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Research article

GENOTYPIC VARIATION FOR RESPONSE TO RHIZOBIAL SP. AND *PIRIFORMOSPORA INDICA* FOR YIELD AND YIELD COMPONENTS IN MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK]

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ABSTRACT: Ten mungbean genotypes were evaluated to estimate the genotypic variation for seed yield components and Nitrogen and Phosphorus uptake after inoculating with three microbial treatments (*Rhizobium*, *Piriformospora indica* and their combined inoculation). Significant genotypic differences for all characters indicated presence of considerable variability. All the microbial treatments and genotype x microbial interaction differed significantly except for maturity, branches/plant and seeds/pod. The traits affected most by *Rhizobium* inoculation in majority of the genotypes were plant height, pods/plant and seed yield. Above 50 per cent *P. indica* infection in roots was observed in eight genotypes, however, its effect was observed only in a few genotypes on plant height, P content in shoot, 100-seed weight and seed yield. The effect of combined inoculation was observed on seed yield only. Effect of all the three inoculants was observed in only MH-810 and MH-721. Maximum response of *Rhizobium* and dual inoculation was observed in MH-421.
Key words: Mungbean, *Piriformospora indica*, *Rhizobium*, Seed yield

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek], also known as green gram, is an important pulse crop of *kharif* season in India. With the advent of short duration varieties, its cultivation is also becoming popular during spring/summer season in northern parts and during *rabi* season in southern parts of India. It is highly nutritious crop and is regarded as a quality pulse due to its rich protein content and excellent digestibility. Besides being a major source of protein for human consumption and high quality crop residue for animal feed, it helps in maintaining the soil fertility through biological nitrogen fixation (Kantar *et al.,* 2007). Being a short duration crop, it fits well into the intensive rice–wheat cropping system of Indo-Gangetic plains of India (Brar *et al.*, 2004). It can also be grown as intercrop with sugarcane, pigeonpea, poplar and orchards. Thus, there is a great scope of increasing area and production of summer mungbean (Sekhon *et al.,* 2007), however, the productivity of mungbean is still very low. Maximization of yield is major objective in all the crops. There are many traits which individually or in combination affect the seed yield in mungbean. Seed yield is a complex character and considering seed yield as unitary trait appears to be faulty and it should be viewed as the end product of component characters like number of branches/plant, number of pods/plant, number of seeds/pod, 100- seed weight, etc. Also, infection of *Rhizobium* and other micro-organisms along with their interaction is of paramount importance in deciding the ultimate yield in mungbean. Moreover, the increasing demand for production of crops and food for a vast population has led to an interest and necessity for the use of biofertilizers for the betterment of these crops and for the soil health.

Rhizobium is the most well known species of a group of bacteria that acts as the primary symbiotic fixer of nitrogen. These bacteria can infect the roots of leguminous plants, leading to the formation of nodules in roots where the nitrogen fixation takes place. The bacterium's enzyme system supplies a constant source of reduced nitrogen to the host plant and the plant furnishes nutrients and energy for the activities of the bacterium. The role of symbiotic nitrogen fixing bacteria in crop productivity is well documented by (Kennedy *et al.*, 2004; Hariprasad and Niranjana, 2009). To improve competitiveness and nitrogen fixing ability of mungbean, other microbial inoculants are used, which includes PSB (phosphate solubilizing bacteria), PGPR (plant growth promoting rhizobacteria) and VAM (vesicular arbuscular mycorrhiza) as elaborated by Dudeja *et al.* (2012). Studies have also been conducted on co-inoculation of *Rhizobium* with other microbes and have shown very encouraging results. VAM perform many different functions such as mineral solubilization and uptake and abiotic stress resistance, however, it cannot be cultivated under laboratory conditions.

Alternatively, a novel plant growth promoting, root colonizing endophytic fungus, *Piriformospora indica* isolated from orchid plants in the Thar desert in India, are able to grow axenically on a variety of simple and complex media (Verma *et al.*, 1998) and mimics most of the beneficial characteristics of VAM. This fungus is extremely versatile in its mycorrhizal associations and its ability to promote plant growth. This fungus functions as a plant growth promoter and biofertilizer in nutrient-deficient soils and also as a bioprotector against biotic and abiotic stresses including root and leaf fungal pathogens and insect invaders; as a bioregulator for plant growth development; bio-agent for the hardening of tissue culture raised plants (Varma *et al.,* 2009). It enhances nutrient uptake, especially of phosphorus and nitrogen (Kumar *et al.*, 2011).

Combined inoculation of *Rhizobium* and *P. indica* has been reported to increase the nodulation, dry matter content, grain yield and nitrogen (N) and phosphorus (P) uptake significantly over the uninoculated control in many crops (Singh *et al.,* 2002), however, no study has been reported on genotypic variability for response to these microbial inoculants in mungbean. Keeping the above facts in view, the present study has been planned to estimate genotypic variation for seed yield component traits for response to *Rhizobium* and *P. indica* in mungbean and also to identify the mungbean genotypes responsive to these inoculations.

MATERIAL AND METHODS

The experiment was carried out at Pulses Research Area of Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar during *kharif* 2013-14. The experimental material consisting of 10 mungbean genotypes *viz*., Basanti, IPM-02-3, MH-318, MH-810, MH-721, MH-560, MH-805, Sattya, MH- 421 and COGG-912 were grown in Factorial RBD with three replications. These genotypes were grown in four rows of 4m length each. Row to row spacing was kept at 30 cm and plant to plant at about 10 cm. The mungbean rhizobial strain MB-703 and fungus *Piriformospora indica* procured from the Department of Microbiology, CCS Haryana Agricultural University, Hisar were used to inoculate the seeds of the above mentioned genotypes and four microbial treatments [Mungbean rhizobial strain MB-703, *P. indica*, combined inoculation of Mungbean rhizobial strain MB-703 + *P. indica* (R+P) and control (uninoculated)] were applied through seed treatment. The initial and N and P status of the soil was analyzed.

Observations were recorded on days to maturity and seed yield on plot basis. Five randomly selected plants from every genotype in each replication were used to record plant height, number of branches/plant, number of pods/plant, number of seeds/pod, 100-seed weight, N and P content in shoot and per cent root infection of *P. indica*. N content was determined in laboratory by Kjeldahl's method (Bremner, 1960) and P content was analyzed by method suggested by John (1970). *P. indica* infection in roots was recorded on five random plants uprooted 55 days after sowing with intact roots. The roots of these were stained with trypan blue dye and their per cent *P. indica* infection was recorded using Phillips and Hayman (1970) method. Analysis of variance of the observations recorded on different characteristics was carried out as per the standard procedure.

RESULTS AND DISCUSSION

Development of high yielding cultivars is the main objective of any breeding programme. Major prerequisite to this is to find sufficient amount of variability from which desired lines can be selected for further manipulation. In the present study, analysis of variance (Table 1) revealed significant genotypic differences for all the characters studied indicating thereby presence of considerable amount of variability for all the characters and thus justifying the use of material. This implied that there is ample scope for selecting superior and desirable genotypes to achieve the ultimate goal. This was in agreement with the findings of many earlier studies done by Singh *et al.* (2006), Rahim *et al.* (2010) and Narasimhulu *et al.* (2013) who also had reported high variability for different traits in mungbean. Microbial treatments differed significantly for plant height, number of pods/plant, 100-seeds weight, N and P content in shoot and for seed yield whereas, non significant differences were observed for days to maturity, branches/plant and number of seeds/pod. Interaction between genotypes and microbial treatments was found significant for plant height, number of pods/plant, 100-seed weight, N and P content in shoot and seed yield meaning thereby that the genotypes interacted differently with different microbial treatments. Ali *et al.* (2000) studied response of three mungbean genotypes to seed inoculation from two different sources on growth and yield components and reported that though the genotypes were significantly affected by inoculants, yet they produced statistically similar growth and yield components except seed weight.

For further comparison of genotypes and microbial treatments and their interactions, two way tables for all the traits were prepared and critical differences were calculated for interpretation of data (Table 2). Inoculation with *Rhizobium* alone resulted in increased plant height in IPM-02-3, MH-318, MH-810, MH-421 and COGG-912 as compared to control whereas, inoculation with *P. indica* alone and combined inoculation (R+P) resulted in increased plant height in IPM-02-3, COGG-912, MH-421 and MH-810 genotypes. Increase in plant height with *Rhizobium* inoculation was also observed by Zahir *et al.* (2010) and Ahmad *et al.* (2013).

Rhizobium inoculation increased number of pods/plant significantly in MH-805, MH-421, MH-810 and Basanti whereas, *P. indica* inoculation increased the number of pods per plant in MH-721, MH-318, MH-805 and MH-810 and their combined inoculation (R+P) in MH-421 and MH-805 genotypes only. Significant effect of *Rhizobium* on number of pods/plant was reported earlier by Zahir *et al.* (2010) and Ahmad *et al.* (2013). Significant increase in 100-seed weight by the inoculation with *Rhizobium* was observed in MH-318 only, however, the inoculation of *P. indica* significantly increased seed weight in MH-318, MH-805, MH-421, MH-721 and MH-560 and their combined inoculation in MH-560, MH-421, MH-721 and MH-318 genotypes. Such results were observed also by Ray and Valsalakumar (2010), Zahir *et al.* (2010) and Ahmad *et al.* (2013). Rhizobial inoculation significantly increased N content in shoots of all the mungbean genotypes studied. *P. indica* inoculation increased N content in shoot of COGG-912, MH-421, MH-560, MH-810 and Basanti, whereas combined inoculation of both increased N content in shoots of MH-421, MH-560, Sattya and COGG-912 genotypes only. Improvement in N content uptake by the inoculation of *Rhizobium* was reported earlier by Bhat *et al.* (2010), Bhat *et al.* (2011), Hussain *et al.* (2012) and Ahmad *et al.* (2013). Increased P content in shoots was found in Basanti, MH-810, Sattya and MH-421 with rhizobial inoculation. *P. indica* increased P content in shoots of MH-560, MH-810, MH-721, MH-318, MH-805, Sattya and COGG-912 genotypes. The combined inoculation of both increased the P content in shoots of MH-560, MH-805, MH-810 and MH-721 only. Significant effect of *Rhizobium* inoculation for P content was also observed by Bhat *et al.* (2010), Bhat *et al*. (2011) and Hussain *et al*. (2012) in mungbean. The seed yield was increased by 3.67 to 13.89 % by rhizobial inoculation in genotypes *viz*., MH-421, MH-810, MH-805, MH-560, MH-721 and MH-318. Similarly *P. indica* inoculation resulted in an increase of seed yield by 3.74 to 6.29% in the genotypes MH-560, Sattya, Basanti, MH-721, MH-810. Genotypes MH-421, Sattya, MH-810, MH-805, MH-318, MH-721 exhibited an increase of 4.60 to 11.73% in seed yield by their combined inoculation. Rhizobia have shown to increase root and shoot weight, plant vigor, nitrogen fixation and grain yield in various legumes (Yadegari *et al*., 2008), Bhat *et al.* (2010) and Bhat *et al.* (2011). Significant effect of *Rhizobium* inoculation for seed yield in mungbean was also reported by many other workers including Bhat *et al.* (2010), Bhat *et al.* (2011), Ahmad *et al.* (2013) and Gosavi (2013), however, no study could be traced genotypic variability in mungbean for response to *P. indica.*

Table 1: Analysis of variance for different characters in mungbean

Source	Degrees of freedom	Mean Squares								
		Days to maturity	Plant height	No. of branches per plant	No. of pods per plant	No. of seed per pod	100 seed weight	N content (shoot)	P content (shoot)	Seed yield
Replications	2	2.772	0.417	0.050	2.352	0.700	0.032	0.021	0.001	172.350
Genotypes (G)	9	27.416**	748.636**	1.285**	122.753**	2.875**	2.062**	0.741**	0.004**	45816.842**
Microbial Treatments (M)	3	3.320	186.585**	0.123	18.912**	0.675	0.332**	1.184**	0.021**	1809.310**
GXM	27	3,410	42.613**	0.129	10.005**	0.724	0.091**	0.284**	0.003**	482.765**
Error	78	2.144	2.644	0.090	2.378	0.785	0.031	0.00611	0.00121	59.553
C.V. (%)		2.08	2.33	14.05	6.50	7.62	4.51	3.03	12.24	7.80

* Significant at p= 0.05 and ** Significant at p= 0.01

The scrutiny of comparative mean performance revealed that, in general the effect *Rhizobium* inoculation was more profound than of *P. indica*. Their combined inoculation though showed improvement over control yet it was not remarkably superior to the *Rhizobium* inoculation alone. A bird eye view on all the traits and genotypes affected by these two inoculants revealed that plant height, number of pods per plant, N content in shoot and seed yield were the traits affected most by *Rhizobium* inoculation in majority of the genotypes. Majority of the genotypes did not show much effect of *P. indica* for most of the traits and it was envisaged only in plant height, 100-seed weight, P content in shoot and seed yield of a few genotypes. The effect of combined inoculation (R+P) could be observed in seed yield only. In a study by Ray and Valsalakumar (2010), arbuscular mycorrhizal fungus was found to be effective in promoting number of pods/plant, number of seeds/pod, biomass and seed yield when applied alone or in combination with the *Rhizobium*, but *P. indica* was not found to be a good synergistic in mungbean. Their observation was based on the use of a single mungbean genotype whereas, genotypic variability in interactions was observed in the present study.

Table 2: Effect of *Rhizobium* and *P. indica* inoculation on various traits in mungbean

Traits	Plant Height (cm)					No. of Pods/plant				
Geno/Tr	**C**	**R**	**P**	**R + P**	**Mean**	**C**	**R**	**P**	**R + P**	**Mean**
Basanti	71.3	69.3	73.7	73.3	71.9	27.2	29.9	26.4	26.8	27.6
IPM-02-3	58.0	74.8	73.6	69.7	69.0	28.3	28.9	26.7	26.5	27.6
MH-318	49.7	61.6	52.9	54.5	54.7	23.2	24.7	26.6	21.4	24.0
MH-810	65.7	75.6	72.4	64.5	69.5	22.7	25.5	25.6	24.8	24.7
MH-721	72.7	70.7	73.1	75.6	73.1	20.7	23.0	25.0	22.7	22.8
MH-560	79.5	77.3	79.8	78.4	78.8	22.0	22.7	22.3	23.8	22.7
MH-805	72.7	74.3	73.6	72.2	73.2	23.7	27.3	26.7	27.2	26.2
Sattya	75.2	72.7	74.8	77.6	75.1	23.1	23.8	25.5	23.4	24.0
MH-421	53.9	64.3	63.6	63.8	61.4	18.0	20.8	20.4	22.1	20.3
COGG-912	57.2	68.6	68.2	71.0	66.2	15.8	15.0	14.4	15.4	15.2
Mean	**65.6**	**70.9**	**70.6**	**70.1**	**69.3**	**22.5**	**24.2**	**24.0**	**23.4**	**23.5**
CD at 5%	**Geno-1.3; Tr-0.8; Int-2.6**					**Geno 1.3; Tr-0.8; Int-2.5**				
Traits	**100-Seed Weight (g)**					**Seed Yield/plot (g)**				
Geno/Tr	**C**	**R**	**P**	**R + P**	**Mean**	**C**	**R**	**P**	**R + P**	**Mean**
Basanti	3.76	3.96	3.97	3.88	3.89	368.3	380.3	386.0	379.3	378.5
IPM-02-3	4.61	4.36	4.36	4.38	4.43	378.3	384.0	366.3	363.0	372.9
MH-318	4.30	4.70	4.87	4.61	4.62	363.0	376.3	370.0	376.0	371.3
MH-810	4.36	4.43	4.62	4.41	4.46	383.0	426.7	397.3	410.7	404.4
MH-721	3.60	3.83	4.05	3.96	3.86	275.3	293.0	290.3	288.0	286.7
MH-560	3.47	3.61	3.92	3.93	3.73	334.0	361.7	355.0	345.0	348.9
MH-805	3.62	3.86	4.11	3.53	3.78	356.7	398.3	367.7	377.7	375.1
Sattya	3.34	3.38	3.53	3.55	3.45	346.7	351.7	367.7	376.0	360.5
MH-421	3.32	3.43	3.78	3.76	3.57	355.3	404.7	367.3	397.0	381.1
COGG-912	3.40	3.56	3.26	3.64	3.47	198.3	193.7	195.0	197.7	196.2
Mean	**3.78**	**3.91**	**4.05**	**3.97**	**3.93**	**335.9**	**357.0**	**346.3**	**351.0**	**347.6**
CD at 5%	**Geno-0.14; Tr-0.09; Int-0.29**					**Geno-6.3; Tr-4.0; Int-12.6**				
Traits	**Nitrogen content in shoot (%)**					**Phosphorus content in shoot (%)**				
Geno/Tr	**C**	**R**	**P**	**R + P**	**Mean**	**C**	**R**	**P**	**R + P**	**Mean**
Basanti	2.55	2.81	2.70	2.53	2.65	0.17	0.16	0.18	0.16	0.17
IPM-02-3	2.57	2.73	2.61	2.47	2.60	0.14	0.14	0.16	0.14	0.15
MH-318	2.43	2.75	2.33	2.40	2.48	0.13	0.14	0.18	0.14	0.15
MH-810	2.78	3.25	3.17	2.68	2.97	0.14	0.14	0.20	0.18	0.17
MH-721	2.65	3.15	2.71	2.53	2.76	0.16	0.17	0.21	0.20	0.19
MH-560	2.76	3.15	3.31	3.17	3.10	0.14	0.18	0.20	0.21	0.18
MH-805	2.71	3.25	2.68	2.73	2.84	0.15	0.23	0.19	0.21	0.20
Sattya	2.41	2.82	2.28	2.78	2.57	0.14	0.16	0.18	0.16	0.16
MH-421	1.88	2.38	2.56	2.32	2.29	0.19	0.20	0.22	0.22	0.21
COGG-912	1.96	2.54	2.68	2.18	2.34	0.13	0.14	0.17	0.16	0.15
Mean	**2.47**	**2.88**	**2.64**	**2.44**	**2.66**	**0.15**	**0.17**	**0.19**	**0.18**	**0.17**
CD at 5%	**Geno-0.07; Tr-0.04; Int- 0.13**					**Geno-0.02; Tr-0.01; Int- 0.04**				

C- Control; R- *Rhizobium*; P- *Piriformospora indica*; NS-Non significant
Geno - Genotype; Tr - Microbial Treatment; Int – Interaction of Geno x Tr

Dual inoculation of *P. indica* and *Rhizobium* resulted in comparatively lesser magnitude of yield and yield attributing components, whereas alone they were able to have positive effects. This shows the lesser compatibility of this endophytic fungus with *Rhizobium* under the environmental conditions in which the experiment was conducted. Incompatibility with rhizobia may be due to environmental conditions or other microbial interactions including native rhizobial interactions. MH-810 and MH-721 were the only two genotypes in which the effect of all the three inoculants was observed. MH-421exhibited maximum effect of *Rhizobium* and combined inoculation, however, there was no effect of *P. indica* on seed yield and other traits. Yield is a more complex trait in pulses because several types of microbes present in the soil interact and affect the plant growth and yield. Information about the genotypic variation in mungbean regarding these is practically limited. Therefore, the knowledge of genotypic variability for root infection to these microbes, N and P uptake in plants and seed yield component traits generated through this study will be of great importance for breeders in formulation and effective execution of future breeding programmes.

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