J. Phytol. Res. 19(2) : 281-284, 2006

RHIZOSPHERE STUDY OF FREE LIVING AND ASSOCIATED DIAZOTROPHS IN *CYNODON DACTYLON* (L.) PERS. AND *DICHANTHIUM ANNULATUM* (FORSK.) STAPF.

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Cynodon dactylon and *Dichanthium annulatum* were collected from 32 localities of twelve districts of Rajasthan. Four types of nitrogen fixing diazotrophic bacteria such as *Azotobacter*, *Derxia*, *Azospirillum* and *Beijerinckia* were isolated from the rhizosphere soil suspension.

Keywords: *Cynodon dactylon*; *Dichanthium annulatum*; Free living and associated diazotrophs; Rhizosphere.

The earlier studies suggest that significant levels of N_2 fixation occur under tropical grass cover. Moore[1] suggested that the plant roots played an important role by supplying the energy supporting the bacterial fixation which depends on close plant-bacterium interdependence. Hardy *et al.*[2] called this relationship "Associative symbiosis." The most widely studied group of free-living plant-associated bacteria is the N_2-fixing genus *Azospirillum*[3]. Dobereiner[4] has reported an associative system of nitrogen fixation in *Paspalum notatum* by *Azotobacter paspali* in the rhizosphere. In natural ecosystems biological nitrogen fixation (by free-living, associated and symbiotic diazotrophs) is the most important source of nitrogen[5,6].

Rajasthan state has diverse ecological regions supporting dominant vegetation of perennial grasses both in western and eastern parts. These grasses are of great fodder value and most of them are C_4 plants. There has not been any study of enumeration of types of nitrogen fixing bacteria (both non-symbiotic and associative-semisymbiotic) in the rhizosphere of these grasses and hence the present study has been undertaken.

The test grasses, *Cynodon dactylon* and *Dichanthium annulatum* were collected with roots and soil intact from 32 localities of twelve districts such as Ajmer, Alwar, Banswara, Bharatpur, Bikaner, Sri Ganganagar, Jaipur, Jaisalmer, Jodhpur, Kota, Sikar and Udaipur covering five ecological regions of Rajasthan.

Four types of nitrogen fixing bacteria were isolated from the rhizosphere, in four different enrichment media of the two test grasses collected from natural and agricultural field soils of twelve districts covering 32 localities in Rajasthan.

The enrichment media for the growth of *Azospirillum* was Sodium Malate Medium[7], for *Azotobacter* Ashby's Mannitol Agar, for *Beijerinckia* Beijerinckia medium[8] and for *Derxia* Derxia medium[9]. *Azotobacter*, *Derxia Azospirillum* and *Beijerinckia* were obtained by plating the rhizosphere soil suspension of the test grasses on the corresponding enrichment media. All the types of nitrogen fixing bacteria were subcultured in slants of corresponding enrichment media, and were purified by streak plate and pour plate methods in succession. The strains of *Azospirillum* which was an endophyte was Koch's postulated on the corresponding test grasses and were reisolated and purified. On the basis of morphological, cultural, and biochemical characteristics, above nitrogen fixing bacteria identified. The colonies of different diazotrophs were counted with a colony counter on different respective enrichment media.

In all, 53 strains of *Azotobacter* (31 from *C. dactylon* and 22 from *D. annulatum*), 53 strains of *Azospirillum* (31 from *C. dactylon* and 22 from *D. annulatum*), 37 strains of *Derxia* (22 from *C. dactylon* and 15 from *D. annulatum*) and 15 strains of *Beijerinckia* (9 from *C. dactylon* and 6 from *D. annulatum*) were isolated from the rhizosphere and subcultured. In the rhizosphere of *C. dactylon* the highest population of nitrogen fixing bacterium (Fig. 1), namely *Azotobacter* was found in Jaipur soil (73.17×10^4). The next in sequence was in Bikaner soil (32.50×10^4), Sikar soil (26.92×10^4), Sri Ganganagar soil (18.40×10^4) and Jodhpur soil (14.64×10^4). The least was found in Udaipur soil (1.33×10^4). As regards the rhizosphere population of *Azospirillum* in *C. dactylon* was concerned, the highest value was in Bikaner soil (66.00×10^4). The next in sequence was Jaipur soil (24.39×10^4), Sikar soil (21.02×10^4) and Sri Ganganagar soil (10.2×10^4). The lowest population of this bacterium was in

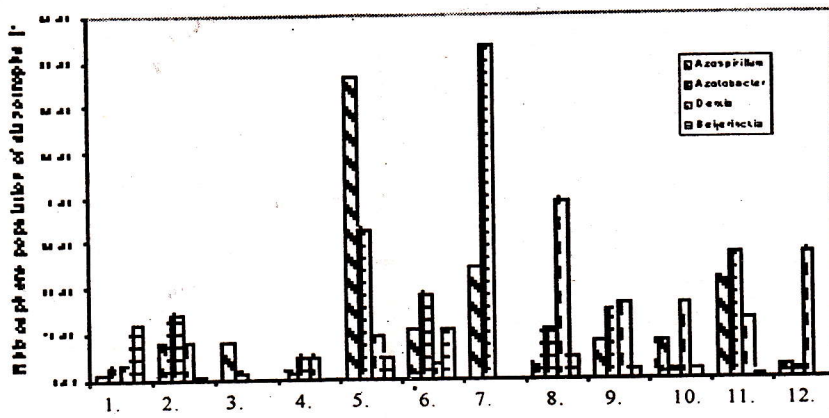

Fig.1. Total population ($\times 10^4$) of free living and associative N_2 fixing Diazotophs in *Cynodon dactylon* collected from different places of Rajasthan.
(1. Ajmer, 2. Alwar, 3. Banswara, 4. Bharatpur, 5. Bikaner, 6. Sriganganagar, 7. Jaipur, 8. Jaisalmer, 9. Jodhpur, 10. Kota, 11. Sikar, 12. Udaipur.)

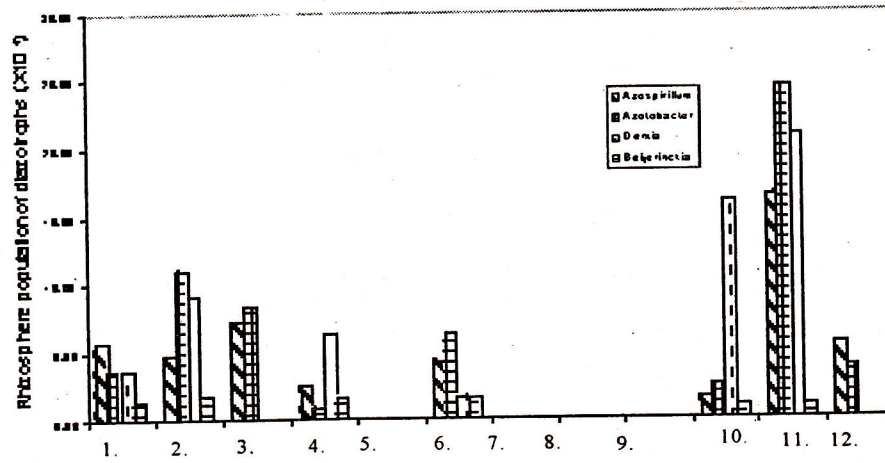

Fig.2. Total population ($\times 10^4$) of free living and associative N_2 fixing Diazotophs in *Dichanthium annulatum* collected from different places of Rajasthan.
(1. Ajmer, 2. Alwar, 3. Banswara, 4. Bharatpur, 5. Bikaner, 6. Sriganganagar, 7. Jaipur, 8. Jaisalmer, 9. Jodhpur, 10. Kota, 11. Sikar, 12. Udaipur.)

Ajmer soil (1.30×10^4). The highest rhizosphere population of *Derxia* in *C. dactylon* was in Jaisalmer soil (38.66×10^4) followed by that in Sikar soil (27.43×10^4), Jodhpur soil (16.07×10^4) and Kota soil (12.58×10^4). The lowest population of *Derxia* was in Rhizosphere of *C. dactylon* in Sri Ganganagar (2.95×10^4). The highest rhizosphere population of *Beijerinckia* in *C. dactylon* was found in Ajmer soil (11.66×10^4). This was followed by that in Sri Ganganagar soil (10.90×10^4), Bikaner soil (5.00×10^4) and Jaisalmer soil (4.66×10^4). The lowest population of *Beijerinckia* was recorded in Alwar soil (0.48×10^4). This analysis of rhizosphere bacterial population in *C. dactylon* showed that Jaipur soil was rich in *Azotobacter*, Bikaner soil in *Azospirillum*, Jaisalmer soil in *Derxia* and Ajmer soil in *Beijerinckia*.

The rhizosphere population of *Azotobacter* in *D. annulatum* (Fig. 2.) was the highest in Sikar soil (24.50×10^4). The same *Azospirillum* was the highest in the same Sikar soil (16.50×10^4). Even the rhizosphere population of *Derxia* in this test grass was the highest (21.00×10^4) in Sikar soil. The rhizosphere population of *Beijerinckia* in *D. annulatum* was very low in the soils of Rajasthan.

However, the highest rhizosphere population of this bacterium in *D. annulatum* was in Alwar soil (1.73×10^4).

Both the test grasses had meager population of *Beijerinckia* in their rhizosphere - in Alwar and Sikar and a heavy population of *Azotobacter*. This could be due either to poor population of *Beijerinckia* and comparatively rich population of *Azotobacter* in the soils of these places of Rajasthan or to the weak or strong stimulatory influence of root exudates to the above bacteria. The rhizosphere population of diazotrophs in *C. dactylon* was more than that in *D. annulatum* vegetating in the same place. According to Rovira[10] this could be definitely due to rhizosphere effect of different test individuals being influenced by their root exudates. Bashan[11] suggested that root exudates possibly govern bacterial motility in the soil. Subba Rao[12] reported that the presence of a high or low rhizosphere population of the specific bacterium in the same test grass at different places was a result of difference in distribution of the above bacterium in different places. The present study also agrees with Roper and Halsall[13], who reported that difference in behaviour of the respective N_2-fixing populations was primarily due to the physical characteristics of the soil affecting the numbers and activities of diazotrophic microorganisms. All the 53 bacterial isolates obtained on Ashby's Mannitol Agar Medium from the rhizosphere soil suspension, of both the test grasses : *C. dactylon* and *D. annulatum* from different localities of Rajasthan were confirmed to be of strains of *Azotobacter chroococcum* because of the morphological cultural and biochemical characteristics: large ovoid or rod-shaped cell, white shining in the beginning, becoming brown to black later, absence of water soluble pigments, cells occurring singly or in pairs, motile, formation of thick walled cysts; Gram-negative, catalase positive and aerobic[14, 15].

The bacterial strains obtained on Sodium Malate Medium from rhizosphere soil suspension of *C. dactylon* were identified as *Azospirillum brasilense* on the bases of cultural and biochemical characteristics : colony white becoming black with age, subsurface white pellicle in semi solid medium, cells rod-shaped or coiled, poor growth on glucose as sole carbon source, non-requirement of biotin for growth alkali production with mannitol, catalase positive, Gram-negative and microaerophilic to begin with becoming aerobic subsequently. Similarly the 22 bacterial strains obtained on Sodium Malate Medium from the rhizosphere soil suspension of *D. annulatum* were identified as *Azospirillum lipoferum* on the bases of cultural biochemical characteristics : colony as white subsurface pellicle in semisolid medium, microaerophilic, subsequent migration to surface becoming black, cells rod-shaped or bent (coiled), motile, good growth on glucose as sole carbon source, requirement of biotin for growth, acid production on mannitol, catalase positive and Gram-negative[7,16].

The bacterial strains isolated on Derxia medium from the rhizosphere soil suspension of both the test grsses (*C. dactylon* and *D. annulatum*) collected from different localities of Rajasthan were identified as *Derxia gummosa* on the basis of cultural and biochemical characteristics : slimy colonies, semi-transparent and smooth colorless brown in the beginning and raised and wrinkled subsequently, acid production, cells singly or in chains, rod-shaped or pleomorphic presence of many lipoid refractive bodies motile when young, cysts very meager, negative to indole production, to nitrate reduction and to catalase activity and Gram-negative[14,15].

The bacterial strains obtained on Beijerinckia medium from the rhizosphere soil suspension of both the test grasses (*C. dactylon* and *D. annulatum*) collected from different localities of Rajasthan were conformed to *Beijerinckia indica* on the basis of cultural and biochemical characteristics : smooth, shining, white colony when young and light brown when old, tough and tenacious slimy, cells single, rod-shaped with rounded ends with large refractive lipoid polar bodies, motile, acid production, aerobic but nitrogen fixation under low oxygen concentration, catalase positive and Gram-negative[14, 15].

Acknowledgement

We feel indebted and thankful to Head, Department of Botany, University of Rajasthan, Jaipur for his kind gesture and consistent help for this study.

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