

EFFECT OF CLIMATIC FACTORS ON THE GROWTH OF RHIZOBIA OF *SESBANIA BISPINOSA* IN THE *IN VITRO* CONDITION

S. GOPALAKRISHNAN* AND A.G. GIRISH*

VHNSN College, MKU, Madurai 625 021, T.N.

ABSTRACT

Screening of *Rhizobium* of *Sesbania bispinosa*, a legume weed from rice growing areas of Kamarajar District, Tamilnadu was done. About 650 plants were uprooted, rhizobia were isolated and they were tested for nitrogenase activity. Out of these the most highly positive isolate, on the basis of nodulation data and nitrogenase activity, DMB R 001 & DMB R 002 were selected and studied further for various climatic factors including different pH, temperature and salinity. The observations indicated that the strains could grow well with the wider temperature range of 28-44°C, salinity range of 0-600 milli molar and pH of 4-11. These results showed that the strains could harvest a good amount of N₂ for legumes in soil.

INDEX WORDS: Rhizobia, *Sesbania bispinosa*, *in vitro*.

The genus *Rhizobium* is categorized as the fast grower with the generation time 2-4 hours and the genus *Bradyrhizobium* categorized as slow grower with the generation time of 6-13 hours. The selection of *Rhizobium* strains in increasing N₂ fixation is very important in view of the need for long term soil fertility and ecological sustainability. Their productivity very much depends on their N₂ fixing capacity in extreme environments. The objective of this study was to isolate and screen the rhizobia of *Sesbania bispinosa* for various environmental conditions in the lab condition.

These experiments were performed in the semi arid areas at Virudhunagar, a town of Tamil Nadu. The temperature of the area where the samples were collected was around 42-45°C, the soil was black and its pH was 8.1-9.2. Plants numbering 650 of the same legume at the age of one month were uprooted and identified as *Sesbania bispinosa*. The efficacy of the nodules was determined by measuring the nitrogenase activity (Acetylene Reduction Assay, ARA) and the rhizobia were isolated and maintained in yeast extract mannitol (YEM) agar medium as described by Vincent (1974). Among the isolates the two most efficient

*Present Address: ICRISAT, Asia Center, Patancheru 502 324 A.P.

determined on the basis of ARA and nodulation were DMB R 001 and DMB R 002. The temperature tolerance of rhizobia was determined by inoculating the cultures in 100 ml of YEM broth in 250 ml erlenmeyer flask and incubating at different temperature viz. 30°C, 36°C, 42°C, 44°C and 50°C under shaker conditions with 150 strokes per minute (Munevar and Wollum 1981). Growths were followed by measuring the absorbance at 600 nano meter on a spectronic 20 and the generation times were calculated. For screening the rhizobia for salinity, the YEM broths were adjusted to different saline levels ranging from 100-1000 milli molar NaCl in steps of 100 milli molar and inoculated with the cultures and incubated at $28 \pm 2^\circ\text{C}$ for 72 hours. Similarly pH of the YEM broths were adjusted from 3 to 11 with an increment of 0.5 using 1N NaOH and inoculated tubes were incubated at $28 \pm 2^\circ\text{C}$ for 72 hours.

All the rhizobia isolated showed the colony size of about 1-3 mm in diameter and growth appeared within 2 days of inoculation. According to Graham and Parker (1966) those rhizobia which grow in 3 days of inoculation are grouped as fast growers and those which grow up to 7 days are slow growers. The nodulation data revealed that 90.18% of the plants showed nodules at an average of 9 per plant.

The effect of different temperatures on the growth of rhizobia showed that, both the isolates could grow well at 30°C, 36°C, 42°C, & 44°C. However

none or very little growth was observed at 50°C. The generation time of the isolates at 30°C were 1.3 & 1.4 hours for the strain DMB R 001 & 002 respectively. The generation time was increased with increase in temperature because elevated temperatures may delay nodule formation and development. Similar results were observed by Lie (1981). High temperature adversely affects the survival and persistence of rhizobia competition, root hair formation adsorption of rhizobia and nodulation (Dudeja and Khurana, 1984). The isolates were able to grow well between a wide pH range of 5-10. Delayed growth was observed at pH 11. The growth under acidic condition of pH 3 & 4 was an interesting observation indicating that the isolates could grow well in acidic soils. Similar results were observed by Munns & Keyser (1981) Yadav and Vyas (1971) in 2 surveys 23 rhizobial isolates from eight diverse legume species reported that all grew well at pH values up to 10. But none of the 17 strains of Bradyrhizobium tested showed significant growth in liquid media at pH 8.5. Similar results observed in our test confirmed that the isolates were the fast growing rhizobia instead of slow growing Bradyrhizobium.

The isolates could tolerate salinity levels up to 600 milli molar indicating that the isolates were highly salinity tolerant and it can grow well in saline soils.

Thus the isolates could grow well in extreme environmental conditions.

pH, salinity temperature indicating that under natural ecosystem, such strains could harvest a good amount of N_2 , for legumes in soils.

REFERENCES

- Dudeja, S.S. and Khurana, A.L. 1989. The pigeonpea *Rhizobium* symbiosis as affected by high root temperature. Effect on nodule formation. J. Exptl. Bot. 40:469-472
- Graham, P.H. and Parker, C.A. 1966. Diagnostic features in the characterization of the root nodule bacteria of legumes. Plant and Soil. 20:383-396
- Lie, T.A. 1981. Environmental Physiology of the legume *Rhizobium* symbiosis. In Nitrogen fixation. Vol. 1: Ecology pp. 104-134 Ed. W.J. Broughton. Clarendon Press Oxford.
- Munevar, F and Wollum, A.G. ii 1981. Effect of high root temperature and *Rhizobium* strain on nodulation, nitrogen fixation, and growth of soybeans. Soil Sci. Soc. Am. J. 45:1113-1120
- Munns, D.N and Keyser, H.H. 1981. Tolerance of rhizobia to acidity aluminum and phosphate. Soil Sci. Soc. AM. J. 34:519:523
- Vincent, J.M 1974. Root nodule symbiosis with *Rhizobium*. In. The Biology of Nitrogen fixation ed. Quispel, A. pp. 265-341. Oxford: North Holland Publishing
- Yadav, N.K. and Vyas, S.R. 1971. Response of root nodule rhizobia to saline, alkaline and acid conditions. J. agric Sci. 41:875-88