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Search for Resistance to Egyptian Broomrape (*Orobanche* Aegyptiaca) in Tomato Germplasm

البحث عن مصادر مقاومة لطفيل الهالوك في محصول البندورة

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Abstract

This experiment was conducted to compare the level of resistance to *Orobanche aegyptiaca* (also known as broomrape) in a collection of sixty wild and cultivated tomato spices. One commercial susceptible variety 'Samara' was used as control. The experiment was performed in the framework of Complete Randomized Block Design (CRBD), with three replicates. Two plants from each accession were transplanted into open field in 2009. Three traits were studied including days to first appearance of *Orobanche* shoots, weakness of tomato plants (vegetative growth) and number of emerged *Orobanche* shoots per tomato plant. Number of *Orobanche* shoots per tomato plant ranged from 3.8 to 9.2 shoots compared with the susceptible check, with an average of 6 emerged *Orobanche* shoots per plant. The accessions were divided into three clusters. Accessions within the first cluster could be considered as the most resistant accessions in the collection since the average number of *Orobanche* shoots per tomato plant was low (4.49) and at the same time tomato plants were very strong (weaknesses value = 2.26). Further histological studies to understand the mechanism of resistance in these accessions would be advisable.

Keywords: Level of Resistance, *Lycopersicon Spp.*, *Orobanche Aegyptiaca*, Tomato.

ملخص

أجريت هذه التجربة بهدف مقارنة مستويات مقاومة مجموعة من أصناف البندورة العادية والبرية لطفيل الهالوك. أستخدم في التجربة 60 نوعا من البندورة العادية والبرية بالإضافة إلى صنف تجاري (سمارة) كشاهد حساس لطفيل الهالوك. أجريت التجربة في بلدة قباطية في محافظة جنين في الموسم الزراعي 2009. زرعت الأشتال في الحقل المكشوف بتاريخ 2009/04/20 وفق النظام العشوائي التام في ثلاث مكررات، ومثل كل نوع من أنواع البندورة بنبتتين في كل مكرر. لم يتم إجراء أي نوع من العدوى لأرض التجربة كونها معروفة بشدة إصابتها بطفيل الهالوك. خلال موسم النمو تم تسجيل موعد ظهور نباتات طفيل الهالوك حول نباتات البندورة، وكذلك قوة النمو الخضري لنباتات البندورة والعدد النهائي لنباتات طفيل الهالوك حول نباتات البندورة وذلك في أخر الموسم الزراعي. أظهرت النتائج تفاوتا في مدى مقاومة أصناف البندورة لطفيل الهالوك حيث تراوح مدى المقاومة ما بين ضعيفة الى متوسطة. كان معدل عدد نباتات طفيل الهالوك الظاهرة حول نباتات الشاهد (صنف سمارة) 6 نباتات، بينما بلغ عدد نباتات الهالوك الظاهرة حول بقية أنواع البندورة المستخدمة في التجربة بين 3.8 – 9.2 وبمعدل 5.7 نبات لكل صنف. أظهرت النتائج أنه يمكن تقسيم أنواع البندورة المستخدمة في التجربة الى ثلاث مجموعات. يمكن إعتبار الأصناف الواقعة ضمن المجموعة الأولى (22 صنف) بأنها أكثر الأصناف مقاومة لطفيل الهالوك حيث بلغ معدل عدد نباتات الهالوك النابتة 4.49 نباتات وبنفس الوقت كانت نباتات البندورة ذات مجموع خضري قوي. نظراً لتأثر طفيل الهالوك بالعوامل الجوية والبيئية الأمر الذي يستدعي إجراء مزيد من التجارب بهدف تأكيد النتائج والعمل على إدخال هذه الأصناف كأصول للبندورة للحد من تأثير طفيل الهالوك على محصول البندورة.

الكلمات المفتاحية: البندورة، الهالوك، درجة المقاومة.

Introduction

Tomato (*Lycopersicon esculentum*) is very important vegetable crop in Palestine where it plays very important role in national agricultural income. Tomato was considered as one of the main elements of Palestinian diets. This importance was achieved from the wide range of usage in cooking, juice and salad in addition to direct (fresh) eating. The total agricultural area in Palestine is around 1,853,951 dunums from which about 24,921 dunums are cultivated with tomato producing 213,212 tons varying from year to year according to environmental conditions and technical management (PCBS, 2009). Tomato production constrained by several difficulties, one of these difficulties is parasitic weeds (Goldwasser, *et al.* 2001, Rubiales, *et al.* 2002); the most dangerous weed is *Orobanche spp.* (Joel, 2000).

The genus *Orobanche* has more than 150 species among which only a few parasitize agronomic crops. Broomrapes vary in host range, some parasitizing a broad range of crops, whereas others are more specific. *O. ramosa* L. has the widest host range, parasitizing many solanaceous crops such as potato (*Solanum tuberosum,* L.), tobacco (*Nicotiana tabacum,* L.) and tomato (*Lycopersicon esculentum*). *O. aegyptiaca* has a host range similar to that of *O. ramosa*, and is also parasitic on carrot (*Daucus carota* L.), legumes such as common vetch (*Vicia sativa* L.), and tomato (Musselman, 1980).

The main type of *Orobanche* affected tomato crop in Palestine is *Orobanche aegyptiaca* (Ministry of Agriculture, unpublished data). As weeds, they cause reductions in crop yield, adversely affect crop quality, and result in loss of cultivated land due to reduced crop alternatives available to farmers. The presence of *Orobanche* in a field may force farmers to plant a less economical, non-host crop or to leave the field fallow. For instance, many productive solanaceous vegetable growing areas had to be abandoned in Palestine due to *Orobanche* infestations and lack of other economically viable crop choices (Ministry of Agriculture, unpublished data).

Management of *Orobanche* is often difficult due to several reasons. These difficulties include the high amount of seed production, viability of seeds in the soil over several years (Cubero & Moreno 1979; Linke & Saxena, 1991; Puzzilli, 1983). Numerous approaches have been tested to control Orobanche such as cultural and mechanical control methods, delayed planting of host crops, soil fumigation, soil solarization, trap and catch-crops, biological control, herbicides, herbicide-resistant genetically engineered crops, tolerant and resistant cultivars. Most of these control measures turned out to be inadequate or difficult to apply or very costly (Foy, *et al.* 1989; Dhanapal, *et al.* 1996; Joel, 2000; Goldwasser, *et al.* 2003; Rubiales, *et al.* 2003; Pérez-de-Luque, *et al.* 2004). Sulfonylurea and imidazolinone herbicides were reported to control *Orobanche*, but in most cases, their application was highly limited by their low crop selectivity (Goldwasser, *et al.* 2001).

Breeding for resistance is the most feasible, economic and environmental friendly method to control pathogens and parasites. However, appropriate effective selection indices and screening methods are still needed to ensure success. Resistance against most parasitic weeds is difficult to access, scarce, of complex nature and of low heritability, making breeding for resistance a difficult task. In spite of these difficulties, significant success has been achieved in some crops (Goldwasser, *et al.* 2001; Rubiales, *et al.* 2002; 2006).

The objective of this experiment is to evaluate the level of resistance in a collection of cultivated and wild tomatoes to *Orobanche aegyptiaca* in open field.

Materials and Methods

Plant material

Sixty accessions belonging to different species of *Lycopersicon* were kindly provided by the CM Rick Tomato Genetics Resource Center, University of California / Davis and Plant Genetic Resources of Canada. Thirty nine of these accessions are cultivated species (Table 1), while twenty one of these accessions are wild species (table Table 2). One commercial susceptible variety ‘Samara’ was used across the experiment as a control variety.

Table (1): Cultivated tomato *(Lycopersicon* spp.) accessions used in the experiment.

No.	Accession Code	*Lycopersicon* spp.	Origin
1	LA0113	*L. esculentum*	Peru
2	LA0126	*L. esculentum*	Ecuador
3	LA0134C	*L. esculentum*	Peru
4	LA0146	*L. esculentum*	Mexico
5	LA0147	*L. esculentum*	Honduras
6	LA0358	*L. esculentum*	Colombia

…continue table (1)

No.	Accession Code	*Lycopersicon spp.*	Origin
7	LA0395	*L. esculentum*	Peru
8	LA0404	*L. esculentum*	Peru
9	LA0466	*L. esculentum*	Chile
10	LA0468	*L. esculentum*	Chile
11	LA0473	*L. esculentum*	Peru
12	LA0477	*L. esculentum*	Peru
13	LA1251	*L. esculentum*	Ecuador
14	LA2283	*L. esculentum*	Peru
15	LA2703	*L. esculentum*	Sri Lanka
16	LA1162	*L. esculentum*	Cuba
17	LA1021	*L. esculentum*	Brazil
18	CN1355	*L. esculentum*	Canada
19	CN17695	*L. esculentum*	Canada
20	CN386	*L. esculentum*	Canada
21	CN74	*L. esculentum*	Canada
22	LA0409	*L. esculentum*	Ecuador
23	LA0168	*L. esculentum var.cerasiforme*	Fr.Oceania
24	LA0172	*L. esculentum var.cerasiforme*	Bolivia
25	LA0292	*L. esculentum var.cerasiforme*	Ecuador
26	LA0349	*L. esculentum var.cerasiforme*	Unknown Origin
27	LA0475	*L. esculentum var.cerasiforme*	Ecuador
28	LA1203	*L. esculentum var.cerasiforme*	Guatemala
29	LA1204	*L. esculentum var.cerasiforme*	Guatemala
30	LA1206	*L. esculentum var.cerasiforme*	Honduras
31	LA1425	*L. esculentum var.cerasiforme*	Colombia
32	LA1426	*L. esculentum var.cerasiforme*	Colombia
33	LA1482	*L. esculentum var.cerasiforme*	Malaysia
34	LA1509	*L. esculentum var.cerasiforme*	Borneo
35	LA1510	*L. esculentum var.cerasiforme*	Mexico

…continue table (1)

No.	Accession Code	*Lycopersicon spp.*	Origin
36	LA1511	*L. esculentum var.cerasiforme*	Brazil
37	LA1512	*L. esculentum var.cerasiforme*	El Salvador
38	LA2660	*L. esculentum var.cerasiforme*	Bolivia
39	LA2702	*L. esculentum var.cerasiforme*	Sri Lanka
40	Samara (control)	*L. esculentum*	Commercia l Variety

Table (2): Wild tomato (*Lycopersicon* spp.) accessions used in the experiment.

No.	Accession Code	*Lycopersicon* spp.	Origin
1	LA0521	*L. cheesmanii*	Ecuador
2	LA0456	*L. chilense*	Peru
3	LA3112	*L. chilense*	Peru
4	LA2695	*L. chmielewskii*	Peru
5	LA1353	*L. hirsutum*	Peru
6	LA2864	*L. hirsutum*	Ecuador
7	CN7544	*L. hirsutum*	Canada
8	CN89	*L. hirsutum*	Canada
9	LA1326	*L. pariflorum*	Peru
10	CN3722	*L. peruvianum*	Canada
11	LA0103A	*L. peruvianum*	Peru
12	LA0103B	*L. peruvianum*	Peru
13	LA1274	*L. peruvianum*	Peru
14	LA1677	*L. peruvianum*	Peru
15	LA2153	*L. peruvianum*	Peru
16	LA0722	*L. pimpinellifolium*	Peru
17	LA1659	*L. pimpinellifolium*	Peru
18	LA2181	*L. pimpinellifolium*	Peru
19	CN18198	*L. pimpinellifolium*	Canada
20	LA0411	*L. pimpinellifolium*	Ecuador
21	LA1586	*L. pimpinellifolium*	Peru

Experiment Design

Field experiment was conducted in naturally heavily infested field with *Orobanche* in Qabatya town near to Jenien district, Palestine during 2009 growing season. All accessions were transplanted to the open field at the 20^{th} of April 2009 in three complete randomized blocks. Each accession was represented by two plants in a single row, 1 m long per replicate, the distance between the represented rows in the same line equal 1 m and between the paralleled lines equal 1.5 m. The susceptible tomato variety ‘Samara’ was planted as a control one every five accessions alternatively with the represented rows. Each replicate occupied $216m^2$ from the experiment field.

Cultural practices

Hand weeding was done two times during the growing season. Irrigation applied during spring through drip irrigation system. Aphid control was done one time by spraying the plants with insecticide (Imidacloprid: 1 cm^3 per liter of water) four weeks after planted.

Data Collection

During the growth period the following data were recorded:

1. Appearance date of first emerged *Orobanche* plants around every accession plants were observed and recorded every two days.
2. The final number of emerged parasite shoots per host plant was accounted at the end of experiment on 10^{th} august, 2009.
3. Plants strength was visually observed at the end of experiment when the emergence of *Orobanche* shoots was stopped using the scale 1-4 where 1 = very strong, 2 = strong, 3 = weak and 4 = dry.

Statistical analysis

Analysis of variance (ANOVA) was conducted by using the SPSS program (version 15). Cluster analysis was conducted by using the complete-linkage method (SPSS, 2002).

Results

The final number of emerged *Orobanche* per host plant

Moderate levels of resistance were found in some species of *Lycopersicon* (Table 3). The susceptible tomato check was infected uniformly across the plot with emerged broomrape plants ranging from 5.8 to 6.4 shoots per tomato plant with an average of 6 emerged broomrape shoots per plant. Broomrape infection on the 60 tomato accessions ranged from 3.8 to 9.2, with an average of 5.7 emerged shoots per host plant.

Table (3): Levels of broomrape infection in tomato (*Lycopersicon* spp.) in the field in 2009, expressed as the percentage of emerged broomrape shoots per plant relative to the susceptible check cv. Samara.

***Lycopersicon* spp.**	**Mean[1]**	**Range[1]**
L. cheesmanii	98	98
L. chilense	101	94-108
L. chmielewskii	112	112
L. esculentum	96	67-150
L. hirsutum	85	73-95
L. pariflorum	118	118
L. peruvianum	97	80-123
L. pimpinellifolium	89	70-114
L. var.cerasiforme	93	64-136
Samara (Susceptible control)	100 (6)	100 (5.8-6.4)

[1] Maximum and minimum percentage of emerged broomrape plants for each species, relative to the susceptible check, tomato cv. Samara (=100%). Real values for Samara in parentheses.

The reaction of the tested accessions in the field ranged from very susceptible to moderately resistant (64% to 150% of the average of their surrounding rows of cv. Samara) (Figure 1).

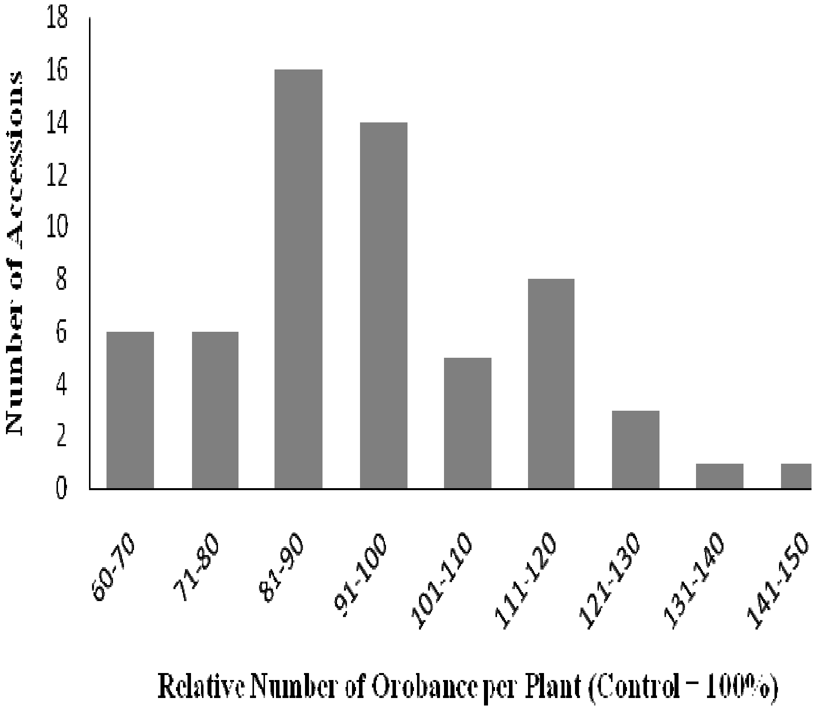

Figure (1): Distribution of the 60 tomato accessions according to the relative number of *Orobanche* shoots per tomato plant.

Most of the accessions studied (about 80%) showed high susceptibility to *Orobanche*. However, *Orobanche* emergence was particularly low in 9 accessions. These accessions could be considered as the most resistant accessions in the collection since they were clustered in the first cluster with a low average number of *Orobanche* shoots per tomato plant and at the same time tomato plants were very strong (Table 4 and Figure 2). Whereas, other 7 accessions showed high level of tolerance to *Orobanche* since the average number of *Orobanche* shoots per tomato plant was high and at the same time tomato plants were very strong (Table 5 and Figure 2).

Table (4): Accessions showed high level of resistance to *Orobanche*.

Accession Code	***Lycopersicon* spp.**	**Relative number of *Orobanche* shoots per plant**	**Plants strength**
CN3722	*L. peruvianum*	80	2
LA0411	*L. pimpinellifolium*	78	2
LA1511	*L. esculentum var.cerasiforme*	77	2
LA1509	*L. esculentum var.cerasiforme*	75	2
LA1353	*L. hirsutum*	73	2
LA0349	*L. esculentum var.cerasiforme*	73	2
LA0475	*L. esculentum var.cerasiforme*	70	2
LA1659	*L. pimpinellifolium*	70	2
LA1425	*L. esculentum var.cerasiforme*	64	2

Table (5): Accessions showed high level of tolerance to *Orobanche*.

Accession Code	**Lycopersicon spp.**	**Relative number of *Orobanche* shoots per plant**	**Plant strength**
LA2703	*L. esculentum*	150	2
LA1206	*L. var.cerasiforme*	136	2
LA0168	*L. var.cerasiforme*	124	2
LA2660	*L. var.cerasiforme*	120	2
LA1426	*L. var.cerasiforme*	120	2
CLA1274	*L. peruvianum*	117	2
LA0292	*L. var.cerasiforme*	111	2

Appearance date of first emerged *Orobanche* plants

By 57 days after planting, the numbers of *Orobanche* shoots that emerged around the susceptible control (Samara) plant were 6 shoots per plant, this infection level causes high damage to the plants, which did not developed normally throughout the experiment. None of the tested accessions showed significant difference from the susceptible control for the days from transplanting to the first appearance of *Orobanche* plants (data not shown).

Weakness level of tomato accessions at the end of experiment

Tomato accessions plants influenced greatly by broomrape infestation. Weakness level of accessions plants was divided visually at the end of experiment to four levels: level one for very strong plants and level four for dried plants, average weakness level of the susceptible control accession plant was high 3.07, means that broomrape emerged around the control accession was absorbed water and minerals from the roots of the control accession and so affected on strength of the control accession leaving it more weak (less strong), some accessions plants more susceptible to the control accession and becomes more weak after infested with broomrape, others are more tolerant to broomrape infestation relative to the control accession, in general none of the tested accessions showed significant difference from the susceptible control accession plant related to the weakness level.

Cluster analysis

Hierarchical cluster analysis was conducted using average linkage (between groups) to conduct a dendrogram. The accessions were divided into three clusters (Figure 2) based on the average of emerged broomrape shoots per host plant and weakness level. Accessions within the first cluster (composed of 22 accessions) could be considered as the most resistant accessions in the collection since the average number of *Orobanche* shoots per tomato plant was low (4.49) and at the same time tomato plants were very strong (weaknesses value = 2.26). Accessions in the second cluster could be considered as susceptible accessions because average number of *Orobanche* shoots per tomato plant was high (5.69) and weaknesses value of tomato plants were high (3.32). While accessions present in the third cluster distinguished as tolerant accessions because average number of *Orobanche* shoots per tomato plant was high (7.38), at the same time weaknesses value of tomato plants were low (2.24) (Table 6).

Table (6): Accessions distribution according to Hierarchical cluster analysis**.**

Cluster	Average Linkage	Average Number of *Orobanche* per Plant	Average Weakness Level of Accessions
1	mean	4.49^{c*}	2.26^{b*}
2	mean	5.69^{b}	3.32^{a}
3	mean	7.38^{a}	2.24^{b}

*Means followed by the same letters within the same columns are not significantly different according to Duncan's multiple range test at 5% level.

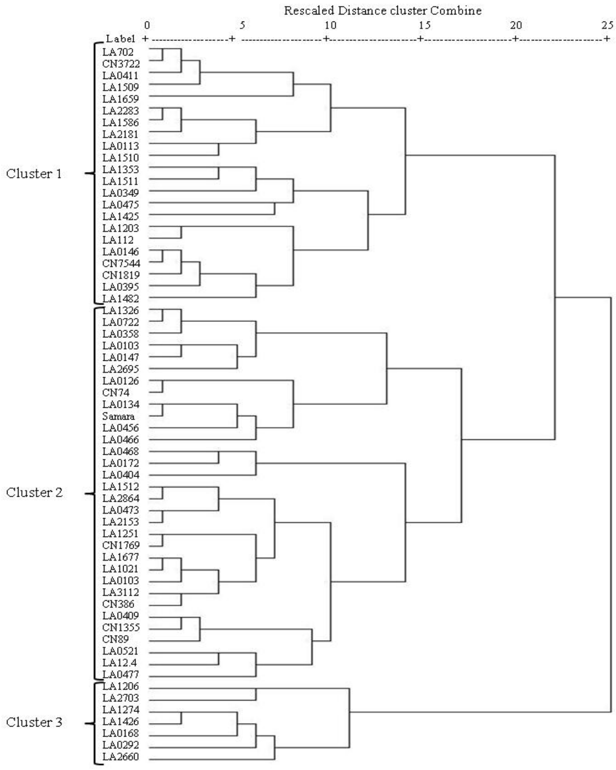

Figure (2): Dendrogram represent complete linkage between accessions groups according to Hierarchical cluster analysis.

Discussion

Egyptian Broomrape is potentially one of the major constrain for tomato cultivation in the Palestinian Territory and mainly through Jenin district (Ministry of agriculture (MoA), personal communication). The lack of resistance and a suitable control method has relegated tomato cultivation in infested areas. The high potential of tomato in Palestinian farming systems reinforces the need to solve the problem. Because most of the recommended control methods have not been successful, the use of resistant cultivars seems to be the most desirable solution. A major problem for breeding of broomrape resistance is the lack of an effective selection criteria and a suitable screening method (Cubero, 1991).

Several indices have been used by different authors to measure the levels of resistance to broomrape, such as total weight of broomrapes per host plant, height of the parasitic shoots, number of broomrapes per unit of grown surface, rate of broomrape reproduction, etc. (Cubero, 1991; Rubiales et al., 2002), but the favorite index for resistance to broomrape is the total number of emerged shoots per host plant (Gil *et al.*, 1987; Cubero, 1991).

The results of the present study indicate that the resistance response of tomato genotypes to *O. aegyptiaca* was not high which is not agreement with the results obtained by El-Halmouch, *et al.* (2006) who reported that some wild relatives, belonging to the *Lycopersicon* genus (*L. pimpinellifolium*, *L. pennellii*, *L. chilense* and *L. hirsutum*), were demonstrated to be completely resistant to *O. aegyptiaca*. Mean while Abu-Gharbieh, *et al.* (1978) reported that wild tomato species were moderately resistant to *O. ramose*. Dalela and Mathur, (1971) evaluate 41 wild tomato species and they found that only one line was moderately resistant to *O. cernua*. Abedeev and Scherbinin, (1982) found that the highly homologous tomato line PZU-11uniformly resistant to *O. aegyptiaca*. However, Foy, *et al.* (1988) reported that PZU-11did not show any resistance to *O. aegyptiaca*. There was no explanation for the reason why PZU-11 did not show any level of resistance to *Orobanche* in the second experiment.

The growth and development of broomrape, like that of the host plant is affected by the environmental conditions. High rainfall and mild soil temperature during December–February favor growth of the crop root system as well as the germination and attachment of broomrape (López-Granados & García-Torres, 1993). Infection is reduced in years with low temperature in winter (Arjona-Berral, *et al.* 1987). For years in which the spring is dry and warm temperatures start early, limiting the host vigor, the emergence of underground broomrape shoots is also hampered. Temperature and moisture influence seed germination, infection and development of broomrape. *Orobanche* seeds germinate in the presence and proximity of roots of a suitable host (Rubiales, *et al.* 2005). During our experiment period, the average temperature and rainfall was 23.9 ºC and 3.2 mm (PMD, 2010) which was very close to the optimum temperature for *Orobanche* germination and growth. Kebreab and Murdoch, (2000) reported that maximum germination of *O. aegyptiaca* occurred at 20–26°C.

Broomrape attack is related to the growth vigor of the host and there is a competition for resources among attachments (Aalders & Pieters, 1987), thus, indices based on size and weight of broomrapes can be misleading. The lower the amount of attachments, the bigger they are, resulting in similar weights of broomrape collected on susceptible and resistant plants (Borg, *et al.* 1994). This is in agreement with our findings where several accessions were strong (high vegetative growth) and at the same time they were heavily infected with *Orobanche* plants. The broomrapes on resistant plants might have a high growth rate and could reach similar or even larger sizes than those of susceptible hosts. As broomrape attack appears to be related to the growth vigor of the host, it is necessary to exclude this misleading effect when interpreting the results, otherwise, we will be unconsciously selecting for plants with reduced plant vigor, reduced root biomass or short growth cycle, which might be agronomically undesirable (Rubiales, *et al.* 2005).

From the results of the present experiment, we can conclude that there was an observed variation between accessions. Several accessions (Table 4 and 5) could be used as a valuable source of resistance in tomato to *O. aegyptiaca* but at the same time more studies should be needed to study the mechanisms of resistance present in these accessions both in the field and controlled conditions. The final number of emerged *Orobanche* and the strength of the vegetative growth of the tomato are two of the most important parameters could be used to detect the level of resistance to *Orobanche* and further studies are needed to study the level of resistance in these accessions at the molecular and histological levels.

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