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Resistance of Some Tomato Species to Orobanche Aegyptiaca

(Comparative study)

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Dedication

This work is dedicated to my brothers, sisters and my friends; the completion of this work was not possible without their support and help.

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الاقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان :

Resistance of Some Tomato Species to Orobanche Aegyptiaca (Comparative study)

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Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name : **اسم الطالب :**

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List of Abbreviations

Abbreviation	Full Name
ANOVA	Analysis-of-Variance
ºC	Degree Centigrade
Cm	Centimeter
CRBD	Complete Randomize Block Design
cv	Cultivar
2,4-D	2,4-Dichlorophenoxy Acetic Acid
2,4-DB	4-(2,4-Dichlorophenoxy)Butyric Acid
EPA	Environmental Protection Agency
kg ha-1	Kilogram Per Hectare
µg	Microgram
mm	Millimeter
mM	Millimole
MOA	Ministry of Agriculture
PCBS	Palestinian Central Bureau of Statistics
PMD	Palestinian Metrological Department
pH	Power of Hydrogen
Spp	Species
SPSS	Statistical Package for the Social Sciences
wk	Week

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Abstract

This study was conducted to compare the level of resistance to *Orobanche aegyptiaca* in a collection of wild and cultivated tomato in open field. Sixty tomato accessions (*Lycopersicon* spp.) and one commercial variety 'Samara' were used. The experiment was conducted in Jenin District, Palestine during the 2009 growing season. The experiment was performed in the framework of a complete randomized block design (CRBD), with three replicates. Two plants from each variety per replicate were transplanted into open field by the 20th of April 2009. No artificial inoculation with broomrape seed was done at the time of transplanting since the field known to be heavily infected with broomrape from the previous growing season when the farmer was planted it with the same commercial tomato (Samara). Three traits were studied including days to first appearance of *Orobanche*, Weakness of tomato plants (vegetative growth) and number of emerged *Orobanche* per tomato plant. Moderate levels of resistance were found in some species of *Lycopersicon.* 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. Broomrape infection on the sixty tomato accessions used in the experiment ranged from 3.8 to 9.2, with an average of 5.7 emerged shoots per host

plant compared with the susceptible tomato check, with an average of 6 emerged broomrape shoots per plant. The accessions were divided into three clusters, the first one composed of 22 accessions, the second one composed of 32 accessions and the third cluster composed of 7 accessions. Each cluster was distinguished by one or more of the measured parameters. 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.

Chapter One

Introduction

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 every year producing 213,212 tons varying from year to year according to environmental conditions and technical management (PCBS, 2009). Many Palestinian farmers depend on tomato production as the main crop in their farms due to its production period (9-11 month in greenhouse and 3 months in open field) and productivity (Ministry of Agriculture, personal communication).Tomato production constrained by several difficulties, one of these difficulties is parasitic weeds (Goldwasser et al., 2000; 2001; Rubiales et al., 2002), the most dangerous weed is *Orobanche spp* ; (Joel, 2000), the main type of *Orobanche* affected tomato crop was called *Orobanche aegyptiaca*. Parasitic plants are those that are dependent on other autotrophic plants for survival, for part or all of their life cycle. Parasitic plants belong to 17 different families, only eight of them are considered weeds. Witchweed (*Striga* spp.) and broomrape (*Orobanche spp.*) are the most economically important parasitic weeds in cultivated crops. This research is focused primarily on management of broomrapes. However, literature on

witchweed and other similar parasitic weeds was cited to illustrate certain aspects of broomrapes biology where research is lacking.

Broomrapes are phanerogamic holoparasites that attack the roots of many dicotyledonous crops. They lack chlorophyll (Baccarini and Melandri, 1967) and obtain carbon, nutrients, and water through haustoria which connect the parasites with the host vascular system.

Broomrapes belong to the family *Orobanchaceae*. The genus *Orobanche* has more than 150 species (Musselman,1980) 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.

The objective of this study is to compare the level of resistance between a collection of cultivated and wild tomatoes to *Orobanche Aegyptiaca* in open field in Qabatya town in 2009 growing season.

Chapter Two

Literature Review

2. Literature Review

2.1. Geographic Distribution

The majority of broomrapes are found in the warm and temperate parts of the northern hemisphere, especially the Mediterranean region (Sauerborn, 1991), but some species have spread to many other parts of the world. *O. aegyptiaca* occurs mainly in south-eastern Europe, northeastern Africa, and the Middle East, whereas *O. ramosa*, which is closely related to *O. aegyptiaca*, is mostly found in the Middle East. *O. cernua* and *O. cumana* are primarily distributed in the Middle East, southern and eastern Europe, and northern Africa. *O. crenata* is restricted to the Middle East.

2.2. Morphology and Biology

Stem of broomrape 15-50 cm, usually branched, yellowish, up to 6-8 mm thick in its midsection, with slight thickening at base. Plant covered with short, glandular hairs. Inflorescence cylindrical, friable, almost equal to or longer than the rest of the stem, up to 25 cm in length. Corolla 25-35 mm in length, tubular, considerably broadened, almost erect, blue-violet or sky blue, lighter at base of tube, whitish, covered with short, sparse, glandular hairs outside, glabrous inside. Calyx 8-14 mm in length, usually very light, whitish, covered with short, glandular hairs. The parasite prefers cultivated (rarely wild-growing) plants of more than 90 species in different families of flower plants.

2.3. Broomrape Ecology

The soil is the main source of the broomrape. Optimum conditions for seed germination in soil are temperatures 18-23°C and humidity 70% to 80%. Seeds of the parasite do not sprout at exceed soil humidity and temperatures less than 10°C or more than 35°C.

2.4. Host Range

Broomrapes usually parasitize only broadleaved plants. Branched broomrape attacks many crops and common weeds. Non-host crops can support infestations because of their weed component, and the production of such crops may carry broomrape seeds.

Some broomrape species have specialized biotypes or races. Plants of the same species attack different sets of hosts in different geographic areas. Susceptibility to attack also depends on the cultivar or genetic characteristics of a particular crop. The plants attacked by branched broomrape around the world include:

2.4.1. Crops and pasture plants*:* bean, broad bean, cabbage, capsicum (peppers), canola, carrot, cauliflower, celery, chickpea, clovers, eggplant, hemp, hops, lentil, medics, onion, parsnip, paprika, pea, pyrethrum, sunflower, tobacco, tomato, potato.

2.4.2. Weeds: amaranths (*Amaranthus*), Bathurst burr (*Xanthium spinosum*), black nightshade (*Solanum nigrum*), capeweed (*Arctotheca*

calendula), cretan weed (*Hedynopsis rhagadioloides*), dandelion (*Taraxacum*), deadnettles (*Lamium* species), Mexican poppy (*Argemone ochroleuca*), skeleton weed (*Chondrilla juncea*), stemless thistle (*Onopordum acaulon*), wild turnip (*Brassica tournefortii*), yellow hawkweed (*Tolpis barbata*).

2.5. Economic Importance.

It is no wondered that broomrapes are called 'halook' in Arabic, which refers to ancient invaders who ransacked Egypt. They cause extensive damage by reducing the yield of parasitized crops. For example, in the former USSR, *O. aegyptiaca* caused a 50% reduction in yield of watermelon [*Citrullus lanatus* (Thunb.) Mansf.] (Panchenko, 1974), 13 to 52% in muskmelon (*Cucumis melo* L.), and 15% in tomato (Kabulov and Tashpulatova, 1974). Yield losses have also been reported in broad bean (Mesa-García and García-Torres, 1984), sunflower (Shalom et al., 1988) and tomato (Cordas, 1973). Although it is hard to make exact estimates of the above yield losses due to the difficulty in creating broomrape-free plots for comparison with infested plots, the potential for loss in crop yield due to broomrape infestations is never over estimated.

Broomrape infestations have been reported to decrease the area under cultivation. Broad bean a major legume crop in Egypt, Morocco, and the Middle East, is subjected to devastation by *O. crenata*. The presence of broomrape plant material in harvested crop production may be reduce its

value or make it unmarketable. For instance, in Israel, the value of hay was reduced due to the presence of broomrape stalks (Foy et al., 1989).

2.6. Growth and Development of Broomrapes

2.6.1. Seeds

Broomrapes are annuals that reproduce by seeds. Seeds are usually dark brown, oval shaped, measure 0.35 x 0.25 mm and weigh 3 to 6 µg (Parker and Riches, 1993). They have a pattern of raised ridges on their surface. There is a hardened testa, surrounding a fatty endosperm that has an undifferentiated embryo at one end. The number of seeds per plant varies from 10^5 to 5 x 10^5, depending on the species.

2.6.2. Germination

Broomrape seed germination occurs only in response to a chemical signal from the host root. Before germination, broomrape seeds must undergo conditioning under suitable temperature and moisture conditions. The conditioning phase may range from five to several days, depending on the species. The requirement of conditioning of the seeds is not completely understood. Following the conditioning phase, the seed produces a ‘germ tube’ or radical in response to a chemical stimulant from the host root. The stability of the chemical stimulant is very short-lived in the soil. Several factors influence germination of broomrapes in the soil including temperature, moisture, pH, nutrients, soil type, and stimulants produced by

host plants. The influence of osmotic stress and temperature on broomrape germination and subsequent development is briefly discussed. Reports of inhibitory effects of nitrogen on the growth of broomrapes, including germination have been common in the literature for many years (Parker and Riches, 1993).

Optimum temperatures for conditioning and germination are different among broomrape species. Studies on the effect of temperature on germination of *O. aegyptiaca*, *O. crenata*, and *O. cumana* indicated that every species had a specific optimum temperature range for germination and development which generally reflected its geographical distribution (Sauerborn, 1991). Kasasian (1973a) showed that optimum temperatures for both conditioning and germination were about 18 ^{5}C for *O. crenata* and about 23 ^{5}C for *O. ramosa.* Although temperature is known to influence germination in broomrape, its effect on subsequent development of the parasitic seedling has not been studied.

2.6.3. Radicle Elongation and Attachment to Host

After germination, the radicle elongates by cell division and extension (Parker and Riches, 1993), and attaches to host roots mainly in the region of root elongation and absorption (Foy et al., 1989). The tip of the radicle enlarges as soon as it attaches to the host root and forms a ‘haustorium’. Dörr and Kollmann (1995) have reported interspecific sieve pores derived from interspecific plasmodesmata at the point where broomrape and the

host cells differentiate into sieve elements. Direct connections between haustorial tissue and the xylem of the host were observed (Dörr and Kollmann 1976; Penny packer et al., 1979). The part of the broomrape seedling outside the root of the host swells to form a tubercle. After 1 to 2 weeks of growth, a shoot bud develops on the tubercle producing a flowering spike which elongates, and emerges above the soil.

2.7. Management of Broomrapes

Management of broomrapes is often difficult due to several reasons. These include the high amount of seed production, viability of seeds in the soil over several years (Cubero and Moreno, 1979), lack of seed germination in the absence of a chemical trigger from a suitable host, vigorous growth habit after emergence, and close association with the host crop. Several means for managing broomrapes have been tried over the years, but all of them with somewhat limited effectiveness.

2.7.1. Mechanical and Physical Methods

2.7.1.1. Hand Weeding and Tillage

Hand weeding is the most commonly practiced method of controlling broomrapes in the developing countries and is recommended only under conditions of light infestations. However, it is time consuming and labor intensive, and only limits seed production. It was reported that three years of hand weeding could control *O. cernua* in tobacco in India

(Krishnamurthy and Rao, 1976), but the problem remained. Tillage is not a feasible control strategy due to the very late emergence of broomrape shoots in the growing season and the risk of crop injury due to close association of the parasite with the host.

2.7.1.2. Deep Inversion Plowing and Fire

Several strategies that physically affect broomrape seeds, such as deep inversion plowing, fire, and soil Solarization have been tried. Placement of seeds at 20-cm depth was observed to cause little emergence of *O. cernua* (Krishnamurthy et al., 1987). However, the buried seeds could be brought up by subsequent tillage. Parker and Riches (1993) propose burning of residue from infested crops to reduce carryover of broomrape seeds back to the soil.

2.7.1.3. Soil Solarization

Solarization is defined as the method of covering moist soil with a clear polyethylene sheet and heating the soil by solar radiation. The temperature of the soil is increased, and the polyethylene cover preserves moisture and at the same time prevents temperature loss. This process can increase the temperature of covered soil by 10 ^{5}C compared to uncovered soil. *O. aegyptiaca* infestations have been reduced by 90 to 100% using Solarization (Jacobsohn et al., 1980). The biggest limitation to this method however, is the high cost of the polyethylene (Foy et al., 1989).

Availability of appropriate machinery and cloud-free sunny days may further restrict use of this method.

2.7.2. Cultural Methods

2.7.2.1 Trap and Catch Crops

Trap and catch crops have been used in crop rotations to reduce the broomrape seed bank in the soil. Trap crops cause germination of broomrape seeds without themselves being attacked (Musselman, 1980), whereas catch crops are susceptible to attack by broomrapes. Several trap crops such as beans (*Phaseolus* spp.) (Abu-Irmaileh, 1982) have been used with varying degrees of success. The use of trap and catch crops to reduce broomrape infestations is limited due to the fact that there are vast amounts of broomrape seeds dispersed in the soil and only a small proportion may be exposed to germination stimulants in the rhizosphere of trap and catch crops (Foy et al., 1989).

2.7.2.2 Sowing Date and Cropping Density

The degree of infestation by broomrapes is closely related to the sowing date of the host crop (Sauerborn, 1991). Delay of the sowing date has resulted in reduced parasitism of broad bean and lentil (*Lens culinaris* Medic.) by *O. aegyptiaca* (Sauerborn, 1991). This strategy takes advantage of the optimum seasonal temperatures of broomrape seed germination, but is useful only when early maturing varieties are available to compensate for

the loss in yield due to the short vegetation period of a conventional variety under late sowing conditions. Increasing density of broad bean reduced competition from *O. crenata* (Pieters and Aalders, 1986) and number of attachments of *O. crenata* (Manschadi et al., 1997). However, increase in other inputs such as seeds, cultivation, fertilizer, and pesticides may result in higher production costs.

2.7.2.3 Host Plant Resistance/Tolerance

Host plant resistance to broomrapes is another approach to limit broomrape infestations. Most of the work on breeding for resistance to broomrapes has been done in sunflower against *O. cernua* and in broad bean against *O. crenata*. In Russia, certain varieties of sunflower were found to be resistant to *O. cernua* in 1912, but the resistance broke down in the late 1920s due to existence of different races of *O. cernua* (Cubero, 1986). Similarly, since the early 1990s, several virulent races of *O. cernua* have emerged in Spain jeopardizing the sunflower industry in Spain (García-Torres et al., 1993). Cubero (1991) has summarized the work done in Italy, Spain, and Egypt which showed various degrees of susceptibility in broad bean to broomrape. Reports of resistance are available in several other crops including tomato (Dalela and Mathur, 1971a). Much effort is required in the study of inheritance of resistance and variability in pathogenicity of the parasite mechanism (Cubero, 1991).

2.7.3. Nutrient Management (Nitrogen)

During their evolution, parasitic plants have acquired the ability to obtain nutrition from host plants and have adapted to prefer less fertile soil conditions (Sauerborn, 1991). Reports of inhibitory effects of nitrogen on the growth of broomrapes, based on field, greenhouse, and laboratories studies, have been common in the literature going back to the 19th century, when farmers were using manure and compost to reduce broomrape growth (Ciccarone and Piglionica, 1979). In general, the ammonium form of nitrogen has been found to be inhibitorier on broomrapes than the nitrate form, while urea has an intermediate effect. Jain and Foy (1992) reported similar results with *O. aegyptiaca* when nitrogen was applied during the preconditioning period. Further, van Hezewijk and Verkleij (1996) have shown that application of ammonium sulfate at 8 mM in combination with a nitrification inhibitor during the conditioning phase reduced germination of *O. crenata*. This effect was more pronounced with 4 mM ammonium sulfate applied with a nitrification inhibitor during the germination phase. Reduced germination and radicle length were observed in *O. ramosa*, grown in association with host crop seedlings in response to application of ammonium nitrate (Abu-Irmaileh, 1994).

In summary, nitrogen has some inhibitory effect on growth of broomrapes. Ammonium form of nitrogen has been found to reduce broomrape parasitism to a greater extent than that caused by either urea or nitrate form.

Further field research is required to accurately predict the magnitude of effect of nitrogen on broomrape infestations.

2.7.4 Biological Methods

A fly, *Phytomyza orobanchia* Kalt., has been used for the control of broomrape in the former USSR and eastern Europe. Several limitations restrict beneficial effects of *Phytomyza*. Tillage may bury broomrape stalks, containing *Phytomyza* pupae, deeper in the soil, thus preventing emergence of adults. Application of insecticides severely decreases the insect population. Parasites of *Phytomyza* reduce the fly population considerably. Crop rotations may also negatively impact survival of *Phytomyza*. Fungi such as *Fusarium oxysporum* var. *orthoceras* gave some control of *O. cernua* (Bedi and Donchev, 1991). Both insects and pathogens such as fungi can be useful in control of broomrape in an integrated management program.

2.7.5 Chemical Methods

Chemical strategies have been used to control broomrapes either directly or indirectly. Direct involvement is by reduction or destruction of broomrape seed reserves in the soil, prevention of or negative influence on the germination of broomrape seeds and attachment to the host root. Measures such as soil fumigation, germination stimulants, and certain preplant or preemergence herbicides act directly on broomrape. Indirect control is aimed at suppressing growth of the parasite after attachment and

penetration of the host root. For this purpose foliage applied herbicides and genetically engineered herbicide-resistant crops are useful.

2.7.5.1 Soil Fumigation

Soil fumigation involves the application of highly volatile compounds into the soil whereby the soil is sterilized. The chemical permeates the soil and kills all soil-borne pathogens including bacteria, fungi, nematodes, and weed seeds. The seeds must be physiologically active to be killed. Fumigants have been widely tested for use in controlling broomrapes. Methyl bromide has been recognized as an effective soil fumigant. It has been routinely used in the US to control localized populations of *O. ramosa* before planting tomato (Wilhelm et al., 1958; 1959). There are several limitations that restrict use of methyl bromide over a large scale. The cost of the chemical as well as the polyethylene sheet needed to cover the treated soil are prohibitively high. A well tilled soil that has been kept moist at 70% field capacity and temperature above 10 ^{5}C are required for productive results after methyl bromide application. Safety gear is recommended for application personnel due to extreme toxicity of the gas. The Environmental Protection Agency (EPA) of the US government has banned the use of methyl bromide due to its toxicity, and a global ban may not be far off. Parker and Riches (1993) caution regarding the risk of bromine residues in produce from methyl bromide treated areas. Further, methyl bromide may temporarily suppress *Rhizobium*, although other soil microflora recover in a few days after treatment (Parker and Riches, 1993).

2.7.5.2 Germination Stimulants

Since broomrape seeds must attach to a host root shortly after germination to survive, any means that would cause seed germination in the absence of a suitable host has potential as a control strategy. This stimulation of seed germination in the absence of a susceptible host is called ‘suicidal germination’ (Eplee, 1975). Ethylene has been found to effectively stimulate witchweed seed germination (Eplee, 1975). In fact, it has been a major component of an integrated witchweed management program in the US from 1956 to 1996. There was limited success when ethylene was used for stimulation of broomrape seed germination (Parker and Wilson, 1986). Foy et al. (1989) reviewed several other compounds including herbicides that have been used to stimulate as well as inhibit germination in broomrape seeds. Germination stimulants, both natural and synthetic, have good potential as effective tools of management of broomrape, but much remains to be learned about their structure, activity, and stability in the soil.

2.7.5.3 Preplant and Preemergence Herbicides

Several herbicides have been tested on broomrape for control during its growth below the soil. Thirteen herbicides tested in tomato in greenhouse experiments, only dichloral urea gave consistent control of broomrape (Saghir and Abu-Shakra, 1971). In a detailed review by Foy et al. (1989), several herbicides were reported to have shown some selectivity against broomrape in a variety of crops.

However, none of these had reliability for field applications. Emergence of two relatively new classes of herbicides, sulfonylureas and imidazolinones, in the early 1990s provided additional options for broomrape control. García-Torres et al. (1989) showed that chlorsulfuron and imazethapyr were effective against broomrape. Kleifeld et al. (1996) were able to control *O. aegyptiaca* effectively in tomato with chlorsulfuron and triasulfuron applied by sprinkler irrigation. Good control of broomrape was obtained by coating seeds of broad bean and pea with imazethapyr, (García-Torres et al., 1996).

2.7.5.4 Postemergence herbicides

Any herbicide that can translocate, without being metabolized, through a host plant into broomrape attached to the host roots has potential for use in broomrape control. This aspect was first demonstrated by Whitney (1972) when 2,4-D applied to the host plant accumulated several times more in *O. crenata* than broad bean. However, the damage to the host was extreme. 2,4-DB is usually used selectively for broadleaf weed control in legumes due to its differential metabolism in legumes and weeds.

Kasasian (1973b) reported for the first time, selective control of broomrape in broad bean with glyphosate, a new nonselective foliar applied herbicide at that time. Rates of 0.2 to 0.3 kg ha^{-1} gave good selective control when sprayed 6 wk after sowing. The duration of the broomrape-broad bean competition is an important factor to be considered in relation to the time

when glyphosate should be applied (Mesa-García and García-Torres, 1985).

The imidazolinone herbicides have been tested for their postemergence use. Imazaquin provided some control of broomrape tubercles in broad bean (Sauerborn et al., 1989). Imazethapyr was used selectively in broad bean (García-Torres and López-Granados, 1991a).

Chapter Three

Materials and Methods

Materials and Methods

3.1. 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 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.

Number	Accession Code	Scientific Name	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
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
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*	Commercial Variety

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

Number	Accession Code	Scientific name	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	CN37227	*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

3.2. 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 e (table 3). Each replicate occupied $216m^2$ from the experiment field.

Table (3): Distribution of the tested and control accessions in CRB during the experiment.

Width (12 m)

Length (18 m)

X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X
X	*	X	*	X	*	X	*
X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X
*	X	*	X	*	X	*	X
X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X
X	*	X	*	X	*	X	*

X: Represent the tested accessions in the experiment.

*: Represent the control accession ‘Samara’ in the experiment.

3.3. 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 litter of water) four weeks after planted.

3.4. 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 10th august, 2009.
3. Plants strength was visually observed at the end of experiment when the emergence of *Orobanche* shoots was stopped. Plants strength divided visually to four levels, very strong, strong, weak and dry.

3.5. 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).

Chapter Four

Results

4. Results

4.1. The final number of emerged *Orobanche* per host plant

Moderate levels of resistance were found in some species of *Lycopersicon* (table 4). 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. 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 compared with the susceptible tomato check, with an average of 6 emerged broomrape shoots per plant.

Table (4): 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.	No. of Accessions	Mean[1]	Range[1]
L. cheesmanii	1	98	98
L. chilense	2	101	94-108
L. chmielewskii	1	112	112
L. esculentum	23	96	67-150
L. hirsutum	3	85	73-95
L. pariflorum	1	118	118
L. peruvianum	6	97	80-123
L. pimpinellifolium	6	89	70-114
L. var.cerasiforme	17	93	64-136
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 level of *Orobanche* infection was high and uniform in the field, with an average of 6 emerged broomrapes per plant of the susceptible check cv. Samara in 2009 trial (table 4). The reaction of the tested accessions and lines in the field ranged from very susceptible to moderately resistant (60% to 150% of the average of their surrounding rows of cv. Samara) (figure 1).

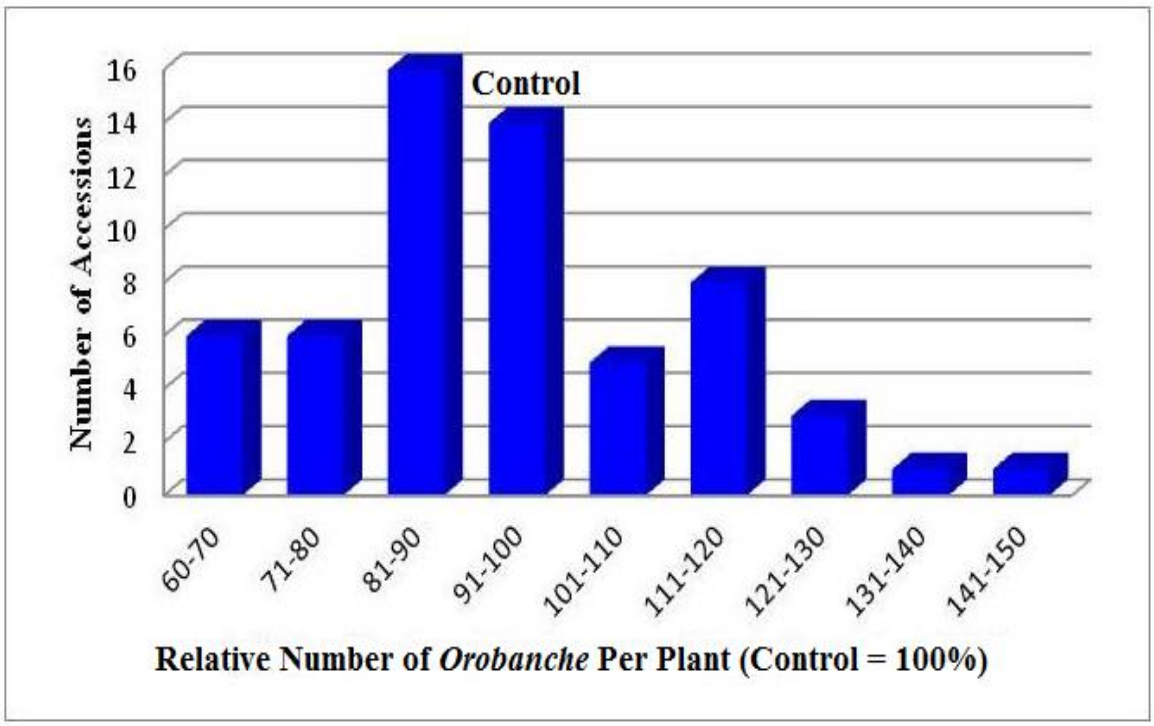

Figure (1): Distribution of the 60 tomato accessions according to the relative number of Orobanche per tomato plant.

Broomrape emergence was particularly low in 12 accessions (table 5). However, most (about 80%) of the accessions studied were very susceptible.

Table (5): Accessions showed the lowest number of emerged broomrape relative to the control accession.

Number	Accession Code	Scientific Name	Origin
1	LA0404	*L. esculentum*	Peru
2	LA0468	*L. esculentum*	Chile
3	LA0172	*L. esculentum var.cerasiforme*	Bolivia
4	LA0349	*L. esculentum var.cerasiforme*	Unknown Origin
5	LA0475	*L. esculentum var.cerasiforme*	Ecuador
6	LA1425	*L. esculentum var.cerasiforme*	Colombia
7	LA1509	*L. esculentum var.cerasiforme*	Borneo
8	LA1511	*L. esculentum var.cerasiforme*	Brazil
9	LA1353	*L. hirsutum*	Peru
10	CN37227	*L. peruvianum*	Canada
11	LA1659	*L. pimpinellifolium*	Peru
12	LA0411	*L. pimpinellifolium*	Ecuador

4.2. 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 cause 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 (Figure 2).

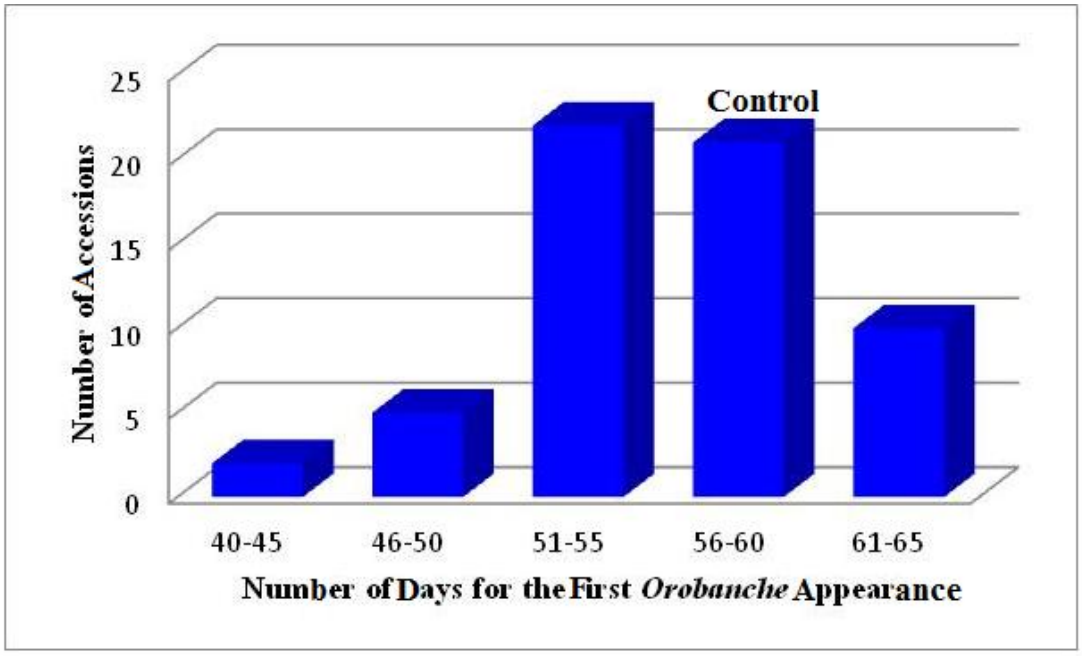

Figure (2): Distribution of the 60 tomato accessions according to the number of days for the first *Orobanche* appearance.

4.3. 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.

4.4. Cluster analysis

Hierarchical cluster analysis was conducted using average linkage (between groups) to conduct a dendrogram (figure 3).

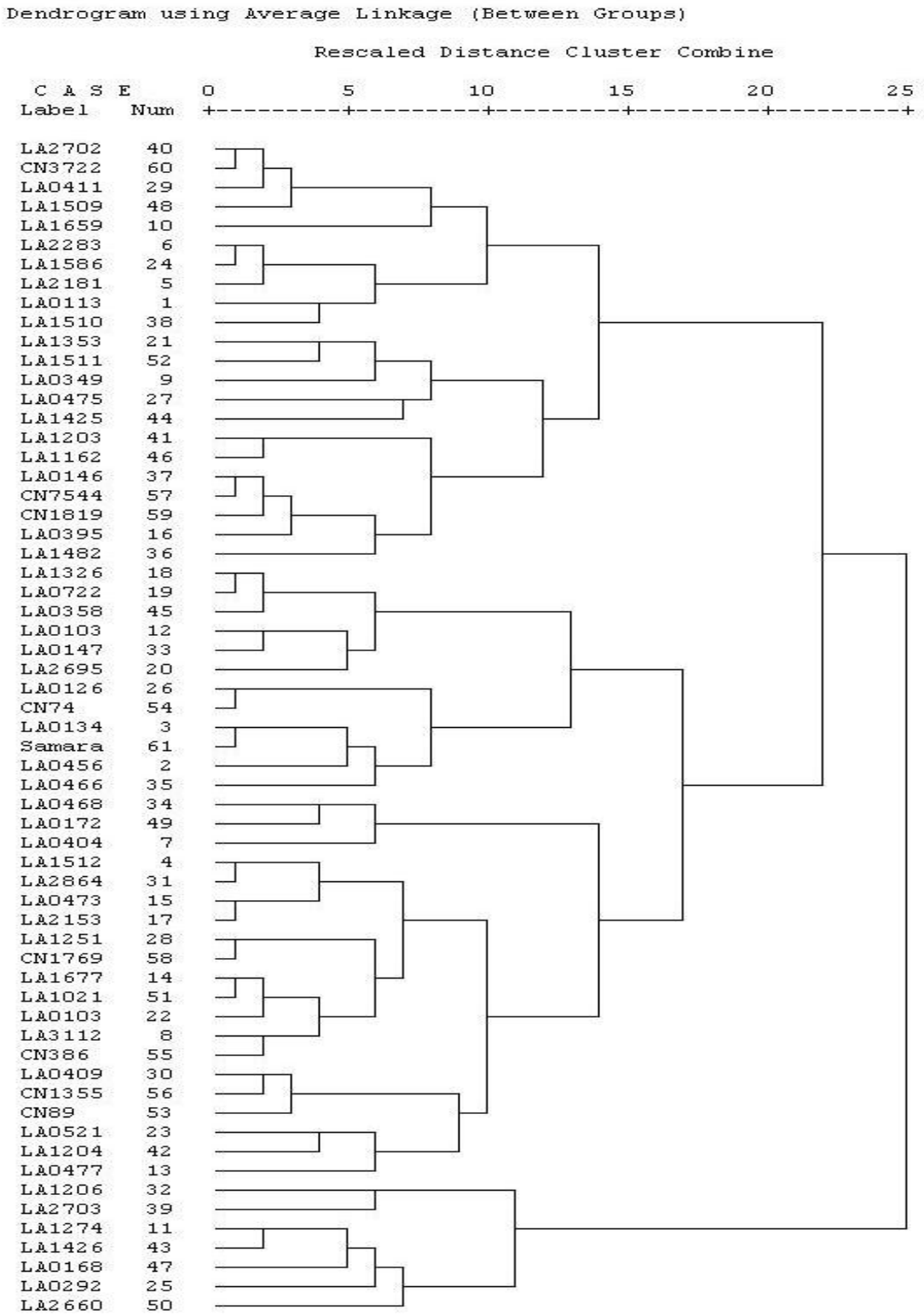

Figure (3): Dendrogram represent complete linkage between accessions groups according to Hierarchical cluster analysis.

The accessions were divided into three clusters according to Hierarchical cluster analysis (figure 3), the first cluster composed of 22 accessions (table 6).

Table (6): Accessions plants present in cluster one according to Hierarchical cluster analysis.

Number	Accession Code	Scientific Name	Origin
1	CN18198	*Lycopersicon pimpinellifolium*	Canada
2	CN37227	*Lycopersicon peruvianum*	Canada
3	CN7544	*Lycopersicon hirsutum*	Canada
4	LA0113	*L.esculentum*	Peru
5	LA0146	*L.esculentum*	Mexico
6	LA0349	*L.esculentum var.cerasiforme*	Unknown Origin
7	LA0395	*L.esculentum*	Peru
8	LA0411	*L.pimpinellifolium*	Ecuador
9	LA0475	*L.esculentum var.cerasiforme*	Ecuador
10	LA1162	*L.esculentum cv.Cuba Plum*	Cuba
11	LA1203	*L.esculentum var.cerasiforme*	Guatemala
12	LA1353	*L.hirsutum*	Peru
13	LA1425	*L.esculentum var.cerasiforme*	Colombia
14	LA1482	*L.esculentum var.cerasiforme*	Malaysia
15	LA1509	*L.esculentum var.cerasiforme*	Borneo
16	LA1510	*L.esculentum var.cerasiforme*	Mexico
17	LA1511	*L.esculentum var.cerasiforme*	Brazil
18	LA1586	*L.pimpinellifolium*	Peru
19	LA1659	*L.pimpinellifolium*	Peru
20	LA2181	*L.pimpinellifolium*	Peru
21	LA2283	*L.esculentum*	Peru
22	LA2702	*L.esculentum var.cerasiforme*	Sri Lanka

The second cluster composed of 32 accessions(table 7) .

Table (7): Accessions plants present in cluster two according to Hierarchical cluster analysis.

Number	Accession Code	Scientific Name	Origin
1	AL0456	*L.chilense*	Peru
2	CN1355	*Lycopersicon esculentum*	Canada
3	CN17695	*Lycopersicon esculentum*	Canada
4	CN386	*Lycopersicon esculentum*	Canada
5	CN74	*Lycopersicon esculentum*	Canada
6	CN89	*Lycopersicon esculentum*	Canada
7	LA0103	*L.peruvianum*	Peru
8	LA0103	*L.peruvianum*	Peru
9	LA0126	*L.esculentum*	Ecuador
10	LA0134C	*L.esculentum*	Peru
11	LA0147	*L.esculentum*	Honduras
12	LA0172	*L.esculentum var.cerasiforme*	Bolivia
13	LA0358	*L.esculentum*	Colombia
14	LA0404	*L.esculentum*	Peru
15	LA0409	*L.esculentum*	Ecuador
16	LA0466	*L.esculentum*	Chile
17	LA0468	*L.esculentum*	, Chile
18	LA0473	*L.esculentum*	Peru

19	LA0477	*L.esculentum*	Peru
20	LA0521	*L.cheesmanii*	Ecuador
21	LA0722	*L.pimpinellifolium*	Peru
22	LA1021	*L.esculentum cv.Santa Cruz*	Brazil
23	LA1204	*L.esculentum var.cerasiforme*	Guatemala
24	LA1251	*L.esculentum*	Ecuador
25	LA1326	*L.pariflorum*	Peru
26	LA1512	*L.esculentum var.cerasiforme*	El Salvador
27	LA1677	*L.peruvianum*	Peru
28	LA2153	*L.peruvianum var.humifusum*	Peru
29	LA2695	*L.chmielewskii*	Peru
30	LA2864	*L.hirsutum f.glabratum*	Ecuador
31	LA3112	*L.chilense*	Peru
32	Samara	samara	Commercial variety

The third cluster composed of 7 accessions (table 8).

Table (8): Accessions plants present in cluster three according to Hierarchical cluster analysis.

Number	Accession Code	Scientific Name	Origin
1	LA0168	*L.esculentum var.cerasiforme*	Fr.Oceania
2	LA0292	*L.esculentum var.cerasiforme*	Ecuador
3	LA1206	*L.esculentum var.cerasiforme*	Honduras
4	LA1274	*L.peruvianum*	Peru
5	LA1426	*L.esculentum var.cerasiforme*	Colombia
6	LA2660	*L.esculentum var.cerasiforme*	Bolivia
7	LA2703	*L.esculentum*	Sri Lanka

Each cluster was distinguished by one or more of the measured parameters. 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). Accessions in the second cluster could be considered as susceptible accessions in this experiment 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 9).

Table (9): 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*}
	No. of accessions	22	22
2	mean	5.69^{b}	3.32^{a}
	No. of accessions	32	32
3	mean	7.38^{a}	2.24^{b}
	No. of accessions	7	7

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

Chapter 5

Discussion

5. Discussion

Egyptian Broomrape is potentially one of the major constraint for tomato cultivation in the Palestinian Territory and mainly through Jenin district (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. Amajor 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 and 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 and 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).

These accessions 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.

Conclusions

From the results of the present experiment, the following conclusions can be drawn:

1. Variation was observed between accessions.

2. Many of these accessions are promising to be used as rootstocks for commercial tomato varieties.

3. 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

4. Further studies are needed to study the level of resistance in these accessions at the molecular and histological levels.

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جامعة النجاح الوطنية
كلية الدراسات العليا

مقاومة أنواع من االبندورة لطفيل الهالوك

(دراسة مقارنة)

إعداد

محمد سليمان محمد صوافطة

إشراف

د. منقذ جميل شتية

قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في الإنتاج النباتي بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين

2012

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الملخص

أجريت هذه التجربة بهدف مقارنة مستويات مقاومة مجموعة من أصناف البندورة العادية والبرية لطفيل الهالوك. أستخدم في التجربة 60 نوعا من البندورة العادية والبرية بإلاضافة 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، وذلك ما تم علمه عندما زرعت الأرض التي أجريت فيها التجربة بنفس صنف البندورة (سمارة) في الموسم الزراعي 2008، حيث كان الانتاج قليلا جدا نتيجة وجود طفيل الهالوك بكثافة حول نباتات البندورة المزروعة.

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.