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Study the Morphometric Characteristics of Vigna Radiata Inoculate with Bradyrhizobium Spp

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Abstract: ***The mung bean (Vigna radiata), alternatively known as the green gram, the genus Bradyrhizoium was created to accommodate rod shaped, slow growing, gram negative, motile bacteria. Research has gradually revealed the molecular mechanism programme of into the leguminous plants. Brady rhizobium Spp from root nodules and cultured on the media in the laboratory morphology and distribution of nodules in the roots of the host plants in relation to different habitats and environmental conditions. All the morphometric parameters registered a better effect with Bradyrhizobium Spp treated plants over control.***

Keywords: Vigna radiata, Bradyrhizobium, leguminous plants

1. Introduction

N2 fixation is one of the biological processes important for development of sustainable agriculture by which the atmospheric N2 is converted to ammonia with the aid of a key enzyme called nitrogenase by which then need for nitrogen can be met more sustainably and economically. The Symbiotic nitrogen fixation (SNF) by soil bacteria /rhizobia in legumes is a crucial entry point for nitrogen into natural and agricultural systems(Graham and Vance,2003).The rhizobia include the genera i.e,*Allorhizobium* (deLajudie*etal.,* 1998), *Azorhizobium* (Dreyfuss *et al.,* 1988), *Bradyrhizobium*(Jordan, 1982), The genus *Bradyrhizobium* was created to accommodate rod shaped, slow- growing, Gram negative, motile bacteria (Vincent *et al.,* 1977) capable of nitrogen fixation and nodule formation on leguminous plants Willems*etal.*,2000)

Biological Nitrogen Fixation

Nitrogen is one of the most important nutrients very essential for growth of crops. Atmosphere contains about 80 per cent of nitrogen by volume in a free state. The major part of the elemental nitrogen that gives its way into the soil is entirely due to its fixation by certain specialized groups of microorganisms. Biological nitrogen fixation by leguminous green manure crops in symbiotic association with *Rhizobium* is a low cost input for rice crop. Ecology and it's physiological and genetically relationship with the roots and nature, morphology and distribution of nodules in the roots of the host plants in relation to different habitats and environmental conditions.

***Rhizobium* - Legume Symbiosis**

Symbiosis is a biological phenomenon involving dynamic changes in the genome, metabolism and signalling network. A multidirectional comprehension of these interactions is required when studying symbiotic organisms.Rhizobia has two different life-styles, either as free-living soil bacteria or as nitrogen-fixing endosymbionts within root nodules of legume host plants. In a well-balanced physiological interaction, the micro symbiotic fixes atmospheric nitrogen and provides ammonia as a nitrogen source to the plant in exchange for a carbon and energy source generated by photosynthesis. Legume crops are important for the development of sustainable agriculture and legume nodules provide an excellent model for studying fundamental aspects of plant–microbe signalling and cell morphogenesis.

***Rhizobium* Characterization**

Many characteristics are useful in the classification and identification of microorganisms. This section briefly reviews some of the most taxonomically important properties, which are characterized into classical and molecular characteristics. The classical approaches to taxonomy such as morphological, ecological, biochemical, bimolecular estimation and genetic characteristics have been employed in microbial taxonomy for many years (Prescott *et al*., 2005). The rhizobia are morphologically characterized as short to medium (0.5-0.9 µm wide x 1-3 µm long), Gram negative rods and motile. The older cells are likely to contain one to several prominent highly refractive granules of poly hydroxybutyrate (PHB) (Fred *etal.,* 1932; Vincent and Date, 1962).

The slow growing and non-acid producing rhizobia have been considered as the ancestral forms of rhizobia, since they are associated with primitive tropical legumes growing in alkaline environment (Vincent, 1970). The genus *Bradyrhizobium* is created to accommodate slow-growing bacteria capable of nitrogen fixation and nodule formation on leguminous plants (Williams *et al.,* 2000).

2. Materials and Methods

In order to compare the effective and in effective root nodules, the root nodulating tropical legumes, Vigna radiata, that produce both effective and in effective nodules were selected. The seeds of *vigna radiata* along with *Bradyrhizobium* strainsS24 (effective) and S24A06 (ineffective) were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore. The obtained rhizobial strains were sub cultured and maintained in yeast extract mannitol agar (YEMA) slants as well as in broth.

Composition of yeast extractmannitol broth (Vincent, 1970)

Mannito - 10.0 g Potassium hydrogen phosphate
- 0.5 g Magnesium sulphate
- 0.2 g Sodium chloride
- 0.1 g Calcium carbonate
- 1.0 g Yeast extract
- 1.0 g Distilled water

Pot culture experiment

Inoculum preparation

About 100ml of brothwastakenina 250ml Erlenmayer flask and 1ml of pure suspension culturecontaining6 $\times 10^{-7}$cells was inoculated. It was kept on a rotary shaker to produce heavily turbid suspension and incubated at 28 ± 2˚C for 4 to 6 days. The population of the test isolate was determined by dilution plate method (Hoben and Somasegaran, 1982). After the quantitative determination of population in the inoculums suspension, the broth cultures (containing 6×10^{-7}cells ml^{-1}) (both effective and ineffective) were mixed with sterilized lignite carrier for seed inoculation.

Seed inoculation

Prior to sowing, the seeds of *Vigna radiata* were mixed with rhizobial culture-carrier material and made air dry. These inoculated seeds were sown in pots which had already been prepared.

Preparation of earthen pots

Soil from fallow pots was mixed well, sieved and filled in earthen ware pots at the rate of 10 kg per pot. The pots were watered to the level of 50 per cent moisture holding capacity of the soil and sterilized in large horizontal autoclave at 20 lbs pressure for 2 h. They were then allowed to incubate in a pot culture house for 4days and the soil in each pot was loosen and mixed well with the help of as toutglassrod.The physico-chemical properties such as EC, pH, bulk density (g/cc) and organic carbon (%) nitrogen, phosphorous, potassium and micronutrients such as zinc, copper, manganese and iron of sterilized soil samples were analysed (Table1) following the methods of Barnes (1959) and Muthuvel and Udhayasoorian (1999).

- Seeds without test isolate – Control (C)
- Seeds treated with test isolate (T)

The *Bradyrhizobium* inoculated seeds were sown in the pot and watered well. The plants were uprooted at different stages (vegetative, flowering and pod filling) of growth for morph metric analysis.

Host response studies

The isolate was test effort shots response effectiveness by conducting morph metric analysis on the host plant and histological and histochemical studies on the effective and ineffective root nodules of host plant.

Morph metric analyses

The morph metric analyses such as root length, shoot length, nodule status and nodule dry weight were done during vegetative, flowering, and pod filling stages, both in control and *Bradyrhizobium* inoculated plants.

Histological studies of root nodules

Collection and preparation of samples for sectioning

There samples of effective and ineffective root nodules were cut and removed from the plant and fixed in FAA (Formalin -5ml+Acetic acid-5ml+70Ethylalcohol-90ml). After 24h offixing, the specimen dehydrated with graded series of tertiary-butyl alcohol (TBA) as per the schedule given by Sass (1940). In filtration of paraffin wax (melting point 58-60˚C) until TBA solution attained super saturation. The specimens were cast into the paraffin blocks.

3. Results

The plants were grown in earthen pots filled with sterilized sandy clay loam soil that contained Nitrogen, Phospurus, Potash etc..., were examined periodically for morph metric analyses such as root and shoot length, number and types of nodules per plant and dry weight of the nodules during vegetative, pod filling and flowering stages. In addition, histological and histo chemical studies of nodules were also carried out.

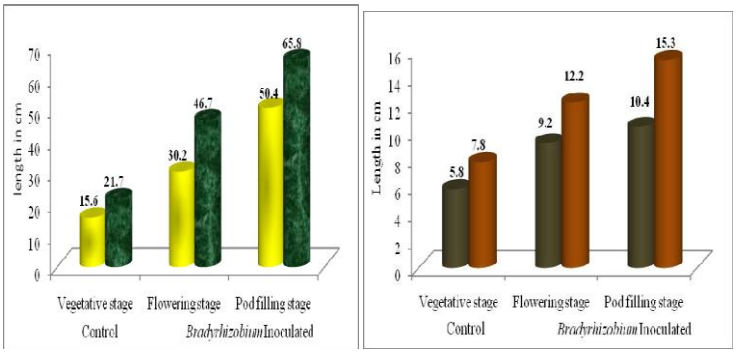
Vigna radiata plants in green house-pot culture experiment

Host response

Morph metric analyses

All the morph metric parameters registered a better effect with *Bradyrhizobium* treated plants over control. The increased height of shoot and length of root were recorded in pod filling stage .During flowering, maximum number of nodules was produced when compared to other stages .Significant differences in nodule number were also noticed. Among the stages, the pod filling stage recorded less number of nodules

Two types of nodules (effective and ineffective) Bradyrhizobium inoculated plants. Effective type were spherical and displayed a pink colour and were grouped in clusters along the roots, ineffective nodules are slightly smaller in size, a white to greenish in colour and were scattered throughout the roots - ineffective type. These two types of nodules were further studied for their internal structure, histochemical and microbiological nature.



Morphometric analysis of *Vigna radiata* inoculated with Bradyrhizobium

4. Discussion

Microbial diversity is considered as one of the most useful resources for bio prospecting and biological nitrogen fixation is one among them. The agricultural importance of nitrogen fixation is not only to provide ammonium to the crops, but also to minimize the pollution of water tables, lakes and rivers. The legume-*Rhizobium* symbiosis and the corresponding physiological adaptations also provide a convenient model for studying aspects of plant-microbe interactions and evolution Provorov, 1994;Quispel,1998). The symbiotic interaction between members of the genera *Rhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium* and *Azorhizobium* and the plants of Leguminosae results in the formation of nitrogen fixing root nodules. Genes from both, the plants and the rhizobia play a role in the establishment and maintenance of this interaction (Bladergroen and Spaink, 1998; Schultze and Kondorosi, 1998).

Root nodules are classified into effective and ineffective based on their effectiveness in nitrogen fixation. Studies on these nodules, particularly on proteome studies, are very little and hence the present investigation. Host response of *Bradyrhizobium*–in terms of morph metric analysis in*V.radiata* as well as anatomical and microbiological studies of root nodules in addition to proteome studies were carried out.

Host response

Morph metric analysis

Seed inoculation of legumes with an efficient rhizobial strains necessary to improve nodulation, N2 fixation, grow than yield of crops (Henzell,1988). Oblisami and Ramaswami (1986) and Rashid et *al.* (1999) reported that an increase in nodules per plants due to application of rhizobial inoculants.

As reported by Vincent (1982), in the present study also two types of nodules such as effective and ineffective were found on the roots of *V.radiata* inoculated with *Bradyrhizobium* .Plants can be infected by a variety of strains of rhizobia differ in their ability to fix nitrogen efficiently.Areal effective strain, as it is called, forms healthy – looking nodules with distinct pink colour; if the nodules are cut open, the interior is distinctly red. Ineffective rhizobia form pallid, sometimes greenish nodules (Vincent, 1982; Postgate,

1998).Similar observations were made in the present study on the nodules of *V. radiata* where, pink and green coloured nodules with internal pink and white tissues respectively were noticed.

Generally, a plant produces more ineffective nodules than effective and these are usually smaller and spread over the whole root system. In ineffective nodules, rhizobia or infection threads are rarely found, the whole nodules being formed of roughly is diametric parenchyma cells, and the development of vascular tracesis restricted(Hardy and Silver, 1974; Rasanen, 2003). Vincent (1982) and Venkataraman and Kannaiyan (1993) also reported that ineffective nodules are generally small and contain poorly developed bacteriod tissue showing accumulation of starch in host cells which donot contain *Rhizobium.*

- Bradyrhizobial strains produced both effective and ineffective nodules in *Vigna radiata* plant
- Both nodules exhibited variations not only in morphology but also in histology and biochemistry.

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