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SYNERGETIC EFFECT OF *Rhizobium* sp. AND *Piriformospora indica* ON BIOENHANCING ACTIVITY, SYMBIOTIC PARAMETERS AND GRAIN YIELD IN PIGEON PEA (*Cajanus cajan* L.)

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ABSTRACT

The synergetic effect of *Rhizobium* sp. (*Cajanus*) and *Piriformospora indica* on bioenhancing activity, symbiotic parameters and grain yield of pigeon pea was evaluated. Nine genotypes were raised during *kharif*, 2013 at the Experimental Farm, HAU, Hisar (India). *Rhizobium* sp. and *P. indica,* alone or co-inoculated, along with untreated control were assessed for response of genotypes to these organisms on various metric traits. Significant differences among the genotypes for the studied traits indicated beneficial effect of both the organisms. Among the treatments, *P. indica* inoculation alone was most effective for most of the characters followed by dual inoculation of *P. indica* and *Rhizobium*. But for symbiotic traits the performance of *Rhizobium* alone was better than dual inoculation or *P. indica* alone. The genotypes Manak, Paras, UPAS 120, Pusa 991 and AH09-47 performed better with respect to symbiotic, growth and yield parameters. Hence, these genotypes could be used as parents in hybridization programme for genetic improvement of pigeon pea.

Key words: *Cajanus cajan*, pigeon pea, *Piriformospora indica*, *Rhizobium* sp., symbiosis

INTRODUCTION

Pigeon pea (*Cajanus cajan* L Millsp.) is one of the major food legumes cultivated in pure and mixed stands in tropical and sub-tropical countries. India is the largest producer of pigeon pea in the world and produces 3.02 million tones of pigeon pea on 3.81 million ha area (about 90% of the world) with productivity of 806 kg ha^{-1} (Anonymous, 2014). Pigeon pea forms root nodules in association with *Rhizobium* sp. (*Cajanus*) and is capable of fixing 41 to 280 kg N ha^{-1} (Red de groups, 2002). However, pigeon pea nodulates poorly in arid and semi-arid tropical regions due to low population density, competition with native strain and poor survival of introduced strains of rhizobia (Dudeja and Khurana, 1989b). The *Rhizobium*-legume symbiosis is the most efficient system which accounts for almost 40% of all biologically fixed nitrogen (Yadav, 2008), estimated at 70 million tones annum^{-1} (Brockwell *et al*., 1995). The symbiotic nitrogen-fixation acts as a renewable and environmentally sustainable source of nitrogen and can supplement or partially replace nitrogenous fertilizer inputs (Peoples *et al*., 1995). However, phosphorus deficiency is an important limiting factor in nitrogen fixation and legume production (Jacobson, 1985). According to Mosse (1973), 75% of the phosphorus applied to crops is not utilized by them. Mycorrhizal inoculation helps in mobilization of unutilized phosphorus. *Piriformospora indica*, an endophytic arbuscular mycorrhizal (AM) fungus, tremendously improves the growth and overall biomass production of diverse hosts including legumes (Varma *et al*., 1999; Prasad *et al*., 2008). It enhances nutrient uptake, especially phosphorus and nitrogen (Varma *et al*., 2001, 2014). The unique feature of this fungus is its axenically culturing ability, a golden lining of AM fungi for the scientist dealing with mycorrhizal research (Kumari *et al*., 2005). Co-inoculation of AM fungus and *Rhizobium* helps in the fixation of nitrogen resulting in a more efficient acquisition of these nutrients by plants.

The interaction studies between AM fungi and *Rhizobium* on legumes have revealed that the growth and yield of soybean increased after inoculation with *Glomus mosseae* in sterilized soil (Bethlenfalvay *et al*., 1987). Co-inoculation of *P. indica* with phosphate-solubilizing bacterium *Pseudomonas striata* on *Cicer arietinum* had synergistic effect on population build up of *P. striata* and plant dry biomass (Meena *et al*., 2010). Synergistic beneficial effects on nodulation, nitrogen fixation and plant growth of AM fungi and *Rhizobium* co-inoculation on different crop plants have also been reported (Hazarika *et al.*, 2000; Kumar and Kumar, 2003). The genetic elements for nodulation and auto-regulation systems in legumes may be involved in the accommodation of root associated microbes other than rhizobia (Seishi *et al*., 2008). In pigeon pea dual inoculation of AM fungus *G. fasciculatum* and *R. phaseoli* under controlled conditions has been reported to significantly increase height, number of nodules, mycorrhizal colonization over control and single inoculation with AM fungus and *R. phaseoli* (Hosmani *et al*., 2011). The present study was undertaken under field conditions to assess the effect of mono- and co-inoculation of AM fungus *P. indica* and *Rhizobium* sp. *(Cajanus)* on pigeon pea.

MATERIALS AND METHODS

The plant materials comprised of 9 pigeon pea genotypes namely Manak, Paras, UPAS 120, H03-41, ICPL 88039, AH09-38, AH09-47, ASJ 123 and Pusa 991 and 4 inoculation treatments i) *Rhizobium* sp. (*Cajanus*) strain CC1021, ii) *Piriformospora indica*, iii) *Rhizobium* sp. + *P. indica* co-inoculation, and iv) control (uninoculated). The *Rhizobium* starin and *P. indica* cultures were were procured from Biofertilizer Production Unit, Department of Microbiology, CCSHAU, Hisar (India). The inoculation was done by pre-sowing treatment of pigeon pea seeds with a peat based culture (having 10^8 cells mL^{-1}). A thin layer of *P. indica* inoculum (consisting of 10 g roots + soil having 100-150 chlamydospores) was placed 2 cm below the soil surface. An uninoculated control was maintained. The seeds of genotypes were obtained from Pulses Section, Deptt. of Genetics & Plant Breeding, CCSHAU, Hisar. The experiments were conducted in *kharif* season 2013 at the Experimental Farm, Pulses Section, CCSHAU, Hisar. The genotypes were raised in a factorial randomized block design with 3 replications per treatment in plots of 4 x 1.8 m size with row to row spacing of 45 cm. The crop was raised following recommended agronomic practices. Observations for all the traits (except yield, flowering and maturity) were recorded on randomly selected 5 plants/ treatment. The data on yield, days to 50% flowering and days to maturity were recorded on plot basis. The plant height, number of branches plant^{-1}, number of pods plant^{-1} and number of seeds pod^{-1} were assessed at the time of maturity; whereas the observation on days to 50% flowering was taken when 50% plants in plots exhibited emergence of inflorescence. The 100-seed weight and seed yield plot^{-1} were obtained after threshing the crop. The number of nodules plant^{-1}, nodule dry weight plant^{-1} and shoot dry weight plant^{-1} were recorded after 45 days of plant growth. The per cent infection by *P. indica* was determined following the method of Phillips and Hayman (1970). Statistical analysis was done following SPAR 2 software (IASRI, 2002). The significance of difference was tested using critical difference at 0.05 probability (P) level.

RESULTS AND DISCUSSION

Effect on symbiotic parameters

Less nodulation was observed in uninoculated (control) and *Piriformospora indica* inoculated plants than *Rhizobium* inoculated plants (Table 1). *Rhizobium* induced more nodulation in all the genotypes. All genotypes differed significantly from each other with genotype AH-09-47 produced maximum 19 nodules plant^{-1} followed by genotypes Manak (17) and UPAS 120 (15). Co-inoculation with *Rhizobium* and *P. indica* resulted in 50% reduction in nodulation compared to solo inoculation with *Rhizobium*. Similarly the nodules dry weight in plants inoculated with *Rhizobium* alone was significantly greater than those co-inoculated with both *P. indica* and *Rhizobium* while the control and *P. indica* inoculation alone treatments recorded minimum nodulation. The genotype AH-09-47 that recorded the maximum number of nodules plant^{-1} also had maximum nodule weight

Table 1: Effect of mono- and co-inoculation of *Rhizobium* sp. (*Cajanus*) and *P. indica* on nodule number and weight in pigeon pea genotypes

Genotypes	Number of nodules plant^{-1}					Nodule dry weight (g plant^{-1})				
	C	P	R	P + R	Mean	C	P	R	P + R	Mean
Manak	4	4	17	9	8	0.04	0.05	0.09	0.05	0.06
Paras	1	2	13	9	6	0.02	0.02	0.08	0.04	0.04
UPAS 120	1	3	15	11	8	0.02	0.03	0.08	0.06	0.05
H 03-41	2	6	10	7	6	0.03	0.05	0.06	0.05	0.05
ICPL 88039	2	2	8	6	4	0.02	0.03	0.05	0.03	0.04
AH 09-38	3	4	7	4	5	0.03	0.05	0.06	0.04	0.05
AH 09-47	2	3	19	12	9	0.03	0.03	0.10	0.07	0.06
ASJ 123	2	3	8	5	5	0.03	0.03	0.05	0.04	0.04
Pusa 991	3	3	11	9	7	0.03	0.04	0.07	0.05	0.05
Mean	2	3	12	8	6	0.03	0.04	0.07	0.05	0.05
SE	G = 0.33; I = 0.22; G x I = 0.66					G = 0.001; I = 0.001; G x I = 0.002				
$CD_{0.05}$	G = 0.93; I = 0.62; G x I = 1.87					G = 0.003; I = 0.002; G x I = 0.005				

C = Control, P = *Piriformospora indica*; R = *Rhizobium* sp.; G = genotype, I = Microbial inoculation

(1.0 g). Yadav *et al*. (2002) also reported a significant increase in the number and weight of nodules plant^{-1} and shoot dry weight plant^{-1} in most of the genotypes of chickpea studied. Osman *et al*. (2011) reported significant increase in nodule number and weight in all the strains used in pigeon pea.

Table 2: Effect of mono- and co-inoculation of *Rhizobium* sp. (*Cajanus*) and *P. indica* on *P. indica* infection and plant height in pigeon pea genotypes

Genotypes	*P. indica* infection (%)					Plant height (cm)				
	C	P	R	P+R	Mean	C	P	R	P+R	Mean
Manak	0.0	80.0	0.0	70.0	75.0	142.0	160.0	147.7	156.7	151.6
Paras	0.0	90.0	0.0	67.0	78.0	151.7	175.7	155.0	158.3	160.2
UPAS 120	0.0	73.0	0.0	60.0	66.0	141.3	179.3	165.0	178.0	165.9
H 03-41	0.0	60.0	0.0	53.0	56.0	140.3	172.7	148.0	155.0	154.0
ICPL 88039	0.0	53.0	0.0	37.0	45.0	132.7	154.3	146.0	159.3	148.1
AH 09-38	0.0	60.0	0.0	47.0	53.0	124.3	161.7	144.7	160.3	147.7
AH 09-47	0.0	70.0	0.0	57.0	63.0	148.3	171.7	161.0	152.0	158.2
ASJ 123	0.0	33.0	0.0	23.0	28.0	145.3	169.3	150.3	156.3	155.3
Pusa 991	0.0	67.0	0.0	40.0	53.0	139.7	167.0	159.3	162.3	157.1
Mean	0.0	65.0	0.0	50.0	57.0	140.6	168.0	153.0	159.8	155.3
SE	G = 1.49; I = 0.99; G x I = 2.98					G = 2.46; I = 1.64; G x I = 4.92				
$CD_{0.05}$	G = 4.21; I = 2.81; G x I = 8.41					G = 6.95; I = 4.63; G x I = ns				

C = Control, P = *Piriformospora indica*; R = *Rhizobium* sp.; G = genotype, I = Microbial inoculation

Table 3: Effect of mono- and co-inoculation of *Rhizobium* sp. (*Cajanus*) and *P. indica* on days to 50% flowering and maturity in various genotypes of pigeon pea

Genotypes	Days to 50% flowering					Days to maturity				
	C	P	R	P+R	Mean	C	P	R	P+R	Mean
Manak	86	76	86	83	83	147	141	148	139	144
Paras	90	74	89	84	84	153	138	151	146	147
UPAS 120	86	76	87	84	83	145	135	140	138	139
H 03-41	89	74	88	87	85	154	136	151	140	145
ICPL 88039	82	74	80	76	78	138	130	137	134	135
AH 09-38	87	72	85	85	82	148	138	146	143	144
AH 09-47	88	78	88	85	85	150	139	148	145	145
ASJ 123	85	75	84	82	82	147	135	145	140	142
Pusa 991	83	70	84	82	80	152	142	148	145	147
Mean	86	74	86	83	82	148	137	146	141	143
SE	G = 0.48; I = 0.32; G x I = 0.96					G = 0.77; I = 0.51; G x I = 1.54				
$CD_{0.05}$	G = 1.36; I = 0.91; G x I = 2.72					G = 2.18; I = 1.45; G x I = 4.35				

C = Control, P = *Piriformospora indica*; R = *Rhizobium* sp.; G = genotype, I = Microbial inoculation

The mycorrhizal infection was higher in *P. indica* inoculated plants than those co-inoculated with *P. indica* and *Rhizobium* (Table 2). The percent infection varied from 33 to 90 in solo *P. indica* inoculated plants while it was between 23 and 70 in co-inoculation treatments. It revealed that co-inoculation with *Rhizobium* caused significant reduction in mycorrhizal infection. Mycorrhizal infection in *Rhizobium* inoculated plants and control was absent in all genotypes. Genotype Paras followed by Manak were highly susceptible while ASJ 123 was highly resistant to mycorrhizal infection. In general, the effect of co-inoculation of *Rhizobium* + *P. indica* was in between the effect of *P. indica* or *Rhizobium* as solo. Jangra and Yadav (2015) found that effect of *Rhizobium* alone was more profound than *P. indica* alone and dual inoculation in moong. Hosmani *et al*. (2011) found that *R. phaseoli* significantly increased the number and dry weight of nodules plant^{-1} in pigeon pea and mycorrhizal infection was higher in the plants which were inoculated with *Glomus fasciculatum* and *Rhizobium*.. This indicated that inoculation of pigeon pea with *Rhizobium* is beneficial for better growth and development with increased nodulation as well as more nitrogen fixation by the plant. Ray and Valsalakumar (2010) reported that in green gram *P. indica* failed to colonize when applied alone, but showed 3% colonization when inoculated along with *Rhizobium*.

Synergetic effect on grain yield and yield components

All the three treatments i.e. *Rhizobium* sp. (*Cajanus*) strain 1021, *P. indica* and *Rhizobium* sp. (*Cajanus*) + *P. indica* influenced the yield and yield attributes of cowpea (pigeon pea) in comparison to the uninoculated control (Table 2-6). However, the effect of *P. indica* inoculation alone was more pronounced for most of the characters studied, followed by dual inoculation and sole *Rhizobium* inoculation. The *P. indica* inoculation alone led to significant early flowering and seed maturity in all the genotypes as compared to the uninoculated control (Table 3). Further, it significantly increased the number of branches plant^{-1} in all the genotypes tested (except genotypes H03-41 and AH09-38), number of seeds pod^{-1} (except genotypes ASJ 123 and AH09-47) and the number of pods plant^{-1} in all the genotype except genotype AH09-38 (Table 4). Similarly, shoot dry weight plant^{-1} and seed yield plot^{-1} significantly increased in all the genotypes (Table 4 & 5). Dual inoculation significantly induced early flowering in genotypes Manak, Paras, ICPL 88039, AH09-47 and ASJ123 and maturity in all the genotypes, except genotypes ICPL 88039 (Table 3). The dual inoculation of *Rhizobium* and *P. indica* significantly enhanced the number of branches plant^{-1} in all the genotypes (except genotype H03-41), number of pods plant^{-1} in all the genotypes (except genotype Pusa 991), number of seeds pod^{-1} in genotypes H03-41, AH09-47 and ASJ 123, shoot dry weight plant^{-1} except in genotype AH09-38 and seed yield plot^{-1} in all the genotypes. The solo *Rhizobium* treatment significantly increased the number of branches plant^{-1} in all the genotypes,

Table 4: Effect of mono- and co-inoculation of *Rhizobium* sp. (*Cajanus*) and *P. indica* on the number of pods plant^{-1} and number of seeds pod^{-1} in various genotypes of pigeon pea

Genotypes	Number of pods plant^{-1}					Number of seeds pod^{-1}				
	C	P	R	P+R	Mean	C	P	R	P+R	Mean
Manak	25	43	34	38	35	4	5	5	4	5
Paras	30	49	35	38	38	4	6	5	4	5
UPAS 120	27	37	29	33	31	4	6	5	4	5
H 03-41	21	34	24	27	27	3	5	6	4	5
ICPL 88039	21	31	23	27	25	3	5	5	3	4
AH 09-38	22	26	33	32	28	3	5	5	4	4
AH 09-47	19	33	21	26	25	3	3	4	5	4
ASJ 123	18	30	22	25	24	3	3	4	5	4
Pusa 991	25	38	29	29	30	4	6	5	4	5
Mean	23	36	28	31	29	3	5	5	4	4
SE	G = 0.99; I = 0.66; G x I = 1.97					G = 0.18; I = 0.12; G x I = 0.35				
$CD_{0.05}$	G = 2.79; I = 1.86; G x I = 5.58					G = 0.49; I = 0.33; G x I = 0.99				

C = Control, P = *Piriformospora indica*; R = *Rhizobium* sp.; G = genotype, I = Microbial inoculation

except genotypes H03-41, AH09-47 and Pusa 991, number of pods plant^{-1} in genotypes Manak and AH09-38, shoot dry weight in genotypes Manak, Paras, AH09-47, ASJ 123 and Pusa 991, number of seeds pod-^{1} in all the genotypes and seed yield plot-^{1} in all the genotypes (except genotype AH09-38 and Pusa 991). Symbiotic interaction was found to be non-significant for plant height and100-seed weight. In general, *P. indica* solo inoculation was found to be the best enhancing yield and its components. Osman *et al*. (2011) found that *Rhizobium* strains TAL 209, TAL 1132 and USDA 3472 significantly increased pigeon pea yield in both the seasons. Hosmani *et al*. (2011) observed a significant increase in yield and dry weight of shoot per plant in pigeon pea for dual inoculation of *Glomus fasciculatum* + *Rhizobium phaseoli* than those plants inoculated with *Rhizobium* alone. Ray and Valsalakumar (2010) reported that dual inoculation of *Rhizobium* and *P. indica* in moong resulted in more number of pods plant-^{1} and 100-seed weight as compared to the uninoculated control; but such increase was non-significant.

In present study *Rhizobium* inoculation alone resulted in significant increase in 100-seed weight (Table 6). Gharib *et al*. (2009) reported that the effect of *Rhizobium* inoculation on the number of branches plant^{-1} on two snap bean cultivars was at par with uninoculated control. Das *et al*. (2012) inoculated a medicinal plant *Coleus forskohlii* with *P. indica* and found that flowering

Table 5: Effect of mono- and co-inoculation of *Rhizobium* sp. (*Cajanus*) and *P. indica* on the number of branches plant^{-1} and shoot dry weight in various genotypes of pigeon pea

Genotypes	Number of branches plant^{-1}					Shoot dry weight (g plant^{-1})				
	C	P	R	P+R	Mean	C	P	R	P+R	Mean
Manak	7	12	11	11	10	9.67	14.02	11.57	13.27	12.13
Paras	8	11	10	10	10	10.68	14.56	13.76	12.06	12.77
UPAS 120	8	11	10	10	10	11.08	15.10	11.34	14.11	12.91
H 03-41	7	8	8	8	8	7.83	11.04	8.14	11.01	9.51
ICPL 88039	6	9	8	9	8	7.74	10.72	8.24	10.33	9.26
AH 09-38	7	8	10	10	9	8.98	11.28	9.68	10.18	10.03
AH 09-47	6	10	7	9	8	8.57	10.74	10.26	9.78	9.84
ASJ 123	5	9	9	8	8	7.25	12.18	9.93	11.93	10.32
Pusa 991	8	11	9	10	9	10.12	13.39	12.45	12.76	12.18
Mean	7	10	9	10	9	9.10	12.56	10.60	11.71	10.99
SE	G = 0.24; I = 0.16; G x I = 0.47					G = 0.21; I = 0.14; G x I = 0.43				
$CD_{0.05}$	G = 0.66; I = 0.44; G x I = 1.33					G = 0.60; I = 0.40; G x I = 1.21				

C = Control, P = *Piriformospora indica*; R = *Rhizobium* sp.; G = genotype, I = Microbial inoculation

Table 6: Effect of mono- and co-inoculation of *Rhizobium sp.* (*Cajanus*) and *P. indica* on seed weight and yield in various genotypes of pigeon pea

Genotypes	100-seed weight (g)					Seed yield (g plot^{-1})				
	C	P	R	P+R	Mean	C	P	R	P+R	Mean
Manak	6.74	7.70	7.40	7.77	7.40	1012.7	1447.3	1307.3	1397.3	1291.2
Paras	6.65	7.20	7.55	7.48	7.22	1174.7	1368.7	1270.0	1314.7	1282.0
UPAS 120	6.38	7.32	7.18	7.81	7.17	1012.7	1253.3	1130.7	1218.7	1153.8
H 03-41	6.71	7.12	7.31	7.24	7.10	874.0	1180.3	1029.3	1179.3	1065.7
ICPL 88039	6.15	7.02	6.74	7.02	6.73	858.0	1098.7	977.0	1037.3	992.7
AH 09-38	6.33	6.90	7.10	7.40	6.93	955.3	1168.7	1026.7	1164.7	1078.8
AH 09-47	6.36	6.61	6.97	7.13	6.77	755.3	1312.0	963.7	1172.3	1050.8
ASJ 123	6.49	7.44	6.95	7.24	7.03	581.3	1225.3	904.0	1034.0	936.2
Pusa 991	6.77	7.78	7.42	7.54	7.38	1044.7	1234.0	1108.0	1139.0	1131.4
Mean	6.51	7.23	7.18	7.40	7.08	918.7	1254.3	1079.4	1184.2	1109.2
SE	G = 0.08; I = 0.05; G x I = 0.16					G = 14.77; I = 9.84; G x I = 29.53				
$CD_{0.05}$	G = 0.22; I = 0.15; G x I = ns					G = 41.73; I = 27.83; G x I = 83.47				

C = Control, P = *Piriformospora indica*; R = *Rhizobium* sp.; G = genotype, I = Microbial inoculation

occurred at least 7 days earlier in colonized plant as compared to non-colonized plant.

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