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Research Paper

Isolation and Characterization of *Rhizobium* and Their Effect on Vigna Mungo L.

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ABSTRACT

Rhizobia are symbiotic bacterial partners forming nitrogen fixing nodules on legumes. It isa gram negative bacterium that fixes nitrogen, bacteria colonize plant cell with root nodules and commonly found in Black ground. In the present investigation Rhizobia isolated from root nodules of Vigna mungo and characterized morphological and biochemically were to as certain in its physiology under the normal condition. The study areas randomized to Thanjavur district of Paravurani, Nadium, Athirampattinam, Puthalur, Kudikkadu and Kattuthottam, In Kattuthottom,Rhizobium strain was found to most effective in positively enhancing the growth of the plant in all morphometric parameters, such as length of plant, fresh weight of the plant, number of nodules, number of root nodules, number of leaves, number of branches, number of pods and weight of seeds were observed.

INTRODUCTION

Soil inhabiting bacteria viz, Rhizobia that form symbiotic relationships with plant legumes species from the atmosphere to form ammonia which is assimilated by the plant. Black gram (Vigna mungo) is the third important pulse crop in India. It is annual pulse crop and native to central Asia. It is also extensively grown in West Indies, Japan and other tropics/subtropical countries. Black gram seeds are highly nutritious containing higher amount of protein (24-26%) and are reported to be rich in potassium, phosphorus and calcium with good amount of sodium. Green gram (Vigna radiata L.) Wilczek (syn: Phaseolus aureus Roxb.) constitutes the important group of grain legumes which form a major source of dietary proteins of high biological value, energy, minerals and vitamins (Taylor et al., 2005). Inoculation of Rhizobium sp. causes a greater increase in growth and yield and the number of nodules per root system is significantly higher in plants inoculated with Rhizobium sp. compared to plants without Rhizobium sp. under field condition (Akhtar et al., 2009). Soil microorganisms produce quite a number of extra cellular enzymes to decompose the complex organic matter before it is absorbed as a source of energy. Seasonal variations in enzymes activities in forest soils are seen to bear correlation with the counts of fungi and bacteria (Kathiresan and Selvam, 2006; Richard et al., 2007).The Rhizobia fix substantial quantities of nitrogen symbiotically between 80 to 150 kg N ha in 90 days. Bacteria that inhabit the rhizosphere were classified on the basis of this effect on plants and the methods they interrelated with roots, a number and structure of root nodules on suitable leguminous host plants. Rhizobacteria that advantage plant growth and development are called plant growth promoting Rhizobacteria (PGPR) (Kloepper 1993), studies of antagonism between Rhizobium strains nodules formation have usually recommended that contain only a single strain (Ullah et al., 2008). Even though dissimilar nodules on the same plant might contain different strains (Koo 2009 and Malika 2004).

Bacteria that inhabit the rhizosphere were classified on the basis of their effects on plant and the method they interrelate with roots, a number of being pathogens while other triggers valuable effects. Rhizobia persuade the structure of root nodules on suitable leguminous host plants. Communications between plants and microbes in the rhizosphere (Rhizobacteria) can obviously affect crop yield. Rhizobacteria that advantage plant growth and development are called plant growth promoting Rhizobacteria (PGPR) (Kloepper, 1993). Studied of antagonism between Rhizobium strains in nodule formation have usually recommended that nodules contain only a single strain (Ullah et al., 2008 and Abd et al., 2000). Even though dissimilar nodules on the same plant might contain different strain (Koo 2009 and Malika 2004).

MATERIALS AND METHODS

Isolation of Rhizobium from Vigna mungo

Strains of Rhizobium sp. were obtained from root nodules of field grown Vigna mungo plants collect from Thanjavur (Dt). Root nodules was sterilize in 0.1% (w/v) sodium hypochloride for 5min immersed in 95% (v/v) ethanol for 10 s, and then washed six times with distilled water and streaked on yeast extract mannitol agar (YEMA) medium containing 0.0025% (w/v) congo red (Vincent, 1970). After 3 days of inoculation at 30°C, single colony was selected and re-streaked YEMA medium for purification.

Identification

Initial inoculums of 108 cell ml-1 were prepared in YEMA with a pH initially adjusted at 6.8. Colony morphology (colour, nature, transparency, borders) was evaluated by streaking a loop of the initial inoculums on YMS medium and allowing the bacteria to grow in the dark at 30o C for 3, 5 and 7 days (Vincent, 1970).

Gram staining

For gram staining the following reagents were prepared; Crystal violet solution (Crystal violet-10 g, Ethyl alcohol-100 ml, Ammonium oxalate-4 g, Distilled water-400 ml); Iodine solution (Iodine-1 g, Potassium iodide-2 g, Ethyl alcohol-25 ml, Distilled water-100 ml); Iodinated alcohol (Iodine solution (b)- 5ml, Ethyl alcohol-95 ml) Counterstain (2.5% safranin in ethyl alcohol-10 ml, Distilled water-100 ml). Gram stained smear were prepared with a loop full of a selected bacterium and spread over on a slide in a drop of water and allowed to dry in air (Graham and Parker 1964). The slide is dried in the vicinity of the flame and allowed to cool and then stained with crystal violet solution as follows: for 1 min followed by rinsing with water and removal of excess water, the slide is then flooded with iodine solution followed by decolourized with iodinated alcohol for one minute, for 5 min the slide is washed in water, drained and counterstained with safranin. Finally the slide is washed in water, drained and air dried and observed under oil immersion (Vincent 1970).

Biochemical Tests

All the collected samples were processed through different biochemical tests viz, Catalase Test, Indole Production Test Methyl Red Test, Vogas Proskauer Test, Citrate Utilization Test as described by (Lowe,1962) and Starch hydrolysis Test, Gelatin liquefaction Test and Motility Test as mentioned by (Arora, 2003) and ONPG Test (O-Nitrophenyl-D Galacto- Pyranoside) as described by (Cappuccino, 2007).

Observation of growth parameters

The pure culture of Rhizobium was mixed with charcoal medi-

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um and used for the inoculation purposes. Each Ten gram seeds of Vigna mungo (Linn.) Hepper T-9 variety was taken for this study. 10 gms of Rhizobium culture was mixed with the seeds and were left for an hour under shade for effective contact and then mixed with heat sterilized garden sand. They were then transferred to earthen pots of 30 cm diameter with unfertilized natural garden soil. In each pot, 20 seeds in charcoal and sand mixture were sown and watered. For controls uninoculated seeds were sown again at the rate of 20 seeds per pot, containing unfertilized natural garden soil. For each pulse crop, the inoculated seeds were grown in twelve pots and uninoculated was grown in another twelve pots. Thus totally replicates were maintained for inoculated and controlled plants. After sowing, the pots were placed in the green house so as to receive uniform light and water. No fertilizers were added to them. After the emergence of the seedlings, the numbers of plants per pot were thinned to four. The plants were regularly and uniformly watered and BHC 10% was applied on the 25th day to word off pests both in treated and non-treated plants. The following growth parameters were studied on the 45th day of growth, 2 plants per pot were removed to study the morphological growth parameters (Salil bose, 1982). Height of the plant, fresh weight, and number of nodules, roots, shoots and leaves were studied and recorded. At 80th day (the completion of growth phase), the plants were harvested and yield characteristics like number of pods, length and weight of pods were studied for V. mungo. Sometimes it is necessary to compare the two sets of data arising from the same population under two different conditions. Measurements are made on each subjects twice once under control conditions and once under experimental conditions. Observation was carried out carefully in every aspect.

Table1: Identification of Rhizobium strains by using selected biochemical test

Name of the Test	Response of strain					
	Stain-1	Stain-II	Stain-III	Stain-1V	Stain-V	Stain-V1
Gram strain	-	-	-	-	-	-
H2S production	-	-	-	-	-	-
NO3 production		+				
Indole production	+	+	+	+	+	+
Methyl Red production	-	+	-	+	-	-
Voges-proskeuer	+	-	-	-	-	+
Citrate Utilization	-		-	-	-	-
Catalase	+	-	+	+	+	+
Urease activity	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Gelatin liquefication	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+
Liquid hudrolisis	+	+	+	+	+	+
Motality	+	+	+	+	+	+

(+)-Negative (-)-Positive

Table2: Effect of Rhizobium inoculums on various growth parameters in Vigna mungo
(T-9 varity) of Thanjavur district

S. No	Name of the area	Length (cms)	Fresh (gms)	No.of roots	No.of roots nodule	No.of leaves	No.of branches	No.of Pods	Weight of 100 seeds (gms)
1	Control	23	29	12	6	15	5	6	8
2	Paravurani	45	35	18	8	15	7	7	9
3	Nadivm	38	40	19	10	20	6	8	10
4	Athirangattinam	30	42	12	12	25	8	9	7
5	Puthalur	52	48	23	10	33	9	10	13
6	Kudidaiu	49	50	20	13	35	10	7	15
7	Kattuthottam	45	52	27	15	40	9	9	18

RESULTS AND DISCUSION

All the positive samples were also streaked on Bromothymol blue (BTB) added Yeast Extract Mannitol (YEM) selective media for further confirmation. Similarly the positive samples from all target areas showed hazy appearance in the motility media and also were positive for catalase, motility and O-nitro phenyl- D-Galacto-pyranoside (ONPG) Tests. The samples found negative for methyl red (MR), voges-proskauer (VP), indole, citrate utilization test, hydrogen sulphide production, urea hydrolysis test and gel liquefication test (Table-2). These findings are in close agreement with (Elsheikh and Wood, 1989) who also characterized the Rhizobium from soil and sunflower root nodules with the same positive biochemical tests. Similarly, the nodulation pattern in forage legume bacteria by screening through the same tests results and characterized Rhizobium strain from the roots of Trigonella foenumgraecum

The Rhizobium-legume symbiosis in arid ecosystems is particularly important for locations where the area of saline soils is increasing and becoming a threat to plant productivity. Legumes, which are usually present in arid ecosystems, may be adapted to fix more N_2 under saline conditions than legumes grown in other habitats.

The results of the present investigation presented in table I and II root nodulating bacteria were isolated from the six different areas of Thanjavur districts. All the strains of Rhizobia were gram-negative did not absorb red colour when authored in YEMA containing congo red dye. The biochemical test for characterization for identification of Rhizobia. The biochemical tests were performed gram straining, H2S production, No3 production, indole production, methyl red reduction, voges proskaur reaction, citrate ultilization, catalyse, urease activity oxidase, gelatin liquefication, starch hydrolysis liquid hydrolysis, and motility test for conformed and identified (Table-1). The Rhizobium inoculated that Vigna mungo (T- a variety) showed to the strain I-IV, the Kattuthottam soil variety possessed greater height, fresh weight, number of roots, greater height, fresh weight, number of roots, total no of nodules, leaves, branches of stems number of pods, and weight of 100 seeds with weight, over their respective controls.

The application of inoculum treatment for Vigna mungo (strain-1) promoted with significantly differed from each other as compared to the control. The peravurani (strain-I) treated with Vigna mungo was length of the plants (23 cm) fresh weight of the plant (35 gms), number of root nodules (8), number of branches (7) when compare to control was less performed. Where as in the case of Kattuthottam (strain-V) treated Rhizobia were highly performed and significantly resulted with all growth parameters including, weight of the seeds became of the application of Rhizobia (strain-VI) and type showed better result.

Different degrees of growth enhancement of the bacteria by the vitamins suggested that the organism required a number of vitamins as cofactors. Biotin and other water soluble vitamins were required for growth of Rhizobium spp. Different legume nodulating rhizobial strains preferred different vitamins sources for IAA production reported (Pallab Kumar Ghosh et al., 2014 and 2015)

All other treatments increased biological yield to variable extent over control. The increased morphometric analysis and yield in Vigna mungo in all treatments might be attributed to high growth owing to the availability of nitrogen in soil and environmental condition.

One the basis of the study, of is concluded that inoculation of soil and seed with Rhizobium was high growth and nodules formation of Vigna mungo.

CONCLUSION

It is concluded that the commercial inocula of Rhizobium used by as in seed inoculation of the pulses namely Vigna mungo (Linn.) Hepper. T-9 variety has resulted in significant increases in growth and yield potential over the respective non inoculated control plants.

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