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FREE LIVING NITROGEN FIXING DIAZOTROPHS IN RHIZOSPHERE OF *LASIURUS SINDICUS*

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ABSTRACT

Samples of *Lasiurus sindicus* grass were collected along with soil from ten different localities of Rajasthan for isolation of free living and associative N_2 fixing diazotrophs. *Azotobacter* and *Azospirillum* were isolated in the cultures. Their counts were found higher in soil samples collected from arid region in comparison to soil samples collected from semi-arid region.

INTRODUCTION

Lasiurus sindicus has a high nutritive value and is preferentially consumed by cattle in the desert. Besides contributing to the development of good rangeland in the Thar Desert, it also has a significant role in stabilizing the blowing sand dunes and expanding desert. Although this agronomically important grass can tolerate prolonged droughts, it does not survive in relatively higher zones of rainfall and faces a serious threat of being a threatened species due to changes in the land-use patterns, increase in soil moisture regimes and overgrazing. The rhizosphere microbiology of endemic grass like *L. sindicus* is important in view of the in situ conservation of biodiversity associated with such niches to sustain delicate ecological processes in the desert ecosystem.

In the western desert region of Rajasthan *L. sindicus* (Sewan grass) plants are known for their involvement in the ecological maintenance by helping in the soil conservation and dune stabilization besides a good fodder. Therefore, it has received a greater attention in the arid and semi-arid sandy plains of North Western hot arid regions of India.

Nitrogen is most vulnerable for microbial transformation (Alexander 1961). Quagliano et al. (1994) isolated free living nitrogen fixing *Azotobacter* spp. from soil samples and studied its various properties. Parker (2007) examined forty-two soils from the Western Australian wheat belt for the presence of *Azotobacter* by microscopic examination of enrichment cultures, and by plate identification on three different selective agar media. After careful microscopic examination, most of the soils appeared to contain *Azotobacter*; culturally distribution was sporadic and varied greatly according to the medium used. Distribution was not correlated with soil type.

Azospirillum is another free living nitrogen fixing bacteria associated with the roots of various grasses, cereals, and tuber plants. *Azospirilla* are generally gram-negative rods which are motile by means of a single flagellum. Bacteria belonging to the genus *Azospirillum* are typically aerobic and have spiral movements, measuring 0.8 to 1.0µm in diameter and 2 to 4µm in length.

Beijerinck (1921) isolated the diazotroph *Azospirillum* and reported it as *Azotobacter spirillum* but Later changed its name to *Spirillum lipoferum* (Beijerinck 1925). Neyra and Dobereiner (1977) described the culture techniques for identification of *Azospirillum*. Tarrand et al. (1978) reclassified the genus *Azospirillum*. Bilal et al. (1990) isolated genus *Azospirillum* readily from roots of many plants using semi-solid nitrogen free malate medium (NFM). These isolates formed fine, white sub-surface pellicle in nitrogen-free malate medium within 24h, which gradually moved to the surface, and exhibited high acetylene reduction rates. Using selected cultural and biochemical tests, most of the isolates were identified as *Azospirillum brasilense*.

Carrillo et al. (2002) reported that inoculation of soil with *Azospirillum* increased crop yield due to nitrogen fixation and improvement in root system that improved water and mineral uptakes of the plant. Akbari et al. (2007) reported that IAA produced by bacteria of the genus *Azospirillum* spp. can promote plant growth by stimulating root formation.

Guimarães et al. (2003) reported that treatment of *Oryza sativa* seeds with diazotrophic bacteria increased nitrogen content in plant and grain yield under conditions of greenhouse.

Gonzalez et al. (2005) documented that *Azospirillum* species belong to the facultative endophytic diazotroph group which colonize at both surface and root interior and this kind of association is considered as the starting point of most ongoing BNF (Biological Nitrogen Fixation) programs with non legume plants worldwide.

Rodrigues et al. (2008) found that inoculation of plants with *Azospirillum* increased their growth and yield significantly. The inoculation of *Azospirillum* in *Zea mays* field increased (26%) productivity. Hossain et al. (2015) conducted experiment and found that *Azospirillum* inoculation could significantly increase all the plant growth parameters including germination. In this communication, we have explored association of *Lasiurus* roots *with Azotobacter and Azospirillum* and important findings have been reported.

MATERIALS AND METHODS

Lasiurus sindicus grass was collected from different localities of Rajasthan. For isolation of free living nitrogen fixing bacteria, plants were collected along with intact rhizosphere soil, brought to the laboratory and enumerated for the rhizospheric bacterial population by serial dilution method. The media used were; Sodium malate medium for *Azospirillum* and Ashby's mannitol agar for *Azotobacter*.

For isolation of associative nitrogen fixing bacteria, *Lasiurus* roots (free from rhizosphere soil) were washed in sterile water for 5 to 6 times, dipped in 1% chloromine -T for 2 to 3 minutes to remove the surface microflora and were washed again in sterile water to remove traces of chloramine -T. The roots were crushed into paste with a mortar and pestle and were suspended in sterile distilled water. One ml of this suspension was plated on sodium malate medium for the isolation of *Azospirillum*. *Azospirillum* and *Azotobacter* were examined for their *in vitro* nitrogen fixing efficiency.

RESULTS AND DISCUSSION

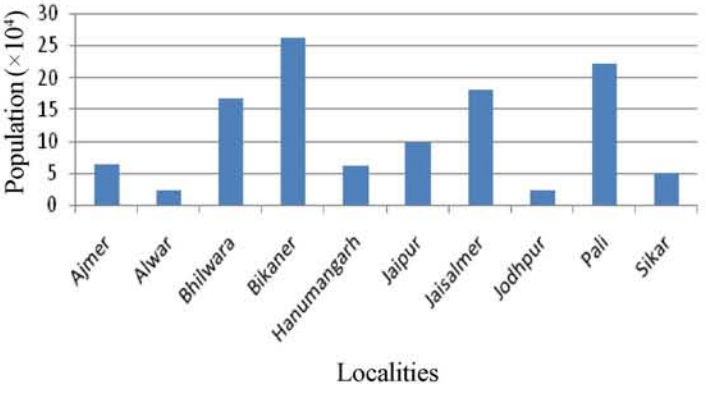
Ten isolates of nitrogen fixing diazotrophs were isolated and studied with regard to their morphological and cultural characteristics and *in vitro* nitrogenase activity. *Azotobacter* colonies were circular or irregular, rough, umblicate, lobate, undulate, shining white in the beginning and were brown to black later. The young cells were large, ovoid and gram-negative. On the basis of morphology, colony characters, physiological and biochemical characters, these isolates were identified as *Azotobacter chroococcum.*

Azospirillum colonies isolated from the cut ends of root bits and crushed roots were circular, smooth, convex, entire and colour less. These turned off-white to black with age, formed sub-surface white pellicles in semi-solid medium. The isolates were catalase positive and formed cysts with age and aerobic, motile, gram negative. On the basis of these above characters, species of *Azospirillum* was identified as *Azospirillum brasilense*.

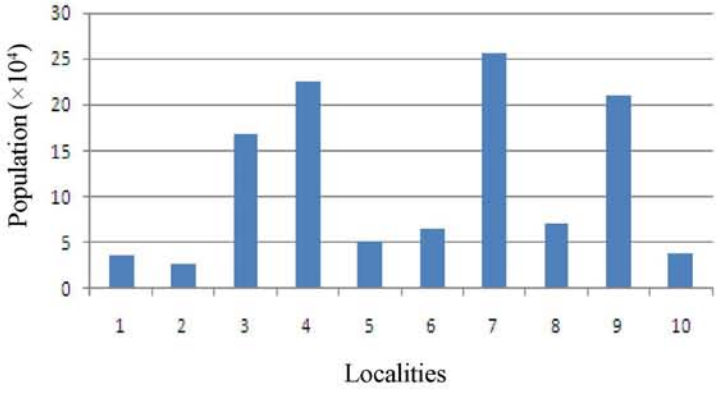
The population of *Azotobacter* in the rhizosphere of *Lasiurus sindicus* was found maximum in Jaisalmer soil (25.64×10^4), followed in decreasing order by Bikaner (22.03×10^4), Pali (21.09×10^4), Bhilwara (16.83×10^4), Jodhpur (7.04×10^4), Jaipur (6.47×10^4), Hanumangarh (5.12×10^4), Sikar (3.89×10^4), Ajmer (3.64×10^4) and Alwar (2.62×10^4) soils (Table 1).

Table 1. Total population ($\times10^4$) of free living associative diazotrophs in the rhizosphere of *Lasiurus sindicus*

Places	*Azotobacter*	*Azospirillum*
Ajmer	3.64	6.43
Alwar	2.62	2.53
Bhilwara	16.83	16.87
Bikaner	22.63	26.09
Hanumangarh	5.12	6.32
Jaipur	6.47	9.94
Jaisalmer	25.64	18.09
Jodhpur	7.04	2.43
Pali	21.09	22.21
Sikar	3.89	5.09



Azospirilium population in different localities of Rajasthan



Azotobacter population in different localities of Rajasthan

The maximum population of *Azospirillum* in the rhizosphere of *Lasiurus sindicus* was found in the soil of Bikaner (26.09×10^4) followed by Pali (22.21×10^4), Jaisalmer (18.09×10^4), Bhilwara (16.87×10^4), Jaipur (9.94×10^4), Ajmer (6.43×10^4), Hanumangarh (6.32×10^4), Sikar (5.09×10^4), Alwar (2.53×10^4) and Jodhpur (2.43×10^4) soils (Table 1).

It is evident that both roots and rhizospehre of *Lasiurus* roots have good populations of free living nitrogen fixing bacteria viz. *Azotobacter* and *Azospirillum* which are responsible for its high productivity in the arid and semi-arid regions of Rajasthan.

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