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STUDIES ON SOME BACTERIAL DISEASES OF *LUPINUS TERMIS*

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ABSTRACT: *The present investigation was carried out to study the effect of Agrobacterium tumefaciens, a soil – borne pathogenic bacteria causing crown – gall disease on lupine plants on plant growth and symbiotic performance of Bradyrhizobium spp. (lupinus) in addition to the nature's biological control of Agrobacterium radiobacter strain k 84.*

Four isolates of Agrobacterium tumefaciens and two selected isolates of Bradyrhizobium sp. (Lupinus) were isolated , identficated and purificated. Inoculation with lupine rhizobia enhanced in general nodule formation , growth of nodular tissue , dry weight of shoots and total nitrogen on all the tested lupine cultivars.

A clear inhibitory effect on nodule formation and their dry weight was observed by inoculation with two spices of Agrobacterium tunefaciens or radiobacter. However , the reduction was more pronounced in the pathogen than that of biocontrol agent. Inoculation with Bradyrhizobium (alone or mixed) and two speices of Agrobacterium led to an increasing of dry weight of shoots , total N-content and seed yield in comparison with pathogen treatment. Serological studies revealed to no dectable rection between two tested organisms (Agrobacterium and Bradyrhizobium)while four common antigenic materials were found to be common between Agrobacterium tumefacies and seed protein of susceptible lupine cultivars (local) , where no common antigen are detected in resistant lupine (foreign). One common precipitin band was found to be common between the Bradyrhizobium sp. (lupine) st. 6 and seed protein of both susceptible and resistant lupin cultivars.

Key words: *Bacterial disease , Agrobacterium tumefaciens , Agrobacterium Radiobacter , Bradyrhizobium sp. Lupinus*

INTRODUCTION

Cultivation of lupine in Egypt has been recorded at early as 2000 BC (Zkukovsky, 1929). Lupine can grow successfully even on poor sandy soils, which gives to lupine adistinct advantage. Lupines make excellent winter growth and high seed yield and are valuable as a winter cover crop to conserve soil fertility and to supply the nitrogen so much needed in crop production. In addition it leaves nitrogen for the following crops (Reeves *et al.*, 1984 ; Armestrong *et al.*, 1997 and Chalk *et al.*, 1993) and Improve soil texture (Rowland *et al.* 1986).

In Egypt , Lupines are widely consumed as green manure and also as dry seeds. According to the records of the central Administration for Agricultural

Economics , Ministry of Agricultural , the areas cultivated were about 6.000 feddan (1998).

Agrobacterium tumefaciens the causal agent of crown gall disease on most dicotyledon plants can affect the symbiotic performance of rhizobium and then legume hosts. Antiboitics could be used (Sasser , 1982) , but they are expensive and , in any case ; the compounds that are valuable for human therapy are not allowed to be used in agriculture. The effective alternative is the use of copper , which is potentially phytotoxic.

Biological control lend it self as a suitable alternative means of antibiotics and chemical control. It relies on the potency of non-pathogenic antagonistic *Agrobacterium raidobacter* strain K84 to (Kerr,1972) colonized the roots and displace the pathogenic A. *tumefaciens*. Inoculation of soil or seeds with such antagonists could be more effective, more enduring, more economic and is more safe for application.

In the light of presumpture evidence this investigation was carried out to study the role of Biological control and inoculation of *Bradyrhizobium* on lupine plants infected with *Agrobacterium* tumefaciens .

MATERIALS AND METHODS

Samples of diseased lupine plants and healthy nodulated plants were collected from four Governorates ,i.e Esmailiya , Kafr El-Sheikh , Minufiya and Gharbiya for isolation of the casual organisms .

A. Laboratory experiments:

A.1.Isolation of causal organisms:

The infected parts were washed under running tap water and then surface sterilized with 95% Ethanol. Tumors were cut into small pices , then crushed with 5 ml of sterile water to prepare a microbial suspension.

This suspension was left for 30 minutes and then streaked on the potato dextrose agar (Kado & Keskett , 1970). The inoculated plates were incubated at 28°c and examined daily to chek up the developing colonies which were streaked on nutrient glucose agar slant.

A.2. Identification of the isolated bacteria:

Isolated bacteria were identified according to their morphological , cultural and physiological characteristies according to Bergey's manual of determinative bacteriology (Krieg and Holt 1984).

A.3. Isolation of Lupine rhizobia:

Nodules were cleaned from adherent particles , surface sterilized by 1%. Hgcls2 solution for 2 minutes , treated with ethyl alcohol for three minutes , washed several times with sterilized distilled water , crushed in few drops of sterilized water and then streak two or three yeast extract mannital agar plates. The developed colonies were used to inoculate yeast mannital agar slant medium(Vicent & Somasegrane , 1985).

A.4. Identification of rhizobial isolates:

All the obtained isolates were subjected to purity tests and all the recommended methods used for identification of rhizobial strains including :

- cultural and biochemical tests
- staining and morphology
- colony characters
- Growth and production of acid or alkali.
- Growth on Glucose peptone agar
- Limits milk test

Preparation of standered inoculum

Lupine rhizobia strains were grown separately in 500 ml plate bottles containing yeast mannitol agar slopes for 7 days. The growth was then washed with YM liquid media into flasks of 750 ml volume containing 400ml of the same medium. Incubation was carried out on rotary shaker (100 rpm) at 28°c for 5 days. Turbidity of the cultures was adjusted colormetrically to obtain optical density 1.6 , at 540 nm (about 10^8 cells /ml)

Pathogenicity test was made using susceptible lupinus termis variety (Giza 2)

B.Greenhouse Pot experiments:

Eleven treatments were conducted on Lupine plants as the following :

1- Control
2- Inoculation with Bradyrhizobium strain 1
3- Inoculation with Bradyrhizobium strain 6
4- Inoculation with Agrobacterium tumefuciens (22)
5- Inoculation with Agrobacterium radiobacter (K84)
6- Inoculation with Bradyrhizobium strain 1+ strain 6
7-Inoculation with Bradyrhizobium strain 1 + A.tum.
8- Inoculation with Bradyrhizobium strain 1 + A.rad
9- Inoculation with Bradyrhizobium strain 6 + A.tum
10- Inoculation with Bradyrhizobium strain 6 + A.rad.
11- Inoculation with A.tum. + A. rad

The same eleven treatments were repated with the other three cultivars of lupine – each treatment was represented by six replicates.

For recording the following parameters at 40 and 80 days after planting

1-Number of nodules / plant
2-Dry weight of nodules / plant (mg / plant)
3-Dry weight of shoots (gam / plant)
4-Total nitrogen content of shoots (mg / plant)

Other three replicates were kept under wire poof greenhouse conditions and irrigated when needed .

At harvest (150 and 140 days after planting for local and foreign cultivars , respectively) the following characters were recorded :
1-Dry weight of shoots / plant (gm) .
2-Dry weight of seeds / plant (gm)
3-Total nitrogen content of shoots (mg / plant) .
4-Total nitrogen content of seeds (mg / plant)

C.Serological studies

Antigen preparation:

Antigen applied for invivo immunization was prepared according to (Vincent , 1970 & Somasegran and Hoben , 1985)

Immunization :

Rabbits , 2-3 kg in weight were immunized according to (Jokey and Erika Karzag , 1968)

-Antigen – antibody reactions:

The serological reactions between the antisera and the antigens were carried out using the double diffusion method.

RESULTS AND DISCUSSION

Results present in table (1) show that the normal cell morphology and are identified as Bradyrhizobium.

Table (1): Morphological of cultural and biochemical characteristics of collected isolates of *Bradyrhizobium* spp (12 isolates).

Test	Result
I. Microscopic examination	
Gram-stain	Negative
Cell shape	Very S, rod
Motility	Motile
II. Growth characteristics	
A. On YEM (media)	
Detectable growth after	6 days
Smooth (s), while (W) convex (C) gumm (G)	S.W.C.
Congo-red reaction (colonies colour).	Colourless
B. In broth (media)	
Produce turbidity	+
C. In litmus milk (media)	
Serum zone with alkaline reaction	-
No serum zone with alkaline reaction	+
D. On glucose peptone agar (media)	
Detectable growth	No growth
Change in pH	-
E. Growth on BTB (media)	-
Acid production (media change to yellow)	+
Alkaline production (media change to blue)	

Results in table (2,3) showed the biochemical and physiological tests of *Agrobacterium* isolates

Table (2): The reaction of isolates Agrobacterium to physiological tests.

Characters	Isolates of *A. tumefaciens*			
	20	21	22	23
1. Catalase activity	+	+	+	-
2. Starch hydrolysis	-	-	-	-
3. Gelatin liquefaction	-	-	-	-
4. Indole formation	+	+	+	+
5. Lipolytic activity	+	+	+	+
6. Production of H_2S	+	+	+	+
7. V.P. test	-	-	-	-
8. Methyl red test (MR)	-	-	-	-
9. Litmus milk test	+	+	+	+
10. Nitrate reaction	-	-	-	-

+ = positive reaction
- = negative reaction

Table (3): Break down of different sugars by four isolates of *A. tumefaciens* inoculated for 5-7 days at 30°C

Tested	Isolates of *A. tumefaciens*			
	20	21	22	23
Glucose	+	+	+	+
Mannose	+	+	+	+
Fructose	+	+	+	+
Sucrose	+	+	+	+
Maltose	+	+	+	+

+ = Positive reaction with production acid without gas.

All the bacterial isolates showed negative results for starch utilization of and gelatin liquefaction , production of H2S indol, Methyle red test , v. p test and nitrate reduction and gave alkaline reaction for catalase , the tested bacterial isolates were able to break down some sugar , i.e, glucose , mannose , fructose , sucrose and maltose as well as produce acid without gas.

Results presented in table (4) show the response of different lupine cultivars to inoculation treatments as shown from these results that the uninoculated treatments faild to from nodules on all the roots of tested lupine cultivars , which indicated that the soil under investigation is free from lupine rhizobia according to (Raza *et al.* 2000) .

The effect of inoculation on nodule numbers as observed after 40 and 80 days from planting time was very clear by all lupine cultivars both including local and foreign cultivars , when inoculated with any of the two *Bradyrhizobia* strain or their combination. On another hand a clear inhibition effect on nodule formation was observed on the inculated plants as a result of their infection will pathogenic bacteria of *Agrobacterium tumefaciens* or the non pathogenic one of *Agrobacterium radiobacter*. It is evident that this reduction of nodule formation reached to its highest values for plants inoculated with double strains inocula and infected with *Agrobacterium tumefaciens* or *Agrobacterium radiobacter* as compared with healthy inoculated plants. The cultivars could be arranged in descending order in their ability to nodulation as follows : Giza 17 Giza 2 > E – 101 > LAG-6.

Reduction in nodule formation as a result of the presence of the two *Agrobacterium* spp may be attributed to the competition of both *Agrobacterium* and Rhi zobium on nodulation sites presents on root plants.

Results in table (5) and (6) showed that the effect of inoculation on dry weight of nodules and dry weight of shoots and their total nitrogen are governed by the number of active nodules formed on the roots or lupine plants. Irrespective of infection treatments with *Agrobacterium tumefaciens* or *radiobacter* results showed high increase in nodule number.

Table (7) showed that the twelve and eight precipitin lines were developed in the homologous reaction of Agrobac: and *Bradyrhizobium* . No detectable reactions were developed in the precipitation system in cross reaction between *Agrobac.* And lupine rhizobia of heterdogous antigens indicating that both the two tested organisms are not closely related , the reaction including the antiserum of Agrobac. tumefaciens strain 22 and the seed protein of susceptible cultivar the obtained results clarify the relationship between *Agrobacterium tumefaciens* and two lupine cultivars one of which showed susceptibility while the other exerted resistance towards the same pathogen (Abd El – Rehim *et al* 1975).These findings support the gene for gene concept suggested by (Flor 1946)

Table (4): Effect of inoculation with *Bradyrhizobium* spp. (lupinus) and *Agrobacterium* spp. on the number of root nodules/plant formed on different lupine cultivars grown under wire proof green house in 2002 season.

Treatments \ Cultivars	Days after planting									
	40								80	
	Giza-1	Giza-2	E-101	LAG-6	Mean	Giza-1	Giza-2	E-101	LAG-6	Mean
Cont. (Uninoculated)	0.00 h	0.00 i	0.00 f	0.00 f	0.00 G	0.00 h	0.00 i	0.00 h	0.00 h	0.00 H
Inoc. *Bradyrhizobium* spp. strain (1)	55..33 c	49.67 c	39.33 c	41.00 c	46.33 C	95.33 c	87.67 c	67.33 c	66.33 c	79.17 C
Inoc. *Bradyrhizobium* spp. strain (6)	57.33 b	51.67 b	44.00 b	44.67 b	49.42 B	97.00 b	90.00 b	70.00 b	68.33 b	81.33 B
Inoc. *Agro. tumefaciens* (22)	2.33 g	3.00 h	0.00 f	0.00 f	1.33 FG	3.00 g	3.00 h	0.00 h	0.00 h	1.50 GH
Inoc. *Agro. radiobacter* (84)	3.00 g	3.00 h	1.33 f	1.67 f	2.25 F	4.33 g	3.33 h	2.00 g	2.00 g	2.92 G
Inoc. Brady: strain 1 + Brady: strain: 6	65.67 a	69.33 a	56.67 a	55.33 a	61.75 A	114.33 a	107.33 a	91.00 a	91.33 a	101 A
Brady. spp. strain (1) + *Agro. tum* (22)	25.67 f	24.67 g	20.67 e	21.33 e	23.08 E	42.33 f	38.00 g	38.00 f	36.33 f	38.67 F
Brady. spp. strain (1) + *Agro rad* (84)	35.67 d	30.33 e	26.00 d	30.67 d	30.67 D	60.67 e	57.67 e	52.00 e	49.67 e	55.00 E
Brady. spp. strain (6) + *Agro tum* (22)	28.67 e	27.67 f	20.67 e	20.00 e	24.25 E	43.33 f	40.33 f	38.67 f	36.67 f	39.75 F
Brady spp. strain (6) + *Agro rad* (84)	37.00 d	33.33 d	27.00 d	31.67 d	32.25 D	63.33 d	61.00 d	65.33 d	52.67 d	58.33 D
Agro. tum (22) + *Agro rad* (84)	3.00 g	3.00 h	1.00 f	1.67 f	2.17 E	3.00 g	3.00 h	0.00 h	0.00 h	1.50 GH
Mean of Cultivars	28.52 A	26.87 B	21.52 C	22.55 D	24.86	47.88 A	44.67 B	37.76 C	36.67 D	41.74

Mean = mean of treatments

Table (5): Effect of inoculation with *Bradyrhizobium* spp. (lupinus) and *Agrobacterium* spp. on the dry weight of nodules (mg/plant) of different lupine cultivars grown under wire proof green house in 2002 season.

Treatments \ Cultivars	Days after planting									
	40					80				
	Giza-1	Giza-2	E-101	LAG-6	Mean	Giza-1	Giza-2	E-101	LAG-6	Mean
Cont. (Uninoculated)	0.00 j	0.00 i	0.00 f	0.00 e	0.00 G	0.00 h	0.00 h	0.00 g	0.00 f	0.00 F
Inoc. *Bradyrhizobium* spp. strain (1)	67.33 b	55.67 b	34.00 b	31.67 b	47.17 B	115.33 b	110.33 c	83.67 c	83.33 b	98.17 B
Inoc. *Bradyrhizobium* spp. strain (6)	65.00 c	56.67 b	34.67 b	32.67 b	47.25 B	116.33 b	115.00 b	87.33 b	84.00 b	100.67 B
Inoc. *Agro. tumefaciens* (22)	2.67 hi	3.00 gh	0.00 f	0.00 e	1.42 FG	4.00 g	3.00 g	0.00	0.00 f	1.75 F
Inoc. *Agro. radiobacter* (84)	2.00 i	2.00 h	1.00 f	1.00 e	1.50 FG	5.67 g	4.67 g	1.00 g	1.00 f	3.08 E
Inoc. Brady: strain 1 + Brady: strain: 6	81.00 a	79.67 a	44.67 a	43.33 a	62.17 A	135.67 a	34.33 a	101.33 a	102.00 a	118.33A
Brady. spp. strain (1) + *Agro. tum* (22)	28.33 g	25.33 f	18.33 e	16.00 d	22.00 E	52.00 f	50.67 f	40.67 f	40.67 e	46.00 D
Brady. spp. strain (1) + *Agro rad* (84)	36.33 e	31.00 d	22.33 d	23.67 c	28.33 D	77.67 d	76.00 e	63.00 e	68.33 d	71.25 C
Brady. spp. strain (6) + *Agro tum* (22)	30.67 f	28.00 e	18.33 e	15.00 d	23.00 E	56.33 e	50.67 f	40.00 f	41.67 e	47.17 D
Brady spp. strain (6) + *Agro rad* (84)	39.33 d	35.33 c	26.33 c	23.67 c	31.17 C	79.67 c	78.33 d	65.00 d	70.33 c	73.33 C
Agro. tum (22) + *Agro rad* (84)	4.00 h	3.67 g	0.67 f	1.00 e	2.33 F	4.33 g	3.00 g	0.00 g	0.00 f	1.83 F
Mean of Cultivars	32.42 A	29.12 B	18.21 C	17.09 D	24.12	58.82 A	56.91 B	43.82 C	44.67 D	51.05

Mean = mean of treatments

Table (6): Effect of inoculation with *Bradyrhizobium* spp. (lupinus) and *Agrobacterium* spp. on dry weight (gm/plant) of shoots of different lupine cultivars grown under wire proof green house after 80 and 120 in 2002 season.

Treatments \ Cultivars	Days after planting									
	80					120				
	Giza-1	Giza-2	E-101	LAG-6	Mean	Giza-1	Giza-2	E-101	LAG-6	Mean
Cont. (Uninoculated)	1.17 g	1.21 g	0.79 g	0.78 f	0.99 G	2.06 h	2.13 f	1.52 g	1.58 f	1.82 G
Inoc. *Bradyrhizobium* spp. strain (1)	4.25 c	4.32 b	3.48 bc	3.26 b	3.83 C	6.58 b	6.21 b	4.52 b	4.48 b	5.45 B
Inoc. *Bradyrhizobium* spp. strain (6)	4.55 b	4.40 b	3.59 b	3.29 b	3.96 D	6.75 b	6.21 b	4.55 b	4.52 b	5.51 B
Inoc. *Agro. tumefaciens* (22)	0.73 i	0.63 h	0.48 h	0.37 g	0.55 H	1.73 i	1.42 g	0.96 h	0.96 g	1.27 H
Inoc. *Agro. radiobacter* (84)	2.15 f	2.16 f	0.82 g	0.83 f	1.49 F	3.91 e	3.13 e	2.42 e	2.28 e	2.94 E
Inoc. Brady: strain 1 + Brady: strain: 6	5.3 a	4.91 a	4.84 a	4.47 a	4.84 A	7.82 a	6.98 a	5.51 a	5.92 a	6.56 A
Brady. spp. strain (1) + *Agro. tum* (22)	2.74 e	2.82 d	2.48 e	2.22 e	2.57 E	3.59 f	3.74 d	3.24 d	2.98 d	3.39 D
Brady. spp. strain (1) + *Agro rad* (84)	3.08 d	3.0 c	3.38 c	2.83 d	3.07 D	5.44 c	5.65 c	4.01 c	3.36 c	4.62 C
Brady. spp. strain (6) + *Agro tum* (22)	2.84 e	2.50 e	2.45 e	2.25 e	2.51 E	3.62 f	3.76 d	3.35 d	3.04 d	3.44 D
Brady spp. strain (6) + *Agro rad* (84)	3.10 d	3.09 c	3.12 d	3.03 c	3.08 D	5.18 d	5.71 c	4.12 c	3.33 c	4.59 C
Agro. tum (22) + *Agro rad* (84)	1.0 h	1.19 g	1.0 f	0.95 f	1.04 G	2.98 g	3.18 e	1.87 f	1.75 f	2.45 F
Mean of Cultivars	2.81 A	2.75 B	2.4 C	2.21 D	2.54	4.52 A	4.38 B	3.28 C	3.11 D	3.82

Mean = mean of treatments

Table (7): Number of precipitin lines formed in cross-reaction between Agrobacterium tumefaciens and Bradyrhizobium spp. (Lupinus) as well as seed proteins of two lupine cultivars.

Antigens Antiserum of	Agro. Tum. St. 22	Lupine rhizobia St. 6	Seed protein of local cultivar (Giza1)	Seed protein of foreign cultivar (E-10)
Agro.tum.st22	12	-	4	-
Brady. Spp.st6	-	8	1	1

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دراسات على بعض الأمراض البكتيرية فى الترمس

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الملخص العربى

أجريت هذه الدراسة لدراسة بعض الأمراض البكتيرية التى تصيب نبات الترمس المنزرعة فى مصر ويمكن تلخيص نتائج البحث المتحصل عليها كما يلى :

–تم عزل وتعريف وتنقية ٤ عزلات من المسبب لمرضى للتدرن التاجى و ١٢ عزله من بكتريا العقد الجذرية المثبتة للأزوت الجوى واجراء الاختبارات المعملية التى شملت الاختبارات المورفولوجية والمزرعية والفسيولوجية وذلك لاستخدامها فى تجارب الصوبة والاختبارات السيرولوجية.

كان تأثير التلقيح البكتيرى بالبيريدى ريزديم واضحا على تكوين العقد الجذرية – عدد ووزن العقد – الوزن الجاف للمجموع الخضرى – المحتوى النيتروجينى عند عمر ١٢٠ يوم ، وذلك على جميع أصناف الترمس المحلية والأجنبية .

أدى العدوى بالاجروباكتريم تيوميفاش والأجر باكتريم رادوباكتر الى انخفاض معنوى فى عدد ووزن العقد – الوزن الجاف للمجموع الخضرى – المحتوى النيتروجينى عند عمر ١٢٠ يوم وكان الانخفاض فى حالة الأجروباكتريم تيوميفاش اكثر وضوحا من الأجروباكتريم رادو باكتر – وذلك على جميع اصناف اكثر من المحلية والأجنبية.

أظهرت النتائج أن التفاعل بين التلقيح بالبريدى ديزويم (منفرد أو خليط) والأجرو باكتريم تيوميفاش والأجرو باكتريم رادو باكتر الى زيادة فى الوزن الجاف للمجموع الخضرى والمحتوى النيتروجينى ومحصول البذرة عند مقاومتها بالمعاملات الملقحة بالأجروباكتريم فقط وهذا يوضح أهمية البيريدى ريزويم فى تحسين صفات المجموع الخضرى.

أوضحت الدراسات السيرولوجية انه لا توجد علاقة بين البيريدى ريزوبيم ليونيس والأجروباكتيرم تيوميفاشن – بالاضافة الى وجود انتجين مشترك بين السيرم المضاد لسلالة بكتريا الترمس مع بروتين الترمس صنف جيزة (١) وصنف E-101 فى حين ظهر وجود أنتجينات مشتركة بين تفاعل السيرم المضاد لسلالة الأجروباكتيرم تيوميفاشن مع بروتين صنف جيزة (١) ولم تظهر أنتجينات مشتركة لبروتين الصنف الأجنبى المقاوم للإصابة E-101 .