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Indication of Tolerance to Broomrapes (*Orobanche crenata* Forsk.) in Wild Lentil Collected from Jordan

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ABSTRACT

The broomrape, *Orobanche crenata* is one of the most serious threats to grain legumes around the world. Cultivated lentil varieties are susceptible to *Orobanche* spp. and can be severely infected by *O. crenata*. Resistance breeding is hampered by scarcity of proper sources of resistance in cultivated lentil. A germplasm collection of 22 wild lentil accessions was screened for resistance to *O. crenata* under laboratory and greenhouse conditions over two seasons (2007 and 2008). A wide range of responses was observed, from high tolerance to susceptibility. The higher levels of tolerance were observed in accessions of *Lens ervoides* and *Lens culinaris* subsp. *orientalis* that were collected from Al Mansourah, Al-sofsafeh, Ain Al Tais and Irbid. Tolerance of these accessions proved to be mainly because of failure to induce germination in Orobanche seeds (accessions 3, 6, 22), and to develop tubercle on lentil roots in accession 3,5, 6 and 22. These results could be of significant importance, but need further verifications by testing them under naturally infested field and if needed to be confirmed by DNA analysis. If the results were confirmed further, these accessions could be used as a source for crossing with cultivated lentils aiming at the development of lentil cultivars tolerant to *Orobanche.*

KEYWORDS: Breeding, Lentil Lines, Jordan, Tolerance, Resistance, Orobanche, Broomrapes.

1. INTRODUCTION

Broomrapes (*Orobanche* spp.) are holoparasitic weeds that cause significant yield and quality losses in many important crops and affect the livelihoods of peoples in many countries around the world (Sauerborn, J. 1991a). The devastating effects of *O. crenata* Forsk., *O. ramose* L., *O. aegyptiaca* Pers., *O. cernua* Loefl., *O. cumana* Wallr., *O. minor* Sm. and *O. foetida* Poir are a major constraint that limits the productivity of faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medick.), tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), sunflower (*Helianthus annuus* L.) and many other crop species (Abu-Irmaileh, 1994; Bayaa, et al., 2000; Parker and Riches, 1993; Rubiales et al., 2006). Yield losses range from 5% to 100% depending on host susceptibility, level of infestation and environmental conditions. Grain legumes are the primary source of protein in the Near East and North Africa (Graham and Carrol. 2003), and people's ability to rely on these crops to provide food security is severely threatened by *Orobanche* infestation (Sauerborn 1991b; Abu-Irmaileh, 2004). Many countries that were once exporters of food legumes and vegetables have been forced to become net importers of these commodities in order to meet local demand, due to *Orobanche* infestation. Lentil production is limited by environmental stresses, diseases and insect pests throughout its distribution. Sources of resistance to several fungal and viral diseases of regional importance are known. In contrast, the parasitic weed broomrapes (*Orobanche* spp.), are significant yield reducers of lentil, no sources of resistance to have been found.

In Jordan, Several *Orobanche* species are widely spread in various agricultural areas including Jordan Valley and the upland attacking many vegetables especially solanaceous, leguminous, cruciferous crops, and also attack some fruit trees. *Orobanche cernua*, *O. ramose/ aegyptiaca*, and *O. crenata* are the major species that may lead to crop failure under heavy infestations (Abu Irmaileh, 1979). Many growers were forced to abandon growing tobacco in their fields around Salt, Madaba and Naour because of heavy broomerape attack. Many growers were forced to shift to less profitable rotational crops than host crops in heavily infested fields with *Orobanche* spp.

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Table 1. Wild lentil lines collected from different parts of Jordan (University of Jordan Wild Lentil Collection (JUWL).

No.	Province	Location	Species	Longitude	Latitude	Altitude M(asl)*
UJWL 1	Ajlune	Bergish	*Lens ervoides*	35° 44' 21"	32° 25' 90"	776.8
UJWL 2	Ajlune	A-safa	*Lens ervoides*	35° 43' 59"	32° 15' 05"	
UJWL 3	Ajlune	Al-sofsafeh	*Lens ervoides*	35° 45' 39"	32° 15' 29"	
UJWL 4	Ajlune	Al-mansourah	*Lens ervoides*	35° 45' 47"	32° 15' 13"	
UJWL 5	Ajlune	Al-mansourah	*Lens ervoides*	35° 46' 41"	32° 15' 56"	
UJWL 6	Ajlune	Ein al-teas	*Lens culinaris subsp. orientalis*	35° 45' 88"	32° 20' 84"	
UJWL 7	Ajlune	Bergish	*Lens ervoides*	35° 44' 21"	32° 25' 90"	776.8
UJWL 8	Ajlune	Bergish	*Lens ervoides*	35° 45' 63"	32° 21' 08"	
UJWL 9	Ajlune	Eshtafenah	*Lens ervoides*	35° 44' 46"	32° 21' 89"	
UJWL 10	Ajlune	Eshtafenah	*Lens ervoides*	35° 44' 46"	32° 21' 89"	
UJWL 11	Ajlune	Eshtafenah	*Lens ervoides*			
UJWL 12	Balqa	Jalaad	*Lens culinaris subsp. orientalis*			
UJWL 13	ICARDA /ILWL 324 Jordan	Tafila	*Lens culinaris subsp. orientalis*	E35° 36'	N30° 46'	1,250
UJWL 14	ICARDA /ILWL 325 Jordan	Ma'an	*Lens culinaris subsp. orientalis*	E35° 29'	N30° 19'	1,400
UJWL 15	ICARDA /ILWL 334 Jordan	Balqa	*Lens ervoides*	E35° 45'	N32° 06'	850
UJWL 16	ICARDA /ILWL 335 Jordan	Balqa	*Lens culinaris subsp. orientalis*	E35° 49'	N32° 10'	290
UJWL 17	ICARDA /ILWL 336 Jordan	Irbid	*Lens ervoides*	E35° 47'	N32° 16'	890
UJWL 18	ICARDA /ILWL 337 Jordan	Irbid	*Lens ervoides*	E35° 45'	N32° 20'	850
UJWL 19	ICARDA /ILWL 338 Jordan	Irbid	*Lens ervoides*	E35° 50'	N32° 18'	1,000
UJWL 20	ICARDA /ILWL 339 Jordan	Irbid	*Lens ervoides*	E35° 48'	N32° 20'	1,250
UJWL 21	ICARDA /ILWL 340 Jordan	Irbid	*Lens ervoides*	E35° 46'	N32° 22'	950
UJWL 22	ICARDA /ILWL 341 Jordan	Irbid	*Lens culinaris subsp. orientalis*	E35° 38'	N32° 36'	50

M (asl) = meters above see level

Various measures have been employed to control Orobanche. While individual control options may lead to some increases in crop yields and to some decreases in the amount of Orobanche seed in the soil, none of the currently available control measures have proved to be completely effective methods of Orobanche management.

Once an area is infected with Orobanche seeds, chemical treatment and husbandry methods cannot control this parasitic weed. The use of resistant cultivars can alleviate the problem (Erskine et al., 1993). Since genetic resistance to Orobanche was unknown in lentils, screening of Jordan lentils lines was undertaken.

Table 2. Mean of number of *Orobanche crenata* attachment on lentil roots grown in season 2007 and 2008

Accession No.	Number of attachments during the season	
	2006- 2007 Mean +/- SE	2007- 2008 Mean +/- SE
UJWL1	2.7 +/-0.62	2.1 +/-0.571
UJWL2	3.29 +/-0.75	1.9 +/-0.722
UJWL3	1.75 +/-0.48	0.8 +/-0.364
UJWL4	2.25 +/-0.63	1.1 +/-0.486
UJWL5	1.4 +/-0.4	1 +/-1
UJWL6	1.5 +/-0.5	0.6 +/-0.245
UJWL7	2.25 +/-0.25	1.3 +/-0.474
UJWL8	2.8 +/-1.4	1.4 +/-0.792
UJWL9	1.83 +/-0.4	1.1 +/-0.348
UJWL10	2.11 +/-0.54	1.6 +/-0.484
UJWL11	2.33 +/-0.72	1.6 +/-0.6
UJWL12	2.17 +/-0.6	1.6 +/-0.565
UJWL13	6.25 +/-1.4	2.3 +/-0.856
UJWL14	2.89 +/-0.87	2 +/-0.707
UJWL15	3.33 +/-1.11	2.5 +/-0.925
UJWL16	5.13 +/-1.17	3.7 +/-1.104
UJWL17	1.67 +/-0.67	1 +/-0.548
UWLJ18	3 +/-0.93	2.3 +/-0.792
UJWL19	3.8 +/-1.46	2.4 +/-1.117
UJWL20	3.6 +/-0.81	2.6 +/-0.724
UJWL21	2.43 +/-0.72	1.2 +/-0.482
UJWL22	1.5 +/-0.5	0.5 +/-0.342
Jordan 1 (check)	6.83 +/-1.22	6.6 +/-1.828

2. MATERIALS AND METHODS

Seeds of twenty two wild lentil accessions collected from different locations in Jordan, and stored for further reference or use in the seed bank at the University of Jordan, and the gene bank of the National Center for Agricultural Research and Extension (NCARE). Information on these accessions is in (Table 1).

To evaluate these accessions for their response to Broomrapes (*Orobanche* spp.) infection, two experiments were conducted: Experiment A under greenhouse condition and Experiment B under laboratory conditions

Table 3. Mean dry weight (g) of *Orobanche crenata* attached to lentil roots grown in season 2007 and 2008

Accession No.	Mean dry weight of *Orobanche crenata*	
	2006- 2007 Mean +/- SE	2007- 2008 Mean +/- SE
UJWL1	0.3 +/-0.13	0.23 +/-0.1017
UJWL2	0.21 +/-0.05	0.15 +/-0.049
UJWL3	0.07 +/-0.02	0.03 +/-0.015
UJWL4	0.26 +/-0.15	0.13 +/-0.08
UJWL5	0.08 +/-0.03	0.05 +/-0.026
UJWL6	0.03 +/-0.01	0.02 +/-0.014
UJWL7	0.13 +/-0.04	0.07 +/-0.035
UJWL8	0.19 +/-0.04	0.1 +/-0.037
UJWL9	0.09 +/-0.05	0.06 +/-0.028
UJWL10	0.31 +/-0.12	0.22 +/-0.092
UJWL11	0.23 +/-0.06	0.14 +/-0.051
UJWL12	0.42 +/-0.16	0.31 +/-0.135
UJWL13	0.35 +/-0.1	0.2 +/-0.07
UJWL14	0.11 +/-0.06	0.07 +/-0.042
UJWL15	0.13 +/-0.05	0.1 +/-0.03
UJWL16	0.58 +/-0.18	0.41 +/-0.144
UJWL17	0.2 +/-0.15	0.12 +/-0.09
UWLJ18	0.1 +/-0.03	0.07 +/-0.024
UJWL19	0.35 +/-0.28	0.22 +/-0.083
UJWL20	0.32 +/-0.11	0.22 +/-0.083
UJWL21	0.35 +/-0.17	0.16 +/-0.089
UJWL22	0.03 +/-0.13	0.006 +/-0.005
Jordan 1 (check)	0.54 +/-0.21	1.53 +/-0.941

Experiment A. Seeds of *Orobanche crenata*, collected from infested crop from previous seasons, were thoroughly mixed in potting media, peat moss, at a rate of 0.2 ml seeds per pot, one liter size, and 20-cm in diameter. Lentil seeds of the twenty two wild accessions were planted in infested pots, at rate of five lentil seeds per pot and grown in greenhouse during winter season 2006-2007. In addition, one local *Orobanche*-sensitive cultivated lentils (Jordan 1), were planted as a check treatment. Each line was planted in 15 pots (i.e. 15 replicates). All pots were placed in the greenhouse in a completely randomized design. The pots were irrigated

daily until *Orobanche* shoots appeared. Lentil plants were checked for *Orobanche* attachments on their roots. The dry weights of *Orobanche* and number of *Orobanche* attachments were recorded per pot.

The experiment was repeated the following season of winter 2007-2008, to verify the obtained results. In addition, Jordan1 lentil seeds were also planted as a check treatment.

Table 4. Percent of germination of *Orobanche crenata* seeds grown with seeds of lentil lines in the laboratory in 2007, 2008

Accession No.	Percent of germination of *Orobanche crenata* seeds (%)	
	2007 Mean +/- SE	2008 Mean +/-SE
UJWL5	21.7	36.8
UJWL6	5.1	6.4
UJWL22	4.4	4.9
Jordan 1 (check	13.5	17.2

Experiment B: This experiment was carried out on January 2008, under laboratory conditions to test the ability of the lines that showed tolerance to broomrape in the previous experiments, to induce seed germination of *Orobanche crenata.* Seeds of the cultivated lentil, Jordan 1, seeds of tolerant accession to *Orobanche* infestation in the previous experiments, accessions 5, 6 and 22, were surface sterilized by soaking in 5% chlorax solution for 10 minutes. Seeds were rinsed in sterilized tap water five to six times. Lentil seeds were placed in 250 ml-Erlenmeyer flasks at 4 seeds of each line per flask, with 4 replicates per lentil line. A volume of 10 mls of *Orobanche* seeds were surface sterilized for 10 minutes in 5% chlorax, then taken out on a filter paper, washed in sterilized tap water several times, then suspended in 200 ml sterilized water in a sterilized beaker. A volume of 5 mls of the *Orobanche* seed suspension plus 20 mls sterilized tap water were added to each flask under a laminar flow hood. Flask mouths were closed by Para film and all flasks were incubated at 20 C in dark for one week to assure lentil seed germination, then lights were turned on for 12 hrs a day. Eight weeks later, several samples were withdrawn from each flask to check for *Orobanche* seed germination, percent of *Orobanche* seed germination was calculated as follows; germinated and non-germinated *Orobanche* seeds were counted under binocular. Seventy five counts were carried out per flask. Lentil roots were also examined for any *Orobanche* attachment. This experiment was repeated to check for consistency.

Standard error of the mean was calculated to separate means of the parameters.

3. RESULTS AND DISCUSSION

Development of improved cultivars with resistance to certain pest is by far one of the most effective methods of pest management if a good source of resistance is available. However resistance against most parasitic plants is scarce, but, significant successes were achieved in some crops, including sunflower cultivars resistant against *O. cumana* (Labrousse et al., 2001). Resistance or tolerance against *Orobanche* spp in wild types was identified in many crops including tomato lines that are resistant against *O. ramose* and *O. aegyptiaca* (Kasrawi and Abu-Irmaileh, 1989); resistance in wild legumes against *O.crenata* (Rubiales, 2003). Similarly, resistance against *O. foetida* has also been identified in faba bean germplasm. Some lines selected against *O. crenata* have also shown a high level of resistance to *O. foetida*, with a high yield potential (Abbes et al., 2007). Tolerance against *O. crenata* was also found in wild lentils (Ferna´ ndez-aparicio *et al*., 2009) and in sunflower (Fernandez-Martinez *et al*., 2000)

Certain wild lentil lines collected from Jordan had shown high level of tolerance to *O.crenata*. There were high significant differences between the entries screened for *Orobanche* tolerance. The most susceptible entry was the local lentil cultivar, Jordan 1.

However, data analysis grouped the tested lines in groups ranging in susceptibility from high susceptibility to tolerant levels. According to number of *Orobanche* attachments (Table 2), and *Orobanche* dry weights that developed on lentils roots (table 3) the most tolerant accessions were; 3, 5, 6, and 22 as these accessions had the lowest values of the mentioned parameters in

experiments of 2007 and 2008. Even though accession number 5 induced higher *Orobanche* seed germination (table 4) than the local susceptible cultivar, but *Orobanche* failed to attach to its roots (tables 2 and 3). This result could indicate that the tolerance to *Orobanche* in this line was the lower ability of the parasite to form attachments.

Experiments on inducing *Orobanche* seed germination during lentil, seed germination showed that seeds from accessions 5, 6 and 22, induced *Orobanche* seed germination in the laboratory at varying degrees (Table 4). The least germination percentage obtained was from accessions 6 and 22. Seeds from accession 3 were not available for testing.

These genotypes could be included in further breeding to incorporate the tolerance feature in high yielding cultivars.

It is worth mentioning that the two tolerant accessions belong to the wild species *Lens culinaris* subsp. *orientalis.* Seeds of accession number 3 were collected from Ajlun area (Ein AlTeas) which is a mountainous area north of Jordan, whereas accession number 22 was collected from Irbid Governorate with moderate elevation.

To the best knowledge of the authors, no previous reports indicated tolerant/resistant to *Orobanche* in Jordanian lentil wild lines. Therefore, these results could be of significant importance, but need further research for their verifications by testing them under naturally infested field and if needed to be confirmed by DNA analysis. If the results were confirmed further, these accessions could be used as a source for crossing with cultivated lentils aiming at the development of lentil cultivars tolerant to *Orobanche,* a serious problem in lentil cultivation and several other important crops which suffer from this parasitic plant.

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دلائل على تحمل الاصابة بالهالوك في خطوط عدس برية جمعت من الأردن

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ملخص

يعتبر الهالوك Orobanche crenata من اخطر الآفات التي تهدد البقوليات حول العالم. أن جميع أصناف العدس المزروعة حساسة جدا للإصابة بهذا النبات المتطفل. ويتوقف تهجين أصناف عدس تقاوم الإصابة بالهالوك على وجود مصدر وراثي للمقاومة. لقد تم جمع اثنين وعشرين خطاً برياً من العدس من مواقع مختلفة من الأردن وتم إجراء الدراسات المخبرية وفي البيوت الزجاجية على مدى موسمين (2007 و2008) ولوحظ أن هناك مدى واسعاً من تحمل الخطوط البرية للإصابة بالهالوك. بينت النتائج ان أعلى درجة تحمل كانت للأجناس التابعة للأنواع Lens ervoides وens culinaris subsp. orientalis والتي جمعت من مناطق المنصورة، الصفصافة، عين التيس وإربد. وكانت أكثر الخطوط تحملا هي الخطوط ذات الأرقام 3، 5، 6، 22. وكان سبب التحمل هو ضعف قدرة بذور الهالوك للإنبات على جذور العدس في الخطوط 3، 6، 22 وضعف إمكانية نمو ممصات الهالوك على الجذور للخطوط 3، 5، 6، و22. تحتاج هذه النتائج إلى مزيد من الدراسات الحقلية تحت ظروف الإصابة الطبيعية، والى دراسات تحليل المادة الوراثية DNA والتي يمكن بعد التأكد من درجة التحمل أو المقاومة للإصابة بالهالوك ادخال الخطوط المذكورة في برنامج تطوير محاصيل عدس مقاومة لهذا النبات المتطفل.

الكلمات الدالة: تهجين، خطوط عدس، الأردن، تحمل، مقاومة، هالوك.

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