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GENETIC VARIABILITY IN GREEN GRAM [*Vigna radiata* (L.) Wilczek] REGARDING RESPONSE TO *Rhizobium* sp. AND *Piriformospora indica* INOCULATIONS

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ABSTRACT

A study was conducted to assess genotypic variations in 10 green gram genotypes for root nodulation, root infection to *Piriformospora indica*, seed yield and N and P uptake. *Rhizobium* sp. strain-MB-703, *P. indica* and their combination were inoculated to green gram seeds. Significant genotypic differences observed for all the characters studied indicated the presence of considerable variability. All the microbial treatments and genotype x microbial interaction differed significantly implying that the genotypes interacted differently with different microbial treatments. The traits affected by *Rhizobium* inoculation in most genotypes were nodules (number and dry weight), N content in seeds and seed yield. *Rhizobium* inoculation increased seed yield by 3.7-13.9% in genotypes MH-421, MH-810, MH-805, MH-560, MH-721 and MH-318. *P. indica* infection in roots was >50% in 8 genotypes and it caused increase in seed yield (3.7-6.3%) only in 5 genotypes *viz*., MH-560, Sattya, Basanti, MH-721 and MH-810. Dual inoculation caused 4.6-11.7% increase in seed yield in genotypes MH-421, Sattya, MH-810, MH-805, MH-318 and MH-721.

Key words: Green gram, nodulation, *Piriformospora indica*, *Rhizobium*, seed yield

INTRODUCTION

Pulses have long been recognized as soil building crops. Their cultivation improves soil quality through beneficial impact on soil biological and physico-chemical properties as they fix atmospheric nitrogen through rhizobia, the primary symbiotic N_2 fixer. This renewable and environmentally sustainable nitrogen can supplement or partially replace nitrogenous fertilizer inputs (Peoples *et al.*, 1995). *Rhizobium* species are host-specific and interact variably with different genotypes of the same species. *Rhizobium* inoculation is a promising biofertilizer as it is cheap, easy to handle and improves plant growth and quality (Giri and Joshi, 2010).

To improve the competitiveness and nitrogen fixing ability of rhizobia, various microbial inoculants like phosphate solubilizing bacteria (PSB), plant growth promoting rhizobacteria (PGPR) and vesicular arbuscular mycorrhiza (VAM) are often used in combination (Dudeja *et al.*, 2012). VAM fungi help in mineral solubilization and uptake as well as impart resistance to abiotic stresses. However, VAM fungi cannot be cultured under laboratory conditions. Alternatively, a novel plant growth promoting, root colonizing endophytic fungus, *Piriformospora indica,* isolated from orchid plants in Thar desert (India), has the ability to grow axenically on a variety of simple and complex media and mimic most of the beneficial characteristics of VAM fungi. This fungus functions as plant growth promoter and biofertilizer in nutrient-deficient soils as well as bioprotector against biotic and abiotic stresses, bioregulator for plant growth development, bio-agent for hardening of tissue culture raised plants and enhances nutrient uptake (Varma *et al.*, 2009). This unique combination of attributes makes it a suitable inoculant of various crops.

Green gram [*Vigna radiata* (L.) Wilczek], is an important pulse crop raised in *kharif*, spring/summer and *rabi* seasons in India. It is highly nutritious and quality pulse due to its rich protein content, excellent digestibility of low flatulence and complementation with staple wheat and rice diet (Khattak *et al.*, 2001). Being a short duration crop, it fits well into intensive rice-wheat cropping system of Indo-Gangetic plains and can be intercropped with sugarcane, pigeonpea, poplar and orchards. The productivity of green gram is low and stability in seed yield is a major problem. Yield is a more complex character in pulses than in cereals as the interaction between plant and microbes affects the ultimate yield. The overall interaction among them, either positive or negative, makes the situation more complex. Infection of *Rhizobium* and other microbes in pulses decides the ultimate yield. Combined inoculation of *Rhizobium* and *P. indica* reportedly increases nodulation, dry matter content, grain yield and nitrogen and phosphorus uptake significantly over uninoculated control in many crops (Dudeja and Duhan, 2005; Yadegari *et al.*, 2008). The information on genotypic variations in green gram regarding response to *Rhizobium* and *P. indica* is sacnty ~~(Bhat et al., 2011; Tariq et al., 2012)~~. The knowledge about the nature and magnitude of genotypic variability for root infection of these microbes, nodulation, nitrogen and phosphorus uptake and seed yield would be of great importance for a breeder in the formulation and execution of a breeding programme. The present study was aimed to estimate genotypic variation in green gram with respect to root infection by *P. indica*, *Rhizobium* nodulation, NP uptake in seeds and yield; and to identify genotypes responsive to these microbes individually and in combination.

MATERIALS AND METHODS

The present study was conducted at CCSHAU, Hisar (India) during *kharif* 2013-2014. The experimental material comprized of ten green gram genotypes *viz*., Sattya, Basanti, MH-421, MH-318 (released varieties of CCS HAU, Hisar), IPM-02-3 (released variety of IIPR, Kanpur), CoGG-912 (released variety of TNAU, Coimbatore), MH-810, MH-721, MH-560 and MH-805 (high yielding green gram genotypes developed at CCS HAU, Hisar). The *Rhizobium* sp. strain MB-703 and fungus *Piriformospora indica*, procured from the Department of Microbiology, CCS HAU, Hisar, were used to inoculate the seeds of genotypes. The seeds were sprinkled with inoculants having inoculum load of 10^9 cells mL^{-1}, @ 0.5 mL kg^{-1} seed and thoroughly mixed. The seeds were shade dried for 4 hr before sowing. The treatments included *Rhizobium* strain MB-703, *P. indica*, *Rhizobium* strain MB-703 + *P. indica* and uninoculated control. The experiment was conducted in a factorial randomized block design with three replications. All these genotypes were sown on July 5, 2013 in 4 rows of 4 m length each. Plant spacing maintained was 10 x 30 cm. The genotypes were harvested in 2^{nd} fortnight of September as per their maturity. The N and P status of soil prior to and at the end of experiment was estimated as per Bremner (1960) and John (1970), respectively.

The seed yield plot^{-1}, number of nodules plant^{-1}, nodule dry weight plant^{-1}, N and P content in seeds and *P. indica* infection in roots were estimated. Five randomly selected plants were gently uprooted 45 days after sowing and nodules from the roots detached and counted. The nodule weight was recorded after oven-drying the nodules at 65^{o}C for 24 hr. Root infection by *P. indica* was noted on 5 random plants uprooted 55 days after sowing. The roots were stained with trypan blue and per cent infection estimated as per Phillips and Hayman (1970). The N content in seeds was determined by Kjeldahl's method (Bremner, 1960) and P content in seeds as per John (1970). The data was analyzed by analysis of variance as per the standard procedure (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

The assessment of genetic variability is the first step in any breeding programme and the progress depends on the extent of genetic variability present for various characters in gene pool. It is crucial for any breeding programme as it provides an opportunity to select desirable genotypes. In present study, the analysis of variance revealed significant genotypic variations in all the characters studied indicating the presence of considerable variability in all the characters studied (Table 1-3). It showed that there is ample scope for selecting superior and desirable genotypes to achieve the ultimate goal. Hakim (2008), Rahim *et al.* (2010), Mehandi *et al.* (2013) and Hozayn *et al.* (2013) reported similar high variability for different traits in green gram. All the microbial treatments and genotype x microbial interaction differed significantly indicating that the genotypes interacted differently with different microbial treatments.

The nodules number per plant increased significantly by rhizobial inoculation and profound effect was observed in genotypes MH-421, IPM-02-3, Basanti, MH-721, MH-318, MH-805 and CoGG-912 (Table 1). Significant effect of *Rhizobium* on nodules dry weight was observed in IPM-02-3, CoGG-912, MH-421 and MH-805 as compared to control. Increase in nodule number and dry weight by *Rhizobium* inoculation observed is in agreement with Dudeja and Duhan (2005); Bansal (2009), Bhat *et al.* (2011), Tariq *et al.* (2012) and Ahmad *et al.* (2013). Significant increase in nodules number by *P. indica* infection was observed only in MH-721 and Basanti while combined inoculation affected only CoGG-912, IPM-02-3 and Basanti. The effect of combined inoculation of *Rhizobium* and *P. indica* on nodules dry weight was seen in genotype CoGG-912 only.

In green gram genotypes Basanti, MH-318 and MH-721 the nodule number was more but its dry weight was less as compared to other genotypes, because these genotypes produced smaller sized nodules. In genotype Sattya, the observations were opposite with greater nodule size but less nodule number. In some genotypes like IPM-02-3, CoGG-912 and Basanti nodulation increased due to *Rhizobium* inoculation, but it did not culminated in yield increase. In genotypes MH-810 and MH-560 no significant increase in nodulation was observed by *Rhizobium* inoculation; however, there was significant yield increase. Thus, it is clear that there is no correlation between number of nodules and seed yield in these genotypes, as in addition to the nodulating efficiency, other factors also contribute to the plant yield.

The rhizobial inoculation increased seed yield by 3.67 to 13.89% in genotypes MH-421, MH-810, MH-805, MH-560, MH-721 and MH-318 (Table 2). Similarly, *P. indica* inoculation enhanced seed yield by 3.7 to 6.3% in genotypes MH-560, Sattya, Basanti, MH-721 and MH-810.

Table 1: Effect of *Rhizobium* and *P. indica* on nodule number and weight in green gram

	Nodules (No. plant^{-1})					Nodules dry weight (mg plant^{-1})				
Geno/Tr	C	R	P	R + P	Mean	C	R	P	R + P	Mean
Basanti	32.4	39.9	35.7	35.3	35.8	301.1	314.1	315.9	313.2	311.1
IPM-02-3	26.7	35.5	27.6	31.5	30.3	275.5	314.0	259.4	277.8	281.7
MH-318	23.0	27.5	24.2	25.0	24.9	272.9	287.9	274.9	274.9	277.6
MH-810	33.8	36.2	36.4	36.6	35.7	365.4	368.0	353.9	373.8	365.3
MH-721	22.6	29.2	26.3	25.4	25.9	266.6	281.7	260.5	281.0	272.5
MH-560	32.2	34.2	30.9	33.2	32.6	356.1	354.4	364.9	364.8	360.0
MH-805	23.9	28.2	24.6	25.2	25.5	269.9	300.1	281.0	276.5	281.9
Sattya	28.3	29.7	26.4	25.4	27.4	280.7	310.6	282.4	271.0	286.2
MH-421	30.4	39.6	32.3	28.6	32.7	280.7	313.3	281.0	266.3	285.3
COGG-912	16.1	19.6	17.0	20.9	18.4	185.2	221.5	200.4	205.8	203.2
Mean	26.9	32.0	28.1	28.7	28.9	285.4	306.6	287.4	290.5	292.5
$CD_{0.05}$	Geno = 1.5; Tr = 0.9; Int = 3.0					Geno = 7.6; Tr = 4.8; Int = 15.3				

C = Control; R = *Rhizobium*; P = *Piriformospora indica*; Geno = Genotype; Tr = Microbial treatments; Int – Geno x Tr interaction

Table 2: Effect of *Rhizobium* and *P. indica* on root infection and seed yield in green gram

Traits	*P. indica* infection in roots (%)					Seed yield (g $plot^{-1}$)				
Geno/Tr	C	R	P	R + P	Mean*	C	R	P	R + P	Mean
Basanti	-	-	62.0	67.7	64.8	368.3	380.3	386.0	379.3	378.5
IPM-02-3	-	-	71.0	51.7	61.3	378.3	384.0	366.3	363.0	372.9
MH-318	-	-	34.7	34.3	34.5	363.0	376.3	370.0	376.0	371.3
MH-810	-	-	81.0	83.7	82.3	383.0	426.7	397.3	410.7	404.4
MH-721	-	-	56.0	64.3	60.2	275.3	293.0	290.3	288.0	286.7
MH-560	-	-	62.3	61.7	62.0	334.0	361.7	355.0	345.0	348.9
MH-805	-	-	56.3	55.7	56.0	356.7	398.3	367.7	377.7	375.1
Sattya	-	-	71.7	68.3	70.0	346.7	351.7	367.7	376.0	360.5
MH-421	-	-	66.7	64.0	65.3	355.3	404.7	367.3	397.0	381.1
COGG-912	-	-	17.3	10.0	13.7	198.3	193.7	195.0	197.7	196.2
Mean	-	-	57.9	56.1	57.0	335.9	357.0	346.3	351.0	347.6
$CD_{0.05}$	Geno-0.9; Tr-0.6; Int-1.8					Geno-6.3; Tr-4.0; Int-12.6				

C- Control; R- *Rhizobium*; P- *Piriformospora indica*; Geno - Genotype; Tr - Microbial treatment; Int – Geno x Tr interaction

Genotypes MH-421, Sattya, MH-810, MH-805, MH-318 and MH-721 exhibited 4.6 to 11.7% increase in seed yield when *Rhizobium* and *P. indica* were co-inoculated. Significant impact of *Rhizobium* on seed yield has been reported by Bansal (2009) and Ahmad *et al.* (2013). Dual inoculation of *P. indica* and *Rhizobium* caused insignificant yield increase than *Rhizobium* alone in most of the genotypes. This showed poor compatibility of endophytic fungus with *Rhizobium* under prevailing environmental conditions. Ray and Valsalakumar (2010) found arbuscular mycorrhizal fungus effective in promoting biomass and seed yield of green gram when applied alone or in combination with *Rhizobium*, but *P. indica* was not found to be a good synergist. This observation was based on a single green gram genotype; whereas genotype to genotype variability in interaction was observed in the present study.

Root infection by *P. indica* inoculation alone or in combination with *Rhizobium* revealed more than 50% infection in genotypes Basanti, IPM-02-3, MH-810, MH-721, MH-560, MH-805, Sattya and MH-421 (Table 2). Only in one genotype MH 810 the infection was >80%; whereas, the remaining had infection between 50-70%. The genotype CoGG-912 exhibited least response to *P. indica* infection in roots followed by genotype MH-318.

Rhizobium inoculation in green gram enhanced seed N content in all the genotypes (Table 3). Increase in nitrogen uptake by *Rhizobium* inoculation has been reported by Dudeja and Duhan (2005),

Table 3: Effect of *Rhizobium* and *P. indica* on nitrogen and phosphorus content in green gram

	Nitrogen content in seeds (%)					Phosphorus content in seeds (%)				
Geno/Tr	C	R	P	R + P	Mean	C	R	P	R + P	Mean
Basanti	2.91	3.50	2.93	3.16	3.13	0.21	0.25	0.26	0.28	0.25
IPM-02-3	3.24	3.55	3.10	3.53	3.36	0.20	0.22	0.18	0.19	0.20
MH-318	3.31	3.62	3.18	3.60	3.43	0.19	0.20	0.22	0.23	0.21
MH-810	3.18	3.77	3.67	3.76	3.60	0.19	0.22	0.20	0.21	0.21
MH-721	3.16	3.82	3.84	3.56	3.60	0.17	0.19	0.21	0.22	0.20
MH-560	3.15	4.02	3.58	3.51	3.57	0.22	0.24	0.21	0.23	0.23
MH-805	3.00	3.33	3.36	3.39	3.27	0.23	0.25	0.22	0.24	0.24
Sattya	3.23	3.55	3.24	3.49	3.38	0.18	0.21	0.20	0.19	0.20
MH-421	3.25	3.45	3.24	3.48	3.36	0.21	0.24	0.22	0.21	0.22
COGG-912	2.10	2.39	2.28	2.63	2.35	0.21	0.23	0.20	0.22	0.22
Mean	3.05	3.50	3.24	3.41	3.30	0.20	0.23	0.21	0.22	0.22
$CD_{0.05}$	Geno-0.05; Tr-0.03; Int-0.11					Geno-0.02; Tr-0.01; Int-0.03				

C- Control; R- *Rhizobium*; P- *Piriformospora indica*; Geno - Genotype; Tr - Microbial treatment; Int - Geno x Tr interaction

Yadegari *et al.* (2008), Bhat *et al.* (2011) and Ahmad *et al.* (2013). *P. indica* inoculation also increased N content in seeds of MH-721, MH-810, MH-560, MH-805 and CoGG-912. Combined inoculation increased seed N content in all the genotypes studied. Increased P content was found in Basanti, MH-810, Sattya and MH-421 with rhizobial inoculation. Our findings are in line with Bhat *et al.* (2011), Tajini *et al.* (2011) and Hussain *et al.* (2012). *P. indica* also increased P content in shoots of MH-560, MH-810, MH-721, MH-318, MH-805, Sattya and CoGG-912 and in seeds of Basanti, MH-721 and MH-318. The combined inoculation of *Rhizobium* and *P. indica* increased the P content in seeds in Basanti, MH-721 and MH-318. No report on effect of *P. indica* and combined inoculation of *Rhizobium* and *P. indica* on N and P uptake in green gram could be traced.

In general the effect of *Rhizobium* inoculation was more pronounced than *P. indica*. The combined inoculation showed improvement over control but was not significantly superior to *Rhizobium* inoculation alone in most of the genotypes. Nodule number and dry weight, seed N content, seed yield were the traits affected by *Rhizobium* inoculation in most genotypes. However, genotypes did not show much effect of *P. indica* for most of the traits except root infection. The significant effect of combined inoculation was observed in seed N content, *P. indica* infection and seed yield. MH-810 and MH-721 were the only genotypes showing promising effect due to *Rhizobium* and combined inoculation. These genotypes can further be used in breeding programmes.

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