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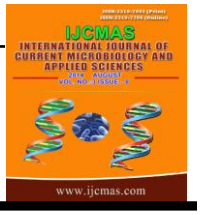

Original Research Article

Influence of PGPR on pigment concentration on *Glycine max* (L). Merr.

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Keywords

PGPR, *Glycine max*, Plant pigments, *Bacillus subtilis*

A B S T R A C T

Plant growth promoting rhizobacteria such as *Pseudomonas fluorescens* and *Bacillus subtilis* are common beneficial microbes of leguminous plants. In this study, the effect of PGPR strains *Pseudomonas fluorescens* and *Bacillus subtilis* when inoculated onto the soybean plant had been demonstrated. It is recently suggested that soil bacteria that indirectly stimulate plant growth be referred to as biocontrol plant growth promoting bacteria while bacteria that directly stimulate plant growth be referred to as plant growth promoting bacteria PGPB. The *Glycine max* is rich protein source for human beings. We inoculate the PGPR stains for to check the growth and pigment characteristics of *Glycine max*. The soybean plant had been singly and co-inoculated with *Pseudomonas fluorescens* and *Bacillus subtilis* resulted in increased shoot and root length, plant dry weight, soil nutritive content and also increased biochemical constituent especially in protein content of the G*lycine max*.

Introduction

Modern agriculture is facing new challenges in which ecological and molecular approaches are being integrated to achieve higher crop yields while minimizing negative impacts on the environment. Diverse groups of soil-borne microbes (eg, root endophytic fungi, mycorrhizal fungi, plant growth promoting fungi and rhizobacteria) exert positive effects on plant growth and survival.

Bacteria especially *Pseudomonads* and *Bacilli* found in the rhizosphere of various leguminous crops, have been found to assist in root colonization by *Rhizobia* and in suppressing soil-borne plant pathogens (Senthilkumar *et al.,* 2009). The interactions between these PGPR and rhizobia may be synergistic or antagonistic and beneficial effects of such interaction may be exploited for enhancing the biological nitrogen fixation and crop yield. There is also report of the presence of plant growth promoting *Bacillus* strains in the root nodules of soybean plants (Yu Ming *et al.,* 2002).

The effect of nine PGPR isolates on plant growth, yield and nutrient uptake of peanut in pots as well as under field conditions for 3 years have been evaluated by Dey *et al.,* (2004).

The observed increases in synthesis of essential oils can be considered as a defensive response to colonization by microorganisms, since several essential oils have antimicrobial properties (Erika *et al.,* 2008).

The present objective is to evaluate PGPR stains are how will increase the pigment content in soybean plant.

Materials and Methods

Bacterial strains

Plant growth promoting rhizobacteria such as *Pseudomonas fluorescens* and *Bacillus subtilis* were used for the present study. The cultures were collected from Tamil Nadu Agricultural University, Coimbatore.

Soybean plant

The soybean (*Glycine max* L. Merr) seeds was used for the present study. It was obtained from agro based farm near Erode, Tamil Nadu.

Bacterial growth and seed inoculation

The rhizobacterial cultures *Pseudomonas fluorescens* and *Bacillus subtilis* were streaked on nutrient agar medium and preserved as slant cultures for future reference. The soybean seeds were inoculated based on the method followed by Sher *et al.*, (2010).

The nutrient broth containing the culture was centrifuged at 8000 rpm at 4° C for about 10 minutes under chilled conditions. Supernatant was discarded and collect the pellets from all the centrifuge tubes and dissolve those pellets in 5 ml of sterile distilled water.

Serial dilution was prepared upto 10^{-8} dilution. The soybean seeds were surface sterilized by dipping in 95% ethanol solution for 30 seconds, following a dip in 0.2% mercuric chloride solution for 2 minutes.

The treated seeds were washed thoroughly with sterilized water to remove disinfectant completely from the seeds. The seeds were inoculated by immersing them for 15 minutes in the broth culture of individual strains containing 10^{-8} cfu ml^{-1}.

Sowing of seed

The soil was also treated with *Pseudomonas fluorescens* and *Bacillus subtilis* by mixing the nutrient broth containing the cultures with it and the treated and untreated seeds were sown at a depth of about 2 cm. The soil mixed with nutrient broth without any culture to which untreated seeds were sown is denoted as T_1. The soil mixed with nutrient broth inoculated with *Pseudomonas fluorescens* to which treated seeds were sown is denoted as T_2. The soil mixed with nutrient broth containing *Bacillus subtilis* to which treated seeds were sown is denoted as T_3. The soil mixed with nutrient broth co-inoculated with *Pseudomonas fluorescens* and *Bacillus subtilis* to which treated seeds were sown is denoted as T_4.

Soybean germination test

The germination rates of soybean seeds were calculated for T_1, T_2, T_3 and T_4 respectively. From this the high percentage of germination rate can be reported.

Shoot and root measurements

The soybean shoot and root length, wet and dry weight of shoot and root, number of leaves, number of nodes, leaf area were measured. For measuring the dry weight, the samples were dried in a oven.

Estimation of photosynthetic pigments

Chlorophyll

Chlorophyll contents was estimated by following the method of Moran and Porath

(1980) using the formulae suggested by Inskeep and Bloom (1985). Fresh leaf discs of 100 mg were cut and placed in a test tube containing 10 ml of N',N-dimethylformamide (DMF) and stored for 24 hours at 4°C.

Carotenoid (Ikan, 1969)

Absorbance values of pigment extracts at 480, 647 and 666 nm were used to find the corrected absorbance (A) for carotenoids.

Estimation of absorbing pigments

Anthocyanin (Mancinelli *et al., 1975*)

Anthocyanin content was extracted in acidified methanol (Methanol: Hcl – 99:1) in 100 mg of leaf material. Extract was kept at 0^0C for 24 hours.

After 24 hours, the content was made upto 10 ml and the absorbance was read at 530 nm. The content of anthocyanin is expressed in absorbance unit.

Flavanoids (Mirecki and Teramura, 1984)

Hundred milligrams of leaves were placed in 80 percent acidified methanol (Methanol : Water : Hcl – 80: 20: 1) for 12 hours in dark at 4^0C to extract flavonoids. Absorbance was read at 315 nm and the content of flavanoid is expressed as absorbance units.

Estimation of soluble protein (Bradford, 1976)

Extraction

Five hundred milligram of leaves were ground in a mortar and pestle with 10 ml of 20 percent Trichloro acetic acid (TCA). The homogenate was centrifuged for 15 minutes at 800 g. The supernatant was discarded and to the pellet, 5 ml of 0.1 N NaOH (400 mg of NaOH was dissolved in distilled water and made upto 100 ml) was added and centrifuged at 800 g for 15 minutes. The supernatant was made upto 10 ml with 0.1 N NaOH and used for estimation of protein content.

Assay

After complete solubilization, 0.1 ml of the extract was added to 5 ml of protein reagent and the contents were mixed by overtaxing. The absorbance at 595 nm was measured after 2 minutes in 3 ml cuvettes against a reagent blank prepared from 0.1 ml of 0.1 N NaOH and 5 ml of protein reagent. Bovine serum albumin fraction V was used as standard.

Protein reagent

Dissolve 100 mg of Commassie brilliant blue G 250 in 50 ml of 95 percent ethanol. To this solution, 100 ml of 85 percent phosphoric acid was added. The resulting solution was diluted to a final volume of one litre. Final concentration in the reagent was 0.01 percent (w/v) Commassie brilliant blue G 250, 4.7 percent (w/v) ethanol and 8.5 percent (w/v) phosphoric acid.

Results and Discussion

The inoculated and the uninoculated soybean seeds were sown in the soil and its growth characteristics in terms of shoot length, root length and number of leaves were observed (Table 1).

The plant pigments such as chlorophyll a, chlorophyll b, total chlorophyll (Moran and Porath 1980) and carotenoid (Ikan 1969) present in the soybean plant were determined and the results were as tabulated below (Table 2). The protein content of the soybean was determined and the amounts were tabulated below (Table 3).

Plant growth promoting rhizobacteria such as *Pseudomonas fluorescens* and *Bacillus subtilis* are common beneficial microbes of leguminous plants. It is frequently suggested that these rhizobacteria may improve phosphorous nutrition, enhance nitrogen uptake, improve disease resistance in their host plants or adaptation to various environmental stresses. The principal objective of this work was to promote the growth of the soybean plant using PGPR *Pseudomonas fluorescens* and *Bacillus subtilis* and also to find whether there is any change in the protein content of soybean.

This study (Table 1) indicates that dual inoculation with *Pseudomonas fluorescens* and *Bacillus subtilis* can increase the plant weight, leaf number as well as the length of the soybean plants more than the uninoculated one.In this connection, Gholami *et al*.,(2009) proved that *Pseudomonas fluorescens* and *Bacillus subtilis* can play an important role in promoting the growth of the plants.

Table.1 Growth characteristics of Soybean plant

Plant	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
Control	21	2.04	10	1.02
T_1	23	5.49	15	3.85
T_2	25	10.54	14	5.02
T_3	56	15.38	30	5.25

Control: Soybean plant without any bacterial culture inoculated.
T1: Soybean plant with *Bacillus subtilis* inoculated.
T2: Soybean plant with *Pseudomonas fluorescens* inoculated.
T3: Soybean plant co-inoculated with *Bacillus subtilis* and *Pseudomonas fluorescens*.

Table.2 Pigments in the soybean plant

Plant	Chlorophyll a (mg/g leaf fr.wt)	Chlorophyll b (mg/g leaf fr.wt)	Total Chlorophyll (mg/g leaf fr.wt)	Carotenoid (mg/g leaf fr.wt)
Control	3.33	1.84	5.01	0.13
T_1	4.49	1.88	5.81	0.16
T_2	6.87	2.15	10.26	0.18
T_3	7.24	3.39	16.07	0.27

Table.3 Protein content of the Soybean

Plant	Protein content (mg/g)
Control	2.05
T_1	3.84
T_2	4.05
T_3	4.26

The data in Table 2 showed that the chlorophyll content of the soybean plant co-inoculated with *Pseudomonas fluorescens* and *Bacillus subtilis* had been increased when compared with the control. This finding supported results from previous studies reporting that nitrogen-fixation by the rhizobacteria can help in improving the chlorophyll content of the soybean plant (Wu *et al.*,2006).

The agriculturally important microorganisms play a remarkable role in nutrient (nitrogen and phosphorous) acquisition for plants. In pursuit of that goal, various workers (Dey *et al.*,2004; Saleh Al-Garni 2006) have used PGPR as single inoculants and in combination with each other's in various plants. These symbiotic organisms have high ability to increase nitrogen, phosphorous and potassium as well as other nutrients in inoculated plants.

In conclusion, plant growth promoting rhizobacteria such as *Pseudomonas fluorescens* and *Bacillus subtilis* are common beneficial microbes of leguminous plants. In this study, the effect of PGPR strains *Pseudomonas fluorescens* and *Bacillus subtilis* when inoculated onto the soybean plant had been demonstrated. The soybean plant had been singly and co-inoculated with the above mentioned strains. The co-inoculation with *Pseudomonas fluorescens* and *Bacillus subtilis* resulted in increased shoot and root length, plant dry weight, soil nutritive content and also increased protein content of the soybean.

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