

REGULAR ARTICLE

Impact of PGPR inoculation on photosynthetic pigment and protein contents in *Arachis hypogaea* L.

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Abstract

The impact of microbial consortium comprising plant development advancing rhizobacteria (PGPR) like *Rhizobium*, *Pseudomonas* and *Bacillus* were tried independently and in blend of *Arachis hypogaea*. The mixes of previously mentioned PGPR strains essentially expanded photosynthetic color (chlorophyll an and b, add up to chlorophyll and carotenoid) and protein content in *A. hypogaea*, when contrasted with the un-inoculated control. The consequences of this study propose that PGPR connected in mix can possibly build the photosynthetic colors and protein substance of *A. hypogaea* which can be a potential tool in increasing the yield in this economically important crop in sustainable way.

Key words: PGPR, *Arachis hypogaea*, photosynthetic, protein

Introduction

India is a nation which primarily depends on agriculture. Horticulture adds to a noteworthy share of national wage. Feasible farming is crucially critical in this day since it offers the possibility to meet the future rural need. Recently, sustainable agriculture is of great interest in almost all regions in India (Basha and Selvaraju, 2015).

When compared to conventional fertilizers and composts, Plant Growth Promoting Rhizobacteria (PGPR) are known to enhance plant development from various perspectives. They improve the soil and plants at the same time in more like an organic way with high sustainability (Singh, 2013). PGPR has been in spotlight among the agriculturists for their advantages on harvest yield. A few researchers have taken after multidisciplinary ways to deal with improve the viability and assortment of components required in expanding the plant development and profitability (Rathore, 2014).

In our previous study, we reported the effects of PGPR on pigments and antioxidant enzyme activities of *Arachis* in seedling stage (Mathivanan et al., 2014).

The aim of this study were to assess the impact of PGPR on photosynthetic pigment and protein content in *Arachis hypogaea* L.

Material methods

Seed material

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.

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Pot culture experiment

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

Pot culture experiment details

Crop : Groundnut (*Arachis hypogaea* L.)

Variety : VRI 2

Design : Complete Randomized Block Design

Sampling days : 25, 50, 75 and 100 DAS

Parameters studied : Photosynthetic pigments (chlorophyll a, b & Total chlorophylls) and Protein.

Seed treatment

The treatment with PGPR was done as described previously (Mathivanan et al., 2014). 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 1×10^9 CFU/ ml⁻¹ and shade dried for 30 min. After shade drying, the seeds were sown.

Pigment analysis

The analysis of Chlorophyll a, b and total chlorophyll were done by the method of Arnon, (1949) and expressed in mg/g fresh weight.

Carotenoid content from the fresh leaves was done by following the method of Kirk and Allen (1965) and expressed in mg/g fresh weight.

Estimation of Protein

Protein was estimated by the method of Lowry et al. (1951) from the shoots and roots separately

Statistical analysis

Statistical significance was assessed at the $P < 0.05$ level using one-way ANOVA and means were separated by Duncan's multiple range test ($P < 0.05$) with the help of SPSS 16 software. Means and \pm standard deviations were calculated from three replicates

Results

Photosynthetic pigment

The PGPR had profound effect on the pigment contents on all the sampling days (25, 50, 75 and 100 DAS). The results are shown in Table. 1-4 for chlorophyll a, b, total chlorophyll and carotenoid contents. The highest chlorophyll

'a', chlorophyll 'b', total chlorophyll and carotenoid content (0.805, 0.740, 1.545 and 0.741 mg/g fr. wt.) were recorded in 75 days old crop plants grown with *Rhizobium* + *Pseudomonas* + *Bacillus*. The lowest chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid content (0.453, 0.315, 0.768, 0.290 mg/g fr. wt.) were recorded in 100 days crop grown without plant growth promoting Rhizobacteria.

Protein

The results on the effect of plant growth promoting rhizobacteria on protein content in root and leaf portion of groundnut at 25, 50, 75 and 100 DAS are shown in Table. 5 and 6. The highest protein 12.549, 13.683, 14.060 and 15.190 mg/g fr. wt. at 25, 50, 75 and 100 DAS were recorded in leaf portion of groundnut grown with *Rhizobium* + *Pseudomonas* + *Bacillus* treatment of PGPR. The lowest protein contents 6.913, 7.155, 7.336 and 7.869 mg/g fr. wt. at 25, 50, 75 and 100 DAS were recorded in the root portion of groundnut crop grown without plant growth promoting rhizobacteria.

Discussion

Chlorophyll

Chlorophyll is a vital segment of plant colors and assumes a crucial part during photosynthesis. Without adequate amount of this pigment, plant cannot perform photosynthesis. It has been demonstrated that chlorophyll assume a vital part in the ATP generation and assurance of fundamental plant constituents (Kochot et al., 1998). Chlorophyll analysis is one of the important biochemical parameters. It is used as an index of plant protection capacity. Chlorophyll 'a', 'b', and total chlorophyll content are indication of photosynthetic and metabolic activity (Wright and Jones, 2006; Hartmann et al., 2009).

We noted an alteration in the pigment contents in groundnut seedlings upon treatment with PGPR on all stages of its growth (25, 50, 75 and 100 DAS). The highest chlorophyll content was recorded in consortium treatment (*Rhizobium* + *Pseudomonas* + *Bacillus*). The highest chlorophyll content was recorded in 75 day old plants when compared with all other sampling days.

Kang et al., (2014) reported increased chlorophyll contents in the PGPR-treated plants under salinity and drought stress in *Cucumis sativus*. The PGPR (*Azospirillum*, *Azotobacter* and *Pseudomonas*) application

increased Chlorophyll 'a', Chlorophyll 'b' and total chlorophyll (Al-Erwy et al., 2016). PGPR inoculations significantly increased the chlorophyll content of strawberry plants (Karlidag et al., 2013). Lenin and Jayanthi (2012) reported that the consortium treatment of *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* enhanced the chlorophyll content of *Catharanthus roseus*. The combined application of *Pseudomonas* sp., *Bacillus lentus* and *Azospirillum brasilense* enhanced the chlorophyll content of *Ocimum basilicum* (Heidari et al., 2011). The growth attributes namely, chlorophyll content, and the total biomass were increased due to PGPR inoculation (Karami Chame et al., 2016). The increased chlorophyll content in plant leaves as a result of bacterial isolate co-inoculation could be due to the increased accumulation of plant nutrition and photosynthesis (Bashan et al., 1990).

Carotenoid

Carotenoid is an accessory pigment in photosynthetic assimilation of plants. The highest carotenoid content was registered in the crop grown in consortium treatment of plant growth promoting rhizobacteria. The highest carotenoid content was recorded in 75 day old plants when compared with all other sampling days. The lowest content was recorded in the crop grown without PGPR.

In lettuce the PGPR (*Azospirillum brasilense* + *Pantoea dispersa*) treatment had a positive effect on plant growth and the contents of carotenoids (Hernandez et al., 2014). Similarly, PGPR and P_2O_5 alone and in combination with each other on soybean (*Glycine max* L.) showed a significant increase in the carotenoid content (Dwivedi and Ram Gopal, 2013). The application of different strains of PGPR treatments showed that the highest value for carotenoid was observed during co-inoculation with *Azospirillum* + *Pseudomonas* in normal and stress

conditions (Ahmadi et al., 2013). The combined treatment of *Rhizobium leguminosarum* + *Bacillus megaterium* + *Bacillus mucilaginosus* enhanced the carotenoid content when compared to all other treatment of black gram (Rajasekaran, 2009).

Biochemical constituents

Protein

Protein is one of the reserve food material utilized by plants for the growth of the seedling. An increase in protein content was recorded in the crop grown in control, single, dual and combined treatment PGPR. Among all treatments, the highest protein content was observed in the plants grown in combined treatment of PGPR. In the experiment, the highest protein content was recorded in 75 DAS and it increased upto harvest stage. The shoot portion of crop contains higher protein content than the root. Significant increase was recorded in groundnut crop grown in *Rhizobium* + *Pseudomonas* + *Bacillus* treatment.

Growth promotion in *Vigna radiata* revealed significant increase in biochemical constituent parameters, viz., protein content, plant treated with *Pseudomonas fluorescens*, *Rhizobium* and phosphate solubilizing bacterial biofertilizers (Dhanya and Adeline, 2014). Alfalfa seeds were treated by PGPR combined with *S. meliloti* enhanced protein content of plants under field conditions (Sarhan and Shehata, 2014). Co-inoculation of PGPR was found much effective for protein contents of maize (Ullah et al., 2013). Adesemoye and Kloepper (2009) compiled the benefits derivable from plant-PGPR interactions with improvements in protein content. Inoculation of PGPR resulted in increased protein content in plants Basu et al. (2008) The application of Bradyrhizobium japonicum also increased the contents of protein in soybean grown with salt stress (Egamberdiyeva et al., 2004).

Table 1. Effect of plant growth promoting rhizobacteria on chlorophyll 'a' content (mg/g fr. wt.) of groundnut (*Arachis hypogaea* (L.)).

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	0.482 ± 0.014 ^e	0.525 ± 0.016 ^f	0.621 ± 0.019 ^d	0.453 ± 0.014 ^f
<i>Rhizobium</i> (T ₁)	0.493 ± 0.015 ^e	0.554 ± 0.017 ^{ef}	0.643 ± 0.019 ^d	0.473 ± 0.014 ^{ef}
<i>Pseudomonas</i> (T ₂)	0.524 ± 0.016 ^{de}	0.576 ± 0.017 ^{def}	0.669 ± 0.020 ^{cd}	0.506 ± 0.015 ^{de}
<i>Bacillus</i> (T ₃)	0.550 ± 0.017 ^{dc}	0.605 ± 0.018 ^{cde}	0.700 ± 0.021 ^{bc}	0.528 ± 0.016 ^{cd}
<i>Rhizobium</i> + <i>Pseudomonas</i> (T ₄)	0.584 ± 0.018 ^{bc}	0.619 ± 0.019 ^{bcd}	0.720 ± 0.022 ^{bc}	0.554 ± 0.017 ^{bcd}
<i>Rhizobium</i> + <i>Bacillus</i> (T ₅)	0.608 ± 0.018 ^{ab}	0.647 ± 0.019 ^{abc}	0.752 ± 0.023 ^{ab}	0.576 ± 0.017 ^{bc}
<i>Pseudomonas</i> + <i>Bacillus</i> (T ₆)	0.632 ± 0.019 ^{ab}	0.669 ± 0.020 ^{ab}	0.782 ± 0.023 ^a	0.603 ± 0.018 ^{ab}
<i>Rhizobium</i> + <i>Pseudomonas</i> + <i>Bacillus</i> (T ₇)	0.660 ± 0.020 ^a	0.696 ± 0.021 ^a	0.805 ± 0.024 ^a	0.630 ± 0.019 ^a
S.Ed.	0.02	0.03	0.03	0.02
CD (P = 0.05)	0.05	0.06	0.06	0.05

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P ≤ 0.05 (L.S.D.)

Table 2. Effect of plant growth promoting rhizobacteria on chlorophyll 'b' content (mg/g fr. wt.) of groundnut (*Arachis hypogaea* (L.)).

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	0.357 ± 0.011 ^g	0.425 ± 0.013 ^g	0.511 ± 0.015 ^g	0.315 ± 0.009 ^f
<i>Rhizobium</i> (T ₁)	0.390 ± 0.012 ^{fg}	0.462 ± 0.014 ^{fg}	0.546 ± 0.016 ^{fg}	0.332 ± 0.010 ^{ef}
<i>Pseudomonas</i> (T ₂)	0.416 ± 0.012 ^{ef}	0.496 ± 0.015 ^{ef}	0.573 ± 0.017 ^{ef}	0.349 ± 0.010 ^{def}
<i>Bacillus</i> (T ₃)	0.457 ± 0.014 ^{de}	0.528 ± 0.016 ^{de}	0.610 ± 0.018 ^{de}	0.370 ± 0.011 ^{cde}
<i>Rhizobium</i> + <i>Pseudomonas</i> (T ₄)	0.486 ± 0.015 ^{cd}	0.566 ± 0.017 ^{cd}	0.644 ± 0.019 ^{cd}	0.388 ± 0.012 ^{bcd}
<i>Rhizobium</i> + <i>Bacillus</i> (T ₅)	0.511 ± 0.015 ^{bc}	0.595 ± 0.018 ^{bc}	0.674 ± 0.020 ^{bc}	0.410 ± 0.012 ^{abc}
<i>Pseudomonas</i> + <i>Bacillus</i> (T ₆)	0.548 ± 0.016 ^{ab}	0.628 ± 0.019 ^{ab}	0.704 ± 0.021 ^{ab}	0.428 ± 0.013 ^{ab}
<i>Rhizobium</i> + <i>Pseudomonas</i> + <i>Bacillus</i> (T ₇)	0.585 ± 0.018 ^a	0.661 ± 0.020 ^a	0.740 ± 0.022 ^a	0.447 ± 0.013 ^a
S.Ed.	0.02	0.03	0.03	0.02
CD (P = 0.05)	0.05	0.06	0.06	0.05

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P ≤ 0.05 (L.S.D.)

Table 3. Effect of plant growth promoting rhizobacteria on total chlorophyll content (mg/g fr. wt.) of groundnut (*Arachis hypogaea* L.).

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	0.839 ± 0.025 ^g	0.950 ± 0.029 ^g	1.132 ± 0.034 ^g	0.768 ± 0.023 ^f
<i>Rhizobium</i> (T ₁)	0.883 ± 0.026 ^{fg}	1.016 ± 0.030 ^{fg}	1.189 ± 0.036 ^{fg}	0.805 ± 0.024 ^{ef}
<i>Pseudomonas</i> (T ₂)	0.940 ± 0.028 ^{ef}	1.072 ± 0.032 ^{ef}	1.242 ± 0.037 ^{ef}	0.855 ± 0.026 ^{def}
<i>Bacillus</i> (T ₃)	1.007 ± 0.030 ^{de}	1.133 ± 0.034 ^{de}	1.310 ± 0.039 ^{de}	0.898 ± 0.027 ^{cde}
<i>Rhizobium</i> + <i>Pseudomonas</i> (T ₄)	1.070 ± 0.032 ^{cd}	1.185 ± 0.036 ^{cd}	1.364 ± 0.041 ^{cd}	0.942 ± 0.028 ^{bcd}
<i>Rhizobium</i> + <i>Bacillus</i> (T ₅)	1.119 ± 0.034 ^{bc}	1.242 ± 0.037 ^{bc}	1.426 ± 0.043 ^{bc}	0.986 ± 0.030 ^{abc}
<i>Pseudomonas</i> + <i>Bacillus</i> (T ₆)	1.180 ± 0.035 ^{ab}	1.297 ± 0.039 ^{ab}	1.485 ± 0.045 ^{ab}	1.030 ± 0.031 ^{ab}
<i>Rhizobium</i> + <i>Pseudomonas</i> + <i>Bacillus</i> (T ₇)	1.245 ± 0.037 ^a	1.357 ± 0.041 ^a	1.545 ± 0.046 ^a	1.077 ± 0.032 ^a
S.Ed.	0.05	0.04	0.05	0.05
CD (P = 0.05)	0.10	0.09	0.10	0.10

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P ≤ 0.05 (L.S.D.)

Table 4. Effect of plant growth promoting rhizobacteria on carotenoid content (mg/g fr. wt.) of groundnut (*Arachis hypogaea* L.).

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	0.304 ± 0.009 ^h	0.376 ± 0.011 ^h	0.448 ± 0.013 ^h	0.290 ± 0.009 ^h
<i>Rhizobium</i> (T ₁)	0.356 ± 0.011 ^g	0.409 ± 0.012 ^g	0.499 ± 0.015 ^g	0.328 ± 0.010 ^g
<i>Pseudomonas</i> (T ₂)	0.394 ± 0.012 ^f	0.436 ± 0.013 ^f	0.549 ± 0.016 ^f	0.362 ± 0.011 ^f
<i>Bacillus</i> (T ₃)	0.433 ± 0.013 ^e	0.459 ± 0.014 ^e	0.586 ± 0.018 ^e	0.401 ± 0.012 ^e
<i>Rhizobium</i> + <i>Pseudomonas</i> (T ₄)	0.456 ± 0.014 ^d	0.504 ± 0.015 ^d	0.632 ± 0.019 ^d	0.445 ± 0.013 ^d
<i>Rhizobium</i> + <i>Bacillus</i> (T ₅)	0.486 ± 0.015 ^c	0.537 ± 0.016 ^c	0.668 ± 0.020 ^c	0.480 ± 0.014 ^c
<i>Pseudomonas</i> + <i>Bacillus</i> (T ₆)	0.505 ± 0.015 ^b	0.568 ± 0.017 ^b	0.705 ± 0.021 ^b	0.501 ± 0.015 ^b
<i>Rhizobium</i> + <i>Pseudomonas</i> + <i>Bacillus</i> (T ₇)	0.539 ± 0.016 ^a	0.593 ± 0.018 ^a	0.741 ± 0.022 ^a	0.528 ± 0.016 ^a
S.Ed.	0.003	0.005	0.005	0.003
CD (P = 0.05)	0.01	0.01	0.01	0.01

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P ≤ 0.05 (L.S.D.)

Table 5. Effect of plant growth promoting rhizobacteria on protein content (mg/g fr. wt.) in leaf of groundnut (*Arachis hypogaea* L.).

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	10.930 ± 0.33 ^e	11.686 ± 0.35 ^h	12.166 ± 0.36 ^h	12.897 ± 0.39 ^g
<i>Rhizobium</i> (T ₁)	11.222 ± 0.34 ^d	11.960 ± 0.36 ^g	12.488 ± 0.37 ^g	13.105 ± 0.39 ^f
<i>Pseudomonas</i> (T ₂)	11.526 ± 0.35 ^c	12.279 ± 0.37 ^f	12.745 ± 0.38 ^f	13.376 ± 0.40 ^e
<i>Bacillus</i> (T ₃)	11.711 ± 0.35 ^c	12.561 ± 0.38 ^e	12.998 ± 0.39 ^e	13.435 ± 0.40 ^e
<i>Rhizobium</i> + <i>Pseudomonas</i> (T ₄)	11.949 ± 0.36 ^b	12.845 ± 0.39 ^d	13.314 ± 0.40 ^d	13.952 ± 0.42 ^d
<i>Rhizobium</i> + <i>Bacillus</i> (T ₅)	12.157 ± 0.36 ^b	13.105 ± 0.39 ^c	13.576 ± 0.41 ^c	14.227 ± 0.43 ^c
<i>Pseudomonas</i> + <i>Bacillus</i> (T ₆)	12.387 ± 0.37 ^a	13.400 ± 0.40 ^b	13.836 ± 0.42 ^b	14.511 ± 0.44 ^b
<i>Rhizobium</i> + <i>Pseudomonas</i> + <i>Bacillus</i> (T ₇)	12.549 ± 0.38 ^a	13.683 ± 0.41 ^a	14.060 ± 0.42 ^a	15.190 ± 0.46 ^a
S.Ed.	0.10	0.01	0.01	0.06
CD (P = 0.05)	0.22	0.03	0.02	0.13

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P ≤ 0.05 (L.S.D.)

Table 6. Effect of plant growth promoting rhizobacteria on protein content (mg/g fr. wt.) in root of groundnut (*Arachis hypogaea* L.).

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	6.913 ± 0.21 ^a	7.155 ± 0.21 ^h	7.336 ± 0.22 ^h	7.869 ± 0.24 ^h
<i>Rhizobium</i> (T ₁)	7.223 ± 0.22 ^g	7.479 ± 0.22 ^g	7.793 ± 0.23 ^g	8.225 ± 0.25 ^g
<i>Pseudomonas</i> (T ₂)	7.410 ± 0.22 ^f	7.734 ± 0.23 ^f	8.224 ± 0.23 ^f	8.735 ± 0.26 ^f
<i>Bacillus</i> (T ₃)	7.675 ± 0.23 ^e	7.969 ± 0.24 ^e	8.636 ± 0.26 ^e	9.112 ± 0.27 ^e
<i>Rhizobium</i> + <i>Pseudomonas</i> (T ₄)	7.938 ± 0.24 ^d	8.266 ± 0.25 ^d	8.998 ± 0.27 ^d	9.574 ± 0.29 ^d
<i>Rhizobium</i> + <i>Bacillus</i> (T ₅)	8.271 ± 0.25 ^c	8.437 ± 0.25 ^c	9.375 ± 0.28 ^c	9.951 ± 0.30 ^c
<i>Pseudomonas</i> + <i>Bacillus</i> (T ₆)	8.541 ± 0.26 ^b	8.788 ± 0.26 ^b	9.660 ± 0.29 ^b	10.466 ± 0.31 ^b
<i>Rhizobium</i> + <i>Pseudomonas</i> + <i>Bacillus</i> (T ₇)	8.759 ± 0.26 ^a	9.021 ± 0.27 ^a	10.145 ± 0.30 ^a	10.868 ± 0.33 ^a
S.Ed.	0.01	0.01	0.01	0.01
CD (P = 0.05)	0.02	0.02	0.01	0.01

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P ≤ 0.05 (L.S.D.)

Conclusion

In the present study, there are significant variations in studied parameters under PGPR treatments in groundnut. There was significant enhancement in pigment and protein contents. This can be further studied for using as a potential tool to increase the yield in this

economically important crop in a sustainable way.

Author contributions

All authors contributed equally in the study and preparation of article. All authors approved the final version of the manuscript for publication.

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