BIODIVERSITY OF RHIZOBIA IN NORTH SINAI DESERT

By

TAREK SAYED RAGAB

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2002.

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APPROVAL SHEET

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ABSTRACT

Acacia saligna trees and 9 different wild herbaceous legumes grown in El-Ahrash protectorate, North Sinai, were surveyed for nodulation. Sixty five rhizobia were isolated from naturally occurring root-nodules of *Acacia saligna* trees and the different wild annuals. According to their cultural, morphological and physiological characteristics as well as SDS-PAGE of total cell proteins, the isolates were phenotypically categorized into two distinct groups. The first includes those isolated from *Acacia saligna* which were differentiated into three distinct clusters. The second group comprises isolates from wild annual legumes; analysis of their phenotypic characters building dendrograms divided them into two distinct clusters. Eight rhizobial isolates representing the various *Acacia*-rhizobia clusters were examined for inoculation of *Acacia saligna* seedlings grown in sandy soil plastic bags under sunlight or shade conditions. After 6 months, all seedlings, whether inoculated or not, formed from 4 to 87 nodules seedling^{-1} with lower number recorded with uninoculated seedlings. In the sunny nursery, Ac22p and Ac18p were the most infective isolates inducing the formation of up to 87 and 75.6 nodules seedlings^{-1} with maxima nodule biomass, shoot and root length and biomass. Shading severely affected nodulation of *Acacia* seedlings either with indigenous or introduced rhizobia. Uninoculated seedlings formed 17 nodules seedling^{-1}under sunlight and 4.4 nodules seedling^{-1}under shade conditions. Seedlings grown in the shaded nursery showed a very poor growth after 6 months. A promoting effect on growth of *Acacia* seedling roots was displayed by the isolates Ac-22p, Ac-23s, Ac-17s and Ac-17f where significantly taller roots were recorded with seedlings drenched with these bacterial inocula. For the first time in Egypt, sugarcane bagasse pith was used as rhizobia inocula carrier. Data recommended pre-sterilization of bagasse pith incorporated in the inocula formula. Survival of rhizobia in pith-based inocula was good or better than that recorded with peat based inocula. Instead of the imported Irish peat moss, the present data recommend sugarcane bagasse pith for rhizobial inoculant formulation.

Key words: Biodiversity, rhizobia, *Acacia saligna*, wild legumes, sugarcane bagasse pith, Sinai desert.

DEDICATION

I dedicate this work to whom my heart felt thanks; to my wife Eman and my daughter Laila for their patience and help, as well as to my father and brothers for all the support they lovely offered along the period of my post graduation.

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INTRODUCTION

The leguminase constitutes one of the largest and most widely distributed families of flowering plants occupying habitats ranging from rain forests to arid zones (Raven and Polhill, 1981). Many members of the group are of considerable ecological and economical significance, due in part to their ability to form nitrogen-fixing symbioses with bacteria from the Rhizobiaceae. *Acacia* species are members of the Leguminosae comprising over 1500 of shrubs and trees with a wide distribution in Africa, Australia, South America and Asia. Their potential as multi-purpose plants in providing fire wood, pulp, timber, forage for cattle and fruit and gum has been well documented (Barnet and Catt, 1991). Most of these woody plants produce nodules and fix nitrogen in an endosymbiotic association with either rhizobia or bradyrhizobia (Njiti and Gliana, 1996). Despite the importance of such tree, there is little information on the nodulation status of the majority of Egyptian *Acacia* trees. The necessity of characterizing indigenous rhizobia cannot be overemphasized. It is crucial to establish which rhizobia nodulate host (s) and how efficiently in terms of N_2-fixation, in order to develop broad host-range inocula for the various Egyptian sites. It has been already shown the wide spread occurrence of indigenous rhizobia and nodulation status of various tree host species. The need arises to isolate and characterize *Acacia* spp. root-nodulating bacteria to select the proper effective and competitive strains tolerant to different environmental stresses prevailing in Egyptian deserts.

Inoculation of legumes with rhizobia is a common international practice, which ensures and maximizes biological nitrogen fixation and enhances the plant yield potentials without using exogenous N fertilizers. Generally, inoculants are prepared by the addition of rhizobial cells to a powdered carrier followed by a period of incubation (Roughley and Plusford, 1982) to allow the development of maximum rhizobia cell numbers. Inoculants are commercially sold in powder, liquid and/or granular form. The powder form (rhizobial and peat powder) is the most popular method for industrial legume inocula production. However, liquid and granular products are available in limited quantities in North America and Europe (Stephens and Rask, 2000). Our objective was to study the feasibility of using pith as a carrier for bacterial inoculants following the common production and application procedures for most peat-based inoculant, peat was used as a reference carrier.

REVIEW OF LITERATURE

Leguminosae, with close to 20,000 species, is the third largest family in the plant kingdom (Doyle and Luckow, 2003). Legumes are important to many agronomic systems due to their symbiotic capacity for biological nitrogen fixation (Vance and Graham, 1995). Symbiosis is an interaction between the host legume root system and specialized soil bacteria, collectively referred to as rhizobia, resulting in the morphogenesis of nodules that harbor these nitrogen-fixing bacteria. There are six main genera of rhizobia, including *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*. Recent reports have expanded the list of nodulating symbiotic bacteria to include strains of *Blastobacter*, *Burkholderia*, *Devosia*, *Ensifer*, *Methylobacterium*, *Ochrobactrum*, and *Ralstonia* (Ngom *et al.,* 2004 and Sawada *et al.,* 2003). Much of our current understanding of legume-rhizobia symbiosis has resulted from coordinated research efforts on model systems using the legumes *Glycine max*, *Medicago truncatulata*, and *Lotus japonicus*, along with their rhizobial symbionts (Choi *et al.,* 2004; Stacey *et al.,* 2004; Young *et al.,* 2003). These legumes have been selected either because of their agronomic importance or due to the practicalities associated with a relatively simple genome that is suitable for genetic manipulation. There are no model systems for woody legumes; however, a substantial amount of scientific information exists describing symbiotic N_2 fixation in woody legumes. One of the most studied genera of woody legumes is *Acacia*.