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Symbiotic Effectiveness of Heat Resistant Mutant Strain of *Rhizobium* sp. (*Cajanus)* on Pigeonpea under Field Conditions

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The heat resistant strain HR-12 of *Rhizobium* sp. (*Cajanus*) was found to be symbiotically superior strain when tested on pigeonpea plants under field conditions in three successive kharif seasons (2004 to 2006). Most of the symbiotic parameters viz; root and shoot dry weight, per cent and total shoot nitrogen and grain yield in pigeonpea plants inoculated with HR-12 showed significantly higher values than the plants inoculated with the wild type (WT) strains PP201 (parent) and PP1021.

Key words: Symbiotic effectiveness, Heat resistant mutant strain, *Rhizobium*-legume symbiosis, Bioinoculant, pigeon pea.

Rhizobium-legume symbiosis is the most efficient system which fixes the greater part of the world's biologically fixed nitrogen, which is estimated at 70 million tones per annum (Brockwell *et al*.1995). The importance of rhizobia is emphasized by the fact that among the legumes infected by these bacteria, are included some of the world's most important pulse crops. Pigeonpea [*Cajanus cajan* (L.) Millsp] is one of the major grain legume crops of tropics and sub-tropics. But, it has poor nodulation in arid and semi-arid tropical regions. Temperature is one of the major factors, which affects rhizobial growth, survival in the soil and symbiosis itself (Boonkerd and Weaver, 1982; Dudeja and Khurana, 1989b). Prevalence of high root temperature in north India during the months of May and June adversely affects root hair formation, adsorption of rhizobia and nodulation in the host plant (Dudeja and Khurana, 1989a, b).

One of the strategies adopted to circumvent the problem of high temperature in the upper layer of soil is to isolate strains/mutants of rhizobia tolerant to elevated temperature and which can fix atmospheric nitrogen at high temperature. Different species and strains of rhizobia differ in their tolerance to high temperature (Karanja and Wood, 1988). Four heat resistant strains of *Rhizobium* sp. (*Cajanus)* were isolated in our lab, out of which the mutant strain HR-12 was found to be most symbiotically effective strain on pigeonpea plants, both in pot house (Nehra *et al*; 2007) as well as in field conditions (Singh and Yadav, 2005). Based on these observations, the present investigation was planned to test the symbiotic performance and yield potential of pigeonpea plants upon inoculation with the heat

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resistant strain HR-12 along with the WT strains PP201 and PP1021 under field conditions in three consecutive kharif seasons of 2004 to 2006.

MATERIAL AND METHODS

Rhizobial strains

The heat resistant mutant strain used in this study was HR-12 (Nehra *et al*.2007), whereas the other two WT strains used were PP201 (parent) and PP1021.

Inoculation of pigeonpea seeds with rhizobial mutant/strains

About 200 seeds of pigeonpea (var.Manak) for each treatment and replication were inoculated with the rhizobial cultures (10^9cells/ml) mixed in the solid carrier charcoal and gur solution (Singh and Yadav, 2005). There were three strains, two WT (PP201 and PP1021) and one heat resistant strain HR-12 with four replications each. The inoculated seeds were then sown in the field in a randomized block design (RBD) under elevated temperature conditions (>40^0C) during June 2004 to June 2006. The data on symbiotic characters (per plant) like root and shoot dry weight, per cent and total shoot nitrogen were recorded 60 days after sowing (DAS) and the yield at final harvest. The per cent shoot nitrogen was determined by micro-Kjeldahl's method (Bremner, 1960).

RESULTS AND DISCUSSION

Symbiotic effectivity during kharif season 2004

The pigeonpea plants inoculated with the heat resistant strain HR-12 (Table 1) showed significantly higher shoot dry weight (97 g/plant) and total shoot nitrogen (314 mg/plant) than that found in uninoculated plants (45.5 g/plant and 152 mg/plant, respectively) and plants inoculated with WT strains, PP201 (51.5 g/plant and 171.250 mg/plant, respectively) and PP1021 (78.250 g/plant and 245.5 mg/plant, respectively). The per cent shoot nitrogen was non-significant.

Table 1. Symbiotic effectivity of heat resistant mutant strain HR-12 and WT strains of *Rhizobium* sp. (*Cajanus*) on pigeonpea during kharif season 2004

Strain/mutant	Shoot dry weight (g/plant)	% shoot nitrogen	Total shoot nitrogen (mg/plant)
UI	45.500	0.319	152.000
PP201 (parent)	51.500	0.333	171.250
HR-12	97.000	0.334	314.000
PP1021	78.250	0.322	245.500
CD at 5 %	9.153	NS	17.312

UI = uninoculated control

Table 2. Symbiotic effectivity of heat resistant mutant strain HR-12 and WT strains of *Rhizobium* sp. (*Cajanus*) on pigeonpea during kharif season 2005

Strain/mutant	Root dry weight (g/plant)	Shoot dry weight (g/plant)	% shoot nitrogen	Total shoot nitrogen (mg/plant)	Yield (q/ha)
UI	1.225	10.005	0.249	24.765	12.482
PP201	1.823	13.683	0.259	34.411	14.101
HR-12	1.760	12.290	0.259	32.694	14.882
PP1021	1.158	9.365	0.277	25.321	14.726
CD at 5 %	0.174	1.484	0.014	3.944	0.703

Symbiotic efficiency during kharif season 2005

The root and shoot dry weights and total shoot nitrogen of pigeonpea plants (Table 2) inoculated with the strain HR-12 (1.76 g/plant, 12.290 g/plant and 32.694 mg/plant, respectively) were significantly higher than those found in uninoculated plants (1.225 g/plant, 10.005 g/plant and 24.765 mg/plant, respectively) and in plants inoculated with the strain PP1021 (1.158 g/plant, 9.365 g/plant, and 25.321 mg/plant, respectively), but not over the plants inoculated with the strain PP201 (1.823 g/plant, 13.683 g/plant, and 34.411 mg/plant, respectively). The yield of plants inoculated with the heat resistant strain HR-12 was significantly higher (14.882 q/ha) than that of uninoculated plants (12.482 q/ha) and plants inoculated with the strain PP201 (14.101 q/ha), but it was at par with that of plants inoculated with the strain PP1021 (14.726 q/ha).

Symbiotic properties during kharif season 2006

The root dry weight (Table 3) of host plants inoculated with the strain HR-12 was significantly higher (1.183 g/plant) than the uninoculated plants (90.737 g/plant) and the plants inoculated with the strain PP1021 (1.018 g/plant), but not with the strain PP201 (1.167 g/plant). The shoot dry weight of plants inoculated with all the three strains was non-significant over one another. The total shoot nitrogen and yield of pigeonpea plants inoculated with the strain HR-12 (23.054 mg/plant and 10.666 q/ha, respectively) was found to be significantly higher than the uninoculated plants (15.476 mg/plant and 6.970 q/ha, respectively) and the plants inoculated with the WT strains PP201 (19.840 mg and 9.895 q/ha)and PP1021 (20.386 mg and 7.958 q/ha).

Table 3. Symbiotic effectivity of heat resistant mutant strain HR-12 and WT strains of *Rhizobium* sp. (*Cajanus*) on pigeonpea during kharif season 2006

Strain/mutant	Root dry weight (g/plant)	Shoot dry weight (g/plant)	% shoot nitrogen	Total shoot nitrogen (mg/plant)	Yield (q/ha)
UI	0.737	7.816	0.196	15.476	6.970
PP201	1.167	7.771	0.252	19.840	9.895
HR-12	1.183	8.790	0.256	23.054	10.666
PP1021	1.018	7.966	0.266	20.386	7.958
CD at 5 %	0.086	NS	0.022	1.391	0.448

Nodulation of a legume under field conditions depends upon the presence of sufficient number of appropriate rhizobia, their multiplication and colonization of root zone and establishment of an effective symbiosis with the host. Temperature is the major impediment for the successful establishment of symbiosis (Boonkerd and Weaver, 1982; Dudeja and Khurana, 1989b). Plants which are dependent on symbiotically fixed nitrogen are more sensitive to elevated temperatures when compared with the plants supplied with inorganic nitrogen (Munevar and Wollum, 1981a). Therefore, it becomes imperative to develop temperature resistant/tolerant rhizobial strains to be used as legume bioinoculants under temperature stress. Temperatures above 40^0 C in the upper 5-6 cm of a soil profile are common during summer season in the northern regions of India (Somasegaran *et al*.1984), where rhizobial inoculants are exposed to temperatures beyond 45^0 C for varying lengths of time. In this direction, heat resistant strains/mutants have been isolated in our lab after a heat shock of 6 hr at 43^0 C in *Rhizobium* sp. (*Cajanus*) (Nehra *et al*.2007). Out of these mutant strains tested in pot house conditions, one mutant strain HR-12 was tested by us in field conditions (Singh and Yadav, 2005) and found to be having higher symbiotic properties than the parent strain PP201. The higher temperature tolerance of mutant strains allows them to survive periods of thermal stress. Under these conditions, the nitrogen fixation activity is shut off, but resumes when temperatures are lowered.

CONCLUSION

It can be concluded from the present study that heat resistant mutant strain HR-12 showed higher symbiotic efficiency and grain yield in pigeonpea plants than the plants inoculated with WT strains PP201 and PP1021 in field conditions in three consecutive kharif seasons (2004 to 2006). This mutant strain has the potential to be used as a bioinoculant in elevated temperature conditions prevailing during summers in north India.

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