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Application of Microbial Biotechnology for Sustainable Legume Production in Desert Conditions

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Abstract

Most of the lands for future agricultural expansion in the Arab countries are located in deserts, which are prevalent in these dry areas of North Africa and West Asia regions. The experience gained by agricultural institutions in these regions for the production of food, feed and fibers overcame the challenges posed by the conditions of dry environment to turn the desert soils into productive agricultural land. Legumes played an important role in this type of agriculture. It is well known that legumes are among the key plants necessary for building the sustainable agricultural production systems particularly in the newly reclaimed soils. The integration of legume-rhizobia biological nitrogen fixation (BNF) technology in desert agriculture reduces the cost of production and preserves underground water from contamination with the excess nitrogen fertilizers required to insure the high productivity. High proportion of soluble nitrogen fertilizers could be leached with irrigation water and reaches the ground water.

The employment of BNF technology to supply host legumes with the nitrogen requirements essential for plant growth, attracted the attention of scientists since early studies of biology and microbiology. However, the dependence on legume/rhizobia symbiotic systems for agricultural expansion in the desert areas requires careful assessment of the performance of these biological systems under the harsh conditions prevailing in the desert environment.

Our studies to quantify the amount of nitrogen fixed by the peanut/*Rhizobium* symbiotic system in sandy soil revealed that the application of rhizobial inoculants in the field through either injection with irrigation water, or as beat- based inoculant before planting, resulted in the fixation of 171 and 186 kg of nitrogen per hectare respectively.

In an assessment of the need for inoculation in widely cultivated legume crops in Egypt, significant positive responses to rhizobial inoculation, with selected previously tested rhizobial strains, were recorded. However, the native rhizobia in the soil were still occupying between 16 to 24% of the total nodules of the field-grown legumes based on the analysis of nodule occupancy using fluorescent antibody (FA) technique.

We studied the factors limiting the response to inoculation in several legumes in sandy soils using advanced molecular biology techniques such as FA and Rep-PCR of rhizobial DNA. The results revealed that the efficiency of nitrogen fixation by inoculant rhizobial strains is determined by the plant cultivar, the presence of high numbers of native rhizobia in the soils, and the amount of nitrogen fertilizers applied. A more profound understanding of these factors and their role in BNF through legume/rhizobia symbiosis is required in order to maximize the benefits from this natural resource particularly under the harsh conditions of newly acclaimed desert soils.

Introduction

Throughout history, agriculture has depended on cropping systems that combined a nitrogen – consuming cereals with nitrogen – fixing legumes. In recent history cereals productivity has dramatically increased under high input systems whilst legume yields have neared a plateau, stagnated or even reduced. This has resulted in unbalanced cereal-legume global production, and thus in higher and unsustainable dependence on N chemical fertilizer inputs which reached $ 20 billion annually (Crouch et al. 2004). Increasing cultivation of legumes will be required to ameliorate environmental degradation, reduce depletion of non-renewable resources and provide adequate nitrogen for sustainable agriculture. Most of the new lands to be opened for legume cultivation in the developing countries are located in the dry desert arrears.

Symbiotic nitrogen fixation (SNF) by legumes plays major role in sustaining crop productivity of marginal lands and in small holders systems. Farmers in dry areas depend on legumes as an important crop in their cropping systems due to the capacity of these plants to fix nitrogen from air by the interaction with nitrogen fixing rhizobia. It is well known that nitrogen is abundant in the atmosphere, but plants cannot directly utilize the elemental nitrogen from the air. Symbiotic nitrogen fixation occurs mainly through symbiotic association of legumes with N_2-fixing rhizobia that convert elemental nitrogen into ammonia. This type of biological nitrogen fixation (BNF) is therefore less costly and more sustainable as compared with nitrogen fertilizes for production of plant proteins. Scientific and technological progress has opened tremendous opportunities for the befit of small farmers.

Most of the lands for future agricultural expansion in the Arab countries are located in deserts, which are prevalent in these dry areas of North Africa and West Asia regions. The experience gained by agricultural institutions in these regions for the production of food, feed and fibers overcame the challenges posed by the conditions of dry environment to turn the desert soils into productive agricultural land. Legumes played an important role in this type of agriculture. It is well known that legumes are among the key plants necessary for building the sustainable agricultural production systems particularly in the newly reclaimed soils. The integration of legume-rhizobia biological nitrogen fixation (BNF) technology in desert agriculture reduces the cost of production and preserves underground water from contamination with the excess nitrogen fertilizers required to insure the high productivity. High proportion of soluble nitrogen fertilizers could be leached with irrigation water and reaches the ground water.

The employment of BNF technology to supply host legumes with the nitrogen requirements essential for plant growth, attracted the attention of scientists since early studies of biology and microbiology. However, the dependence on legume-rhizobia symbiotic systems for agricultural expansion in the desert areas requires careful assessment of the performance of these biological systems under the harsh conditions prevailing in the desert environment.

Substantial information is available on the net benefits of BNF by legume-rhizobia symbiosis. However, this biological system is particularly sensitive to various environmental stresses. In desert areas the major stress factors is the drought associated with the scarcity of water availability. It is reported that most of the stress factors including drought influence all physiological process in plants (Serraj et al. 1998, Sinclair and Serraj, 1995, Singleton et al. 1982, VanHoorn et al. 2001). Rupela and Rao (1987) also showed that legume-*Rhizobium* symbiosis is particularly sensitive to drought. This stress factor may impair the development of root hairs and the site of entry of rhizobia to the host. It is generally agreed that rhizobial strains are relatively more tolerant to the drought stress than the plant host (Williams and Mallorca, 1984). A deeper root system with enhanced water uptake capacity is considered the major plant developmental trait for drought avoidance in legume crop native to desert and arid conditions (Gregory et al. 1994).

This paper illustrates our research efforts on the integration of BNF by legume-rhizobia symbiotic systems in several legumes adapted for growth in arid and desert environments. The analysis of the factors involved in the success or failure of SNF systems provide the knowledge that facilitates development of appropriate management options for sustainable legume production in desert conditions.

Materials and methods

This paper covers a wide range of greenhouse and field-testing of rhizobial strains performance in desert soils. The performance was assessed by the application of various techniques. For the quantification of the amount of nitrogen fixed under field condition the ^{15}N isotope dilution technique was used (Burris and Miller, 1941, Fried and Middelboe, 1977, Amarger et all. 1979).

The competition for nodulation between native and inoculants rhizobial strains was assessed by fluorescent antibody (FA) technique (Somasegaran and Hoben, 1994). For studying the factors limiting the response to inoculation in several legumes in sandy soils, the advanced molecular biology techniques, such as Rep-PCR of rhizobial DNA was applied (Louws et al. 1994) in addition to FA analysis of nodule occupancy.

Results and discussion

Sustainable agricultural production is attractive system because it is supposed to cause least damage to the environment while ensuring high productivity. This is where SNF becomes an indispensable component of sustainable farming systems.

Improvement in SNF would come from selection and ability to use successfully better strains of N_2-rhizobia. Where the naturally occurring strains in the soil are proven to be highly effective, inoculation likely to be not necessary, which may be the case in many areas where particular legumes have been cultivated successfully over many years. In other words, the benefit from inoculation is not easy in soils that harbor high numbers of very competitive but ineffective N_2- fixing strains, but should not be regarded as impossible.

We participated in a world wide *Rhizobium* Ecology Network (WREN) supported by NSF to establish prediction model for inoculation success for economically important legumes (Moawad et al. 1994). It is recognized that native soil population of rhizobia can be characterized functionally for nitrogen fixation potential by determining their numbers and effectiveness (Singleton and Travers, 1986). The MPN counts of rhizobia nodulating the tested legumes and the effectiveness of the native rhizobia for these legumes are presented in Table 1.

Table 1: MPN of rhizobia in soil and its effectiveness

Host plants	Counts g^{-1} soil	Effectiveness
Glycine max	0.0	0.0
Trifolium alexandrenum	1X 10^2	55.0
Vicia faba	5X 10^2	70.0
Phaseolus vulagaris	0.0	0.0
Vigna unguiculata	4.2X 10^2	60.0

*Percentage of pink nodules fromed by native population in Untreated plots

The table shows that the tested soils were void of rhizobia for soybean and beans. However, rhizobia nodulating faba bean and clover occurred in densities of 100 cell/g. and for cowpea 10.000 cells/g. The date show that the effectiveness of rhizobia was high within the native population of faba bean rhizobia (70%) and less for cowpea and clover rhizobia being 60% and 55% respectively.

The response to inoculation in these legumes in pot experiments (Table 2) show significant yield responses to rhizobial inoculation with selected strains of rhizobia with soybean, beans, cowpea, lentil and clover. No significant differences were found between inoculated and N-fertilized treatments. No yield response was found in faba bean either inoculated or fertilized with nitrogen. The data show the importance of rhizobial inoculation in the studied legumes with the exception of faba bean which showed no response to inoculation.

Table 2: Response of six legume crops to rhizobial inoculation (yield Tons/ha)

Treatments	Soybean	Beans	Cowpea	Faba bean	Lentil	Clover
Uninoculated	2.16 a	0.76 a	1.20 a	3.02 a	1.27 a	24.87 a
Inoculated	3.25 b	0.99 b	1.99 b	3.06 a	1.78 b	27.47 b
900 kg N/ha	3.22 b	1.07 b	2.08 b	3.15 a	1.82 b	27.65 b

The competitive ability of inocula strains (Table 3) shows that the inoculant strains dominated nodules of soybean, beans, cowpea, clover and lentil. However, most of faba bean nodules were formed from the indigenous rhizobia, which likely are very efficient in N_2 fixation.

Table 3: Competition for nodulation between inoculant strains and native rhizobia in several legumes.

Host legume	Strain nodule occupancy (%)	
	Inoculant	Native
Soybean	100	0
Beans	100	0
Cowpea	64	36
Clover	56	44
Faba bean	4	96
Lentil	68	32

The quantification of SNF in the field is of great importance to establish nitrogen fertilization requirements. Nonetheless, the estimation of nitrogen fixed by various legume-rhizobia symbiotic systems under field conditions is not an easy task. The problem becomes more complicated if one wants to assess the N_2 fixing capacity of particular potential inoculant strain in a field with native background rhizobia. In this case the N_2 fixed may come from two sources; the inoculant strain and/or the native strains existing in the soil. The ^{15}N isotope dilution technique (Fried and Middleboe, 1977) is the only available approach for the quantification of N_2 Fixation in the field. We used this approach in several field studies (Moawad et al., 1989, 1998).

The opening of new desert areas for legume cultivation usually creates unique opportunities for accurate measurement of N_2fixation with the first field cultivation with legume crop with no need for isotope technique. In such soils the success of nodulation depends primarily on the introduction of inoculant strain to the host legume.

The quantification of N_2 fixed can be calculated by comparing the total nitrogen in both inoculated and uninoculated fields (difference method).

This approach was applied in one peanut filed trial in newly reclaimed soil at Salheia desert near Ismailia, Egypt. The rhizobial field application was done using two separate approaches; peat-based inoculants and liquid inoculants injection with pivot irrigation system. The cells of the three recommended rhizobial strains were equally mixed for inoculant preparation. Both inoculation techniques were capable of supplying peanut under field conditions with enough nitrogen to support good growth and yield with high net N_2 gains (Table 4). Dart and Krantz (1977) reported that peanut can fix appreciable amounts of nitrogen and estimated as much as 240 kg N/ha.

Table 4: Yield and N_2fixation in field grown peanut using two inoculation techniques

Inoculation	Yield kg ha^{-1}	N_2fixed kg N ha^{-1}
Uninoculated	1855 a*	23 a
Peat-based	5398 b	186 b
Liquied	4425 b	171 b

*Values with different letters within the same vertical column are significantly different.

In soils with history of cultivation of certain legume particularly with repeated inoculation the competition for nodulation play significant role in SNF. It is reported that the background native rhizobia in the field may adversely affect the chances of nodulation by the desired inoculant strains (Moawad and Schmidt, 1984, Moawad and Bohlool, 1984, Moawad et al. 1988, 1991). Table 5 shows that the comprehensive screening programs to identify the highly fixing strains of rhizobia for specific host faces the challenge of native strains which limits the benefits of SNF capacity of the selected strains when inters into competition with these native soil rhizobia. In the two legume crops listed in this table the native rhizobial strains occupied substantial proportion of nodules indicating that inoculant strains were not capable of nodulating the host legume.

Phaseolus bean is among the legume crops which grow very will in sandy and sand loam soils. The symbiotic and competitive performance of two highly effective rhizobia was tested with two cultivars; Bronco and Giza 6. Data in Table 6 show that strain CE3 was the best nitrogen fixer with cv. Bronco, whereas strain Ph. 163 was better with cv. Giza 6.

Table 5: Nodule occupancy by native rhizobial strains in several legume cultivars using FA analysis.

Legume	% of nodules	Reference
Lentil		Moawad et al. 1994.
Giza 9	24	
Giza 370	28	
Beans		
Dual	50	Moawad and Wafaa, 2002.
Paultista	20	
Smamtha	30	

Table 6: Response of two bean cultivars to rhizobial inoculation

Treatments	cv. Bronco		cv. Giza 6	
	Shoot Dry weight g/plant	N uptake mg/plant	Shoot Dry weight G/plant	N uptake mg/plant
Uninoc.	4.83 b*	135.2	4.16 c	158.9
+N**	9.03 a	307.0	8.08 a	315.1
Ph.163	2.96 b	109.5	5.55 b	199.8
CE3	9.54 a	282.2	4.32 c	113.4

*Values labeled with the same letters are not significantly different using Duncan's multiple-range test.
**Fertilized with nitrogen.

Two techniques were applied to assess the success of nodulation by inoculant strains with the two bean cultivars. These techniques are FA and REP-PCR fingerprinting. The results from both techniques were close (Table 7). None of the strains was able to take all the nodule sites on both cultivars. Strain CE3 gave 40 – 50 % nodulation on cv. Bronco, whereas strain Ph. 163 gave 30-40% on Giza 6. same nodule occupancy was found with strain Ph. 163 on cultivar bronco (30-40%).

The native rhizobia occupied 50% of nodules on Bronco and much more on Giza 6. These data the importance of selection of the proper rhizobial strains for use as inocula in different bean cultivars, to insure the nodulation by desired strain.

The benefit from SNF system can be increased with the in depth studies of the performance of selected rhizobial strains under the prevailing environmental conditions. The more we study these biological systems the better we can sustain legume production in arid and semi arid conditions to open new agricultural land in the marginal arid soils particularly desert which dominate the future potential land reserve for production of food and feed for ever increasing population in these regions.

Table 7: Nodule occupancy (%) by inoculant strains with two bean cultivars.

Treatments	Bronco		Giza 6	
	163	CE3	163	CE3
Uninoc.				
+N**	0	0	0	0
Ph #163	30 (40)*	0 (0)	**30 (40)	0
CE3	0 (0)	50 (40)	0 (0)	20 (10)

*Numbers in side parenthesis are % nodule occupancy using FA.
**Numbers without parenthesis are % nodule occupancy using REP-PCR fingerprinting.

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