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Antagonistic Effects of Plant Growth Promoting Bacteria Against Bacterial Diseases of Black Gram

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ABSTRACT

Vigna mungo, the black gram, urad bean, mash kalai, uzhunnu parippu, ulundu paruppu, minapa pappu, Uddu (in Kannada) or black matpe is a bean grown in South Asia. Like its relative, the mung bean, it has been reclassified from the Phaseolus to the Vigna genus. The product sold as black lentil is usually the whole urad bean, whereas the split bean (the interior being white) is called white lentil. It should not be confused with the much smaller true black lentil (Lens culinaris).Black gram originated in South Asia, where it has been in cultivation from ancient times and is one of the most highly prized pulses of India. It is very widely used in Indian cuisine. In India the black gram is one of the important pulses grown in both Kharif and Rabi seasons. This crop is extensively grown in southern part of India, northern part of Bangladesh and Nepal. In Bangladesh and Nepal it is known as mash daal. It is a popular daal (legume) side dish in South Asia, that goes with curry and rice as a platter. Black gram has also been introduced to other tropical areas such as the Caribbean, Fiji, Mauritius, Myanmar and Africa. It is an erect, suberect or trailing, densely hairy, annual bush. The tap root produces a branched root system with smooth, rounded nodules. The pods are narrow, cylindrical and up to six cm long. The plant grows 30–100 cm with large hairy leaves and 4–6 cm seed pods. While the urad dal was, along with the mung bean, originally placed in Phaseolus, it has since been transferred to Vigna.present study is based on antagonistic effects of plant growth promoting bacteria against bacterial diseases of black gram.

KEYWORDS: *black gram, Vigna mungo, antagonistic, bacterial, diseases, growth, promoting, cultivation*

Introduction

The charcoal rot of Black gram caused *by Macrophomina phaseolina* (Tassi) Goid. is an important disease affecting Black gram production areas in India. Although considerable research related to the biology and ecology of *Macrophomina* has been conducted, it continues to cause huge economic losses in many crops. No single control measures are effective or not feasible under farmer's conditions. *Pseudomonas fluorescens* were assessed for their ability to reduce the growth of M. phaseolina under laboratory conditions. Root rot caused by *Macrophomina phaseolina* (Tassi) [1,2]Goid is one of the most important fungal diseases of Black gram. It inflicts series economic loss to the crop. It was reported to result in a loss of 28.6 per cent in black gram yield. It is an important disease of broad range of crops particularly in regions with warm and dry weather conditions. *M. Phaseolina* is primarily soil borne in nature, with heterogeneous host specificity i.e., the ability to infect monocots as well as dicots and non-uniform distribution in the soil. The pathogen is seed-borne and seed-to-seedling transmission has been documented in infected seeds. *Macrophomina* infection causes both pre- and post-emergence plant mortality. The disease symptom starts initially with yellowing and drooping of the leaves. The leaves later fall off and the plant dies within a week.

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Dark brown lesions are seen on the stem at ground level and bark shows shredding symptom. The affected plants can be easily pulled out leaving dried, rotten root portions in the ground. The rotten tissues of stem and root contain a large number of black minute sclerotia. Under above circumstances, it became inevitable to develop a bio-based, ecofriendly, biodegradable microbial antagonists in order to control plant pathogens. Biological control using antagonistic microorganisms offers a practical and economical alternative for the management of plant pathogens. Biological control is eco-friendly,[3,4] does not leave any residual toxicity, besides being cost effective and can be successfully exploited in the framework of integrated disease management. The bio control agents were isolated from rhizosphere soil by serial dilution using king's B medium for *Pseudomonas*. The plant growth-promoting activity of the bio control agents was assessed based on the seedling vigour index by the standard roll towel method. Bacterial antagonists tested increased the germination per cent and increased the growth parameters of black gram seedling when compared to control. Application of *P. fluorescence* not only controlled dry root rot but also promoted plant growth, and this gives them an advantage over use of chemicals.[5,6]

Discussion

To improve the growth of black gram by inoculating with of halophilic bacteria under salinity stress. Four PGPHB bacteria, viz., *Bacillus safensis* strain Lewis_Bac_3 (HB-5), *Pseudomonas stutzeri* strain MN1 (HB-13), *Staphylococcus xylosus* strain C5 (HB-18) and *Pseudomonas* sp. were inoculated to black gram seeds to evaluate their plant growth promoting ability at 4 dS m-1 and pH >8.5.

Root nodules of *Vigna mungo*

Increase in root length, plant height, and number of branches have been reported in consortium treatment indicating that salinity does not affected black gram[7,8] photosynthesis and nutrient absorption in consortium treatment. Corroborating evidence revealed higher nodulation and total nitrogen and phosphorous content in the same treatment, in comparison with control. Other investigation also aimed to provide a safer and more effective biocontrol method. *Pseudomonas* strains of had an antibacterial spectrum and the *Bacillus* strain had a broad antimicrobial spectrum against many tested pathogenic bacteria . We further found that many bacterial strains had effective biocontrol efficacy against bacterial disease under greenhouse conditions and *P. fluorescens* strain had the best performance.In other studies also statistical analysis showed that two Pseudomonas spp. StT2 and StS3 were the most effective with 65.1 and 73.9 percent biocontrol efficacy, as well as 87.3 and 98.3 percent yield increase, respectively. Potential

antagonistic bacterial strain StS3 showed maximum homology to Pseudomonas sp. as determined by 16S rRNA gene sequencing. These results suggest that bacterial isolates StS3 and StT2 have excellent potential to be used as effective biocontrol agents promoting plant growth with reduced disease incidence.[9,10]

In an experiment Rhizosphere engineering is one of the means to increase the competitive survivability of rhizobial bio-inoculants. This can be achieved by altering the rhizospheric community structure with a co-compatible and co-operative microbial partner. There exists a proto-cooperative mode of interaction between the non-rhizobial endophytes (NRE) and the endosymbiont Rhizobium, which can be unraveled to enhance their competitive survivability. In the present investigation, 8 isolates of nodule associated bacteria (NAB) and 1 rhizobial species were isolated from the root nodules of black gram (*Vigna mungo* L.,) cultivar VBN6. Among the NAB isolates, 75% were grampositive bacilli belonging to the phylum firmicutes. The promising NABs that showed maximum PGP features were phylogenetically affiliated as *Bacillus subtilis* NANEB1 and *Paenibacillus taichungensis* TNEB6. Among them, *P.taichungensis* TNEB6 registered significant IAA production (15.39 µg.ml-1), siderophore (10.7%), ammonia, HCN and also ACC deaminase activity (94.3 mg-1 hr-1). Interestingly, 75 % of the NAB isolates could solubilize phosphorous. Furthermore, *P.taichungensis* TNEB6, when co-inoculated with *Rhizobium* sp showed significant plant growth promotion with respect to enhanced root and shoot length. The vigor index was also maximum in the treatment that received co-inoculation of *Rhizobium* sp and *P.taichungensis* (1812)

Results

A research clearly demonstrated the existence of putative nodule associated bacterial endophytes and there exists a co-operative mode of interaction. The phylum firmicutes are predominant in nodule niche and posses significant PGP traits such as IAA, siderophore, ammonia, HCN production, P solubilization and ACC-deaminase activity. The co-inoculation of promising NAB strains *Paenibacillus taichungensis* with the symbiont Rhizobium increased the vigor index of black gram (8 DAS). Hence, the results paved a way to develop a co-inoculum of multifaceted highly co-operative endophytes, in order to increase pulse productivity and thereby sustains soil health.[11]

Black gram is one of the important pulses grown in both Kharif and Rabi seasons. Black gram is being cultivated under irrigated, rainfed as well as rice fallow condition, after the harvest of samba/thaladi paddy. Rice fallow black gram grows in the residual soil moisture, which is broadcasted 7-10 days before the harvest of paddy crop and allowed to germinate and grow. However, yield recorded in this ecosystem is highly variable mostly and depends on the management practices followed. The yield ranged from 200-400 kg/ha. The poor yield of rice fallow black gram is attributed by many factors. Among the various factors cause for poor yield of black gram, occurrence of disease and drought is one of the important factors for reducing yield. As there is no field preparation for sowing, the crop residue and weed are severe as source for the inoculum. Important diseases noticed in the rice fallow black gram are root rot, leaf spot, powdery mildew, viral diseases like yellow mosaic and leaf crinkle. The eco-friendly approaches such as biological control and host resistance induction have gained much attention in the past decade as a way of reducing the use of chemical products in agriculture. In these circumstances, use of microbes could play an important role in the management of biotic and abiotic stresses. Among different microbes, PGPR bacteria may play important beneficial roles in the metabolism and physiology of the host plant, including promotion of plant growth, inhibiting strong fungal activity, accumulation of pathogenesis related protein, deposition of cell wall barrier, inhibit growth of pathogens. Fluorescent pseudomonads have received attention throughout the global science because of their catabolic versatility, excellent root-colonizing abilities and their capacity to produce a wide range of antifungal metabolites. Hence, the present study was undertaken to test the efficacy of the endophytic *P. fluorescens* TRRI 1 formulation for the management of rice fallow black gram diseases. On the other hand, ethylene is

synthesized in plant tissue from precursor 1-aminocyclopropane-1-carboxylic acid (ACC) during biotic and abiotic stress conditions. Which in turn retarded root growth and caused senescence in crop plants . Interestingly, PGPR strains possess the enzyme ACC deaminase can cleave the plant ethylene precursor ACC and thereby lower level of ethylene in a developing seedling or stressed plant . The ACC deaminase containing biocontrol agents markedly lowered the level of ACC in the stressed plants. Thereby limiting the amount of stress ethylene synthesis and hence the damage to the plant. Inoculant on the outside of seed may be knocked off during seeding and/or desiccates and dies before making it into the soil. Polymer coating has been used to improve inoculants viability and shelf life with legume seed. Polymer is a film coating chemical normally applied over seeds without significantly increasing the size or weight of seed. The film formulations consist of a mixture of polymer, plasticizer and colourants that are commercially available as ready to use liquids or as dry powders . This kind of plasticizer polymer form flexible film that prevents dusting off and loss of biocontrol agent/fungicide during handling and is readily soluble in water (hydrophilic) so as not to impede with normal germination . Film coating provides protection from the stress imposed by accelerated ageing, which include fungal invasion. Keeping this view, current study is aiming to manage the diseases in the rice fallow black gram by using ACC deaminase producing Pseudomonas sp with polymer coated seed.[12]

Conclusions

Globally, crop growth protection and health is continuously challenged by emerging, reemerging and endemic plant pathogens. Rhizospheric microbes that suppress plant pathogens could be used as biocontrol agents and may be considered as alternative to chemical pesticides. There are several mechanisms for beneficial microorganisms to protect plants from biotic and abiotic stress. Plant growth promoting rhizobacteria (PGPRs) are known as beneficial bacteria for plant growth and yield. One PGPR group are the ACC deaminase positive (ACC+) bacteria which degrade 1-aminocyclopropane-1- carboxylic acid (ACC), the plant produced per-cursor to ethylene. Studies have shown that ACC+ bacteria, in association with plant roots, can improve plant growth under abiotic stress (e.g., drought, salinity, heavy metals) by reducing concentrations of stress ethylene. Current study, growth promotion by ACC positive fluorescent pseudomonads polymer coated black gram under in vitro condition showed improvement in plant growth parameters over untreated seeds. In this study, we evaluated the ACC positive fluorescent pseudomonads under rice fallow condition black gram cultivation. Rice fallow black gram grows in the residual soil moisture, which is broadcasted 7-10 days before the harvest of paddy crop and allowed to germinate and grow. Major problem in rice fallow crop is drought and occurrence of disease. This study demonstrates the effectiveness of rhizobacteria containing ACC deaminase for inducing drought tolerance and consequently improving the growth of black gram plants under rice fallow (water stress) conditions.

The biocontrol *Pseudomonas* strains TRRI 1, TRRI 15 and TRRI19 produced green gram seedlings with a significantly higher vigour index, 7426.6, 6942.2 and 6874.3, respectively. The reference biocontrol strains Pf1, TDK1 and Py15, whose vigour index was 7010.5, 7038.4 and 7024.3 respectively. Interestingly, TRRI1 strain produced seedlings with a high vigour index, Seedling dry matter production, No. of nodules and a higher germination percentage, than seedlings treated with the other two strains. The untreated control seedlings had the lowest vigour index, 6435.4 . Visual observation from this study, we observed early germination (within 2 days) in all the pseudomonas polymer coated seeds as compared to the normal seeds .

Inoculation with the polymer coated ACC deaminase containing PGPR caused significant alleviation of biotic and abiotic stress and consequently improving the yield of the black gram. Formulation of PGPR bacteria may have practical application in biological promotion of plant growth characteristics which can potentially replace the use of chemical fertilizers. The use and application of such bioformulations in the fields can result in the reduction of application of harmful chemicals, protect

the environment and biological resources and can be an important component of integrated pest management (IPM) that can help the growers to achieve a sustainable agricultural system.[13]

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