TEMPERATURE TOLERANCE AND EFFECTIVENESS OF *RHIZOBIUM* STRAINS INFECTING PIGEONPEA *(CAJANUS CAJAN* L.) HOST

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SUMMARY

Nineteen *Rhizobium* and *Bradyrhizobium* strains isolated from pigeonpea and screened for their ability to grow at high temperature in liquid medium showed that optimum temperature of growth at for 7 strains was 30°C and for 5 strains 37°C. While other strains like PP 1008, PH 8866, PH9038 and PP309 were able to grow under wide range of temperatures upto 42°C. Thermotolerant variant of strain PP2015-1 was able grow upto 44°C, however, optimum temperature remained same as that of parent strain. No correlation could be observed between thermotolerance and its efficiency with pigeonpea host grown in the range of 35 to 42°C.

Key words: *Temperature tolerance, pigeonpea, rhizobium.*

INTRODUCTION

High soil temperature prevalent in tropical soils is a major constraint for biological nitrogen fixation by legume crops. Pigeonpea *(Cajanus cajan)* nodulates poorly in Northern region of India (Khurana and Dudeja, 1981). Different reasons for its poor nodulation were investigated and higher soil temperature which may reach even upto 50°C in upper 5 cm soil layer was responsible for poor nodulation of pigeonpea. High temperature adversely affected the survival, persistence of pigeonpea rhizobia, competition, rhizosphere colonization, root exudation, chemotaxis, growth of rhizobia and hydrolytic enzyme production (Dudeja and Khurana, 1988 a, b), root hair formation, adsorption of rhizobia and nodulation (Dudeja and Khurana, 1989), and flavonoid production by pigeonpea (Raghuwanshi *et al.*, 1994). Some modification of the symbiotic properties of *Rhizobium* strains at higher temperature has also been reported (Rennie and Kemp, 1986; Singh and Khurana, 1992). It has been reported that effect of root temperature on nodulation and dinitrogen fixation in legumes is modified by the strain of *Rhizobium* (Arayangkoon *et al.*, 1990). Different species and strains of *Rhizobium* differ in their tolerance to high temperature (Karanaja and Wood, 1988) and selection of strains for temperature tolerance has been suggested as a means of overcoming temperature stress. Therefore, tolerance to high temperature is a desirable property for *Rhizobium* strains. Keeping these objectives in view thermotolerance in native pigeonpea rhizobial population was determined in relation to their ability to fix N_2 in symbiotic association with pigeonpea host.

MATERIALS AND METHODS

Fifteen fast growing *(Rhizobium* sp.) and 4 slow growing (Bradyrhizobum sp.) strains infecting pigeonpea were used. These bacterial strains were isolated from pigeonpea (PH8806, PP2016-2, PP2016-3, PP2021-3, PH8222, PP309, PH9038 (PH9022), PP219, A7, PP304, PP1008, PH8866, PP2015-1, PP2015-1 (TT), PBH8/7, PH8666, P149, cluster bean (PG-3) and the mungbean (S24). Isolate PP2016-2 and PP2016-3 were two different isolates picked from a single nodule. All these isolates were maintained on yeast extract mannitol (YEM) aga. medium (Vincent, 1970).

One hundred ml of the YEM broth in 250 ml Erlenmeyer flasks was sterilized and inoculated with different isolates at 1% level. The flasks were incubated at 30, 37, 42 and 44°C under stationary conditions with occassional shaking and growth was determined by optical density using Systronics 106 Spectrophotometer at 600 nm and for uniformity maximum OD in each case was taken as 100 per cent. Thermotolerant variant of one strain PP2015-1 able to grow at 44°C was selected by progressively increasing the temperature from 30 to 44°C and serially transferring the inoculum.

Sandy soil which was used had pH (soil : water 1 : 1) 8.0, electrical conductivity 0.28 mmhos dsm^{-1}, organic C 0.43%, total N and P 0.038% and 885 ppm. About 8 kg of soil was filled in earthen pots. Seeds of pigeonpea cv. Manak were treated with 2.0 ml inoculum (10^6 -10^7 cells/ml) and were sown in triplicate. After germination five plants in each pot were maintained and irrigated with water as and when required. Plants were uprooted after 60 days of growth and observations were recorded. Nitrogenase (ARA, acetylene reduction assay) activity was determined in intact nodules. Nodules were detached from the roots after determination of nitrogenase activity, oven dried at 80°C till constant dry weight of nodules and plants parts were recorded. Total nitrogen contents was estimated by Kjeldahl's steam distillation method (Bremner, 1960).

RESULTS AND DISCUSSION

Results on final growth of fast growing (after 4 days) and slow growing (after 7 days) rhizobial strains infecting pigeonpea were recorded. Results showed that majority of the strains like PH8806, PP2016-2, PP2016-3, PP2021-3, PG3, A7 and PBH8/7 the optimum growth was at 30°C, while there were other strains which showed better growth at 37°C and this included PH8222, PP219, PP309, S24 and PH8666 (Fig. 1 and 2).

The rhizobial strains PP1008 and PH8866 grew well

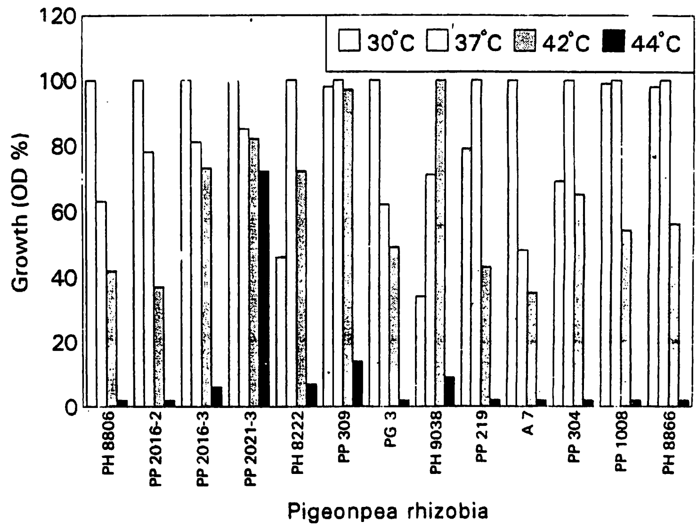

Fig. 1. Growth of *Rhizobium* spp infecting pigeonpea host in liquid medium at elevated temperature.

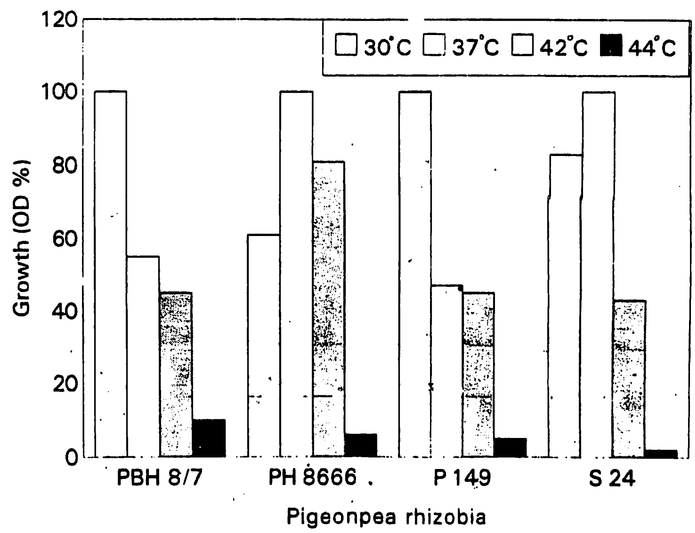

Fig. 2. Growth of *Bradyrhizobium* spp infecting pigeonpea host in liquid medium at elevated temperature.

at 30°C as well as at 37°C and PH9038 showed good growth even upto 42°C while PP309 was able to grow at wide range of temperature regimes. Thermotolerant variant of *Rhizobium* strain PP2015-1 selected through enrichment technique was able to grow upto 44°C. However, its optimum growth was 30°C as in case of parent strain. Screening of rhizobia infecting pigeonpea for growth at different temperature showed that different stains varied

Table I : Efficacy of *Rhizobium* and *Bradyrhizobium* strains varying in thermotolerance in symbiotic association with pigeonpea host

	Treatment	Nodule number/ plant	Nodule fresh weight (g/plant)	Root dry weight (g/plant)	ARA (μ moles of acetylene (reduced/g/hr)	Plant dry weight (g/plant)	Total plant N content (mg/plant)
Rhizobium strains							
1.	Uninoculated	0	0.00	0.27	0.00	0.35	25.0
2.	PH-8806	61	0.14	0.76	33.85	1.53	112.0
3.	PP-2016-2	25	0.10	0.24	52.30	0.66	35.0
4.	PP-2016-3	45	0.06	0.36	67.78	0.71	80.5
5.	PP-2021-3	34	0.06	0.20	135.44	1.10	63.0
6.	PH-8222	34	0.09	0.35	42.98	1.16	56.0
7.	PP-309	60	0.15	0.35	78.62	1.47	105.0
8.	PG-3	30	0.15	0.58	42.92	0.57	52.5
9.	PH 9038 (PH 9022)	29	0.15	0.14	21.74	0.14	45.5
10.	PP-219	40	0.08	0.30	19.60	0.22	73.5
11.	A-7	40	0.11	0.29	42.38	0.48	70.0
12.	PP-304	36	0.15	0.46	20.87	1.25	63.9
13.	PP-1008	31	0.08	0.20	34.24	0.66	45.5
14.	PH-8866	5	0.09	0.43	34.39	1.49	94.0
15.	PP-2015-1	22	0.39	0.56	24.00	1.45	88.0
16.	PP-2015-1 (TT)	35	0.54	0.60	25.00	1.48	90.0
Bradyrhizobium strains							
17.	PBH-8/7	9	0.02	0.35	64.80	1.37	28.0
18.	PH-8666	59	0.12	0.28	31.20	1.37	87.5
19.	P-149	49	0.86	0.49	58.77	0.96	77.0
20.	S-24	3	0.15	0.75	14.68	1.49	84.0
	SE (m)	2.12	0.03	0.03	0.56	0.05	0.99
	CV	8.89	27.90	11.80	2.03	8.30	2.50
	CD at 5%	6.28	NS	NS	1.64	NS	2.93

in their optimum temperature for growth and also for the maximum permissive temperature. Similarly differences in tolerance of high temperature among species and strains of *Rhizobium* has been reported (Karanaja and Wood, 1988). No specific trend in thermotolerance for *Rhizobium* or *Bradyrhizobium* strains infecting pigeonpea was observed. Both types showed similar trends. In the present study a thermotolerant pigeonpea rhizobial variant could be selected. Its optimum temperature remained same as that of its parent (30°C), however, this was able to equally grow well even at 44°C. This indicates that the strain has acquired thermotolerance.

Efficiency of all these rhizobial strains was determined under pot culture conditions using pigeonpea as host. Maximum nodule number was observed by three strains PH8806, PP309 and PH8666 with 61, 60 and 59 nodules/ plant, respectively (Table I). Strain P149 recorded highest nodule biomass followed by thermotolerant variant PP2015-1 (TT). Maximum ARA activity was shown by strain PP2021-3, whereas root and shoot weight by strain PH8806. Similarly this strain recorded maximum plant nitrogen followed by strain PP309. The differences in nodule number, ARA and plant nitrogen contents was statistically significant between different strains. However, the efficacy of these strains was not correlated with their thermotolerance.

The efficacy of these *Rhizobium* strains infecting pigeonpea was determined under pot culture conditions and the natural root temperature during the experiment remained in the range of 35 to 42°C. Such temperatures has been reported to be detrimental to nodule formation or N_2 fixation (Dudeja and Khurana, 1989; Dahiya *et al.*, 1981). In the present study no correlation between the thermotolerance/thermotolerant mutant having acquired thermotolerance with their efficacy to form nodules and fix nitrogen could be established. Rustogi *et al.*, (1996) also reported that *Acacia nilotica* inoculated with strains tolerant to 40 and 45°C showed comparable efficacy to temperature sensitive strains. Alternatively, selection of starvation tolerant particularly to C, N and P and than selection of temperature tolerant mutants could prove better (Thorne and Williams, 1997) or some other procedure should be used to enhance heat resistance of *Rhizobium* (Abdel Gadir and Alexander, 1997). The efficacy of such strains/mutants could prove better under the higher temperature conditions even upto 50°C in upper 5 cm soil depth in the north India.

REFERENCES

Abdel Gadir, A.H. and Alexander, M. (1997). Procedures to enhance heat resistance of *Rhizobium. Plant Soil*, **188** : 93-100.

Arayangkoon, T., Schomberg, H.H. and Weaver, R.W. (1990). Nodulation and N_2 fixation of guar at high root temperature. *Plant Soil*, **126** : 209-213.

Bremner, J.M. (1960). Determination of nitrogen in plants by Kjeldhal method. *J. Agric. Sci.*, **55** : 11-13.

Dahiya, J.S., Khurana, A.L. and Dudeja, S.S. (1981). Evaluation of pigeonpea rhizobia. Proc. International Pigeonpea Workshop ICRISAT, Hyderabad, Vol. II, pp. 373-379.

Dudeja, S.S. and Khurana, A.L. (1988 a). Effect of high temperature on root exudation in pigeonpea-*Rhizobium* symbiosis. *Indian J. Microbiol.*, **28** : 193-198.

Dudeja, S.S. and Khurana, A.L. (1988 b). Effect of high root temperature on chemotaxis of *Bradyrhizobium* sp. *Cajanus* towards pigeonpea root exudates. *Ann. Biol.*, **4** : 66-69.

Dudeja, S.S. and Khurana, A.L. (1989). The pigeonpea-*Rhizobium* symbiosis as affected by high root temperature. Effect on nodule formation. *J. Exp. Bot.*, **40** : 469-472.

Karanaja, N.K. and Wood, M. (1988). Selecting *Rhizobium phaseoli* strains for use with beans *(Phaselous vulgaris* L.) in Kenya : Tolerance of high temperature and antibiotic resistance. *Plant Soil*, **112** : 15-22.

Khurana, A. L. and Dudeja, S.S. (1981). Field population of rhizobia and response to inoculation, molybdenum and nitrogen fertilizer in pigeonpea. Proc. International Pigeonpea Workshop. ICRISAT, Hyderabad. Vol. II, pp. 381-386.

Raghuwanshi, A., Dudeja, S.S. and Khurana, A.L. (1994). Effect of temperature on flavonoid production in pigeonpea *[Cajanus cajan* (L.) Millsp.] in relation to nodulation. *Biol. Fertil. Soil*, **17** : 314-316.

Rennie, R.J. and Kemp. G.A. (1986). Temperature sensitive nodulation and N_2 fixation of *Rhizobium leguminosarum* biovar. *phaseoli* strains. *Can. J. Soil. Sci.*, **66**: 217-224.

Rustogi, N., Badhwar, S., Sharma, P.K. and Dogra, R.C. (1996). Temperature tolerance and efficiency of *Rhizobium* sp. *(Acacia)* in symbiosis with *Acacia nilotica*. In : Resource Management in Agriculture. (Dogra, R.C., Behl, R.K. and Khurana, A.L. eds.) CCS Haryana Agric. University, Hisar and Max Muller Bhavan, New Delhi. pp. 107-112.

Singh, K. and Khurana, A.L. (1992). Effect of temperature and moisture on the survival and nodule inducing ability of *Rhizobium* sp. pigeonpea. *Exptl. Ecol.*, **10** : 730-733.

Thorne, S.H. and Williams, H.D. (1997). Adaptation to nutrient starvation in *Rhizobium leguminosarum* bv *phaseoli* : Analysis of survival, stress resistance and changes in macromolecular synthesis during entry to and exit from stationary phase. *J. Bacteriol.*, 179: 6894-6901.

Vincent, J.M. (1970). A manual for the practical study of root nodule bacteria. IBH (International Biological Programme) *Handbook 15*.