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RESPONSE OF THREE LENTIL (*Lens culinaris* M.) CULTIVARS TO *RHIZOBIUM* AND AZOTOBACTER INOCULATION UNDER LOW DOSE OF N-FERTILIZATION

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ABSTRACT

A field experiment was carried out at Sakha Agricultural Research Station in 1998/99 and 1999/2000 seasons to study the effect of N-fertilization and inoculation with *Rhizobium* and Azotobacter on nodulation, N_2-fixation and yield of Giza 51, Sinai 1 and Line 3693 lentil cultivars. Treatments of nitrogen zero and 15 kg/fed. (1 fed. = 0.42 ha) and four treatments of inoculation were used, uninoculated, inoculated with *Rhizobium*, Azotobacter and *Rhizobium* + Azotobacter.

The results indicated that, the new lentil line 3693, N-fertilization with starter dose (15 kg/fed.), and mixed inoculation with *Rhizobium* and Azotobacter gave the highest number and dry weight of nodules and dry weight/plant. The interactions effect between mixed inoculation with *Rhizobium* and Azotobacter with lentil line 3693 or N-fertilization gave the highest values of these traits.

The two cultivars, Giza 51 and line 3693 superior to Sinai-1 cultivar in yield and yield components in the two growing seasons. While, the data showed that insignificant response to N-fertilization on yield and its components. Mixed inoculation of Rhizopium and Azotobacter significantly increased seed yield and all tested components. Nitrogen seed content was increased by N-fertilization and bacterial inoculation. The interactions between lentil cultivars, N-fertilization and bacterial inoculation on yield and its components were not significant.

INTRODUCTION

Lentil is an important pulse crop in Egypt. The cultivated area of it decreased from year to year of the last seasons, as a result to the high competition between lentil and other winter crops in the old lands.

Nitrogen is one of the important factors improving yield of lentil. The optimum level of nitrogen that maximized lentil yield was 33 kg/hectare (Nassib *et al.*, 1984). Hammouda *et al.* (1991) found that, modules number and dry weight increased due to nitrogen fertilization alone at 20 kg/fed.

Inoculation with rhizobia is essential agronomic practice for ensuring adequate nitrogen nutrition of legume crops such as lentil. Number and dry weight of nodules and also seed yield increased by rhizobial inoculation [Kumar *et al.* (1993), Khurana and Sharma (1995) and Shah *et al.* (1996)].

Under appropriate conditions, Azotobacter can enhance plant development and promote the yield of several agriculturally important crops in different soils and climatic regions, Becking (1992).

Positive effect of combined inoculation with *Rhizobium* plus Azotobacter have been reported for different forage and grain legumes, and

were related to the favourable influence of the free lowing diazotrophic bacteria on nodule weight and nitrogen content [Sanoria and Malik (1981), Malik and Sanoria (1981), Abdalla and Hegazy (1990) and Teledo (1996)].

Therefore, the present study was undertaken to investigate the effect of N-fertilization, inoculation with *Rhizobium* and Azotobacter on nodulation, yields and nitrogen seed content for the three new cultivars of lentil.

MATERIALS AND METHODS

A field experiment was conducted at Sakha Agricultural Research station, Egypt, during the two growing seasons 1998/1999 and 1999/2000. A split split-plot design with four replications was used with cultivars (Giza 51, Sinai-1 and Line 3693) as main plots, nitrogen treatments (zero and 15 kg/feddan) as sub-plots and inoculation treatments as sub sub-plots. Inoculation treatments includes four treatments:

1.Uninoculated. 2. Inoculated with *Rhizobium*
3. Inoculated with Azotobacter. 4. Inoculated with *Rhizobium* + Azotobacter.

Each sub sub-plots contained six rows 30 cm apart and 3 meters long. Inoculation, lentil seeds were mixed, immediately before sowing in the form of local peat inoculum. Nitrogen was applied at sowing. Other cultural practices were done as usual.

Two plant sampling were taken during each growth season at 60 and 90 days after sowing. At each plant sampling, the modules of 10 plants were separated, counted, dried and weighted and dry weight/plant was determined also. For measuring the studied yield components, ten guarded plants were taken from each plot at harvest. A central four rows were harvested each plot to determine seed yield ardab (160 kg) per feddan (4200 m^2). In the dry seeds total nitrogen was determined also.

The statistical analysis was done as a split split design (Snedecor and Cochran, 1981) using L.S.D. Test for comparison between means.

RESULTS AND DISCUSSION

The effect of inoculating lentil plants of different cultivars with *Rhizobium leguminosarum* biovar viceae, or free N_2-fixing Azotobacter in a form of single or combined inocula on nodule formation and its dry weight, dry weight of plant, yield and yield components and total nitrogen accumulated as well as their interactions were determined and recorded in Table (1-5).

1. Number and dry eight of nodules and dry weight of plant:

Mean values for number and weight of nodules per plant and the average dry weight of plant are given Table (1). There were significant differences between cultivars for each of the three characters. Roots exhibited nodules in all instances with any inoculum applied. Data showed that the new line 3693 had the highest mean number of nodules per plant followed by Giza 51, while Sinani 1, was the lowest. The corresponding values were 14.1, 12.9 and 11.0 nods/plant, respectively after 60 (DAS). It was also

observed that by aging of the plants from 60 to 90 days the number of nodules has also increased. This difference in nodule number was reflected in the total dry weight of nodular tissue per plant, as it followed a pattern similar to that of nodule number.

Application of increasing levels of nitrogen showed that the typical effect of N-fertilizers on biological N_2-fixation, stimulate for the lower levels of nitrogen. As shown in Table (1), the highest nodule numbers were recorded when 15 kg N/fed. were applied in comparison with control treatments. This was true at the two investigation periods of 60 and 90 days from sowing. Such results indicates that the available nitrogen was the main factor limiting plant growth.

The inoculation treatments showed a positive correlation and the highest number of nodules was recorded for co-inoculation of lentil with *R. legumenosarium* bv. viceae plus plant growth promoting Azotobacter, when compared with lentil inoculated with *Rhizobium* or Azotobacter only (Table 1). The synthesis and excretion of plant growth regulators by Azotobacter in the rhizosphere should explain the ability of rhizobial strain to promote the growth and nodulation of lentil (Burns *et al.*, 1981). These could be observed clearly from the nodulation pattern of plants (Table 1). As the average number of nodules per plant raised from 23.5 and 9.5 due to inoculation with *Rhizobium* and Azotobacter (the average of two seasons after 90 DAS) to 27.0 in co-inoculation treatment.

The above mentioned results find confirmation with those observed by Khurana and Sharma (1995), Nassib *et al.* (1984), Kumar *et al.* (1993), Malik and Sanoria (1981), Abdalla and Hegazy (1990) and Ghabrial (1990).

Interaction effect was detected between cultivars and inoculation treatments on number of nodules/plant at 60 days in the two seasons and at 90 days in the first season only, nodules dry weight at 60 and 90 days from sowing in the first season and dry weight/plant at the two sampling in the two seasons (Table 2). Line 3693 gave the high values for the three studied characters with mixed inoculation of Rhizobium and Azotobacter. Khurana and Sharma (1995) reported that, the interaction between cultivars and *Rhizobium* strains was not significant.

Regard to interaction effect between N-fertilization and inoculation treatments, the significant interactions was observed for nodules dry weight at 90 days from sowing in the first season and dry weight per plant at 90 days also, in the two seasons (Table 3). The optimum results were recorded for mixed inoculation *Rhizobium* and Azotobacter and the starter dose of nitrogen (15 kg/fed.). The same result was obtained by Hammouda *et al.* (1991).

Table (1): Effect of cultivars, N-fertilization and bacterial inoculation on nodules number, dry weight and dry weight plant at 60 and 90 days from sowing in 1998/99 and 1999/2000 seasons.

Treatments	1998/99 season						1999/2000 season					
	At 60 days			At 90 days			At 60 days			At 90 days		
	Nodules		D.W./ plant gm.	Nodules		D.W./ plant gm.	Nodules		D.W./ plant gm.	Nodules		D.W./ plant gm.
	No.	Weight mg.		No.	Weight mg.		No.	Weight mg.		No.	Weight mg.	
A- Cultivars												
Giza 51	14.84	14.14	0.27	18.22	18.62	0.94	1.22	8.08	0.22	12.53	14.04	1.29
Sinai-I	12.31	11.66	0.23	14.25	15.36	0.90	10.88	7.47	0.20	13.9	14.15	1.35
L. 369	16.34	15.97	0.27	19.56	20.79	0.96	13.53	8.34	0.22	16.91	18.79	1.55
L.S.D. 0.05	0.80	0.92	0.02	0.90	0.85	0.02	0.81	0.12	0.003	1.47	1.62	0.08
B- N-fertilization												
Zero	13.71	13.08	0.25	16.10	16.57	0.90	10.73	7.73	0.21	13.04	14.31	1.31
15 kg/fed.	15.27	14.77	0.26	18.58	19.94	0.96	12.35	8.20	0.22	15.31	17.02	1.48
L.S.D. 0.05	0.48	0.56	0.03	1.00	1.02	0.01	0.60	0.12	0.03	0.76	1.12	0.07
C- Inoculation												
Uninoculation	3.00	2.80	0.24	3.88	4.02	0.83	1.92	7.24	0.18	3.33	3.96	0.23
Rhizobium	19.79	18.61	0.25	25.13	26.53	0.96	17.58	8.18	0.23	22.00	23.84	2.13
Azotobacter	9.42	8.92	0.24	11.21	11.69	0.90	6.33	7.72	0.21	7.75	8.99	0.852
Rh. + Az.	25.79	25.35	0.29	29.17	30.78	1.04	20.33	8.71	0.21	23.63	25.86	2.41
L.S.D. 0.05	0.81	0.980	0.03	1.05	0.84	0.02	0.85	0.15	0.04	1.04	0.98	0.11
Interaction												
AB	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
AC	*	*	*	*	*	*	*	N.S	*	N.S	N.S	*
BC	N.S	N.S	N.S	N.S	*	*	N.S	N.S	N.S	N.S	*	N.S

Table (2): Effect of interaction between cultivars and bacterial inoculation on number and dry weight of nodules (mg)/plant and dry weight/plant (gm) at 60 and 90 days from sowing in 1998/99 and 1999/2000.

Dry weight/plant												
Cultivars	1998/99 season						1999/2000 season					
	60 days			90 days			60 days			90 days		
Inoc.	G. 51	Si. 1	L. 3693	G. 51	Si. 1	L. 3693	G. 51	Si. 1	L. 3693	G. 51	Si. 1	L. 3693
Uninoc.	0.24	0.22	0.24	0.84	0.81	0.85	0.18	0.16	0.19	0.12	0.21	0.37
Rh.	0.26	0.23	0.26	0.96	0.93	0.98	0.23	0.22	0.23	2.05	2.23	2.09
Az.	0.25	0.23	0.25	0.91	0.88	0.91	0.21	0.21	0.20	2.56	0.80	1.08
Rh. + Az.	0.32	0.24	0.32	1.03	0.97	1.10	0.24	0.23	0.25	2.43	2.13	2.67
L.S.D. 0.05	0.05			0.03			0.01			0.19		
Nodules numbers												
Uninoc.	4.25	2.00	2.75	5.25	2.75	3.63	1.00	1.75	3.00			
Rh.	20.25	16.00	23.13	26.56	18.88	30.00	16.38	18.13	18.25			
Az.	9.00	8.50	10.75	11.25	10.25	12.13	4.38	5.88	8.75			
Rh. + Az.	25.88	22.75	28.75	29.88	25.13	32.50	19.13	17.75	24.13			
L.S.D. 0.05	1.41		1.82		1.48							
Nodules weight (ng)												
Uninoc.	3.95	1.88	2.57	5.37	2.79	3.91						
Rh.	18.46	14.98	22.40	26.70	21.07	31.81						
Az.	8.90	7.62	10.26	11.50	10.86	12.72						
Rh. + Az.	25.24	22.16	28.67	30.90	26.73	34.71						
L.S.D. 0.05	1.57	1.45										

Table (3): Effect of interaction between N-fertilization and bacterial inoculation on nodules weight at 90 days in 1998/99 season and dry weight plant at 90 days in 1998/99 and 1999/2000 seasons.

Inoculation	Nodules weight (mg)				Dry weight/plant (gm)							
	1998/99				1998/99				1999/2000			
N- fertilization	Uninc.	Rh.	Az.	Rh. +Az.	Unino.	Rh.	Az.	Rh. +Az.	Unino.	Rh.	Az.	Rh. +Az.
Zero	3.57	23.88	10.73	28.10	0.82	0.93	0.88	0.97	0.22	1.96	0.75	2.30
15 kg/fed.	4.48	29.18	12.65	33.46	0.85	1.00	0.92	1.17	0.24	2.29	0.88	2.52
L.S.D. 0.05	1.18				0.02				0.16			

Yield and yield components:

Data of yield and its components and seed nitrogen content as affected by cultivars, nitrogen fertilization and inoculation in 1998/99 and 1999/2000 seasons, are presented in Tables 4 and 5, respectively.

Data as affected by cultivars showed clearly that the two lentil cultivars Giza 51 and line 3693 were superior to Sinai 1 cultivar in plant height, number of branches and pods/plant, seed and straw yields/plant seed yield ardab/fed. and also seed nitrogen content in both seasons. Similar results were obtained by Khurana and Sharma (1995).

The data showed that no significant response to nitrogen fertilization on yield and yield components in the two growing seasons. While nitrogen fertilization increased seed nitrogen content also, in the two seasons (Tables 4 and 5). Hammouda *et al.* (1991) and Kumar *et al.* (1993) found that the same results.

Table (4): Effect of cultivars, N-fertilization and inoculation on yield and yield components in 1998/99 season.

Treatments	Plant height cm	No. of branches/ plant	No. of pods/ plant	No. of seeds/ plant	Seed yield/ plant	Straw yield/ plant	Seed yield ard./fed.	N% seeds
A. Cultivars								
Giza 51	36.20	1.75	23.80	29.74	0.70	1.41	3.98	3.27
Sinai 1	29.44	1.09	18.23	23.27	0.83	0.99	3.57	3.20
L., 3693	37.60	1.52	25.66	32.08	0.83	1.43	4.04	3.45
L.S.D. 0.05	3.27	0.26	2.74	3.46	0.04	0.09	0.45	0.02
B. N-fertiliz.								
Zero	34.83	1.47	23.16	29.25	0.80	1.28	3.92	3.26
15 kg/fed.	34.00	1.43	21.96	27.47	0.78	1.27	3.81	3.35
L.S.D. 0.05	N.S	N.S	N.S	N.S	N.S	N.S	N.S	0.02
C. Inoculation								
Uninoculation	33.65	1.18	19.49	23.54	0.66	1.13	3.59	2.65
Rhizobium	34.95	1.48	24.63	31.97	0.87	1.42	4.07	3.60
Azotobacter	33.74	1.59	21.00	25.25	0.68	1.15	3.56	3.23
Rh. + Az.	35.33	1.56	25.13	32.09	0.93	1.40	4.26	3.74
L.S.D. 0.05	N.S	0.24	1.49	2.33	0.06	0.11	0.31	0.02
Interactions								
AB	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
AC	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
BC	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

Table (5): Effect of cultivars, N-fertilization and inoculation on yield and yield components in 1999/2000 season.

Treatments	Plant height cm	No. of branches/ plant	No. of pods/ plant	No. of seeds/ plant	Seed yield/ plant	Straw yield/ plant	Seed yield ard./fed.	N% seeds
A. Cultivars								
Giza 51	48.12	3.39	57.49	58.42	2.27	1.94	6.52	3.13
Sinai 1	33.96	1.85	34.24	51.90	1.87	1.22	3.76	3.03
L., 3693	50.08	3.13	57.41	62.50	2.05	1.95	6.11	3.26
L.S.D. 0.05	3.32	0.62	15.92	4.46	0.43	0.40	0.47	0.05
B. N-fertiliz.								
Zero	44.27	2.84	48.18	57.82	1.94	1.62	5.53	3.11
15 kg/fed.	43.83	2.74	51.25	57.39	2.19	1.78	5.40	3.18
L.S.D. 0.05	N.S	N.S	N.S	N.S	N.S	N.S	N.S	0.04
C. Inoculation								
Uninoculation	42.68	2.46	43.97	48.66	1.70	1.49	4.62	2.46
Rhizobium	45.92	3.26	54.24	66.27	2.26	1.83	5.85	3.41
Azotobacter	42.20	2.31	43.69	49.50	1.89	1.59	5.15	3.12
Rh. + Az.	45.42	3.13	56.96	66.00	2.41	1.90	6.24	3.57
L.S.D. 0.05	2.31	0.38	10.26	8.57	0.29	0.18	0.35	0.03
Interactions								
AB	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
AC	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
BC	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

Inoculation with mixed culture of *Rhizobium* and Azotobacter followed by inoculation with *Rhizobium* only significantly increased seed yield and all tested components in the two growing seasons. Also, inoculation gave the higher values of seed nitrogen content than uninoculation in the two growing

seasons. These findings are in great accordance with those obtained by Malik and Sanoria (1981), Sanoria and Malik (1981), Abdalla and Hegazy (1990) and Shah *et al.* (1996). No interactions effect were detected between cultivars, nitrogen fertilization and inoculation on any studied characters. Hammouda *et al.*, 1991 reported that the interaction between N-fertilization and Rh. inoculation was not significant with seed and straw yields.

In conclusion, increases in N_2-fixation potential should be possible in lentil through positive effect of combined inoculation with *Rhizobium* and Azotobacter and superior host x biofertilizers combinations.

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إستجابة ثلاثة أصناف من العدس للتلقيح البكتيرى والتسميد الآزوتى

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***قسم بحوث المحاصيل البقولية ـ معهد المحاصيل ـ مركز البحوث الزراعية ـ الجيزه**

****قسم بحوث الميكروبيولوجى الزراعية ، معهد الأراضى والمياه ـ مركز البحوث الزراعية ـ الجيزه**

أقيمت هذه الدراسة فى مزرعة محطة البحوث الزراعية بسخا فى موسمى الزراعة ١٩٩٨/١٩٩٩م ، ١٩٩٩/٢٠٠٠م لدراسة تأثير التسميد النيتروجينى واللقاح البكتيرى سواء بالريزوبيا أو الازوتباكتر أو مخلوط منهما على العقد البكتيرية والأزوت المثبت بواسطتها وكذلك تأثيرها على أصناف العدس الجديدة وهى جيزه٥١ ، سيناء١ والسلالة رقم ٣٦٩٣.

أظهرت النتائج أن السلالة الجديدة ٣٦٩٣ من العدس ، التسميد الآزوتى بالجرعة التنشيطية ١٥كجم/فدان من الآزوت وكذلك المعاملة المختلطة بالريزوبيا والآزوتباكتر أعطت أعلى القيم من حيث عدد ووزن العقد البكتيرية وكذلك الوزن الجاف للنبات. كما أعطت المعاملة المختلطة من الريزوبيا والازوتباكتر أعلى القيم لتلك الصفات السابقة مع السلالة الجديدة من العدس وهى ٣٦٩٣ أو المعاملة التى تم تسميدها بالجرعة التنشيطية من الآزوت وهى ١٥ وحدة أزوت/فدان.

كما أظهرت النتائج أن صنفى العدس جيزه٥١ ، السلالة ٣٦٩٣ تفوقتا فى محصول البذور ومكوناته. فى حين أظهرت النتائج أن التسميد الازوتى ليس له تأثير معنوى على محصول العدس من البذور ومكوناته. كما أعطت معاملة اللقاح المخلوطة من الريزوبيا والازتباكتر تفوقا عاليا فى المحصول ومكوناته على باقى المعاملات. كما زاد محتوى البذور من النيتروجين بالتسميد الآزوتى وكذلك اللقاح البكتيرى. وكان التفاعل بين أصناف العدس وكلا من التسميد الآزوتى واللقاح البكتيرى غير معنوى التأثير على المحصول ومكوناته.