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Isolation and Identification of Rhizobia From Wild Legumes in Blue Nile and Gezira States

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Abstract:

A field survey was carried out during 1998 in some parts of the Blue Nile and Gezira States for the purpose of collecting samples of wild legumes for isolation and identification of their associated rhizobia. *Rhizobium* strains were isolated using YEMA media and presumptive tests were carried out. Seedlings of some leguminous plants representing the main cross-inoculation groups were inoculated with isolates from the wild legumes.

The results showed that all collected wild legumes were found to bear nodules on their roots except those of the *Cassia* spp. All isolates were found to be fast-growing and acid-producing rhizobia. The cross-inoculation classification of the isolates showed that isolates from *Phaseolus trilobus* plants can be grouped with *Rhizobium leguminosarum* bv. *Viceae* and *Rhizobium* spp. Isolates from *Sesbania sesban* and *Indigofera articulata* can be grouped with *Rhizobium leguminosarum* bv. *Viceae*, *Rhizobium leguminosarum* bv. *Phaseoli*

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and *Rhizobium spp.* Moreover, isolates from *Indigofera articulata* can be clustered with *Rhizobium meliloti* group. Isolates from *Citurcu lanatus* plants in Gezira area can be grouped with *Rhizobium leguminosarum* bv. *Phaseoli* and *Rhizobium* spp. Isolates from *Crotalaria senegalensis* and *Desmodium dichotomum* can be classified as *Rhizobium* spp.

None of the isolates can be grouped with *Rhizobium leguminosarum* bv. *Trifolcae*, *Bradyrhizobium lupinus* or *Bradyrhizobium japonicum*. It was also noticed that although all isolates are fast-growing, they formed nodules on roots of *Vigna unguiculata* and they are belonging to the miscellaneous cowpea group.

Introduction:

Leguminous crops are of great importance as a source of protein for man and animal. They have the ability to fix nitrogen through the symbiotic relationship with the soil bacteria of the genus *Rhizobium*. The compatibility between rhizobia and plant species shows a considerable level of specificity whereby the concept of cross-inoculation groups has been developed (Vincent, 1970). A cross-inoculation group is a group of legumes that are invaded by rhizobia isolated from any member of the group. Wild legumes are known to be nodulated naturally by indigenous rhizobia inhabiting the soil. These rhizobia are expected to be adapted to environmental conditions like their symbiont plants. Accordingly, these rhizobia can serve as adaptive inoculant rhizobia if they prove to be highly effective in nitrogen fixation with their homologous crop legumes.

This study aims at isolation, identification and preservation of rhizobia isolated from wild legumes, and further to test their ability to fix nitrogen with their homologous crop legumes.

Materials and Methods:

A field survey was carried out during 1998 in some parts of the Blue Nile and Gezira States whereby samples of wild legumes have been collected. The whole plants were pulled up carefully without detaching the nodules, if any. Plants were labelled and taken to the laboratory of the Biofertilization Department of the National Centre for Research. Plants were then identified and the incidence of root nodules was observed. Nodules were then detached and the interior colour of representative nodules from each plant was observed. Then rhizobia from nodules of each plant were isolated (Somasegaran and Hoben, 1994). Typical *Rhizobium* colonies were then streaked in Yeast Extract Mannitol Agar (YEMA) with Congo Red or Bromothymol Blue. A third set of Petri dishes containing Glucose Peptone Agar (GPA) was also streaked by the isolates. The dishes were then incubated at 28° C. for 10 days. Growth was observed daily.

A broth inoculant from each isolate was used to inoculate seedlings of some leguminous plants grown in potted sterile sand five days after emergence. The plants used were: *Glycine max*, *Lupinus ternis*, *Vicia faba*, *Trifolium* sp., *Phaseolus vulgaris*, *Medicago sativa* and *Vigna unguiculata* representing the 7 major cross-inoculation groups (Tate, 1995). Pots were grown in 3 replicates for each plant genus. Samples were taken 45 days after planting where the occurrence of nodulation in each plant was observed and accordingly the isolates were grouped.

Results and Discussion:

The plants collected from the study area are shown in Table 1. No nodules were observed in the roots of any *Cassia* (*Senna*) collected from the two areas. This genus belongs to the subfamily Caesalpinoideae. It is known that of the leguminous species examined in many parts of the world, 97% of the Papilionoideae subfamily, 90% of the Mimosoideae and only 23% of the Caesalpinoideae have been found to bear nodules (Allen and Allen, 1991; de Faria et al., 1989). Other reports also noticed that the Caesalpinoideae members are the least in supporting nodulation (Sprent, 1989). The *Cassia* (*Senna*), in different parts of Sudan have been reported not to bear nodules (ENRRI, 1998).

All studied members of the *Papilionoideae* subfamily were found to bear nodules on their roots (Table1). According to the field observations, all nodules proved to be active in nitrogen fixation since the interior colour of the collected nodules was pink indicating the formation of leghaemoglobin (Sprent, 1989). Generally, nodulation in wild legumes in Blue Nile State was heavier than in those collected from Gezira State. This might be attributed to environmental factors, particularly the heavier rains in Blue Nile State.

The results of the presumptive test showed that none of the isolates could grow in Glucose Peptone Agar, they were all fast-growing, acid-producing, formed colonies in 3-5 days on YEMA, did not absorb Congo Red and showed the typical morphology of *Rhizobium* colonies on YEMA (Somasegaran and Hoben, 1994) (Table 2).

Table 1: Wild legumes collected and the status of nodulation

Host plant	Local name	Subfamily	Area of collection	Nodulation	Interior colour of nodules
Phaseolus trilobus	Phillipsara	*Papilionoideae*	Blue Nile	+	Pink
Desmodium dichotomum	Abu-Areeda	"	"	+	"
Crotalaria senegalensis	Fartaga	"	"	+	"
Clitorea ternata	Clitoria	"	"	+	"
Indigofera articulata	Dahaseer	"	"	+	"
Cassia tora (*Senna tora*)	Kawal	*Caesalpinoideae*	"	–	na*
Cassia occidentalis (*Senna senna*)	Soureeb	"	"	–	na
Cassia senna	Sena-makka	"	"	–	na
Clitorea ternata	Clitoria	*Papilionoideae*	Gezira	+	Pink
Indigofera articulata	Dahaseer	"	"	+	"
Sesbania sesban	Sesban	"	"	+	"
Phaseolus trilobus	Phillipsara	"	"	+	"
Cassia tora (*Senna tora*)	Kawal	*Caesalpinoideae*	"	–	na
Cassia occidentalis (*Senna occidentalis*)	Soureeb	"	"	–	na

Table 2: Result of the presumptive test of isolates

Host plant	Area of collection	Growth rate	Growth reaction	Growth on GPA*
Phaseolus trilobus	Blue Nile	Fast	Acidic	–
Desmodium dichotomum	"	–	"	–
Crotalaria senegalensis	"	"	"	–
Clitoria ternatea	"	"	"	–
Indigofera articulata	"	"	"	–
Clitoria ternatea	Gezira	"	"	–
Indigofera articulata	"	"	"	–
Sesbania sesban	"	"	"	–
Phaseolus trilobus	"	"	"	–

* - no nodules developed

The results of the plant infection test showed that rhizobia isolated from *Phaseolus trilobus* plants from both sites formed nodules on *Vicia faba* and *Vigna unguiculata* plants (Table 3) which suggests that they can be grouped with *Rhizobium leguminosarum* bv. *Viceae* and *Rhizobium* spp.

Isolates from *Sesbania sesban* and *Indigofera articulata* formed nodules on roots of *Phaseolus vulgaris*, *Vicia faba*, and *Vigna* unguiculata. Hence, they can be grouped with *Rhizobium leguminosarum* bv. *Viceae*, *Rhizobium leguminosarum* bv. Phaseoli and Rhizobium spp. Moreover, isolates from *Indigofera articulata* formed nodules on roots of Medicago sativa which makes them belong to the *Rhizobium meliloti* group. A previous study of

Rhizobium isolates indicated that one isolate had the ability to nodulate both alfalfa and common bean (Lindly *et al.*, 1985).

Isolates from *Clitoria ternatea* plants in Gezira area formed nodules on roots of *Phaseolus vulgaris* and *Vigna unguiculata* and they can be grouped with *Rhizobium leguminosarum* bv. *Phaseoli* and *Rhizobium* spp. While isolates from *Clitoria ternatea* plants in Blue Nile area formed nodules on roots of *Vicia faba*, *Medicago sativa* and *Vigna unguiculata*. Isolates from *Crotalaria senegalensis* and *Desmodium dichotomum* formed nodules only on roots of *Vigna unguiculata* and accordingly they can be classified as *Rhizobium* spp.

None of the isolates can be grouped with *Rhizobium leguminosarum* bv. *Trifolcae*, *Bradyrhizobium lupinus* or *Bradyrhizobium japonicum* since none of them formed nodules on roots of plants representing these groups. It was also noticed that although all isolates are fast-growing, they formed nodules on roots of *Vigna unguiculata* and they are belonging to the miscellaneous cowpea group which is widely abundant in soils of the tropics (Giller and Wilson, 1993). This result is in agreement with the findings of other research studies which indicated that isolates from wild legumes belong to the miscellaneous cowpea group (Gandhi and Gosbole, 1990; Nimbalkar, 1986).

Table 3: Cross-inoculation of isolates with representative legumes

Host plant	Source	*Glycine max*	*Lupinus termis*	*Vicia faba*	*Trifolium* sp	*Phaseolus vulgaris*	*Medicago sativa*	*Vigna unguiculata*
Phaseolus trilobus	Blue Nile	-	-	+	-	-	-	+
Desmodium dichotomum	"	-	-	-	-	-	-	+
Crotalaria senegalensis	"	-	-	-	-	-	-	+
Clitorea ternatea	"	-	-	+	-	-	+	+
Indigofera articulata	"	-	-	+	-	+	+	+
Clitorea ternatea	Gezira	-	-	-	-	+	-	+
Indigofera articulata	"	-	-	+	-	+	+	+
Sesbania sesban	"	-	-	+	-	+	-	+
Phaseolus trilobus	"	-	-	+	-	-	-	+

+ nodules developed - no nodules developed

The presence of isolates from wild legumes cross-inoculating *Phaseolus vulgaris* is an important finding since it is known that this crop is poorly nodulated and rarely forms effective symbiosis with rhizobia (Herridge and Bergersen, 1988). Isolates from the wild legumes might serve as a solution for this problem and might prove to be effective in nitrogen fixation with *Phaseolus vulgaris*. This necessitates extensive research on the compatibility of isolates from wild legumes with leguminous crops and their ability to form effective symbiosis with their homologous crop(s).

The results of the experiment showed that some of the isolates belong to more than one cross-inoculation group. This finding reflects the weakness of the cross-inoculation classification of rhizobia which has been criticized by many researchers (Segovia *et al.*, 1993). This entails that other systems of classification should be studied and adopted for the identification and classification of rhizobia.

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