on the species.

The morphological parameters

On 15th day of seed germination, randomly five plants from each replicates for all three treatments were selected and observed for all morphological parameters. In case of wheat, maximum root length of 8.02 cm was recorded with T1 and T2 whereas it was 5.6 cm for C respectively. A maximum shoot length of 32 cm and 33 cm was recorded in case of both *B.subtilis*(T1) and *P.aeruginosa* (T2). Whereas for control (27.1 cm), the shoot length was considerably lower than T1 and T2. (Table 4, fig3).

In case of maize, maximum root length was observed for T2 (6.67 cm), for T1 it was 3.33cm and for C it was 3cm. Shoot length of 14 cm was recorded in case of T1 and 10 in case of T2. The fresh root weight was maximum in case of T1 i.e. 0.43 gm where as for T2 it was quite less i.e. 0.36 gm and for C (0.31 gm). Fresh shoot weight was maximum in case of T1 i.e. 0.26 gm which is greater than the fresh shoot weight of T2 (0.24 gm) and C (0.16 gm). (Table 5, fig4). Gholami *et al.*, 2009 studied effect of PGPR on germination of maize with six bacterial strains *P.putida* R-168, DSM291, *P. fluorescens* strain R-93, DSM 50090, *A. lipoferum* DSM 1691 and *A.brasilense* DSM 1690. Inoculation of maize seeds with all bacterial strains significantly increased the plant height (14.3 % - 21.7%). The most effective strain was *P.fluorescens* DSM50090 which increased 100 seed weight up to 44% over control.

Evaluation of seed vigour index (S.V.I.)

Bacillus (T1) treated wheat showed highest vigour index (3680) which is followed by *P.aeruginosa* (T2) i.e 3485 whereas (C) showed 2086 SVI. In case of maize, it was led by *Bacillus subtilis* (T1) i.e 1700 and *Pseudomonas aeruginosa* (T2) 1224. But control (C) bears the lowest vigour index 672 (Table 6). Wang *et al.*, 2010 analyzed the quantitative changes of plant defence enzymes and phytohormone in biocontrol of cucumber *Fusarium* wilt by *Bacillus subtilis* B579. Higher content of IAA, an important plant growth regulator, was detected in B579 treated plants and plant growth promoting ability (Vigour Index 4,177.53).

Chlorophyll content

Highest chlorophyll content was observed in *Bacillus subtilis* (T1) treated wheat and maize with 20mg and 12mg respectively. It was followed by *P. aeruginosa* (T2) in wheat and maize with 9.4mg whereas in C had least chlorophyll content i.e. (5mg). (Table7, fig5). Srichaikul *et al.*, (2011) and Singh *et al.*, (2011) showed important roles of chlorophyll in plant physiology and their role in nutrition. Chlorophyll also plays important role in detoxification, digestion, excretion and decreasing allergens.

Effect of PGPRs on soil macronutrients

pH

pH was same 6.5 for T1, T2 and C. Soil falling between ph 5.0 to 6.0 is generally suitable for most of common crops. On 15th day it was observed that pH declined in case of C i.e. 5.5. (Table8)

Nitrogen content

Nitrogen is an essential element for plant growth and productivity. PGPR fix atmospheric nitrogen and provide it to plants by both symbiotic and non symbiotic manner. The nitrogen content available in pots treated with all three treatments C, T1 and T2 recorded on the day of inoculation was <50kg/acre (L1). It is observed that the nitrogen concentration increases from L1 and L2 (L2= 50-99kg/acre) for both *P.aeruginosa* and *B.subtilis* where as it remains same for C. (Table8)

Phosphorous content

Phosphorous is the most important key nutrients of plants next to nitrogen,The phosphorus content available in pots treated with T1 and T2 treatments recorded on the day of inoculation was in the range of 8 – 10 kg/acre (M2) whereas C was in the range of 11-15kg/acre (H1). It was observed that on the 7th day the phosphorus concentration decreases from M2 to L2 in all the three cases i.e. C, T1 and T2. (L2 = 1 to 3 Kg/Acres). The value on the 15th day again increases to M2 in case of *P.aeruginosa* and *B.subtilis* whereas for control it remains same. (Table8)

Potassium content

The potassium content available in pots for C and T1 treatment recorded on the day of inoculation was in the range of 121-150kg/acre (H1) whereas T2 was in the range of >155 kg/acre (H2). On the seventh day this conc. decrease to M1 (50-80 kg/acre) for T1 and T2 whereas for C it was same (H1). The value again increases to H1and H2 for T1 and T2. And C was declined to the range of M1. (Table 9) Biswase *et al.*, (2000) confirmed that Rhizobia inoculation improves nutrient uptake and growth of low land rice. Six rhizobial diazotrophs isolated from a wide range of legume hosts were investigated to determine their growth-promoting activities in low land rice (*Oryza sativa* L.) during 1997. Inoculation with *Rhizobium leguminosarum* E11, *Rhizobium* sp. IRBG74, and *Bradyrhizobium* sp. IRBG271 increased rice grain and straw yields by 8 to 22 and 4 to 19%, respectively, at different N rates.

Conclusion

Both the PGPR strains are quite efficient as they produce IAA, solubilise inorganic phosphates and produce ammonia. They induce higher germination rate, high vigour index, chlorophyll content and better morphological features in crops.

Table 1. Quantification of IAA, phosphate solubilisation test ammonia production.

PGPR (Plant Growth Promoting Rhizobacteria)	IAA Conc. µg/ml		Phosphate Solubilization test	Ammonia Production Test
	With tryptophan	Without tryptophan	Diameters of clear zone	
<i>Pseudomonas aeruginosa</i>	15µg/ml	3µg/ml	4mm	+
<i>Bacillus subtilis</i>	18µg/ml	5µg/ml	5mm	+



Plate1(a) Phosphate solubilisation test



Bacillus subtilis



Pseudomonas aeruginosa

Plate1(b) Ammonia Production Test

Table2. Per cent Seed germination of Wheat and Maize under Paper Towel Method

Days	Control		Treatments			
	Wheat C (Mean ±SD)	Maize C (Mean±SD)	Wheat T1 (Mean±SD)	Maize T1 (Mean±SD)	Wheat T2 (Mean±SD)	Maize T2 (Mean±SD)
3 rd	15.00±6.92	20.00±0.00	40.30 ±6.30	26.66±11.54	36.66±6.35	26.66±11.54
6 th	30.30±5.50	46.60±11.50	69.60 ±12.70	46.67±11.55	52.66±6.65	46.70±11.55
9 th	45.00±0.00	60.00±0.00	88.60 ±11.50	80.00±0.00	80.66±6.35	60.00± 0.00
12 th	70.00±6.92	66.60±11.50	96.10 ±7.10	96.60±5.78	89.66±10.01	73.33±11.54
15 th	80.60 ±6.35	86.60±11.50	100.00±0.00	100.00 ±0.00	96.67±5.78	80.00±20.00

Figure2. Percentage of Seed germination in Wheat and Maize under paper towel method

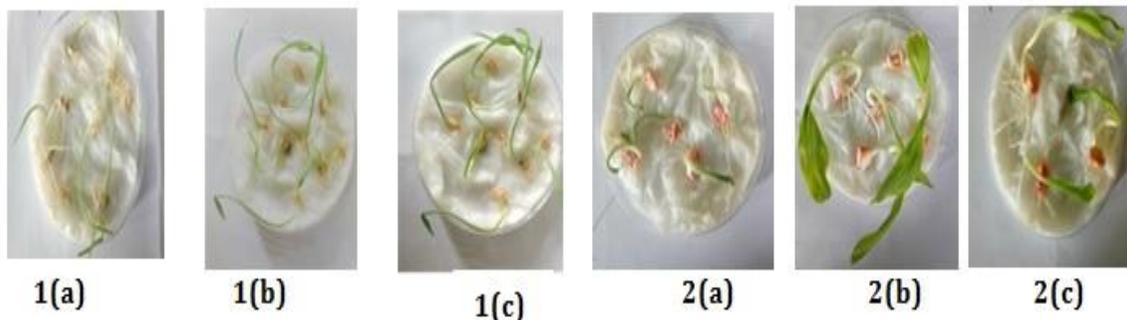
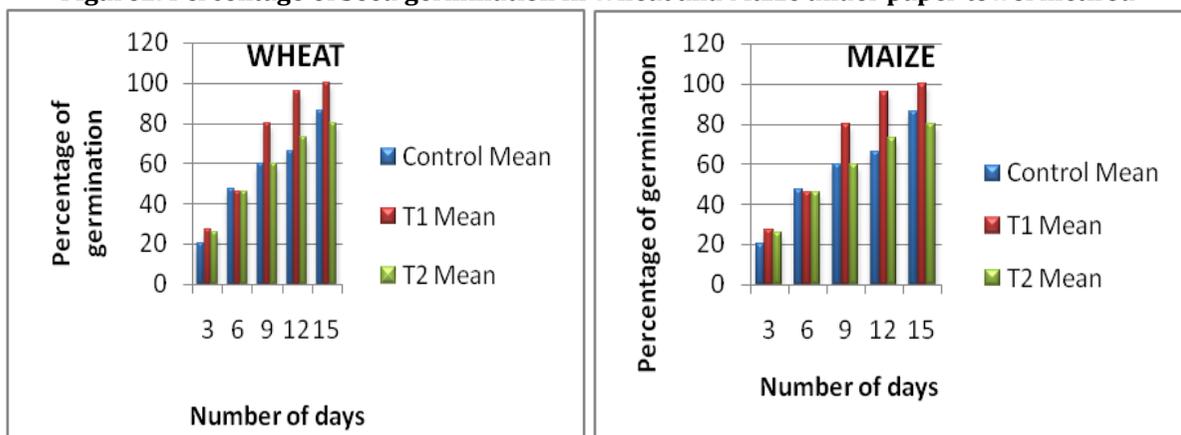


Plate2. Germination of wheat (1) and maize (2) seeds at Day15

Table3. Percentage of seed germination of wheat and Maize under pot trial method

Days	Control		Treatments			
	Wheat C (Mean ±SD)	Maize C (Mean ±SD)	Wheat T1 (Mean±SD)	Maize T1 (Mean±SD)	Wheat T2 (Mean±SD)	Maize T2 (Mean±SD)
3 rd	10.66±2.31	9.33±2.30	20.00±0.00	16.00±4.00	10.67±2.31	8.00 ±4.00
6 th	16.00±4.00	12.00±4.00	40.00±0.00	32.00±4.00	17.33±2.31	21.33±2.30
9 th	20.00±0.00	16.00±4.00	53.33±6.11	56.00±4.00	36.00±4.00	34.66 ± 2.31
12 th	30.66±2.31	25.33±6.11	80.00±8.00	73.30±6.11	66.66±9.23	54.66±6.11
15 th	64.00±6.92	42.66±4.62	92.00±10.58	85.33±4.61	85.33±12.22	68.00±4.00

Figure2. Percentage of seed germination of Wheat and Maize under pot trial method

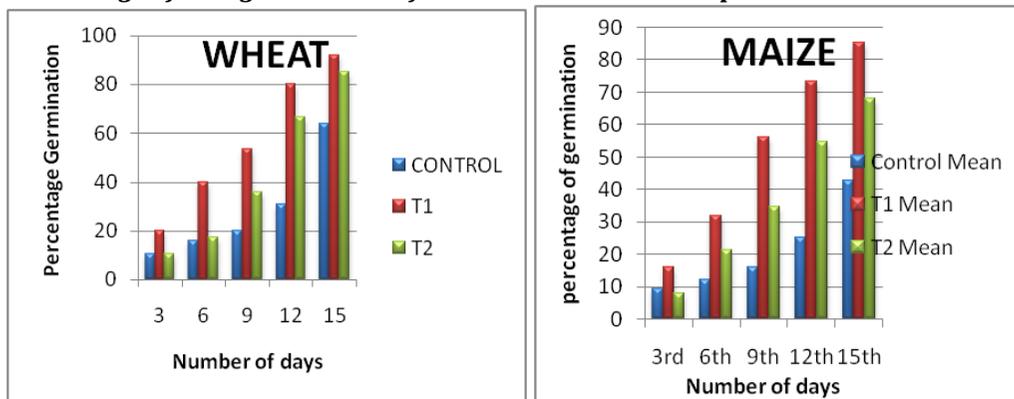


Table4. Morphological parameters of wheat after 15 days

Parameters	Control Mean±S.D	T1 Mean±S.D	T2 Mean±S.D
Root length(cm)	5.6±0.1	8.0±0.5	8.25±0.25
Shoot length(cm)	27.0±0.5	32.0±2.0	33.0±1.0
No. of leaves (n)	3.0±1.0	3.0±1.0	2.0±0.0
No. of roots (n)	4.0±0.0	11.0±1.0	13.0±1.0
Root girth(mm)	3.25±1.75	1.65±0.35	1.5±0.5
Shoot girth(mm)	2.2±0.5	1.5±0.1	0.25±0.05
Fresh root weight (gm)	0.05±0.01	0.27±0.01	0.25±0.01
Fresh shoot weight(gm)	0.14±0.01	0.15±0.01	0.155±0.005
Dry root weight(gm)	0.004±0.001	0.004±0.002	0.0045±0.0005
Dry shoot weight(gm)	0.024±0.001	0.0205±0.0005	0.0197±0.0001

Table5 Morphological parameters of Maize after 15 days

Parameters	Control Mean±S.D	T1 Mean±S.D	T2 Mean±S.D
Root length(cm)	3.33±0.58	3.30±1.04	6.67±0.57
Shoot length(cm)	13.33±0.58	14.00±1.00	10.00±1.00
Number of leaves (n)	2.67±0.57	3.33±0.57	2.67±0.57
Number of roots(n)	5.33±0.58	9.33±1.53	7.33±0.58
Root girth(mm)	1.33±0.58	1.67±0.29	1.33±0.29
Shoot girth(mm)	1.67±0.58	2.67±0.28	2.00±0.50
Fresh root weight(gm)	0.31±0.02	0.43±0.03	0.36±0.02
Fresh shoot weight(gm)	0.17±0.01	0.26±0.02	0.23±0.03
Dry root weight(gm)	0.08±0.01	0.096±0.001	0.093±0.001
Dry shoot weight(gm)	0.0077±0.0005	0.008±0.001	0.0083±0.0005

Figure3 Morphological parameters of Wheat seedlings after 15 days.

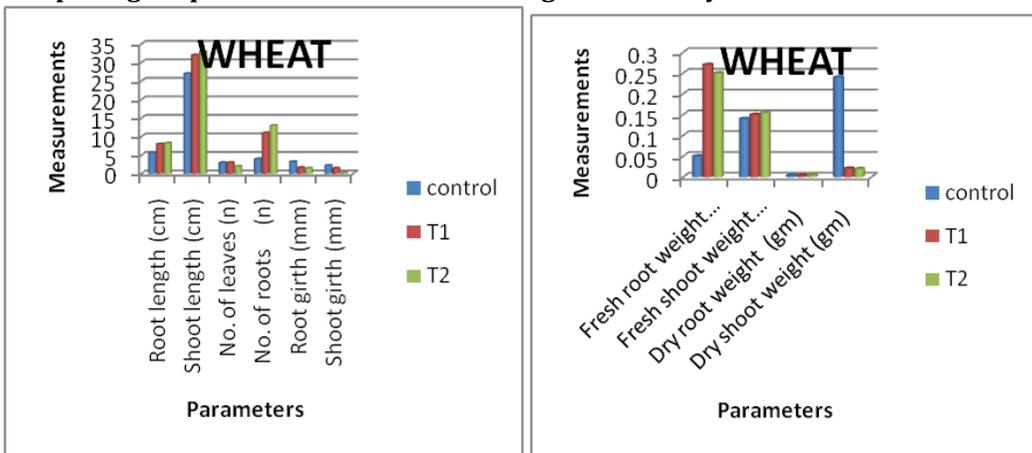


Figure4 Morphological parameters of Maize seedlings after 15 days.

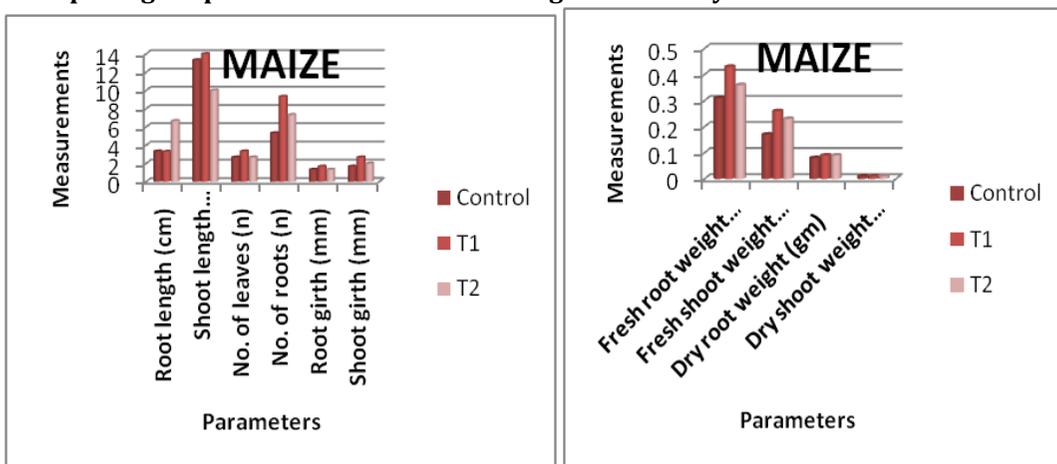


Table 7. Evaluation of seed vigour index (S.V.I)

Crops	Isolates	Per cent germination (%)	Seed Vigour Index (per cent germination × seedling length)
Wheat	<i>B. subtilis</i> (T1)	92%	3680
	<i>P.aeruginosa</i> (T2)	85%	3485
	Control (C)	64%	2086
Maize	<i>B. subtilis</i> T1	85%	1700
	<i>P.aeruginosa</i> T2	68%	1224
	Control C	42%	672

Table7. Chlorophyll content of Wheat and Maize.

Crops	Control (mg)			T1(mg)			T2(mg)		
	Chla	Chlb	Total	Chla	Chlb	Total	Chla	Chlb	Total
Wheat	2.390	3.485	5.875	7.0553	13.7628	20.8181	3.4571	5.9997	9.4568
Maize	1.743	3.287	5.030	3.4829	8.7477	12.2300	3.2378	6.1371	9.3749

Figure5. Chlorophyll content of Wheat and Maize.

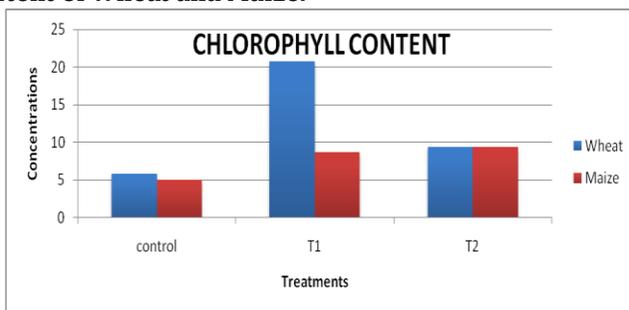


Table8. Testing of soil samples w.r.to. pH, N, P and K content

Test	Days	Control	T1	T2
pH	0	6.5	6.5	6.5
	7 th	6.5	6.5	6.5
	14 th	5.5	6	6.5
Nitrogen (kg/Acre)	0	40	40	40
	7 th	40	99	99
	14 th	40	50	80
Phosphorus (Kg/Acre)	0	11	10	10
	7 th	3	3	3
	14 th	3	10	10
Potassium (Kg/Acre)	0	121	121	155
	7 th	121	50	80
	14 th	50	125	155

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