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EVALUATION OF SOME LUPINE MUTANTS PATHOLOGICALLY AND AGRONOMICALY

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ABSTRACT

The present studies were carried out at Etay El-Baroud Agricultural Research Station during 2006/2007 and 2007/2008 of growing seasons in addition to a pot experiment under controlled conditions during 2007/2008. M14 and M15 generations of six mutant lines derived from three radiated varieties (Giza-1, Giza-2 and Dijon-2) in addition to varieties Belpies-9 and Sohag 2 were evaluated against pre-, post-emergence damping-off, root rot and wilt diseases. Under artificial infection with *Fusarium oxysporum* f. sp. *lupini*, mutant lines number 23, 33 and 37/3 in addition to Giza- 1 and Dijon- 2 had the lowest pre- emergence damping- off % with averages of 0.00% for the mutant 23 and 6.95% for the other four genotypes. In case of the post-emergence damping- off, the mutant line number 37/3 and varieties Giza-1, Dijon- 2 and Behpis-9 were not affected (0.00%). The mutant lines 23, 37/3 followed by Giza-1 and Dijon-2 had the highest survival plants percentages. The disease index of wilted plants ranged between 1.26 and 2.07 and showed no significant differences between the all tested genotype. The mutant lines number 23, 33, 35/4 and 37/3 showed the highest values of shoot length, plant fresh weight, plant dry weight and seed yield/pot compared to their origin varieties. Under filed conditions, the best values of the pre-, post- emergence damping- off, root rotted and wilted plants% and survival plants% were cleared with the mutant lines number 23 and 37/3 in addition to Dijon-2 and Sohag-2 in both seasons. Also, the mutant lines number 37/3 and 23 in addition to Sohag-2 had the best values of number of pods /plant, weight of 100 seeds and seed yield/plot in the two growing seasons.
Keywords : lupine, Mutant lines, Damping-off, *Fusarium* wilt.

INTRODUCTION

Lupine (*Lupinus albus* L.) is a food legume, a winter crop, high in protein value and has many benefits for human and animal nutrition as well as its efficient in nitrogen fixation system and application in many medical industries (Hamblin *et al*.,1993 and Julier *et al*.,1994).

Nowdays, white lupine makes a minor contribution to Egypt grain legume production as its exploitation has been restricted due to several factors including low yield, late maturity and susceptibility of the commercial varieties to wilt disease caused by *Fusarium oxysporum* f. sp. *Lupini* in Egypt causing drastic losses in seed yield reaching 60% in many growing areas (Abd- El- Kader, 1983; Yassen, 1994; Christiansen *et al*., 1999; Raza *et al*., 1999 and El- Sayad and El- Barougy, 2002).

Fusarium oxysporum, Fusarum solani, Fusarium avenaceum, Pythium sp*., Rhizoctonia solani* and *Macrophomina phaseoline* have been frequently reported as the causal fungal pathogens causing damping- off, root rot and wilt diseases of lupine (El-Awadi *et al.*, 1997; El- Barougy and El- Sayad, 2003; Amer and El- Shennawy, 2005; Zian, 2005 and Baraka *et al*., 2006)

The objective of this work aimed to evaluate different lupine cultivars and its mutants to damping-off and wilt diseases in pot experiment and under field experiment conditions.

MATERIALS AND METHODS

Pot Experiment:

Isolation and identification :

Wilted lupine plants were collected from Etay El-Baroud Agric. Res. Station Farm. Roots were first washed in running tap water and cut into small pieces, then surface sterilized in 5% sodium hypochlorite solution for three minutes. The sterilized plant parts were rinsed several times in sterilized distilled water and dried between sterilized filter paper and directly placed on PDA medium in Petri dishes which were incubated at 25°C for 5 -7 days (El-Sayad and El- Barougy, 2002). *Fusarium oxysporum*, the pathogenic fungi caused the wilt symptoms, was isolated and purified on PDA according to Nelson *et al*.(1983) and Smith (1965).

Preparation of fungal inoculum and soil infestation:

Sterilized sorghum medium (250g sorghum grains /1000 ml bottle and enough water to cover the sorghum) was used to prepare fungal inoculum. The medium was autoclaved then inoculated with *F. oxysporum* and incubated at 27°C for 15 days (Barak *et al*., 2006).

The soil was sterilized with 5% formalin solution and left for 2 weeks for formalin evaporation. Pots (30 cm in diameter) filled with sterilized soil and then infested with the fungal inoculum at the rate of 3% (w/w) of the soil weight. The infested soil was mixed thoroughly and watered for one week. Non infested soil was used as control.

M14 and M15 generations of six mutant lines (22/2, 23, 33, 35/4, 37/3 and 7) derived from three radiated varieties (Giza-1, Giza-2 and Dijon-2) in addition to varieties Belpis-9 and Sohag-2 were evaluated against *Fusarium oxysporum* f. sp*. lupini* (Table A after El-Sayad and El- Barougy, 2002). Seeds were sown at the rate of four seeds/pot. Randomized complete block design with six replicates for each lupine genotype were used.

Table A : Characteristics of mutants assessed in the present study (after El-Sayad and El- Barougy, 2002).

Variety	Mutant No.	Mutagen dose	Characteristics	No. of Line
Giza-1	-	-	Egyptian variety control	-
	22	2.5 K R*	High yield and high protein	2
	23	2.5 K R	High yield, early maturing, dark blue flowers and resistant to *Fusarium* wilt.	1
Giza-2	-	-	Egyptian variety control	-
	33	2.5 KR	High yield and tolerant to *Fusarium* wilt.	1
	35	10 KR	Dwarf, early maturing, small seeds and resistant to *Fusarium* wilt.	4
	37	40 KR	High yield, early maturing, large seeds, high protein and resistant to *Fusarium* wilt.	3
Dijon-2	-	-	French variety control.	-
	7	5 KR	High yield, white seed, early maturing and resistant to *Fusarium* wilt.	1

*** KR is a dosage of gamma ray**

Pre and post emergence damping-off were recorded after 25 and 50 days from sowing but the assessment of *Fusarium* wilt, survived plants were removed after 90 days form sowing date, washed and diagnosed. They were scored for disease on 0-5 scale described by Hassan (1992)as the following: 0 = no infection, 1= very weak infection (tiny discoloration covering 10% of root surface area), 2 = weak infection (tiny necrotic lesions covering 11-25% of root surface area), 3 = moderate infection (medium size lesions with corky tissues covering 26-50% of root surface area), 4 = sever infection (well developed large necrotic lesion covering 51-75% of root surface area) and 5 = very sever infection (complete death of plant show large necrotic lesions covering more then 75% of root surface area). Disease index (DI) was determined by the equation:

$DI = [\sum (a.b) /5k] x100$

Where : a = number of infection plants in each category.

b = the numerical number given to each category ranged from 0-5 as mention above.

K = the total number of the examined plants.

At the end of the experiment, disease index, shoot length (cm), shoot fresh weight (g), shoot dry weight (g) and seed yield /pot (g) were determined.

Field Experiments:

The field experiments were carried out at Etay El-Baroud Agric. Res. Station farm during the growing seasons 2006/2007 and 2007/2008. Randomized complete block design with three replicates was used. Each experimental plot (5.2m^2) had three ridges, 60 cm in between and three meters long. Planting took place on one side/ridge with two seeds/ hill, 20 cm a part. Fertilization and irrigation were carried out as it recommended. The commercial cultivars and six lupine mutants mentioned before were evaluated under filed conditions against pre- and post emergence damping-off disease after 25 and 45 days from sowing date in addition, the percentage of root rotted and wilted plants after 90 days.

At harvest, five guarded plants were taken at random from each experimental plot, on which the following characters were recorded: plant length (cm), number of pods / plant and 100 seeds weight (g). Also, seed yield/plot (g) was determined.

Statistical analysis:

The obtained data were statistically analyzed according to Snedecor (1961)and means were compared using LSD at the 0.05 level of probability.

RESULTS AND DISCUSSION

Under Egyptian conditions, lupine (*Lupinus alblus* L.) plants suffer from infection by soil borne fungi causing a high percentage of seedling damage and plant mortality leading to a considerable yield losses (Baraka *et al*., 2006).

Pot experiment:

Fig.(1) shows lupine wilt symptoms caused by *Fusarium oxysporum* f. sp. *Lupini* preisolated from diseased plants. Data in Table (1) appeared pre-, post- emergence damping – off, survival plants and disease index of wilted plants. Mutant lines 23 of Gize- 1 and 33, 35/4 and 37/3 of Gize-2 gave lower pre- emergence damping- off than their source varieties. Also, mutant lines number 33 and 37/3 had no post- emergence damping- off in addition to Giza-1 and Dijon-2. Generally, data of pre and post emergence damping off reflected on the survival plants percentages where mutant lines numbers 23 of Giza-1 and 33, 35/4 and 37/3 of Giza-2 gave the highest values compared with their source varieties although the increase in case of the mutant 23 did not reach the limits of significance as compared with its source variety.

Fig. (1): **Lupine wilt symptom caused by *Fusarium oxysporum* f. sp. *Lupini* (left) and healthy plants (right).**

Table (1): Pre-, post-emergence damping-off, survival lupine plants% and disease index of wilted plants of the tested lupine genotypes under pot experiment conditions.

Parameter / Genotype	Damping- off		Survival plants %	Disease index of wilted plants
	Pre -	**Post -**		
Giza-1	6.95	0.00	93.05	1.67
Mutant 22/2	6.95	4.16	88.89	1.87
Mutant 23/1	0.00	3.12	96.88	1.52
Giza-2	20.24	2.29	77.47	2.07
Mutant 33/1	6.95	0.00	93.05	1.52
Mutant 35/4	13.89	3.12	82.99	2.02
Mutant 37/3	6.95	0.00	93.05	1.26
Dijon-2	6.95	0.00	93.05	1.87
Mutant 7/1	13.89	2.34	83.77	1.62
Belpis 9	20.83	0.00	79.17	1.61
Sohag 2	34.72	1.88	63.40	1.62
L.S.D 0.05	11.99	0.39	6.71	N.S

On the other hand, the differences between the all genotypes were not significant in case of the disease index of the wilted plants and it ranged between 1.26 and 2.07. In this concern, El Sayad and El- Barougy (2002) reported that mutant lines number 7 of Dijon-2, 23 of Giza-1 and 37/3 of Giza -2 were more resistant to wilt disease caused by *F. oxysporum* f. sp. *Lupini*, whereas, they gave the highest percentages of survived plants in M7 and M8 generations at the end of the growing season under artificial infection conditions in the pot experiment.

Table (2) represents the means of shoot length (cm), whole plant fresh weight (g), whole plant dry weight (g) and seed yield/pot (g) for lupine plants sown in infested and noninfested soil. The statistical analysis showed significant differences between some mutant lines and their source varieties and also between some mutant lines and others. In case of the infested soil with *F. oxysporum* f. sp*. Lupini*, mutant lines 23, 33, 35/4 and 37/3 gave the highest parameters compared with their source varieties. On the contrary, mutant line 7 had less values compared to its source variety (Dijon 2). Concerning the noninfested soil, the mutant lines number 23,33,35/4 and 37/3 gave the highest values of the previous four parameters compared with their source varieties except in case of the mutant 37/3 which had the lowest value with the plant fresh weight. These results are in harmony with those reported by Gataulina (1996) and El-Sayad and El-barougy(2002).

Table (2): Effect of *Fusarium oxysporum* f.sp.*lupini* on some lupine genotypes growth parameters (pot experiment).

Parameter	Shoot length (cm)		Plant fresh wt (g)		Plant dry wt (g)		Seed yield / pot (g)	
Genotype	**D**	**H**	**D**	**H**	**D**	**H**	**D**	**H**
Giza-1	47.0	60.0	5.68	8.36	3.85	4.24	3.37	5.95
Mutant 22/2	44.5	52.6	4.92	8.07	3.08	5.22	2.63	5.03
Mutant 23/1	53.5	52.6	8.14	10.15	5.24	5.48	3.55	6.58
Giza- 2	47.5	53.9	4.36	9.52	3.58	5.05	2.40	4.80
Mutant 33/1	58.4	59.3	9.21	15.80	5.06	9.08	3.20	6.81
Mutant 35/4	52.5	53.2	5.22	13.87	3.74	6.17	2.84	5.13
Mutant 37/3	58.3	56.2	6.85	7.55	5.05	5.49	4.23	6.01
Dijon - 2	47.9	42.3	8.09	9.67	4.83	6.32	3.94	6.09
Mutant 7/1	44.9	47.5	4.86	7.96	3.89	5.46	2.47	4.37
Belpis - 9	51.7	58.5	10.95	13.17	5.14	7.13	4.82	7.42
Sohag - 2	47.0	56.3	5.85	11.41	4.14	6.64	2.58	3.62
L. S.D 0.05								
G	3.56		1.33		0.67		0.50	
I	N.S		0.57		0.29		0.21	
GxI	5.03		1.87		0.95		0.70	

G : genotype **D: diseased plants**
I : infection **H: healthy plants**

Filed experiments:

Data in Table (3) show pre-, post emergence damping-off, root rotted and wilted plants% and survival plants% at the end of the two growing seasons. The mutant lines number 23,33 and 37/3 had the lowest values of pre-emergence damping - off compared to their source varieties in the two growing seasons but the reduction was significant with 23 in the first season and with 37/3 in both seasons. On the other hand, mutant lines number 37/3 and 33 had the lowest values for the post- emergence damping-off compared to Giza 2 (their source variety) significantly in both season, in addition to the mutants number 22/2 and 23 at the second season. The mutant lines number 37/3 and 23 showed less percentages of root rotted and wilted plants compared to their source variety (Giza 2) in both seasons in addition to the mutant number 33 in the second season. The data mentioned before reflected in the survival plants where Dijon 2 had the highest value followed by mutant 37/3, mutant 23 and Sohag 2 with averages of 77.68, 73.43, 69.63 and 68.29%, respectively in the first season. In the second season, the same trend was cleared with light differences in the arrangement where mutant 37/3 had the highest value followed by mutant 23, Dijon 2 and Sohag 2 with averages of 83.79, 82.45, 79.32 and 73.14%, respectively. These results are in general agreement with those reported by El-Sayad and El- Barougy (2002) where they mentioned that survival plants under natural field infection of mutant lines number 22/2 and 23 of Giza-1 as well as mutant lines number 33 and 37/3 of Giza-2 moreover mutant line number 7 of Dijon-2 showed the highest percentages of survived plants at the end of the growing season compared to the other mutant lines and their source varieties in both M7 and M8 generations. Also, data in the same table exhibited that Sohag- 2 was promising for *Fusarium* wilt tolerance where its survival plants in the two growing seasons were 68.29 and 73.14%. In contrast, Belpis-9 recorded the lowest values (33.03 and 41.51%). In this respect, induced lupine mutants with resistance to *Fusarium* wilt through the application of gamma rays were previously obtained by Debelyi *et al*. (1978); Golovchenko (1987); Gataulina (1996) and El- Sayad and Barougy (2002).

Table (3): Evaluation of some lupine genotypes against damping-off, root rot and wilt diseases and survival plants% during 2006/2007 and 2007/2008 growing seasons under filed conditions.

Genotypes	**2006 / 2007**				**2007/2008**			
	damping-off pre-emergence %	**damping-off post-emergence %**	**root rotted and wilted plants %**	**survival plants %**	**damping-off pre-emergence %**	**damping-off post-emergence %**	**root rotted and wilted plants %**	**survival plants %**
Giza-1	26.66	7.78	8.15	57.41	15.55	10.00	3.57	70.88
Mutant 22/2	32.22	11.11	9.55	47.12	29.99	8.89	4.87	56.24
Mutant 23/1	15.55	8.89	5.93	69.63	11.11	4.45	2.03	82.45
Giza-2	33.33	12.22	8.38	46.07	25.56	15.55	4.92	57.30
Mutant 33/1	32.22	7.76	8.40	51.62	20.00	8.89	3.90	67.21
Mutant 35/4	35.55	17.78	10.98	35.69	27.78	15.55	6.71	49.96
Mutant 37/3	18.89	5.56	2.12	73.43	12.22	2.23	1.76	83.79
Dijon-2	11.11	7.78	3.43	77.68	11.11	6.67	2.90	79.32
Mutant 7/1	22.22	12.22	8.22	57.34	18.89	7.78	3.90	69.43
Belpis-9	37.78	17.78	11.41	33.03	35.55	12.28	7.38	41.51
Sohag-2	18.89	8.89	3.93	68.29	16.67	6.67	3.53	73.14
L.S.D 0.05%	8.24	4.46	3.96	7.85	8.15	4.56	2.23	6.21

Data presented in Table (4) indicated that plant length of all mutant lines decreased compared to their source varieties in the first season, but in the second one the length of the mutant lines number 22/2, 23 of Giza-1 as well as 35/4 and 37/3 of Giza-2 gave the highest values although the differences were not significant except between 22/2 and Giza-1. On the other hand, there are significant differences between some mutants in both growing seasons.

In the first season, mutant lines number 23,37/3,33 and 35/4 gave the highest number of pods/plant compared to their source varieties. At the same time Sohage-2 had the higher number of pods/ plant than the other mutants and varieties. In the second season, the same trend appeared where the mutant lines number 37/3,33 and 23 had the highest number of pods/plant compared to their source varieties. Bovtramovich and Bovtramovich(1981) and El- Sayad and Barougy (2002) reported that the increase of seed yield in most induced mutants was mainly due to the increase in number of pods/ plant.

Table (4): Means of plant length cm (A), number of pods/plant (B), 100 seeds weight g (C) and yield/plot g (D) for some lupine genotypes in two growing seasons 2006/2007 and 2007/2008 (field experiment).

Season	2006 / 2007				2007/2008			
parameter / Genotype	A	B	C	D	A	B	C	D
Giza-1	71.00	6.00	24.20	214.75	48.00	4.25	18.99	186.25
Mutant22/2	66.25	5.51	26.51	160.00	60.75	4.65	22.80	158.25
Mutant 23/1	71.00	6.10	32.42	186.50	51.75	5.35	20.80	201.50
Giza- 2	76.25	4.05	25.00	131.25	58.75	5.45	20.51	156.50
Mutant 33/1	67.75	4.90	21.30	173.25	56.50	5.75	20.21	172.50
Mutant 35/4	62.00	4.40	24.28	112.50	61.25	4.85	17.70	117.50
Mutant 37/3	75.00	5.60	26.12	235.75	62.25	8.45	22.87	228.75
Dijon-2	63.50	5.35	25.03	236.25	57.50	4.95	21.17	191.00
Mutant 7/1	59.50	5.20	22.55	182.75	56.00	4.10	19.57	181.25
Belpis-9	62.00	3.50	22.92	105.00	59.00	4.10	22.49	105.75
Sohag-2	72.00	6.60	27.31	194.75	50.50	5.25	22.70	194.25
L. S.D 0.05%	4.96	0.46	3.60	7.71	4.94	0.89	1.38	13.51

Also, in the first season mutant lines number 32/1, Sohag-2, mutant lines number 22/2 and 37/3 gave the highest weight of 100 seed (g), which Belips-9 and the mutant lines number7 and 33 gave the lowest values. In the second season, the mutant lines number 37/3, 22/2 and varieties Sohag-2 and Belpis-9 gave the highest weight of 100 seed. On the other hand, mutant line number 35/4,Sohag-2 and the mutant number 7 gave the lowest values. These results are in harmony with those mentioned by El- Sayad and Barougy (2002).

Finally in the first growing season, Dijon-2 had the highest seed yield/plot followed by the mutant line number 37/3 and Giza-1, but in the second season the mutant line number 37/3 had the highest seed yield/plot followed by the mutant line number 23, Sohag-2 and Dijon-2. In this respect, El- Sayad and Barougy (2002) reported that the mutant lines number 7, 22/2, 23, 35/4 and 37/3 gave the highest values in the seed yield in the M7 and M8 under Ismailia governorate environmental conditions.

It can be concluded that some mutant lines are suitable under El-Behera governorate environmental conditions and still gave the highest values in some a gronomic characters and in resistance of damping-off and wilt disease as the mutant line number 37/3.

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تقييم بعض طفرات الترمس مرضياً ومحصولياً

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أجريت هذه الدراسة الحقلية بمزرعة محطة البحوث الزراعية بإيتاي البارود خلال موسمى الزراعــة ٢٠٠٦/ ٢٠٠٧ ، ٢٠٠٧/ ٢٠٠٨ إضــافة لتجربــة أصــص خــلال الموســم الزراعــى ٢٠٠٧/٢٠٠٨ ثم تقييم ست طفرات مشتقة من ٣ أصناف (جيزة -١ ، جيزة -٢ ، ديجون-٢) بالإضافة للصنفين بلبيس -٩ ، سوهاج -٢ . تحت ظروف العدوى الصناعية بفطر فيوزاريوم أوكسيسبورم و وجد أن الطفرات رقم ٢٣ ، ٣٣ ، ٣٧ / ٣ بالإضافة للصنفين جيزة -٢ ، ديجون-٢ كانت الأقل حدوثا فى أعفان ما قبل الظهور بنسب تراوحت من صفر – ٦,٩٥ % . أما بالنسبة لأعفان ما بعد الظهور فلم تصب الطفرة ٣٧ / ٣ والأصناف جيزة -١ ، ديجون -٢ ، بلبيس -٩ . أظهرت الطفرتان ٢٣، ٣/٣٧ يليهما الصنفان جيزة -١ ، ديجون -٢ أعلى نسبة مئوية للنباتات السليمة. فى شدة حدوث الإصابة بالذبول لم توجد أي فروق معنوية بين الطفرات وكانت تتراوح بين ١,٢٦ – ٢,٠٧ . أظهرت الطفرات ٢٣ ، ٣٣ ، ٣٥ / ٤ ، ٣٧ / ٣ أعلى قيم فى طول المجموع الخضرى ، الوزن الطازج للنبات الكلى ، الوزن الجاف للنبات الكلى ، محصول البذور / أصيص مقارنة بالأصناف المستنبطة منها. تحت الظروف الحقلية أظهرت الطفرتان ٢٣،٣٧ /٣ والصنفان ديجون -٢ ، سوهاج -٢أدنى نسبة لحدوث أعفان ما قبل وما بعد الظهور وأعفان الجذور والذبول وأعلى نسبة للنباتات السليمة فى كلا موسمى الزراعة. وجد أن الطفرتان ٢٣ ، ٣٧ /٣ بالإضافة للصنف سوهاج -٢ أعطت زيادة فى عدد القرون / نبات ، وزن ١٠٠ بذرة ومحصول البذور لكل قطعة تجريبية خلال موسمى الزراعة.