International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 08-10-2021, Accepted: 23-10-2021, Published: 09-11-2021
Volume 6, Issue 6, 2021, Page No. 180-184

An innovative consortium based bacterial endophytic liquid formulation and its effect on the growth and yield of groundnut var. VRI-2

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Abstract
Groundnut the King of oilseeds is popularly called as wonder nut and poor men's cashew nut. India exports groundnut kernels, shell, handpicked selected (HPS) groundnut and its oil cake forms. Groundnut plays a pivotal role in the oilseed economy of India. Endophytes are wide range of microorganisms that live in host plants without causing diseases. Endophytic bacteria, helps the plants directly by plant growth promotion strategies by providing phytohormones to the plant, controlling ethylene levels in the plant by ACC deaminase synthesis and distributing key nutrients like N, P, iron, and other elements from the environment. They can also promote plant growth and development by activating a variety of novel mechanisms, such as IAA production, phosphate solubilizing activity, and siderophore activity. Besides enhanced plant growth, they also protect plant health through different methods. Anticipating these advantages this research was designed and conducted to isolate and characterize endophytic bacteria from different crop plants, that could help in enhancing its productivity and thereby food security. The chosen endophytic bacterial isolates were evaluated for the PGPR activity such as nitrogen fixation, indole acetic acid (IAA) production, GA_3 production, mineral phosphate solubilization, and siderophore (iron sequestering chemicals) production. The pot culture experiment was carried out in the Department of Agricultural Microbiology Annamalai University, Annamalai Nagar, Tamil Nadu. A Completely Randomized Design with three replicates and nine treatments were compared and each pot was considered one experimental unit. The effect of different treatments on plant growth parameters like plant height, root length, shoot length, germination percentage, vigour index, number of pods plant, Test weight seed yield and dry matter production (DMP) in Groundnut was assessed. The maximum germination percentage (96.21), vigour index (1624), plant height (56.42cm), root length (10.21cm), and shoot length (8.51cm) was recorded in treatment T_2 (100 % RDF) it was on par the treatment T_9 (*Lysinibacillus* sp. + *Rhizobium* + *Bacillus megatherium* + 50 % RDF) by recording the germination percentage (94.85), vigour index (1585), plant height (54.68cm), root length (9.84cm), and shoot length (8.23cm) was recorded. The maximum DMP production, number of pods plant, Test weight and kernel yield in Groundnut was recorded in T_2 (100 % RDF) were 54.21 (g plant $^{-1}$), 38.29, 51.99 (g plant $^{-1}$) and 227 (g plant $^{-1}$) and this was on par with T_9 (*Lysinibacillus* sp. + *Rhizobium* + *Bacillus megatherium* + 50 % RDF) like DMP production, number of pods plant, Test weight and seed yield is 52.65 (g plant $^{-1}$), 37.42 (g), 50.41 (g plant $^{-1}$) and 225 (g plant $^{-1}$).

Keywords: endophytes, PGPR, liquid formulation, consortium and groundnut

Introduction

Groundnut (*Arachis hypogeae L.*) is an important edible oilseed crop. The seed contains upto 55 per cent oil and 25 per cent protein. Groundnut oil is mostly useful in cooking as well as the production of edible fats and soaps. After the oil is extracted, the cake is mostly used as animal and poultry feed. The seeds are eaten whole as raw, boiling, or roasted kernels, and processed into a variety of confectionary products. The haulm is a good source of nutrition for animals. It is a South American native that originated in Central Brazil and is now cultivated all over the world between the latitudes of 40 N and 40 S. Almost every tropical and subtropical country in the globe grows groundnut on a big scale. India, China, Nigeria, Sudan, and the United States are the top groundnut producers. It is grown in 27.94 million hectares worldwide; it contributes overall production upto 47.09 million tonnes of seeds and its productivity ranges upto 1686kg/ha to world oil seed production (FAO STAT,2020). In India groundnut is grown in 5.02 million hectares; it contributes overall production upto 6.69 million tonnes (Gujarat, Rajasthan, Tamil Nadu) and its yield ranges upto 1393kg/ha oil seed production. In Tamil Nadu grown in 0.34 million hectares; production upto 0.88 million tonnes; and its yield ranges upto 2620 kg/ha (Directorate of Economics and Statistics, 2019).

The realm of eco-friendly agriculture has been flourishing with potential alternatives of harmful agrochemicals in last few decades. In the rhizosphere, endosphere, phyllosphere, and distinct ecological niches, microbes with significant PGPR, biocontrol, and abiotic stress reduction capacities are being investigated (Abbamondi *et al*., 2016) [1]. Endophytes among these have been shown to have potential application in agriculture. Bacterial endophytes can be found in a variety of plant components and have a positive impact on the host plant (Lodewyckx *et al*., 2002) [24]. They provide a wide range of benefits to their host plants, including nitrogen fixation, mineral solubilization, siderophore, phytohormone, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, secondary metabolites, enzymes, antibiotics, and the ability to induce systemic resistance and tolerance. etc. (Lodewyckx *et al*., 2002; Sahu *et al.*, 2016) [24, 33]. Endophytes have been shown to improve the

performance of other beneficial bacteria such as Rhizobium (Bai *et al*., 2003) [4]. Further, mineral nutrients in the rhizosphere have been reported to be solubilized by endophytic inoculants (Otieno *et al*., 2015) [30]. This improves crop performance while also reducing the need for greater amounts of chemical fertilisers. They also influence the plant physiology and performance, and improve plant growth and development by regulating phytohormone levels in the plants (Khan *et al*., 2013; Szilagyi-Zecchin *et al*., 2014) [23, 34].

Endophytic diazotrophic bacteria have been reported to be responsible for delivering biologically fixed N to their host plants, and their hunt for an environmentally acceptable and cost-effective alternative for N fertilisers is currently focusing on them. Endophytic bacteria, in theory, help plants develop either directly or indirectly. ACC deaminase synthesis reduces ethylene levels in plants directly, (Long *et al*., 2008) [25], or by removing key nutrients from the environment, such as N, P, Fe, and others (Glick *et al*., 1999) [10]. The indirect way involves the suppression of plant pathogens through the production of antagonistic secondary metabolites or by a combination of different mechanisms including niche competition (Gupta *et al*., 2012; Bacon and Hinton 2006) [14, 3]. Endophytic bacteria have been tested in field and greenhouse environments for N fertiliser savings and improved plant growth and production (Yanni and Dazzo, 2010; Govindarajan *et al*., 2008; Muthukumarasamy *et al*., 2007) [36, 12, 28].

Materials and Methods

Isolation and identification of endophytic bacteria from different crop plants

Five endophytic bacterial strains were isolated from different parts of the crop plants *viz.*, leaf, stem, and root. Morphological and biochemical characterization was carried out for the tentative identification of the bacterial species. The efficiency of the different endophytic bacterial isolates was examined for its nitrogen fixation, IAA, GA3, phosphate solubilization, and siderophore production. Among the different isolates *Lysinibacillus* sp., performed best when compared to all the other isolates. The best strain was morphologically characterized by 16S rRNA sequencing and the sequenced gene was submitted to the NCBI gene bank and was allotted with the deposition number (OK 036788). All the above studies were carried out by Arunachalam and Sivasakthivelan (2021).

Compatibility test between endophytic bacterial isolates

Dual culture technique

Compatibility among *Lysinibacillus* sp., *Rhizobium*, *Bacillus megatherium* was tested by following the dual culture technique (Dennis and Webster, 1971).

Preparation of liquid formulation of endophytic bacterial consortium

For the preparation of liquid formulation, the method suggested by Manikandan *et al*. (2010) was followed. The most effective isolate *Lysinibacillus* sp., *Bacillus megatherium* and *Rhizobium* were identified in the present study. Among these, *Lysinibacillus* sp., and *Bacillus megatherium* were multiplied on Luria broth and *Rhizobium* on YEMA broth. The mother culture of *Lysinibacillus* sp., *Bacillus megatherium* and *Rhizobium* were inoculated individually into respective broth and incubated at room temperature (28 ± 2°C). Further, the respective broths were added with glycerol at 2 per cent level. After the incubation period, the formulation was assessed for adequate CFU following serial dilution plating technique and the formulation thus prepared was sealed in plastic containers and used for further studies.

Liquid based formulation for combination studies

For assessing the efficacy of combination of different endophytic bacteria in the liquid based formulation of *Lysinibacillus* sp., *Bacillus megatherium* and *Rhizobium* were prepared separately and equal amounts of individual preparations with the adequate number of colony forming units (CFU) were mixed well just before use.

Seed treatment with different endophytic bacteria as single, dual and as consortium

Seeds of groundnut variety (VRI 2) were surface sterilized with two per cent sodium hypochlorite for 30 seconds, rinsed in sterile distilled water and dried overnight. Five ml of endophytic bacterial consortium based liquid formulated inoculum was taken in a Petri dish with 100 mg carboxy methyl cellulose (CMC) was used as an adhesive substance. Seeds were they, soaked in endophytic bacteria as single, dual and as consortium suspension for 2 hour and air dried overnight in a sterile Petri dish and for bacterization the selected endophytic isolate *Lycinibacillus* sp., *Bacillus megatherium* and *Rhizobium* were used for the treatment. The treated seeds were dropped directly into the potted soil that had already been prepared. In each pot, four 5 cm digs were made, with five bacterized seeds distributed at random to each dig. For combination treatments T0 through T7, seed-treated pots were prepared separately.

Pot culture experimental design

A Completely Randomized Design (CRD) with three replicates and nine treatments was fixed and a total of 27 pots were used, and each pot was considered as one experimental unit. The treatments details were described in Table 2. The pots were filled with a mixture of sandy loam and clay soil. The soil was tested for their physico-chemical properties in the department of soil science and Agricultural chemistry laboratory, Faculty of Agriculture, Annamalai University. Soil sample of the pot mixture was analysed and the reports shows the soil was loamy in texture with a pH 6.98, electrical conductivity of 0.120 dsM^{-1}, organic carbon content of 9.12 %, available nitrogen 114 kg ha^{-1}, available phosphorus 3.26 kg ha^{-1} and available potassium 79.41 kg ha^{-1}.

Germination percentage

Germination percentage was computed by recording total number of groundnut plants germinated against number of seeds sown in each plot on seventh day after sowing.

Vigour index

The vigour index was calculated on 15 DAS using Abdul Balli and Anderson's recommended method (1973).

Germination % X Shoot length = Vigour Index

Plant height, root, shoot length and dry weight

Plant height was recorded from the ground level to the tip of the plant on 25, 50, 75 DAS and at harvest and expressed in

cm. The plants were uprooted on 20 DAS and their root length, shoot length, and dry weight were measured.

Dry matter production (DMP)
Plants were removed at random from each treatment without damaging the roots and washed. The samples were sun dried initially for 24 hours and subsequently oven dried at 80º C to attain a constant weight. Then the DMP was recorded at harvest and expressed in g plant $^{-1}$ at harvest stage.

Test weight
One hundred filled seeds were randomly collected from the plants of each treatment plot and their weight was recorded in grams (g).

Kernel yield
The pods from each treatment were harvested, threshed, sun dried to attain 14 per cent moisture, weighed and the seed yield was expressed in g plant $^{-1}$

Statistical analysis
The data on the various parameters studied during the course of investigation were analysed statistically as per the procedure suggested by Panse and Sukhatme (1978).

Result
The effect of different treatments on plant growth parameters and yield parameters like plant height, root length, shoot length, germination percentage, vigour index, dry matter production (DMP) number of pods plant, Test weight and kernel yield of Groundnut were assessed and presented in Table 2 and 3. The maximum germination percentage (96.21), vigour index (1624), plant height (56.42), root length (10.21), and shoot length (8.51) was recorded with the treatment T_2 (100 % RDF) which was on par with the treatment T_9 (*Lysinibacillus* sp. + *Rhizobium* + *Bacillus megatherium* + 50 % RDF) with the germination percentage (94.85), vigour index (1585), plant height (54.68cm), root length (9.84cm), and shoot length (8.23cm) was recorded. These treatments were followed by T_6 (*Lysinibacillus* sp. + *Rhizobium* + 50 % RDF) with the germination percentage (91.31), vigour index (1532), plant height (51.59cm), root length (9.01cm), and shoot length (7.61cm). The minimum germination percentage (76.67), vigour index (1191), plant height (40.03cm), root length (5.37cm), and shoot length (5.13cm) was recorded in T_1 (control). Similarly the maximum DMP production, number of pods plant, Test weight and kernel yield in Groundnut was recorded with T_2 (100 % RDF) were 54.21 (g plant $^{-1}$), 38.29, 51.99 (g plant $^{-1}$) and 227 (g plant $^{-1}$), which was on par with the treatment T_9 (*Lysinibacillus* sp. + *Rhizobium* + *Bacillus megatherium* + 50 % RDF) by recording higher DMP production, number of pods plant, Test weight and kernel yield of 52.65 (g plant $^{-1}$), 37.42 (g), 50.41 (g plant $^{-1}$) and 225 (g plant $^{-1}$), respectively. These treatment were followed by T_6 (*Lysinibacillus* sp. + *Rhizobium* + 50 % RDF) DMP production, with the number of pods plant, Test weight and kernel yield of 50.29 (g plant $^{-1}$), 35.11(g), 48.34 (g plant $^{-1}$), and 218 (g plant $^{-1}$), respectively. The minimum DMP production, number of pods plant, Test weight and seed yield in Groundnut was recorded in 31.42 (g plant $^{-1}$), 21.02 (g), 40.32 (g plant $^{-1}$), and 180 (g plant $^{-1}$), respectively.

Table 1: Details of treatment schedule

Treatments	Treatment details
T_1	Control
T_2	100 % RDF
T_3	*Lysinibacillus* sp. + 75 % RDF
T_4	*Rhizobium* + 75 % RDF
T_5	*Bacillus megatherium* + 75 % RDF
T_6	*Lysinibacillus* sp. + *Rhizobium* + 50 % RDF
T_7	*Rhizobium* + *Bacillus megatherium* + 50 % RDF
T_8	*Bacillus megatherium* + *Lysinibacillus* sp. + 50 % RDF
T_9	*Lysinibacillus* sp. + *Rhizobium* + *Bacillus megatherium* + 50 % RDF

Table 2: Effect of endophytic bacterial isolates on the germination percentage, vigour index, plant height, root length and shoot length of groundnut

Tr. no.	Treatment	Germination percentage	Vigour index	Plant height (cm)	Root Length (cm)	Shoot Length (cm)
T_1	Control	76.67	1191	40.03	5.37	5.13
T_2	100 % RDF	96.21	1634	56.42	10.21	8.51
T_3	*Lysinibacillus* sp. + 75 % RDF	81.04	1403	42.42	7.90	5.78
T_4	*Rhizobium* + 75 % RDF	83.93	1448	43.87	7.52	6.12
T_5	*Bacillus megatherium* + 75 % RDF	86.67	1489	45.12	7.12	6.27
T_6	*Lysinibacillus* sp. + *Rhizobium* + 50 % RDF	91.31	1532	51.59	9.01	7.61
T_7	*Rhizobium* + *Bacillus megatherium* + 50 % RDF	91.02	1556	50.46	8.73	7.43
T_8	*Bacillus megatherium* + *Lysinibacillus* sp. + 50 % RDF	90.83	1525	49.23	8.52	7.10
T_9	*Lysinibacillus* sp. + *Rhizobium* + *Bacillus megatherium* + 50 % RDF	94.85	1585	54.68	9.84	8.23
	SEd	1.60	23.42	1.01	0.22	0.19
	CD	3.28	48	2.08	0.45	0.39

Means with same alphabets are statistically on par by Duncan's Multiple Range Test (DMRT) at 5% level

Table 3: Effect of endophytic bacterial isolates on the number of pods plant, test weight, kernel yield and dmp production of groundnut

Tr. No.	Treatment	Plant DMP at harvest (g plant $^{-1}$)	Number of pods plant^{-1}	Test weight (g)	Kernel yield (g plant $^{-1}$)
T_1	Control	31.42	21.02	40.32	180
T_2	100 % RDF	54.21	38.29	51.99	227
T_3	*Lysinibacillus* sp. + 75 % RDF	44.85	30.43	45.12	200
T_4	*Rhizobium* + 75 % RDF	45.89	30.89	45.72	203

T_5	*Bacillus megatherium* + 75 % RDF	46.14	31.24	46.21	205
T_6	*Lysinibacillus* sp. + *Rhizobium* + 50 % RDF	50.29	35.11	48.34	218
T_7	*Rhizobium* + *Bacillus megatherium* + 50 % RDF	49.92	35.02	47.29	216
T_8	*Bacillus megatherium* + *Lysinibacillus* sp. + 50 % RDF	48.16	34.23	46.21	213
T_9	*Lysinibacillus* sp. + *Rhizobium* + *Bacillus megatherium* + 50 % RDF	52.65	37.42	50.41	225
	SEd	0.96	0.64	0.79	2.31
	CD	1.93	1.28	1.62	4.73

Discussion

Bacterial endophytes have been shown to have a beneficial effect as observed on a variety of crops, such as rice (Rho *et al.*, 2018), legumes (Bai *et al.*, 2003) [4], wheat (Herrera *et al.*, 2016) [16], cotton (Zhou, 2015) [38], sugarcane (Aguiar *et al.*, 2016) [2], maize (Gond *et al.*, 2015) [11], fruits (Kannan *et al.*, 2015 [21], vegetables (Xie *et al.*, 2016) [35], flowers (Engel *et al.*, 2016) [8], *etc.*

Studies reported on the use of *Lysinibacillus sphaericus* ZA9 shown its potential for plant growth promotion and biotic stress management. The increased levels of IAA (697 g/ml), siderophore, hydrolytic enzymes, HCN synthesis and the ability to solubilize phosphorus, potassium, and silicon were observed.

Further the treatment of this bacteria resulted in increased shoot development in tomato and cucumber, also known to produce antifungal compounds such as 2-pentyl-4-quinolinecarboxylic acid and 1-methyl cyclohexene, (Naureen *et al.*, 2017) [29]. Several studies have found that *Lysinibacillus* sp. has the ability to promote plant growth through phosphate solubilization and phytohormone synthesis (Hardoim *et al.*, 2008) [15]. These reports could have favoured and contributed for the better growth and yield of groundnut crop, as brought out in the present study.

Conclusion

The isolates of endophytic bacteria *viz.*, *Bacillus*, *Rhizobium*, *Klebsiella*, *Pseudomonas*, *Azospirillum*, and *Lysinibacillus* sp. from different crop plants were subjected to morphological and biochemical characterization and *invitro* plant growth promoting activity were assessed for its efficiency to produce IAA, GA_3, siderophore production, and phosphate solubilization. From the studies, it could be concluded that among the different bacterial endophytes REB 2 (*Lysinibacillus* sp.) isolated from rice root was found to perform best in producing IAA, GA_3, and phosphate solubilization with better siderophore production, and hence this it is made as consortium with other well-known bio inoculants *viz Rhizobium* and *Bacillus megatherium i*n liquid formulation could be recommended as an effective plant growth promoting bio inoculant for groundnut as it improved the growth and yield attributes in Groundnut when compared to single, dual or conventional chemical fertilizers.

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