

6. Summary and Conclusion

6.1 SUMMARY

6.1.1 A total number of eighty seven wild leguminous samples were collected from 3 agroclimatic zones of Western Maharashtra and examined for their nodulation status. When the list of nodulated plants was compared with the global list of nodulated leguminous species it was found that nineteen species (one from Caesalpinioideae, seventeen from Papilonoideae and one from Mimosoideae) were first reports for nodulation. They comprised one climber, thirteen shrubs and five arboreal trees.

6.1.2 The morphological studies of nodules of these species showed that nodules exhibited 6 different shapes viz., globose, elongate, bifurcate, fan shaped, coralloid and siamese and the nodule size ranged between 2 to 16 mm. The colour of the nodule varied from light brown to dark brown and in one case blackish nodules were observed.

6.1.3 Root nodule bacteria associated with all the 19 species were isolated on Yeast extract Mannitol agar. Based on rate of growth and colony diameters after 8 days of incubation they were put into 2 groups viz., 5 in the fast growing group and 14 in the slow growing group. The colonies of fast and slow growing group appeared within 2-3 days and within 5-6 days of incubation respectively and they attained colony diameter ranging between 8 to 11 mm and 1 mm respectively after 8 days

of incubation.

6.1.4 In the examination of the isolates for their identification

- a) Ketolactose test, Nile blue reduction test, growth in Hofer's alkaline medium and on Congo red Yeast extract medium showed that they did not belong to genus *Agrobacterium*.
- b) The absence of growth on Glucose Peptone agar after 2 days of incubation showed that the nonrhizobial contaminants were not present.

6.1.5 The biochemical and physiological tests carried out to further differentiate fast and slow growing strains showed that:-

- a) Growth on YEMA : The fast growing strains produced acid while slow growing strains produced alkali.
- b) Litmus milk reaction : Only fast growing strains produced serum zone.
- c) Carbohydrate utilization : Of the 20 carbohydrates tested fast growing strains utilized 16 while slow growing strains utilized only 8 carbohydrates.
- d) Sensitivity to antibiotics : Of the 21 antibiotics tested, fast growing strains were sensitive to 11 to 13 antibiotics while slow growing ones to 4 to 6 antibiotics.
- e) Gelatinase activity : Only fast growing strains showed gelatinase activity.
- f) Salt tolerance : Only fast growing strains were able to tolerate 1.5 and 2.0% salt concentration.

g) Growth at different pH values : At pH 4.5 only slow growing strains showed growth while at pH 9.0 only fast growing strains were able to grow.

6.1.6 In the serological tests with agglutination method :

- a) based on cross agglutination reactions the 19 strains were put into 11 different serogroups.
- b) Cross reactions were not observed between fast and slow growing strains.

6.1.7 In the serological tests with immunodiffusion method :

- a) heat treatment of antigens was found necessary for appearance of somatic bands.
- b) Sonication of cells released internal antigens.
- c) Slow diffusing somatic band was found strain specific since it was produced only in the homologous reactions.
- d) Cross reactions were not observed between fast and slow growing strains.

6.1.8 In the symbiotic tests of the rhizobial strains :

- a) All the strains were able to nodulate the authentic host of cowpea cross inoculation group i.e. Siratro (Macropitilium atropurpureum).
- b) All the strains except one fast growing strain Cakk and one slow growing strain Alpb effectively nodulated cowpea (Vigna unguiculata L. Walp.) plants. Two fast growing strains viz., Espb and Prcy and two slow growing strains viz., Mump and Tptb

- gave significantly higher dr, weights of plant tops than the homologous strain of cowpea viz. VK10.
- c) The specific nitrogenase activity of the cowpea plants inoculated with 19 rhizobial strains ranged between 6409 to 33494 n moles of ethylene produced/g weight of nodule/hour.
- d) In the unsterilized soil containing indigenous rhizobia, fast growing strains viz., Erst, Esbp, and Prcy and 3 slow growing strains, viz. Dlnk, Mump, and Tptb, gave significantly higher dry weights of plants over uninoculated control.
- e) The competitive ability of the rhizobial strains with the indigenous rhizobia in the soil to form nodules on the cowpea plants ranged between 33.3 to 66.7%. The maximum competitiveness was shown by the slow growing strain, Dlnk.

6.1.9 The 5 fast growing strains show similarity to fast growing species of rhizobia viz. R.leguminosarum, R.phaseoli, R.trifolii and R.meliloti in biochemical and physiological characteristics and their symbiotic characteristics relate them to slow growing cowpea rhizobia.

6.2 CONCLUSIONS :

The data collected in the present investigation showed that :

6.2.1 Nodulation on 19 wild leguminous species is a first report.

6.2.2 Based on the cultural characteristics the 19 rhizobial strains associated with these leguminous species could be divided in two groups viz., 5 in the fast growing group and 14 in the slow growing group. It is further seen that fast growing strains are distinctly different from the slow growing ones with respect to their biochemical and physiological characteristics.

6.2.3 Fast growing rhizobial strains are serologically distinct from slow growing strains and the 19 strains formed 11 serogroups on the basis of cross agglutination tests.

6.2.4 Three fast and 3 slow growing strains showed effective nitrogen fixation and competitiveness, so they have got a potential to enhance the yield of cultivated pulse legumes like cowpea.