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The Effect of Plant Growth Promoting Rhizobacteria on Groundnut (*Arachis hypogaea* L.) Seed Germination and Biochemical constituents

S.Mathivanan*, A.L.A.Chidambaram, P.Sundaramoorthy, L.Baskaran and R.Kalaikandhan

Department of Botany, Annamalai University, Annamalai Nagar, Chidambaram- 608 002, TamilNadu, India
Corresponding author

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A B S T R A C T

This study aimed at assessing the effect of three plant growth promoting rhizobacteria (PGPR) either singly or in combination on groundnut under pot culture experiment. Seeds were inoculated with single and combined solution of 10^9 CFU/ml of rhizobacteria. Seeds were not inoculated for the control variant. The highest germination percentage, seedling length, vigour index, dry weight, photosynthetic pigment, biochemical studied and enzyme studied was obtained with the combination of *Rhizobium* + *Pseudomonas* + *Bacillus* as compared to the uninoculated control. The result of the study indicate the potential of harnessing the benefit combination of plant growth promoting rhizobacteria to improve the germination and biochemical content of groundnut.

Introduction

Oil seed crops have been the backbone of agricultural economy of India. India is one of the largest producers of oilseeds in the world. Indian Council of Medical Research (ICMR) recommended consumption of 20 g edible oil /day/person by 2020 A.D. India requires around 20.3 million tonnes of edible oil (Karunaakaran *et al*., 2009). Oil seed are grown over an area of 24.7 million hectares with total production of 33 million in the world (Thamaraikannan *et al*., 2009). Today, these crops are cultivated on about 16.5 million hectors with a total production of nearly 10 million tonnes.

In India groundnut is cultivated in an area of 6.29 million hectares with an average productivity of 1010kg ha^{-1} and it is low when compared to the world average yield of 1640 kg ha^{-1}. The crop production application of plant protectants and farming practices need to be changed without affecting the yield or quality of the crops with the advancement of new generation technologies. PGPR are known to improve plant growth in many ways when compared to synthetic fertilizers, insecticides and pesticides. They enhance crop growth and

help in sustainability of safe environment and crop productivity. The rhizospheric soil contains diverse types of PGPR communities, which exhibit beneficial effects on crop productivity.

Experimental

Seed materials

The seeds of groundnut (*Arachis hypogaea* L.) var. VRI- 2 were obtained from Regional Research Station of Tamil Nadu Agricultural University, Virudhachalam, Cuddalore District, Tamil Nadu, India.

Plant Growth Promoting Rhizobacteria

Plant growth promoting rhizobacteria (*Rhizobium*, *Pseudomonas* and *Bacillus*) were obtained from the Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalainagar, Tamil Nadu.

Pot culture experiment

Pot experiments were conducted in Botanical Garden, Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu.

Seed treatment

The seeds of groundnut were surface sterilized with 80 percent ethanol and 0.1 percent mercuric chloride and washed the seeds with sterile distilled water for 3 to 4 times. The seeds were mixed with carrier based plant growth promoting rhizobacteria, either as individual organisms or consortium of organisms separately having a cell load of $1\chi10^{9}$ CFU/ ml^{-1} and shade dried for 30 min. After shade drying, the seeds were sown.

Germination percentage

The number of seeds germinated in each treatment was counted on 7^{th} day after sowing. Three replicates were maintained for each treatment. The total germination percentage was calculated by using the following formula.

$$\text{Germinationpercentage} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Seedling growth (cm/seedlings)

Ten seedlings were randomly selected from each treatment for recording the seedling growth. The growth of the seven days old groundnut seedlings were measured by using a centimeter scale and the values were recorded.

Vigour index (Abdul-Baki and Anderson)

Seedling height and germination percentage were recorded and vigour index was calculated as per the procedure.

Vigour index = Germination percent ×Total length of seedling (cm)

Dry weight (g/seedling)

The same seedlings used for seedling growth measurement were kept in a hot air oven at 80°C for 24 hrs. Then, the seedlings were kept in desiccators for sometime. Their dry weight was taken by using an electrical single pan balance and the average values were expressed in g/seedling.

Biochemical analyses

The photosynthetic pigments such as chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll and carotenoid and the

biochemical contents such as protein, amino acid and sugars and enzyme activities catalase, peroxidase and polyphenol oxidase were analyzed in both the seedlings and plants grown in the pot culture experiments. In case of pot experiments, the test crop were randomly collected periodically (25, 50, 75 and 100 DAS) and separated into root and leaf they were used for biochemical content analyses.

Chlorophyll (Arnon, 1949)

Five hundred mg of fresh leaf material was ground with a mortar and pestle with 10 ml of 80 percent acetone. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved and the residue was re-extracted with 10 ml of 80 percent acetone. The supernatant was saved and the absorbance values were read at 645 and 663 nm in a UV-Spectrophotometer. The chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents were estimated and expressed in mg/g fresh weight basis

Carotenoid (Kirk and Allen, 1965)

The same plant extract used for chlorophyll estimation was also used for carotenoid estimation. The acetone extract was read at 480 nm in a UV Spectrophotometer. The carotenoid content was calculated by using the following formula and it is also expressed in mg/g fresh weight

Estimation of Protein (Lowry *et al.*, 1951)

One ml of the extract was taken in a 10 ml test tube and 5 ml of reagent 'c' was added. The solution was mixed and kept in darkness for 10 minutes. Later, 0.5 ml of Folin phenol reagent was added and the mixture was kept in dark for 30 minutes. The sample was read at 660 nm in a UV Spectrophotometer.

Estimation of amino acids (Moore and Stein, 1948)

One ml of the extract was pipette out into a test tube. A drop of methyl red indicator was added. The sample was neutralized with one ml of 0.1N sodium hydroxide. To this, one ml of ninhydrin reagent was added and mixed thoroughly. The content of the test tube was heated for 20 minutes in a boiling water bath. Five ml of the dilute solution was added and heated in water bath for 10 minutes. The test tubes were cooled under the running water and the contents were mixed thoroughly. Blank was prepared with one ml of distilled water (or) ethanol. The absorbance was read at 570 nm in a UV – Spectrophotometer.

Estimation of sugars (Nelson, 1949)

One ml of extract was taken in a 25 ml marked test tube 1 ml of reagent 'C' was added. Then, the mixture was heated for 20 minutes at 100°C in boiling water bath, cooled and 1 ml of arsenomolybdate reagent was added. The solution was thoroughly mixed and diluted to 20 ml with distilled water. The sample was read at 520 nm in a UV Spectrophotometer.

Enzyme activity

The activities of catalase, peroxidase and polyphenoloxidase in groundnut were estimated and recorded in the plants grown in pot culture experiment in 7^{th} DAS at all the sampling days (25^{th}, 50^{th}, 75^{th} and 100 DAS).

Catalase (Machly and Chance, 1967) H_2O_2 Oxido reductase

One gram of leaf sample was homogenized in 10 ml of 0.1 N phosphate buffers (pH7),

and centrifuged at 4^0C for 10 minutes at 10,000 rpm. An aliquot of one ml of the supernatant of the enzyme extract was added to the reaction mixture containing. One ml of 0.01M H_2O_2 and 3 ml of 0.01M phosphate buffer. The reaction was stopped after incubation of 5 minutes at 20° C by adding 10 ml of one percent H_2SO_4. The acidified medium without or with the enzyme extract was titrated against 0.005 N $KMNO_4$ and catalase enzyme activity expressed as 'n' moles of H_2O Utilize (units min/mg/protein).

Peroxidase (Kumar and Khan 1982)
Hydrogen peroxidant oxireductase

Assay mixture of peroxidase contained 2 ml of 0.1 M phosphate buffer (pH 6.8). 1 ml of 0.001 M pyragallol, 12 ml of 0.005 hydrogen peroxidases and 0.5 ml of enzyme extract. The solution was incubated for 5 minutes at 25°C, after which the reaction was terminated by adding 1 ml of 2.5 N of sulphuric acid. The amount of purpurogallin formed was determined by reading the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N of sulphuric acid. The activity was expressed in unit = 0.1 absorbance mg/ protein / min.

Polyphenol oxidase (Kumar and Khan (1982)

(O- Diphenol: O2 Oxido reductase, EC 1.10.3.1)

Assay mixture for Polyphenoloxidase contained 2 ml of 0.1M phosphate buffer (pH 6.0), 1 ml of 0.1M catechol and 0.5 ml of enzyme extract. This was incubated for 5 min at 25°C, after which 1ml of 2.5N H_2SO_4 was added and stopped the reaction. The absorbance of the purpurogallin formed was read at 495 nm. To the blank, 2.5N H_2SO_4 was added to the same assay mixture. PPO activity is expressed in U mg^{-1} protein (U=Change in 0.1 absorbance min^{-1} mg^{-1} protein). For all the enzymatic calculations, protein was determined by the method of Bradford (1976), using Bovine Serum Albumin (BSA, Sigma, USA) as the standard.

Table.1 Effect of various treatment of plant growth promoting rhizobacteria on seed germination, growth, vigour index and dry weight of groundnut (*Arachis hypogaea* L.) seedlings

Treatments	Germination percentage	Seedling length (cm/seedling)	Vigour index (seedling length × germination percentage)	Seedling dry weight (g/seedling)
Control (**T_0**)	80	5.6 ± 0.17	448.0	0.89 ± 0.027
Rhizobium (**T_1**)	84	6.3 ± 0.19	529.2	1.02 ± 0.031
Pseudomonas (**T_2**)	87	7.0. ± 0.21	609.0	1.16 ± 0.035
Bacillus (**T_3**)	89	7.6 ± 0.23	676.4	1.29 ± 0.039
Rhizobium + Pseudomonas (**T_4**)	92	8.1 ± 0.24	745.2	1.42 ± 0.043
Rhizobium + Bacillus (**T_5**)	94	8.6 ± 0.26	808.4	1.54 ± 0.046
Pseudomonas + Bacillus (**T_6**)	96	9.0 ± 0.27	864.0	1.67 ± 0.050
Rhizobium + Pseudomonas + Bacillus (**T_7**)	98	9.5 ± 0.29	931.0	1.82 ± 0.055

Mean ± Standard deviation

Table.2 Effect of various treatment of Plant growth promoting rhizobacteria on chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid contents (mg/g fr. wt.) of groundnut (*Arachis hypogaea* L.) seedlings

Treatments	Chlorophyll 'a'	Chlorophyll 'b'	Total Chlorophyll	Carotenoid
Control (T_0)	0.454± 0.014	0.384± 0.012	0.838± 0.025	0.232± 0.007
Rhizobium (T_1)	0.492± 0.015	0.407± 0.012	0.899± 0.027	0.259± 0.008
Pseudomonas (T_2)	0.545± 0.016	0.429± 0.013	0.974± 0.029	0.287± 0.009
Bacillus (T_3)	0.600± 0.018	0.448± 0.013	1.048 ± 0.031	0.301± 0.009
Rhizobium + pseudomonas (T_4)	0.648 ± 0.019	0.467 ± 0.014	1.147± 0.034	0.348± 0.010
Rhizobium + Bacillus (T_5)	0.672 ± 0.020	0.483± 0.014	1.155± 0.035	0.421± 0.013
Pseudomonas + Bacillus (T_6)	0.697 ± 0.021	0.497± 0.015	1.194± 0.036	0.460± 0.014
Rhizobium + Pseudomonas +Bacillus (T_7)	0.764± 0.023	0.549± 0.016	1.313± 0.039	0.570± 0.017

Mean ± Standard deviation

Table.3 Effect of various treatment of Plant growth promoting rhizobacteria on protein, amino acid and sugar contents **(mg/g fr. wt.)** of groundnut (*Arachis hypogaea* L.) seedlings

Treatments	Protein	Amino acid	Sugar
Control (T_0)	12.890± 0.386	4.807± 0.144	3.209± 0.096
Rhizobium (T_1)	14.768± 0.443	5.107± 0.153	3.724± 0.112
Pseudomonas (T_2)	16.807± 0.504	5.427± 0.163	4.271± 0.128
Bacillus (T_3)	18.647± 0.559	5.807± 0.174	4.668± 0.140
Rhizobium + Pseudomonas (T_4)	20.722± 0.622	6.019± 0.181	4.970± 0.149
Rhizobium +Bacillus (T_5)	22.022± 0.660	6.309± 0.189	5.380± 0.161
Pseudomonas + Bacillus (T_6)	24.587± 0.737	6.593± 0.198	5.712± 0.171
Rhizobium +Pseudomonas+ Bacillus (T_7)	26.459± 0.794	7.847± 0.235	6.070± 0.182

Mean ± Standard deviation

Table.4 Effect of various treatment of plant growth promoting rhizobacteria on catalase, peroxidase and polyphenol oxidase of groundnut (*Arachis hypogaea* L.) seedlings

Treatments	Catalase units/min/g/fr.w)	Peroxidase (units/min/g/fr.w)	Polyphenol oxidase (units/min/g/fr.w)
Control (T_0)	8.64± 0.259	10.46± 0.314	1.84± 0.055
Rhizobium (T_1)	8.92±0.268	11.02± 0.331	1.96± 0.059
Pseudomonas (T_2)	9.28± 0.279	11.74± 0.352	2.10± 0.063
Bacillus (T_3)	9.55± 0.289	12.36± 0.371	2.24± 0.067
Rhizobium + Pseudomonas (T_4)	9.82± 0.295	12.90± 0.387	2.36± 0.071
Rhizobium +Bacillus (T_5)	10.14± 0.304	13.40± 0.402	2.48± 0.074
Pseudomonas + Bacillus (T_6)	10.42± 0.313	13.96± 0.419	2.60± 0.078
Rhizobium +Pseudomonas+ Bacillus (T_7)	10.75± 0.323	14.50± 0.435	2.76± 0.083

Mean ± Standard deviation

Result and Discussion

Germination studies

Germination percentage, growth and dry weight of groundnut seedlings as influenced by the application of Plant growth promoting rhizobacteria, such as control, *Rhizobium*, *Pseudomonas*, *Bacillus*, *Rhizobium* + *Pseudomonas*, *Rhizobium* + *Bacillus*, *Pseudomonas* + *Bacillus* and *Rhizobium* + *Pseudomonas* + *Bacillus* treatment/seeds are presented in Table 1. The highest germination percentage (98%), seedling growth (9.5 cm/seedling), vigour index 931 (seedling length χ germination percentage) dry weights (1.82 g/seedling) were recorded in groundnut seedlings grown with *Rhizobium* + *Pseudomonas* + *Bacillus* treatment. The lowest germination percentage (80%) seedling growth (5.6 cm/seedling), vigour index 448.0 (seedling length χ germination percentage) and dry weight (0.89 g/seedlings) were recorded in groundnut seedlings grown without Plant growth promoting rhizobacteria treatment. The combined application of the microbes enhanced seed germination and plant growth better than individual application. (Yadave *et al*., 2013; Noumavo *et al*., 2013). A commercial soil amendment containing a mixture of four PGPR (*Azospirillum lipoferum*, *Azotobacter chroococcum*, *Pseudomonas fluorescence* and *Bacillus megaterium*) was evaluated for impact on germination rate and vigour index compared with the control (Lenin and Jayanthi, 2012). The effect of plant growth promoting rhizobacteria on seed germination, seedling growth and yield of field grown maize were to be significantly enhanced (Gholami *et al*., 2009).

Photosynthetic pigments

The effect of Plant growth promoting rhizobacteria on the photosynthetic pigment contents of groundnut seedlings are presented in Table 2. The highest photosynthetic Pigments such as chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid content (0.764, 0.544, 1.313, 0.570 mg/g fr. wt.) of groundnut seedlings were recorded in *Rhizobium* + *Pseudomonas* + *Bacillus* treatment. The lowest chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid contents (0.454, 0.384, 0.838, 0.232 mg/g fr. wt.) were recorded in groundnut seedlings grown without Plant growth promoting rhizobacteria. Farinaz Vafadar *et al*. (2014) reported that the effect of single, dual, or triple inoculation of Plant Growth Promoting Rhizobacteria on the promotion of plant growth, NPK and Chlorophyll content of *Stevia* plants. The combined inoculation of Plant Growth Promoting Rhizobacteria showed that higher total chlorophyll content and corresponding increase in chlorophyll pigment in the Rajmash plant (Mishra *et al*., 2013).

Biochemical studies

The results on the effect of Plant growth promoting rhizobacteria and biochemical studies in groundnut are presented in Table 3. The highest protein, amino acid and sugar (25.587, 7.847 and 4.970 mg/g fr. wt.) are recorded in groundnut seedlings grown in *Rhizobium* + *Pseudomonas* + *Bacillus* treatment.

The lowest protein amino acid and sugar contents (18.647, 5.207 and 3.009 mg/g fr. wt.) are recorded in groundnut seedlings grown without Plant growth promoting rhizobacteria. Akbari *et al*. (2011) reported that the protein content was improved by the inoculation of PGPR, as compared to the control. Basu *et al*. (2008) have also reported that protein content was increased by the inoculation of PGPR, when compared to the control treatment.

Enzyme activity

The results on the effect of plant growth promoting rhizobacteria and enzyme studies in groundnut are presented in Table 4. The highest catalase, peroxidase and polyphenoloxidase (10.75, 14.50 and 2.76 units/min/g/fresh weight) are recorded in groundnut seedling grown in combination of plant growth promoting rhizobacteria treatment. The lowest catalase, peroxidase and polyphenoloxidase content (8.64, 10.46 and 1.84 units/min/g/fresh weight) are recorded in groundnut seedling grown without plant growth promoting rhizobacteria treatment. PGPR application has induced plant defence enzyme (such as phehylamine ammonia –lyase, peroxidase and polyphenoloxidase) activities in the leaf and root of, *Piper betle* L (Lavania *et al*., 2006). The enhanced activities of the enzymes trough the application of PGPR are reported in *Antheraea assama* (Unni *et al*., 2008)

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