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ASSESSMENT OF FABA BEAN *RHIZOBIUM* DIVERSITY IN SHARKIA GOVERNORATE AREAS BASED ON SYMBIOTIC TRAITS OF BIOLOGICAL NITROGEN-FIXATION

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ABSTRACT: In order to contribute for the optimization of biological nitrogen fixation (BNF) associated with faba bean plants, this study aimed to analyze the diversity of rhizobial populations (*Rhizobium leguminosarum* bv. *viciae*) in eight areas of the Sharkia governorate. Symbiotic traits of biological nitrogen-fixation were determined for a collection of rhizobia captured using faba bean as a host plant. A computer numerical analysis of data was carried out. An assessment of diversity among rhizobial isolates based on symbiotic traits of biological nitrogen fixation (no. of nodules/plant, dry weight of nodules, nitrogenase activity, dry weight of roots and shoots as well as their total nitrogen content) showed that the highest distance value was found between isolates RZ11 and RK11. The minimum genetic distance was found between RR13 and RA21 also between RB2 and RB3 isolate. The data indicated that isolates RZ11 and RK11 were the most distant isolates. The obtained dendrogram discriminates all the *Rhizobium leguminosarum* bv. *viciae* populations examined. Diversity analysis of the studied 27 *Rhizobium* populations showed high levels of genetic diversity among these populations and sub-divided them into nine main groups with subdivision into sub-groups and confirmed that the isolate RZ11 was the most divergent. The data indicated that symbiotic traits of biological nitrogen fixation can provide a relatively unbiased method of quantifying genetic diversity among isolates of indigenous populations of rhizobia nodulating faba bean in Sharkia soils. This study provides information on the diversity of indigenous populations

of rhizobia (*Rhizobium leguminosarum* bv. *viciae*), which has practical implications for applying biological nitrogen fixation in plant production.

Key words: Biological nitrogen fixation, numerical analysis, *Rhizobium* diversity.

INTRODUCTION

The Leguminaceae is one of the largest families of plant kingdom. The principal legume species have defined sites of origin and these coincide with the diversification centers for their specific symbiotic bacteria. These nitrogen-fixing bacteria, which form nodules on the roots or stems of the plants, belong to different bacterial lineages (*Rhizobium, Bradyrhizobium, Sinorhizobium* and *Azorhizobium*) related to other nonsymbiotic bacteria. A remarkable characteristic of these bacteria is their large genetic diversity (Triplet and Sadowsk, 1992). *Rhizobium* is a genus of soil bacteria whose members are able to establish a nitrogen fixing symbiosis with legumes (Hirsh, 1992 and Moschetti *et al.*, 2005). The inoculation of cultivated legume plants with specific rhizobial isolates is recommended to maximize the contribution of biological nitrogen fixation (BNF) to the nitrogen status of the host plant (De Oliveira *et al.*, 1999).

Faba bean (*Vicia faba* L.) is an important legume food crop in many developing countries, and substantial research programmes are being carried out to improve its yield, disease resistance and nutritional value. It is used for seed and as an inexpensive source of protein in the diets of people in Egypt. Faba bean may produce substantial yield with half or even less amount of nitrogen fertilizers due to symbiotic relationship with the nitrogen-fixing bacteria *Rhizobium leguminosarum* bv. *viciae* (Swelim *et al.*, 1997).

In order to increase seed yield, the inoculation of faba bean with appropriate rhizobial isolates represent an agriculturally sustainable approach. However, the presence of indigenous rhizobia in soil may represent a barrier to efficient inoculation with commercially available isolates because indigenous isolates are often better adapted to the prevailing soil and climate conditions (Strecker, 1994). Therefore, the evaluation of

diversity within indigenous rhizobial population is important for inoculation, and for the screening of novel, highly effective inoculant isolates (*De Oliveira et al.*, 1999). The diversity of *Rhizobium leguminosarum* bv. *viciae* has usually been determined by phenotypic and/or genotypic characterization of isolates isolated from legume root nodules (Esperanza Martínez-Romero, 2003; Zilli *et al.*, 2004, Abd El-Basit *et al.*, 2007 and Blazinkov *et al.*, 2007). Knowledge on diversity among indigenous rhizobium isolates could help to improve faba bean biological nitrogen fixation.

MATERIALS AND METHODS

Rhizobium Isolates

In previous work (Salem *et al.*, 2006), a total of twenty-seven isolates of the nitrogen-fixer *Rhizobium leguminosarum* bv. *viciae* were collected from root nodules of faba bean (*Vicia faba* L.) plants grown in different locations in Sharkia governorate. The collected samples represented eight locations: Zagazig (Z), Fakous (F), Abo-Kibeer (K), El-Hessenia (H), Kafr-Sakr (R), Abo-Hammad (A), Belbase (B) and Salhia (S). The tested rhizobial isolates have shown their different efficiencies on plant growth represented by their nodulation and N_2-fixation parameters of faba bean (*Vicia faba* L.) plants. The most important traits of rhizobial isolates were selected and presented in Table (1).

Data Analysis

To study clustering pattern among the studied *Rhizobium leguminosarum* bv. *viciae* isolates based on symbiotic traits, the data generated from these variations were recorded and analyzed. Genetic distance, calculated as an Euclidean metric distance, was computed between all pairs of populations. The Euclidean metric distance between two populations is equivalent to their total number of observed differences. Genetic distance values between isolates were used to produce a dendrogram of the relationships among *Rhizobium leguminosarum* bv. *viciae* populations by the unweighted pair-group method with arithmetic averages (UPGMA) as suggested by Sneath and Sokal (1973). The cluster analysis and dendrogram construction were performed according to SPSS (1995).

Table 1. Shoot and root dry weight (g/plant), total nitrogen content, nitrogenase activity in nodulation test of faba bean plants (Salem *et al.*, 2006)

Isolates	Dry weight shoot (g/plant)	Dry weight root (g/plant)	Total nitrogen shoot (mg/plant)	Total nitrogen root (mg/plant)	No. of nodules/plant	Dry weight of nodule (mg/plant)	Nitrogenase activity μ molC_2H_4/h/g dry nodule
RR11	1.43	0.60	55.63	12.30	43.30	86.66	2.95
RR13	1.04	0.28	34.32	5.12	36.70	103.33	2.92
RR23	1.49	0.34	53.49	6.22	116.70	110.00	2.59
RR31	0.84	0.33	31.50	6.37	47.00	116.66	1.89
RF12	1.32	0.33	45.14	5.78	45.00	123.33	1.51
RF13	1.36	0.45	55.62	10.44	36.00	123.33	3.95
RF23	1.19	0.34	44.15	7.31	64.00	100.00	3.60
RF31	1.24	0.33	47.00	6.93	66.00	26.67	3.85
RF32	1.04	0.34	40.77	6.63	83.30	16.66	3.23
RH13	1.21	0.44	41.38	8.58	87.70	123.33	2.41
RH21	0.97	0.41	35.60	7.18	10.30	50.00	2.47
RH31	0.95	0.27	36.67	5.94	92.00	93.33	3.83
RA21	0.93	0.28	31.81	5.40	25.70	116.66	2.49
RA33	1.28	0.53	43.26	8.90	35.00	130.00	1.22
RZ11	1.65	0.46	69.96	12.56	46.70	90.00	7.21
RZ22	1.47	0.49	61.30	11.27	56.70	140.00	5.01
RZ23	1.37	0.56	44.25	9.46	69.30	143.33	1.27
RK11	0.86	0.20	23.74	3.06	71.70	73.33	0.56
RK12	1.31	0.43	53.58	9.59	49.00	70.00	4.53
RK13	0.96	0.32	35.23	5.60	42.30	150.00	1.56
RK21	1.35	0.34	44.55	5.88	89.70	123.33	1.35
RK23	1.02	0.33	32.33	5.31	101.70	116.66	1.27
RK31	0.98	0.24	33.52	4.39	41.70	30.00	1.76
RS3	1.12	0.30	43.90	5.58	61.00	46.66	2.78
RB1	1.19	0.42	44.74	8.06	66.00	113.33	1.04
RB2	1.37	0.34	57.13	7.89	50.00	70.00	4.33
RB3	1.29	0.33	51.21	6.77	43.30	76.66	3.98

RESULTS AND DISCUSSION

Analysis of genetic divergence in faba bean *Rhizobium* populations can provide some interesting information about differentiation, adaptability and interrelationships of isolates. The magnitude of genetic distances measured the extent of genetic diversity between the *Rhizobium leguminosarum* bv. *viciae* isolates. The dissimilarity distance values among the studied *Rhizobium leguminosarum* bv. *viciae* populations based on symbiotic traits of biological nitrogen fixation are presented in Table 2. Data in this Table show the effect of inoculation with 27 isolates of *Rhizobium leguminosarum* bv. *viciae* on plant dry weight, nodulation, nitrogenase activity and nitrogen content in broad bean plants (Salem *et al.*, 2006). The distance values were found to have the range from 1.9 (most related) to 15.3 (distantly related). The highest distant value (15.3) was found between isolates RZ11 and RK11, followed by a value of 13.3 between RZ11 and RK21 and between RZ11 and RK31. These data confirm that these *Rhizobium leguminosarum* bv. *viciae* populations were the most distant genotypes. This result indicated that these populations showed significant genetic differentiation. The minimum genetic distance (1.6) was found between RR13 and RA21 and between RB2 and RB3 isolates of *Rhizobium leguminosarum* bv. *viciae*. The data presented in Table 2 indicate that the RZ11 was the most divergent isolate from all other isolates.

The dendrogram produced from genetic distances between *Rhizobium leguminosarum* bv. *viciae* isolates is shown in Figure 1. Diversity analysis of the 27 *Rhizobium* isolates found high levels of genetic diversity among these populations and sub-divided them into eight main groups with subdivision into 12 sub-groups. Cut off point at 5.5 dissimilarity points (Euclidean genetic distances) was fixed as minimum dissimilarity. The obtained dendrogram discriminates all the *Rhizobium leguminosarum* bv. *viciae* populations examined.

The grouping pattern and distribution of *Rhizobium leguminosarum* bv. *viciae* isolates into different clusters is given in Table 3. Cluster VIII was the largest having six isolates (RR23, RH13, RK21, RK23, RB1 and

Table 2. Genetic Euclidean distances among different *Rhizobium leguminosarum bv. viciae* isolates based on studied symbiotic traits of biological nitrogen-fixation

Rhizobia	RR11	RR13	RR23	RR31	RF12	RF13	RF23	RF31	RF32	RH13	RH21	RH31	RA21	RA33	RZ11	RZ22	RZ23	RK11	RK12	RK13	RK21	RK23	RK31	RS3	RB1	RB2
RR13	8.8																									
RR23	9.3	8.1																								
RR31	8.2	6.8	7.7																							
RF12	7.7	3.5	6.7	7.8																						
RF13	4.4	6.7	8.3	8.0	5.8																					
RF23	6.9	4.0	5.5	5.0	4.3	4.8																				
RF31	8.0	6.6	7.7	2.0	7.6	8.1	5.2																			
RF32	9.1	7.3	7.7	3.1	8.4	9.5	6.2	2.8																		
RH13	6.7	6.3	4.7	7.5	5.1	5.8	3.8	7.5	7.8																	
RH21	7.7	5.3	10.9	6.2	6.8	7.3	6.4	6.3	7.0	8.6																
RH31	9.6	5.1	5.3	5.9	6.3	8.1	3.7	5.7	5.5	4.9	8.4															
RA21	9.3	1.9	9.3	8.1	4.0	7.1	5.2	8.1	8.7	7.1	5.6	6.4														
RA33	5.2	6.2	8.6	9.3	4.3	4.8	6.0	9.1	9.9	5.3	7.0	8.6	6.2													
RZ11	7.0	11.1	10.8	9.4	10.9	6.2	8.6	9.3	11.1	10.0	11.1	10.9	11.9	10.4												
RZ22	5.4	8.8	8.3	9.4	7.8	3.2	6.3	9.5	10.8	6.3	10.1	8.8	9.2	6.7	5.4											
RZ23	5.7	7.7	6.9	9.8	5.4	5.7	6.3	9.7	10.1	3.8	9.3	8.2	8.0	3.2	10.6	6.3										
RK11	12.2	5.6	8.6	8.1	6.7	11.2	7.3	7.9	7.0	8.2	8.1	6.1	6.2	9.5	15.3	13.0	10.1									
RK12	4.4	6.3	7.7	4.9	6.6	4.2	4.3	4.7	6.2	6.2	6.1	6.6	7.4	6.6	5.8	5.4	7.3	10.1								
RK13	9.5	4.0	8.3	9.6	3.5	7.1	5.6	9.6	10.0	6.0	7.7	6.8	3.5	5.4	12.6	9.0	6.4	6.9	8.5							
RK21	8.7	5.9	3.7	7.9	4.0	7.5	4.6	7.7	7.9	3.4	9.2	4.9	6.8	6.3	11.7	8.3	5.2	6.6	7.6	5.5						
RK23	10.2	6.3	4.9	8.2	5.7	9.2	5.4	8.2	7.7	4.4	9.5	4.3	7.0	7.7	13.3	10.1	6.8	5.3	8.9	5.8	3.1					
RK31	10.5	5.3	9.8	5.3	7.1	10.1	6.8	5.1	5.1	9.0	5.1	6.8	6.3	9.4	13.3	12.3	10.8	4.8	7.9	8.4	8.2	8.0				
RS3	8.5	4.8	7.0	2.9	5.9	7.8	4.2	2.9	3.4	6.7	5.3	4.7	6.2	8.1	10.6	9.7	8.9	5.7	5.4	7.6	6.3	6.5	3.4			
RB1	6.4	5.4	5.8	7.5	3.5	5.7	4.3	7.5	7.7	3.5	7.1	6.1	5.9	3.8	10.7	7.4	3.8	7.2	6.3	4.6	3.7	4.8	7.8	6.0		
RB2	6.3	5.8	7.1	4.1	6.1	5.0	3.9	4.4	6.1	6.7	6.3	6.2	6.9	7.4	6.7	6.3	8.1	9.3	2.7	8.0	7.1	8.5	7.2	4.5	6.2	
RB3	7.0	4.7	7.4	4.2	5.4	5.2	3.2	4.4	6.3	6.4	5.5	5.8	5.7	7.0	7.9	7.0	8.1	8.4	3.9	7.0	6.7	7.8	6.3	4.0	5.9	2.4

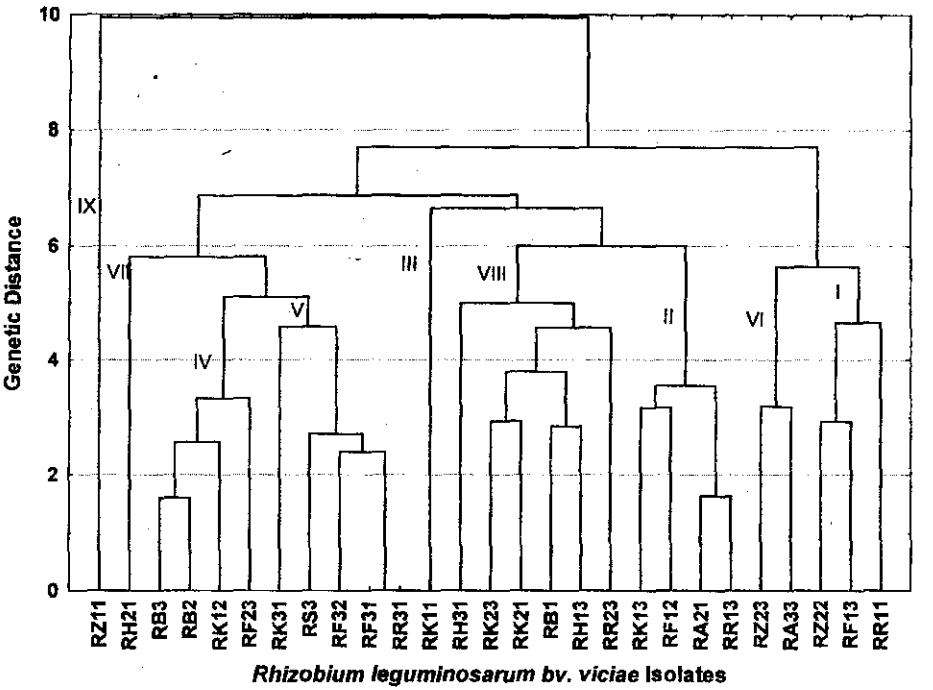

Fig. 1. Dendrogram of studied 27 *Rhizobium leguminosarum bv. viciae* isolates based on symbiotic traits of biological nitrogen-fixation

RH31), while clusters III, VII and IX had single isolates. Cluster V consisted of five isolates (RR31, RF31, RF32, RS3 and RK31). Cluster I contained three isolates (RR11, RF13 and RZ22). Cluster II consisted also of four isolates (RR13, RF12, RA21 and RK13).

The average intra-cluster and inter-cluster genetic distances are presented in Table 4. The maximum distance between clusters was between clusters IX and IV, followed by that between clusters IX and VIII, suggesting wide diversity between them. The minimum inter-cluster distance was observed between clusters IV and V, which was followed by clusters VI and VIII, indicating close relationship among the isolates included. Generally, the magnitude of inter-cluster distances reflects the diversity exists among the studied *Rhizobium leguminosarum* bv. *viciae* isolates. These results indicated wide genetic diversity between the studied *Rhizobium leguminosarum* bv. *viciae* isolates. The intra-cluster distance ranged from 0.00 (clusters III, VII and IX) to 0.90 (cluster I), indicating that the three isolates in cluster I are the most heterogeneous. Therefore, these three isolates may be important sources of genetic variation within this collection of *Rhizobium leguminosarum* bv. *viciae* isolates. In fact, analysis of isolates with the same geographic origin imply a low genetic diversity, since these populations may have exchanged genetic materials. The similarity observed between some isolates could be ascribed to *Rhizobium* populations sharing a common ancestral population and geographical region.

The existence of such a wide genetic diversity among indigenous rhizobial populations were similarly reported by (Palmer and Young, 2000; Maatallah *et al.*, 2002; Zilli *et al.*, 2004; Blazinkov *et al.*, 2007; Zhang *et al.*, 2007).

The data indicated that symbiotic traits of biological nitrogen fixation can provide a relatively unbiased methods of quantifying genetic diversity among *Rhizobium leguminosarum* bv. *Viciae* isolates. Several studies applied cluster analysis of *Rhizobium leguminosarum* bv. *viciae* isolates based on DNA polymorphism in order to identify the distribution of genetic diversity within the species (Maatallah *et al.*, 2002 and Blazinkov *et al.*, 2007). They concluded that *Rhizobium leguminosarum* bv. *viciae*

Table 3. Grouping pattern of the studied 27 *Rhizobium leguminosarum bv. viciae* isolates

Cluster	No. of isolates	*Rhizobium leguminosarum* isolates falling in cluster
I	3	RR11, RF13 and RZ22
II	4	RR13, RF12, RA21 and RK13
III	1	RK11
IV	4	RB2, RB3, RF23 and RK12
V	5	RR31, RF31, RF32, RS3 and RK31
VI	2	RA33 and RZ23
VII	1	RH21
VIII	6	RR23, RH13, RK21, RK23, RB1 and RH31
IX	1	RZ11

Table 4. Intra-cluster (in bold) and inter-cluster genetic distances among eight clusters of the studied 27 *Rhizobium leguminosarum bv. viciae* isolates

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX
Cluster I	**0.901**								
Cluster II	2.186	**0.586**							
Cluster III	3.061	1.609	**0.000**						
Cluster IV	1.870	1.323	1.680	**0.853**					
Cluster V	1.831	1.810	1.607	1.146	**0.616**				
Cluster VI	1.713	1.408	2.406	1.700	2.090	**0.544**			
Cluster VII	2.217	1.678	1.755	1.999	1.544	2.100	**0.000**		
Cluster VIII	2.193	1.589	2.027	1.047	1.804	1.366	2.568	**0.625**	
Cluster IX	2.475	1.801	3.115	4.392	3.177	2.348	3.221	3.258	**0.000**

populations isolated from faba bean nodules in various areas of Sharkia governorate are both phenotypically and genetically diverse.

So, this study provides an information on the diversity of indigenous populations of rhizobia nodulating faba bean in Sharkia soils which has practical implications for applying biological nitrogen fixation in plant production.

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تحديد التباعد الوراثى لريزوبيا الفول البلدى فى مناطق محافظة الشرقية على أساس تثبيت النتروجين الحيوى التكافلى

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تهدف هذه الدراسة الى تحليل التباعد الوراثى لعشائر من ريزوبيا الفول البلدى (*R. leguminosarum* bv *viciae*) فى ٨ مناطق بمحافظة الشرقية وذلك لتعظيم الاستفادة من تثبيت النتروجين الحيوى المصاحب لنباتات الفول البلدى.

تمت دراسة الصفات التكافليه لتثبيت النتروجين الحيوي فى مجموعه من العزلات باستخدام الفول البلدى كنبات عائل. أجرى تحليل عددى بالحاسب الآلى للبيانات بأستخدام المسافات الوراثيه وتم عمل رسم توضيحى يوضح القرابة الوراثية للعزلات (دندوجرام) باستخدام طريقة تحليل المجموعات UPGMA. وقد أوضح تحليل التباعد الوراثى بين العزلات الريزوبيه على أساس الصفات التكافلية لتثبيت النتروجين الجوى (عدد العقد/النبات، الوزن الجاف للعقد، نشاط انزيم النتروجينيز، الوزن الجاف للجذور والافرع الخضرية وأيضا النتروجين الكلى للجذور والأفرع الخضرية). ووجد أن أكبر قيمه للتباعد الوراثى كانت بين العزليتين RKII, RZII وكانت أقل قيمه للتباعد الوراثى بين كل من العزلتين RA21, RR13 وبين RB3, RB2. وأوضحت البيانات أن كل من العزلتين RKII, RZII كانتا الاكثر تباعدا بين العزلات. وقد أمكن للرسم التوضيحى دندوجرام المتحصل عليه أن يفرق بين كل عزلات.

R. leguminosarum bv *viciae* المختبرة. وقد أظهر تحليل التباعد لعشائر الريزوبيا المدروسة وجود مستويات عالية من التنوع الوراثى بين هذة العشائر وقسمها الى ٨ مجموعات رئيسيه وأكد أن RZII كانت العزلة الاكثر تباعداً من بقيه العزلات.

و تبين النتائج أن صفات تثبيت النيتروجين الحيوي التكافلى يمكن أن تقدم طريقة غير متحيزة لتقدير كميه التنوع الوراثى بين العزلات المحلية لريزوبيا الفول البلدى فى أراضى محافظة الشرقية – كما تقدم هذه الدراسة معلومات عن التباعد الوراثى بين العشائر المحلية بين الريزوبيا والتى قد تكون لها تطبيقات عملية فى مجال استخدام تثبيت النتروجين الحيوى فى الإنتاج النباتى.