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Resistance to *Phytophthora infestans* in tomato wild relatives

Ramadan Ahmed Arafa[1,3], Olfat Mohamed Moussa[2], Nour Elden Kamel Soliman[2], Kenta Shirasawa[3], Said Mohamed Kamel[1] and Mohamed Tawfik Rakha[4,5*]

[1]Plant Pathology Research Institute, Agricultural Research Center, Giza, 12619, Egypt.
[2]Department of Plant Pathology, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt.
[3]Kazusa DNA Research Institute, 2-6-7 Kazusa-Kamatari, Kisarazu, Chiba 292-0818, Japan.
[4]Horticulture Department, Faculty of Agriculture, University of Kafrelsheikh, Kafr El-Sheikh 33516, Egypt.
[5]World Vegetable Center, P. O. Box 42, Shanhua, Tainan 74199, Taiwan.

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Late blight of tomato (*Solanum lycopersicum* L.) caused by the heterothallic oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most destructive and serious diseases of tomato in cool and wet environments. Tomato breeders have developed late blight-resistant tomato lines and cultivars based on *Ph* resistance genes derived from *S. pimpinellifolium*, but resistance can be short-lived because *P. infestans* is highly diverse and can readily develop virulence towards the *Ph* resistance genes. Studies were carried out to assess the resistance level of four tomato genotypes and 48 wild relatives of cultivated tomato to *P. infestans*. The highest late blight resistance was detected in *S. habrochaites* accessions LA1777, LA1352, LA2855, LA1347, LA1718 and LA1295, with disease severities ranging from 4.5 to 13.5%. Interestingly, tomato genotypes containing *Ph*-2 and *Ph*-3 had significantly lower disease severity indices compared with the susceptible control 'Super Strain B' when inoculated with a highly virulent isolate. However, when a different isolate was used in 2014, the *Ph-2* and *Ph-3* containing tomato genotypes were as susceptible as 'Super Strain B'. The overall results demonstrate that LA1777, as previously reported, had a high level of resistance against all isolates of *P. infestans* and is a useful genetic resource for future tomato breeding programs.

Key words: Tomato, late blight, *Phytophthora infestans*, disease resistance, *Ph*-genes, *Solanum habrochaites*.

INTRODUCTION

Economically, tomato (*Solanum lycopersicum* L.) is the fourth most important crop in the world after rice, wheat, and soybean (Nowicki et al., 2013), with global production of 162 million tons and a net value of more than $62 billion in 2014 (FAO, 2017). In Egypt, the tomato crop production value was estimated at $1.7 billion in 2014

*Corresponding author. E-mail: mohamed.rakha@worldveg.org.

(FAO, 2017). Diseases caused by different organisms including fungi, bacteria, virus, and nematodes can limit tomato production. Late blight, caused by the hemibiotrophic oomycete *Phytophthora infestans* (Mont.) de Bary, is considered a major threat to tomato production in tropical and subtropical regions (Lima et al., 2009; Elsayed et al., 2012). The pathogen attacks all above-ground parts of the plant including leaves, petioles, stems and fruit at any growth stage, causing blights, necrosis, blotches and rots that reduce yield and fruit quality (Lievens et al., 2004). The disease can spread and kill plants rapidly when favorable environmental conditions of high humidity and low temperature (18°C) prevail (Haq et al., 2008; Stroud et al., 2016).

P. infestans is a highly diverse pathogen that can reproduce sexually and asexually. During asexual reproduction *P. infestans* produces lemon-shaped sporangia, while sexual reproduction results in the production of large, thick-walled oospores capable of surviving for several years in soil or plant debris (Smart and Fry, 2001). The widespread potential of sexual reproduction may increase the risk of host resistance breakdown, due to the development of new aggressive races and genotypes (McDonald and Linde, 2002). Recently, the genetic diversity in *P. infestans* is most often characterized using simple sequence repeat (SSRs) markers (Cooke and Lees, 2004; Lees et al., 2006; Li et al., 2010, 2013). The data is used to assign genotypes to isolates, for example in Europe Cooke et al. (2012) uses a system where 2 to 25 alleles per locus were detected in many European isolates using 11 SSR markers. These genotypes, however, do not reflect information regarding the specific race of the pathogen, with the same SSR genotype containing different races (Li et al., 2012)

Late blight management strategies include the use of several fungicides. Apart from the economic impact of their use, fungicides have been shown to reduce efficacy and resistance problems, particularly formulations containing metalaxyl (mefenoxam) (Randall et al., 2014; Saville et al., 2015; Montes et al., 2016). It can be difficult to detect *P. infestans* in the field during the initial stages of infection, from where it can rapidly develop into severe epidemics due to the pathogen's short life cycle. Most fungicides are ineffective once the pathogen had been established in the field. Host resistance has the potential for being a key component in managing late blight of tomato; it would reduce fungicide use and provide cost-effective, environmentally safe management strategies against the pathogen.

A few resistant varieties of tomato have been developed through the introgression of resistance genes obtained from wild tomato species (Panthee and Gardner, 2010). To date, five late blight resistance genes have been identified. *Ph-1*, a single dominant allele located on chromosome 7, confers resistance to *P. infestans* race 0 but it has been overcome by new races of the pathogen.

The second gene, *Ph-2*, a partially dominant allele located on chromosome 10, confers partial resistance to some *P. infestans* isolates and often fails in the presence of aggressive isolates. *Ph-3*, a single dominant allele located on chromosome 9, provides increased resistance against some aggressive isolates like Pi-16 from Taiwan that can overcome *Ph-1* and *Ph-2* (Irzhansky and Cohen, 2006). However, *Ph*-3 can also be overcome by some isolates (Kim and Mutschler, 2006; Chen et al., 2008; de Miranda et al., 2010). A new resistant gene, *Ph-5,* has been identified recently on chromosome 1 and 10, and confers strong resistance to several *P. infestans* isolates including those overcoming the aforementioned four resistance genes (Foolad et al., 2008). Currently, the *Ph*-2 and *Ph*-3 genes are available in tomato breeding lines (e.g. 'NC1 CELBR', 'NC2 CELBR') and hybrid cultivars (Gardner and Panthee, 2010; Zhang et al., 2014). The continuous cycle of resistance being overcome by new *P. infestans* races warrants continuous efforts to identify additional sources of resistance from commercial cultivars, or other wild relatives of tomato in order to improve future breeding programs. The objectives of this study were to (i) investigate the level of resistance to *P. infestans* in wild relatives *S. habrochaites*, *S. pennellii*, *S. pimpinellifolium* and *S. peruvianum* and (ii) determine whether tomato accessions containing late blight resistance genes (*Ph*-1, *Ph*-2 and *Ph*-3) could provide acceptable resistance to *P. infestans* isolates present in Egypt.

MATERIALS AND METHODS

Plant material and growth condition

Forty-eight wild tomato accessions were evaluated, including 24 accessions of *S. pimpinellifolium*, 12 accessions of *S. habrochaites*, eight accessions of *S. pennellii*, and four accessions of *S. peruvianum*, along with four accessions of *S. lycopersicum* containing *Ph* resistance genes. Seed was obtained from the C. M. Rick Tomato Genetics Resource Center (TGRC), University of California, Davis (LA numbers). Tomato variety 'Super Strain B', received from the Horticulture Research Institute, Agricultural Research Center (ARC), Egypt, which is known to be susceptible to late blight, was included as a control. In the greenhouse (25±2 °C, 16/8 h day/night), seeds of all accessions and the susceptible control were sown in 209-cell seedling trays containing 40 ml of peat moss-vermiculite mixture (1:1 volume) per cell plug. Plants were watered daily and fertilized weekly with N: P: K 15-15-15. Five weeks after sowing, seedlings of accession and control plants were transplanted into 20 cm pots containing potting soil, which were used for whole plant assays in 2013 and 2014. Seven-week-old plants were moved from the greenhouse to a growth room (20 ± 2°C, 90% relative humidity (RH), 16/8 h day/night) at the Plant Pathology Research Institute at ARC for inoculation assays.

Isolate selection and maintenance

Fourteen *P. infestans* isolates were collected from naturally infected tomato plants from 2013 to 2014 epidemics occurring in Beheira,

Table 1. Geographic locations where *Phytophthora infestans* isolates were collected in Egypt, which were evaluated for virulence.

P. infestans isolates	County	District	Genotype	Year	Mean disease severity (%)
EG_1	Kafr El Sheikh	Baltem	23_A1_10	2013	45.0±2.9^{c}
EG_1.1	Kafr El Sheikh	Baltem	23_A1_10	2013	44.7±2.6^{c}
EG_2	Kafr El Sheikh	Sidi Ghazy	23_A1_10	2013	22.7±1.5^{e}
EG_3	Kafr El Sheikh	Sakha	23_A1_10	2013	44.7±2.6^{c}
EG_4	Qalubiya	Qaliub	23_A1_12	2013	30.0±2.9^{de}
EG_5	Ismailia	Kasasen	Unknown	2013	75.0±2.9^{b}
EG_6	Beheira	Nubaria	Unknown	2013	37.3±1.5^{cd}
EG_6.1	Beheira	Nubaria	Unknown	2013	41.7±5.8^{cd}
EG_7	Kafr El Sheikh	Sakha	23_A1_12	2013	99.0±0.6^{a}
EG_8	Kafr El Sheikh	Sidi Ghazy	23_A1_10	2013	80.0±1.7^{b}
EG_9	Beheira	Badr	Unknown	2014	96.7±3.3^{a}
EG_10	Beheira	Badr	Unknown	2014	78.3±9.3^{a}
EG_11	Kafr El Sheikh	Sakha	Unknown	2014	100.0±0.0^{a}
EG_12	Beheira	Kom Hamada	Unknown	2014	100.0±0.0^{a}

Means disease severity rating followed by ± standard error. Means followed by different letters are significantly (P = 0.05) different, whereas means followed by the same letter are not significantly different.

Kafr El Sheikh, Qalubiya, and Ismailia counties in Egypt (Table 1). The susceptible 'Super strain B' control was artificially inoculated with the 10 *P. infestans* isolates collected in 2013, as described for the germplasm testing, in order to select the most virulent isolate for late blight screening. Among the 10 evaluated isolates, EG_7 was the most virulent (Table 1). Isolate EG_7 was identified as genotype 23_A1_12 and mating type A1 based on identification carried out by the laboratory of Dr. David E. L. Cooke at the James Hutton Institute, Dundee, UK. This isolate was chosen as the inoculum source in the first round of germplasm tests conducted in 2013 and 2014. In 2014, another four *P. infestans* isolates were collected from naturally infected tomato plants (Table 1). These isolates were used to re-test germplasm from the first round of testing, due to the severe epidemics they caused in tomato fields in 2014.

Inoculum production

Sporangia and zoospores were produced as described by Chen et al. (2008). Tomato leaves collected from 6-week-old plants of the susceptible 'Super strain B' genotype were placed on moist filter paper in 140 mm sterilized Petri plates. The abaxial surface of the leaves were injured at the center using a sterile 10 µl micropipette tip and inoculated with 30 µl of a sporangial suspension obtained from 20-day-old rye agar plates. Leaflets were incubated for 48 h at 18°C in darkness, followed by incubation at 18°C for 10 days with a 12-h photoperiod. Subsequently, tomato leaflets were placed in a glass beaker containing 500 mL sterilized distilled water and gently shaken using a vortex to dislodge sporangia from the leaflets. The suspension was filtered through four layers of sterile muslin cloth. The concentration of the sporangia was determined using a haemocytometer, and was adjusted to 15×10^{4} sporangia/ml. The suspension was chilled at 4°C for 2 to 4 h prior to inoculation to encourage zoospore release from the sporangia.

Disease assessment using *P. infestans* isolate EG_7

Eight-week-old plants of 48 wild accessions along with four tomato genotypes containing *Ph* genes and a susceptible control were inoculated with *P. infestans* isolate EG_7 using whole plant assays. Plants were sprayed with a suspension of *P. infestans* zoospores using a hand sprayer until complete leaf coverage and excess run-off was observed. The inoculated plants were covered with a plastic tunnel to increase humidity and kept at 16 to 18°C in the dark in a growth chamber for 24 h under 100% RH. The inoculated plants were then grown at 18 to 20°C and 90% RH with a 12 h photoperiod for 7 to 12 days. Plants were arranged following a completely randomized design with five replicate plants per accession.

The experiment was conducted twice from November to January in 2013 and 2014. Plants were evaluated individually at 10 days post-inoculation (dpi) by visually scoring the disease severity using a scale of 0 to 6 as described by Chen et al. (2014), where 0 indicates no symptoms (immune); 1 indicates <5% leaf area affected and small lesions (highly resistant); 2 indicates 6 to 15% leaf area affected and restricted lesions (resistant); 3 indicates 16 to 30% leaf area affected and/or water-soaked flecks on stems (moderate resistant); 4 indicates 31 to 60% leaf area affected and/or a few stem lesions (moderate susceptible); 5 indicates 61 to 90% leaf area affected and expanding stem lesions (susceptible); 6 indicates 91 to 100% of leaf area affected, extensive stem damage, or a dead plant (highly susceptible).

Reassessment of disease resistance using *P. infestans* isolates EG_9, EG_10, EG_11 and EG_12

The four *P. infestans* isolates collected in 2014 were used to confirm the resistance identified in S. *habrochaites* accession LA1777, and to re-access the response of the five tomato accessions containing *Ph* resistance genes against different *P. infestans* isolates. The susceptible control 'Super Strain B' was also included in the re-testing. Preparation of isolates and disease assessments were carried out as described previously. The experiment was repeated twice only with LA1777 along with the susceptible control 'Super Strain B' inoculated with isolate EG_11.

Statistical analysis

Statistical procedures were performed using the statistical software SAS (version 9.1; SAS Institute, Cary, NC). The percentage of late blight severity was transformed using Arcsine square root transformation. Back-transformed data are presented in tables. All data were subjected to one-way analysis of variance (ANOVA). Mean separations were determined using the Tukey-Kramer honestly significant difference (HSD) test ($P = 0.05$).

RESULTS

Isolate selection

To identify the most virulent isolate, 10 *P. infestans* isolates collected from tomato fields in 2013, were artificially inoculated on the susceptible control 'Super Strain B' under controlled greenhouse conditions. All *P. infestans* isolates infected the susceptible control, with disease severity ranging from 99% for the most aggressive isolate EG_7 to 22.7% for the least aggressive isolate EG_2 (Table 1). In addition to isolate EG_7, isolates EG_5 and EG_8 also exhibited high virulence causing disease severities of 75 and 80%, respectively. Based on these results, *P. infestans* isolate EG_7 was selected and used to further screen late blight resistance in 48 tomato wild tomato relatives along with tomato genotypes containing the *Ph* genes and the susceptible control.

Disease assessment using *P. infestans* isolate EG_7

Late blight severity on the 48 wild accessions, four tomato genotypes containing *Ph* genes, and the susceptible control 'Super Strain B', was evaluated using a whole plant assay under controlled greenhouse conditions (Table 2). The ANOVA revealed highly significant differences among treatments ($P < 0.0001$). Results from our two experiments conducted in 2013 and 2014 were similar. No genotype was immune to *P. infestans* EG_7 and all susceptible control plants had a 100% blight severity (dead). Mean disease ratings of all tested accessions eight weeks after sowing ranged from 4.5 to 100% (Table 2). Lower disease severities were identified for *S. habrochaites* accessions LA1777, LA1352, LA2855, LA1347, LA1718 and LA1295, with disease severities ranging from 4.5 to 13.5%. *S. lycopersicum* accessions containing *Ph-2* and *Ph-3* genes had significantly lower disease severity values, whereas LA3152 (*Ph*-2), LA3151 (*Ph*-2) and LA4286 (*Ph*-3) were moderately resistant. Conversely, all *S. peruvianum* and *S. pennellii* accessions were susceptible or highly susceptible to isolate EG_7. The majority of the evaluated *S. pimpinellifolium* accessions were either susceptible or highly susceptible to EG_7, whereas LA 1269 and LA1578 were moderately resistant with disease severity 22 and 23%, respectively. No correlation was seen between the geographic origin of tomato genotypes and their resistance to *P. infestans*. For example, all resistant accessions in this study originated from Peru and Ecuador, but many susceptible accessions also came from these regions.

Reassessment of disease resistance using *P. infestans* isolates EG_9, EG_10, EG_11 and EG_12

The late blight resistance identified in the 2013 and 2014 experiments in *S. habrochaites* accession LA1777 and the *S. lycopresicum* genotypes containing *Ph* genes was verified using a new set of *P. infestans* isolates including EG_9, EG_10, EG_11 and EG_12, which were collected from tomato epidemcis in 2014 (Table 3). Responses to individual isolates varied. Genotypes inoculated with the aggressive isolate EG_12 had 13.3 to 100% disease ratings, while genotypes inoculated with EG_10, the least aggressive isolate, had disease severities of 0 to 78.3% (Table 3). LA1777 was the most resistant genotype, exhibiting the lowest disease severity values against all selected isolates of *P. infestans* compared to other genotypes containing *Ph* genes and the susceptible control, with disease severity means ranging from 0 to 13.3%. The susceptible control had a significantly higher disease severity compared to LA1777 and tomato genotypes containing *Ph* genes when inoculated with isolates EG_10 and EG_11. Interestingly, all *S. lycopersicum* accessions containing *Ph-2* and *Ph-3* genes were found to be moderately resistant or resistant to isolates EG_9 and EG_10, but were susceptible to isolates EG_11 and EG_12 with mean severity ratings > 68.3%.

DISCUSSION

Late blight is one of the most devastating diseases of tomato, especially in cool and moist environments. In Egypt, it is almost impossible for grower's to produce tomatoes from November to February, when cool and moist weather predominate and favour *P. infestans* epidemics. Knowledge on the effectiveness of resistance genes in existing cultivars can help growers in Egypt to manage late blight. Efforts must thus continue to assess the stability of resistance and whether such cultivars are adaptable to all tomato-growing regions.

Resistance to *P. infestans* has been discovered in numerous wild tomato species, including *S. pimpinellifolium*, *S. habrochaites* and *S. pennellii* (Conver and Walter, 1953; Turkensteen, 1973; Moreau et al., 1998; Chunwongse et al., 2002; Smart et al., 2007; Li et al., 2011). To the best of our knowledge, cultivars

Table 2. Mean late blight disease severity ratings for 48 wild tomato accessions and controls inoculated with *P. infestans* isolate EG_7.

Taxa and accession	Origin	Mean disease severity (%)[z]			Disease response[y]
		2013	2014	Mean combined	
S. habrochaites[#]		45.2±5.0	43.9±4.8	44.5±1.7	
LA1777	Peru	2.0±1.2^{l}	7.0±1.2^{hi}	4.5±0.9^{i}	HR
LA1352	Peru	9.0±1.8^{k}	5.0±2.2^{i}	7.0±1.8^{hi}	R
LA2855	Ecuador	9.0±3.3^{k}	6.0±3.7^{i}	7.5±3.3^{hi}	R
LA1347	Peru	10.0±2.2^{k}	7.0±2.0^{hi}	8.5±1.3^{ghi}	R
LA1718	Peru	12.0±1.2^{jk}	9.0±1.9^{hi}	10.5±1.2^{gh}	R
LA1295	Peru	14.0±1.9^{jk}	13.0±2.0^{gh}	13.5±1.7^{g}	R
LA2196	Peru	40.0±1.6^{gh}	65.0±2.2^{cde}	52.5±1.6^{de}	MS
LA1252	Ecuador	76.0±1.9^{cde}	84.0±2.5^{abcd}	80.0±2.1^{bc}	S
LA1772	Peru	90.0±2.7^{abcd}	77.0±2.0^{abcde}	83.5±1.7^{abc}	S
LA1223	Ecuador	90.0±2.2^{abcd}	82.0±2.0^{abcd}	86.0±1.9^{ab}	S
LA1378	Peru	95.0±2.2^{abc}	85.0±2.3^{abcd}	90.0±1.4^{ab}	S
LA0094	Peru	95.0±0.0^{abc}	87.0±2.0^{abcd}	91.0±1.0^{ab}	HS
S. pennellii[#]		87.6±2.6	83.9±2.0	85.7±1.6	
LA1367	Peru	54.0±2.5^{fg}	68.0±3.7^{bcde}	61.0±1.9^{de}	S
LA1340	Peru	72.0±2.6^{def}	63.0±2.0^{de}	67.5±2.1^{cd}	S
LA0716	Peru	85.0±2.7^{abcd}	84.0±2.5^{abcd}	84.5±2.4^{ab}	S
LA1674	Peru	91.0±4.0^{abcd}	89.0±5.1^{abc}	90.0±3.6^{ab}	S
LA1303	Peru	100.0±0.0^{a}	88.0±1.2^{abcd}	94.0±0.6^{ab}	HS
LA1302	Peru	100.0±0.0^{a}	89.0±1.0^{abc}	94.5±0.5^{ab}	HS
LA0751	Peru	99.0±1.0^{ab}	91.0±2.5^{ab}	95.0±0.8^{ab}	HS
LA1649	Peru	100.0±0.0^{a}	99.0±1.0^{ab}	99.5±0.5^{a}	HS
S. pimpinellifolium[#]		88.7±2.0	89.4±1.9	89.0±1.2	
LA1269	Peru	20.0±2.7^{ij}	24.0±2.9^{fg}	22.0±2.3^{f}	MR
LA1578	Peru	19.0±2.5^{ij}	27.0±2.0^{f}	23.0±1.2^{f}	MR
LA1478	Peru	77.0±2.0^{bcde}	82.0±2.6^{abcd}	79.5±1.8^{bc}	S
LA1594	Peru	86.0±2.9^{abcd}	90.0±2.7^{abc}	88.0±1.5^{ab}	S
LA2646	Peru	85.0±1.6^{abcd}	92.0±2.6^{ab}	88.5±1.3^{ab}	S
LA0443	Ecuador	95.0±0.0^{abc}	88.0±1.2^{abcd}	91.5±0.6^{ab}	HS
LA1586	Peru	91.0±3.3^{abcd}	92.0±3.4^{ab}	91.5±3.2^{ab}	HS
LA1561	Peru	94.0±2.9^{abc}	90.0±2.7^{abc}	92.0±1.7^{ab}	HS
LA0413	Ecuador	91.0±2.9^{abcd}	93.0±3.7^{ab}	92.0±2.9^{ab}	HS
LA2147	Peru	95.0±1.6^{abc}	91.0±2.5^{ab}	93.0±1.7^{ab}	HS
LA1579	Peru	93.0±2.0^{abcd}	96.0±2.4^{a}	94.5±2.0^{ab}	HS
LA1617	Peru	97.0±2.0^{abc}	93.0±4.3^{ab}	95.0±2.3^{ab}	HS
LA1633	Peru	95.0±1.5^{abc}	96.0±2.4^{a}	95.5±1.4^{ab}	HS
LA2391	Peru	98.0±1.2^{abc}	96.0±1.9^{a}	97.0±0.9^{ab}	HS
LA3123	Ecuador	98.0±2.0^{abc}	96.0±2.5^{a}	97.0±1.2^{ab}	HS
LA3161	Mexico	98.0±2.0^{abc}	99.0±1.0^{a}	98.5±1.5^{a}	HS
LA1246	Ecuador	98.0±1.2^{abc}	100.0±0.0^{a}	99.0±0.6^{a}	HS
LA0375	Peru	99.0±1.0^{ab}	100.0±0.0^{a}	99.5±0.5^{a}	HS
LA0114	Peru	99.0±1.0^{ab}	100.0±0.0^{a}	99.5±0.5^{a}	HS
LA1237	Ecuador	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	HS
LA1469	Peru	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	HS
LA1256	Ecuador	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	HS
LA1242	Ecuador	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	HS
LA1343	Peru	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	HS
S. peruvianum[#]		97.3±1.2	96.8±1.2	97.0±1.3	

Table 2. Contd.

LA1935	Peru	92.0±3.4^{abcd}	93.0±3.7^{ab}	92.5±2.9^{ab}	HS
LA0446	Peru	97.0±2.0^{abc}	97.0±2.0^{a}	97.0±1.2^{ab}	HS
LA2581	Chile	97.0±2.0^{a}	97.0±2.0^{a}	98.5±1.0^{a}	HS
LA1333	Peru	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	HS
S. lycopresicum[#]		47.8±3.1	48.2±3.5	48.0±2.3	
LA3152 (*Ph-2*)		27.0±3.0^{hi}	24.0±2.9^{fg}	25.5±1.5^{f}	MR
LA3151 (*Ph-2*)		26.0±4.3^{i}	26.0±5.6^{fg}	26.0±3.2^{f}	MR
LA4286 (*Ph-3*)		28.0±2.5^{hi}	33.0±3.5^{f}	30.5±2.7^{f}	MR
Table 2. continued					
LA2009 (*Ph-1*)		58.0±5.6 ^{ef}	58.0±5.6^{e}	58.0±4.1^{de}	MS
Super Strain B		100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	HS

[z]Means of visually disease severity ratings throughout 2013 and 2014 experiments followed by ± standard error (± SE; n=5). Means followed by different letters are significantly (*P* = 0.05) different. [y]Disease response: HS = highly susceptible, S = susceptible, MS = moderately susceptible, MR = moderately resistant, R = resistant, and HR = highly resistant.

Table 3. Mean late blight disease severity ratings for *Solanum habrochaites* accession LA1777 compared to controls inoculated with four different *P. infestans* isolates (EG_9, EG_10, EG_11 and EG_12).

Accession code	Resistance gene	***P. infestans* isolates**			
		EG_9	**EG_10**	**EG_11**	**EG_12**
L A2009	*Ph-1*	98.3±1.7^{a}	41.7±4.4^{ab}	96.7±3.3^{ab}	85.0±2.9^{a}
LA3151	*Ph-2*	36.7±3.3^{bc}	31.7±8.3^{cb}	86.7±3.3^{bc}	80.0±15.3^{a}
LA3152	*Ph-2*	21.7±1.7^{c}	0.0±0.0^{e}	95.0±2.9^{ab}	83.3±12.0^{a}
LA1269	*Ph-3*	40.0±5.8^{bc}	11.7±1.7^{d}	68.3±7.3^{c}	81.7±11.7^{a}
LA4286	*Ph-3*	53.3±3.3^{b}	21.7±6.0^{cd}	83.3±6.7^{c}	78.3±9.3^{a}
LA1777	Unknown	1.7±1.7^{d}	1.7±1.7^{e}	6.7±1.7^{d}	13.3±3.3^{b}
Super Strain B	None	96.7±3.3^{a}	78.3±9.3^{a}	100.0±0.0^{a}	100.0±0.0^{a}

Means of visually disease severity rating followed by ± standard error (± SE; n=5). Means followed by different letters are significantly (*P* = 0.05) different.

commonly grown or introduced in Egypt have not been assessed for their resistance to local populations of *P. infestans*. Therefore, a large number of accessions and genotypes were screened for their resistance to late blight using a highly virulent isolate EG_7 under controlled greenhouse conditions. Our results showed that *S. habrochaites* accessions LA 1777, LA1352, LA2855, LA 1347, LA1718 and LA1295 exhibited high levels of resistance to late blight, with LA1777 being the most resistant. Similar results have been reported in *S. habrochaites* accessions by Abreu et al. (2008) in BGH6902, Brouwer et al. (2004) in LA2099, and Li et al. (2011) in LA1777.

Although the resistance mechanism is unknown, these accessions have high densities of glandular trichomes type IV and VI (Muigai et al., 2003; Momotaz et al., 2010; Bergau et al., 2015), which are often able to secrete exudates with antifungal activities as has been shown in a wild potato species (*S. berthaulltii*) that are resistant to *P. infestans* (Lai et al., 2000). Additional resistance mechanisms in LA1777 could be the secretion of a wide range of proteins that have been shown in other plants to play a role in the degradation of microbial cell walls, and in blocking pathogen-released elicitors (Ferreira et al., 2007). The resistance present in LA 1777 will, however, be difficult to confer to tomato since *S. habrochaites* is a distant relative of cultivated tomato and challenges such as self-incompatibility, segregation distortion, and linkage drag complicate the transfer of useful genes into tomato (Covey et al., 2010; Elizondo and Oyanedel, 2010; Labate and Robertson, 2012; Haggard et al., 2013). The sequencing of the tomato genome will be helpful in this regard since it has resulted in the availability of thousands of molecular markers that can facilitate the mapping and introgression of beneficial genes from wild species into cultivated tomato.

Tomato genotypes containing *Ph*-2 and *Ph*-3 genes, LA3152, LA3151 and LA4286, demonstrated acceptable

levels of resistance when inoculated with *P. infestans* EG_7. These genes have thus far been used in commercial cultivars and may directly benefit farmers by reducing their dependence on fungicides to control *P. infestans,* thus lowering their production cost. These results support previous reports indicating that *Ph*-2 and *Ph*-3 confers resistance to *P. infestans. Ph*-2 is a completely dominant gene that confers partial resistance to *P. infestans* and in this case isolate EG_7, and *Ph*-3 is a partial dominant allele that confers stronger resistance to *P. infestans* when it is homozygous. Notably, resistance to *P. infestans* EG_9, and EG_10 isolates exhibited by accession LA3152 (containing *Ph*-2) was significantly higher than the resistant accessions LA3151, LA1269 and LA4286 containing *Ph*-2 or *Ph*-3, suggesting the presence of unknown resistance gene(s) potentially involved in resistance to late blight in accession LA3152. Further, *P. infestans* is a highly diverse pathogen that can develop virulence toward the *Ph* resistance genes (Goodwin et al., 1995; Bradshaw et al., 2006; Chen et al., 2014). The fluctuation of *P. infestans* populations may be due to sexual recombination and transposable elements (Vetukuri et al., 2012). Based on the results of this study, tomato accessions possessing *Ph*-2 and *Ph*-3 resistance genes were susceptible to *P. infestans* isolates EG_12 and EG_11, whereas LA1777 had a high level of resistance against all five evaluated *P. infestans* isolates. In addition, the tomato accession possessing the resistance gene *Ph*-1 was also susceptible to all five *P. infestans* isolates EG_7, EG_9, EG_10, EG_11, and EG_12.

The differences in the effect of *Ph* genes against different *P. infestans* isolates could be due to isolates used in this study probably having different race compositions. For example, isolates EG_2 and EG_7 were collected from the same county, but differed in their virulence to the host plant. Differences between these isolates have significant implications for local tomato breeding programs against *P. infestans* in Egypt, where the diversity of pathogen populations, the existence of different physiological races within *P. infestans*, and polygenic inheritance can hamper breeding efforts (Singh and Singh, 2006; Zhang and Kim, 2007; Harbaoui et al., 2011; Pule et al., 2013; Tian et al., 2016). Further studies are required to characterize *P. infestans* populations, define races that infect tomato, and determine their spatial structure in order to deploy tomato breeding programs effectively in Egypt.

Only two accessions of *S. pimpinellifolium*, LA1269 and LA1578, were found to be moderately resistant. These red-fruited accessions are closely related to tomato and therefore fewer backcrosses may be required to introgress late blight resistance into tomato compared to the green-fruited species *S. pennellii* and *S. habrochaites* (Rick, 1971; Peralta et al., 2008). LA1269, also known in the literature as L3708, was described as being resistant to multiple strains of *P. infestans* (Chunwongse et al., 2002). Such resistance has been linked to the *Ph*-3 gene and *qPh*2.1 QTL, which confers resistance to isolate Pi733 in Taiwan (Black et al., 1996; Chen et al., 2014). Although breeders might be interested in attaining high levels of resistance, the use of partially resistant cultivars can be useful and can help to reduce the number of fungicide applications (Stevenson et al., 2007).

Interestingly, some accessions found to be resistant in other studies were moderate or susceptible in this study. For example, *S. pennellii* LA716 was reported as highly resistant by Eshed and Zamir (1994) and Smart et al. (2007), but was found to be susceptible in this study with a mean rating of 5.0. Conversely, the most resistant *S. habrochaites* accession LA1352 identified in this study was highly susceptible to aggressive *P. infestans* isolate T1, 2, 3, 4 in Taiwan, with a mean rating 6. Differences in resistance in different studies could be due to the use of different experimental designs or more likely differences in *P. infestans* races. Simple sequence repeat was recently conducted (Li et al., 2013) genotyping 40 isolates of *P. infestans* collected from different regions in Egypt from 2012 to 2014, which revealed genetic variability among *P. infestans* isolates (Arafa et al., unpublished data). This could have implications for resistance screening. However, since SSR genotypes cannot be linked to race composition, race typing will also have to be conducted to determine the extent of variation in races in Egypt and their relevance in resistance screenings.

The results of the study show that resistance in *S. habrochaites* accession LA1777 was higher than the resistance conferred by *Ph*-2 and *Ph*-3 genes, indicating that the LA1777 genome contains a different source of late blight resistance. This is in agreement with the study of Li et al. (2011), who also found that LA1777 has a good level of resistance to several isolates of *P. infestans*. Li et al. (2011) has identified five loci derived from LA1777 (*Rlbq*4a, *Rlbq*4b, *Rlbq*7, *Rlbq*8 and *Rlbq*12) associated with lesion size, which were co-localized with previously described QTLs from *S. habrochaites* LA2099 except QTL *Rlbq*4b (Brouwer et al., 2004). Pyramiding resistance alleles from LA1777 and available late blight resistance genes such as *Ph*-2 and *Ph*-3 in cultivated tomato could enhance the durability of resistance to late blight.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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REFERENCES

Abreu FB, Da Silva DJH, Cruz CD, Mizubuti ESG (2008). Inheritance of resistance to *Phytophthora infestans* (Peronosporales, Pythiaceae) in a new source of resistance in tomato (*Solanum* sp. (formerly *Lycopersicon* sp.), *Solanales*, *Solanaceae*). Genet. Mol. Biol. 31:493-497.

Bergau N, Bennewitz S, Syrowatka F, Hause G, Tissier A (2015). The development of type VI glandular trichomes in the cultivated tomato *Solanum lycopersicum* and a related wild species *S. habrochaites*. BMC Plant Biol. 15:289.

Black LL, Wang TC, Hanson PM, Chen JT (1996). Late blight resistance in four wild tomato accessions: Effectiveness in diverse locations and inheritance of resistance. Phytopathology 86:S24.

Bradshaw J, Bryan G, Ramsay G (2006). Genetic resources (including wild and cultivated *Solanum* species) and progress in their utilization in potato breeding. Potato Res. 49:49-65.

Brouwer DJ, Jones ES, St Clair DA (2004). QTL analysis of quantitative resistance to *Phytophthora infestans* (late blight) in tomato and comparisons with potato. Genome 47: 475-492.

Chen AL, Liu CY, Chen CH, Wang JF, Liao YC, Chang CH, Tsai MH, Hwu KK, Chen KY (2014). Reassessment of QTLs for late blight resistance in the tomato accession L3708 using a Restriction Site Associated DNA (RAD) linkage map and highly aggressive isolates of *Phytophthora infestans*. PLoS ONE 9:e96417.

Chen CH, Sheu ZM, Wang TC (2008). Host specificity and tomato-related race composition of *Phytophthora infestans* isolates in Taiwan during 2004 and 2005. Plant Dis. 92:751-755.

Chunwongse J, Chunwongse C, Black L, Hanson P (2002). Molecular mapping of the Ph-3 gene for late blight resistance in tomato. J. Hort. Sci. Biotech. 77:281-286.

Conver RA, Walter JM (1953). The occurrence of a virulent race of *Phytophthora infestans* on late blight resistance tomato stocks. Phytopathology 43:344-345.

Cooke DEL, Lees AK (2004). Markers, old and new, for examining *Phytophthora infestans* diversity. Plant Pathol. 53: 692-704.

Cooke, DEL, Cano LM, Raffaele S, Bain RA, Cooke LR, Etherington GJ, Deahl KL, Farrer RA, Gilroy EM, Goss EM, Grunwald NJ, Hein I, MacLean D, McNicol JW, Randall E, Oliva RF, Pel MA, Shaw DS, Squires JN, Taylor MC, Vleeshouwers VGAA, Birch PRJ, Lees AK, Kamoun S (2012). Genome analyses of an aggressive and invasive lineage of the Irish potato famine pathogen. PLoS Pathog. 8:e1002940.

Covey PA, Kondo K, Welch L, Frank E, Sianta S, Kumar A, Nuñez R, Lopez-Casado G, Van Der Knaap E, Rose JK, McClure BA (2010). Multiple features that distinguish unilateral incongruity and self-incompatibility in the tomato clade. Plant J. 64:367-378.

De Miranda BEC, Suassuna ND, Reis A (2010). Mating type, mefenoxam sensitivity, and pathotype diversity in *Phytophthora infestans* isolates from tomato in Brazil. Pesq. Agropec. Bras. Bras. 45:671-679.

Elizondo R, Oyanedel E (2010). Field testing of tomato chilling tolerance under varying light and temperature conditions. Agricultura técnica 70:552-558.

Elsayed AY, Silva DJHD, Carneiro PCS, Mizubuti ESG (2012). The inheritance of late blight resistance derived from *Solanum habrochaites*. Crop Breed. Appl. Biotechnol. 12(3):199-205.

Eshed Y, Zamir D (1994). A genomic library of *Lycopersicon pennellii* in *L. esculentum*: a tool for fine mapping of genes. Euphytica 79:175-179.

FAO (2017). FAOSTAT statistics. Food and agriculture organization of United Nations.

Ferreira RB, Monteiro S, Freitas R, Santos CN, Chen Z, Batista LM, Duarte J, Borges A, Teixeira AR (2007). The role of plant defense proteins in fungal pathogenesis. Mol. Plant Pathol. 8:677-700.

Foolad MR, Merk H, Ashrafi H (2008). Genetics, genomics and breeding of late blight and early blight resistance in tomato. Cri. Rev. Plant Sci. 27:75-107.

Gardner RG, Panthee DR (2010). NC 1 CELBR and NC 2 CELBR: Early blight and late blight resistant fresh market tomato breeding lines. Hort. Science 45:975-976.

Goodwin SB, Sujkowski LS, Fry WE (1995). Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. Phytopathology 85:669-676.

Haggard JE, Johnson EB, Clair DAS (2013). Linkage relationships among multiple QTL for horticultural traits and late blight (*P. infestans*) resistance on chromosome 5 introgressed from wild tomato *Solanum habrochaites*. G3: Genes| Genomes| Genet. 3:2131-2146.

Haq I, Rashid A, Khan SA (2008). Relative efficacy of various fungicides, chemicals and biochemicals against late blight of potato. Pak. J. Phytopathol. 21(1): 129-133.

Harbaoui K, Harrabi M, Vleeshouwers VGAA, Hamada W (2011). Virulence patterns of *Phytophthora infestans* isolates using R differential set of *Solanum demissum*: a useful tool to identify pathogen races in Tunisia. Tun. J. Plant Prot. 6:1-10.

Irzhansky I, Cohen Y (2006). Inheritance of resistance against *Phytophthora infestans* in *Lycopersicon pimpinellifolium* L3707. Euphytica 149:309-316.

Kim MJ, Mutschler MA (2006). Characterization of Late Blight Resistance Derived from *Solanum pimpinellifolium* L3708 against Multiple Isolates of the Pathogen *Phytophthora infestans*. J. Amer. Soc. Hort. Sci. 131:637-645.

Labate JA, Robertson LD (2012). Evidence of cryptic introgression in tomato (*Solanum lycopersicum* L.) based on wild tomato species alleles. BMC Plant Biol. 12:133.

Lai A, Cianciolo V, Chiavarini S, Sonnino A (2000). Effects of glandular trichomes on the development of *Phytophthora infestans* infection in potato (*S. tuberosum*). Euphytica 114:165-174.

Lees AK, Wattier R, Shaw DS, Sullivan L, Williams NA, Cooke DEL (2006). Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. Plant Pathol. 55:311-9.

Li J, Liu L, Bai Y, Finkers R, Wang F, Du Y, Yang Y, Xie B, Visser RGF, van Heusden AW (2011). Identification and mapping of quantitative resistance to late blight (*Phytophthora infestans*) in *Solanum habrochaites* LA1777. Euphytica 179:427-438.

Li Y, Cooke DEL, Jacobsen E, van der Lee T (2013). Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*. J. Microbiol. Methods 92:316-322.

Li Y, Govers F, Mendes O (2010). A new set of highly informative markers for *Phytophthora infestans* population analysis assembled into an efficient multiplex. Mol. Ecol. Resour. 10:1098-105.

Li Y, van der Lee TA, Evenhuis A, van den Bosch GB, van Bekkum PJ, Förch MG, van Gent-Pelzer MP, van Raaij HM, Jacobsen SWE, Govers F, Vleeshouwers VG, Kessel GJ (2012). Population dynamics of *Phytophthora infestans* in the Netherlands reveals expansion and spread of dominant clonal lineages and virulence in sexual offspring. G3: Genes| Genomes| Genet. 2:1529-40.

Lievens B, Hanssen IRM, Vanachter ACRC, Cammue BPA, Thomma BPHJ (2004). Root and foot rot on tomato caused by *Phytophthora infestans* detected in Belgium. Plant Dis. 88:86.

Lima MA, Maffia LA, Barreto RW, Mizubuti ESG (2009). *Phytophthora infestans* in a subtropical region: survival on tomato debris, temporal dynamics of airborne sporangia and alternative hosts. Plant Pathol. 58:87-99.

McDonald BA, Linde C (2002). The population genetics of plant

pathogens and breeding strategies for durable resistance. Euphytica 124:163-180.
Momotaz A, Scott JW, Schuster DJ (2010). Identification of Quantitative Trait Loci conferring resistance to *Bemisia tabaci* in an F2 population of *Solanum lycopersicum* × *Solanum habrochaites* accession LA1777. J. Am. Soc. Hort. Sci. 135:134-142.
Montes MS, Nielsen B, Schmidt S, Bødker L, Kjøller R, Rosendahl S (2016). Population genetics of *Phytophthora infestans* in Denmark reveals dominantly clonal populations and specific alleles linked to metalaxyl-M resistance. Plant Pathol. 65:744-753.
Moreau P, Thoquet P, Olivier J, Laterrot H, Grimsley N (1998). Genetic mapping of Ph-2, a single locus controlling partial resistance to *Phytophthora infestans* in tomato. Mol. Plant Microbe In. 11:259-269.
Muigai SG, Bassett MJ, Schuster DJ, Scott JW (2003). Greenhouse and field screening of wild *Lycopersicon* germplasm for resistance to the whitefly *Bemisia argentifolii*. Phytoparasitica 31:27-38.
Nowicki M, Kozik EU, Foolad MR (2013). Late blight of tomato. pp. 241-265. In: R. K. Varshney and R. Tuberosa (ed.). Translational genomics for crop breeding. John Wiley & Sons Ltd.
Panthee DR, Gardner RG (2010). 'Mountain Merit': A late blight-resistant large-fruited tomato hybrid. Hort. Sci. 45:1547-1548.
Peralta IE, Spooner DM, Knapp S (2008). Taxonomy of wild tomatoes and their relatives (*Solanum* sect. *Lycopersicoides*, sect. *Juglandifolia*, sect. *Lycopersicon*; *Solanaceae*). Syst. Bot. Monogr. 84:1-186.
Pule BB, Meitz JC, Thompson AH, Linde CC, Fry WE, Langenhoven SD, Meyers KL, Kandolo DS, Van Rij NC, McLeod A (2013). *Phytophthora infestans* populations in central, eastern and southern African countries consist of two major clonal Lineages. Plant Pathol. 62:154-165.
Randall E, Young V, Sierotzki H, Scalliet G, Birch PR, Cooke DE, Csukai M, Whisson SC (2014). Sequence diversity in the large subunit of RNA polymerase I contributes to mefenoxam insensitivity in *Phytophthora infestans*. Mol. Plant. Pathol. 15:664-676.
Rick CM (1971). The tomato Ge locus: Linkage relations and geographic distribution of alleles. Genetics 67:75-85.
Saville A, Graham K, Grünwald NJ, Myers K, Fry WE, Ristaino JB (2015). Fungicide sensitivity of US genotypes of *Phytophthora infestans* to six oomycete-targeted compounds. Plant Dis. 99: 659-666.
Singh PH, Singh BP (2006). Population dynamics of metalaxyl sensitive and resistant strains of *Phytophthora infestans* under controlled environment. Potato J. 33:162-163.
Smart CD, Fry WE (2001). Invasions by the late blight pathogen: renewed sex and enhanced fitness. Biol. Invasions 3:235-243.
Smart CD, Tanksley SD, Mayton H, Fry WE (2007). Resistance to *Phytophthora infestans* in *Lycopersicon pennellii.* Plant Dis. 91:1045-1049.
Stevenson WR, James RV, Inglis DA, Johnson DA, Schotzko RT, Thornton RE (2007). Fungicide spray programs for defender, a new potato cultivar with resistance to late blight and early blight. Plant Dis. 91:1327-1336.
Stroud JA, Shaw DS, Hale MD, Steele KA (2016). SSR assessment of *Phytophthora infestans* populations on tomato and potato in British gardens demonstrates high diversity but no evidence for host specialization. Plant Pathol. 65:334-341.
Tian YE, Yin JL, Sun JP, Ma YF, Wang QH, Quan JL, Shan WX (2016). Population genetic analysis of *Phytophthora infestans* in northwestern China. Plant Pathol. 65:17-25.
Turkensteen LJ (1973). Partial resistance of tomatoes against *Phytophthora infestans,* the late blight fungus, The Netherlands: Wageningen University, Ph D thesis P. 88.
Vetukuri RR, Åsman AK, Tellgren-Roth C, Jahan SN, Reimegård J, Fogelqvist J, Savenkov E, Söderbom F, Avrova AO, Whisson SC, Dixelius C (2012). Evidence for small RNAs homologous to effector-encoding genes and transposable elements in the oomycete *Phytophthora infestans*. PLoS ONE 7:e51399.
Zhang C, Liu L, Wang X, Vossen J, Li G, Li T, Zheng Z, Gao J, Guo Y, Visser RGF, Li J, Bai Y, Du Y (2014). The *Ph‐3* gene from *Solanum pimpinellifolium* encodes CC‐NBS‐LRR protein conferring resistance to *Phytophthora infestans*. Theor. Appl. Genet. 127:1353-1364.
Zhang X, Kim B (2007). Physiological races of *Phytophthora infestans* in Korea. Plant Pathol. J. 23:219-222.