

Related wild species for breeding of tomato resistant to early blight disease (*Alternaria solani*)

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Abstract. Tomato (*Solanum lycopersicum* L.) is susceptible to many fungal diseases, including early blight of foliage caused by the necrotroph fungus *Alternaria solani*. Frequent application of fungicide is the major component to keep the disease low. Resistant tomato cultivar is the most desirable as it can reduce the cost of disease control significantly. So far, sources of resistance to early blight can only be found in wild relative species of tomato, and few of them have been used in traditional breeding. Unfortunately, tomato lines bred from wild donor parent still exhibit poor horticultural performances like low yield, and late maturity, and indeterminate plant habit, which hinders the release of these lines directly as cultivars. The quantitative expression and polygenic control of the early blight resistance trait, as well as the influence of plant developmental stages and environmental conditions, complicate phenotypic selection in traditional breeding. Genotypic selection by using closely linked-markers to the resistance loci is thus preferred, but mapping of early blight resistance QTL in interspecific crosses of tomato have not obtained markers which are useful for marker-assisted breeding. This review presents sources of early blight resistance in wild species of tomato and efforts in dissecting early blight resistance QTL via linkage analysis with molecular markers. Strategies to obtain closely-linked markers and genomics-assisted breeding to facilitate the introgression of useful resistance genes to cultivated tomato are discussed.

Keywords: tomato, early blight, wild related species, marker-assisted breeding.

1. Introduction

The cultivated tomato, *Solanum lycopersicum* L. section *Lycopersicon* (formerly *Lycopersicon esculentum* Mill.) [1] is susceptible to over 200 diseases [2]. Fungal diseases are the most important threat to tomato productivity and can increase production costs by 30% due to application of fungicides alone [3]. Early blight (EB) caused by the necrotroph fungus *Alternaria solani* Ellis and G. Martin, is the most frequent and widely distributed foliar diseases of tomato especially in areas with high rainfall and humidity [4]. About 15–20 fungicidal sprays must be applied per season to achieve reasonable control of EB [5]. Therefore, improvement of cultivars with increased fungal resistance is still the primary goal of private and public tomato breeding program [6].

EB, as the name implies, is strongly associated with tomato with early maturing type. Older senescing leaves and plants at fruiting stage or with heavy fruit load are more susceptible to the disease [4]. Consequently, early-maturing cultivars are more susceptible to EB than medium- or late-



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maturing cultivars because leaves of early-maturing cultivars tend to senesce earlier in the growing season [7]. Currently, no early maturing tomatoes have adequate EB resistance under field epidemics and therefore breeders are still developing highly resistant cultivar with desirable horticultural performances [8].

Sources of EB resistance are not known in cultivated tomato but can be found in its wild relative species. However, their utilization in the development of resistant tomato cultivar have been restricted by incompatibility barrier, the quantitative expression of EB which makes difficult the selection of the best and promising progenies, and negative linkage drag introgressed from the donor parent [9, 10, 11]. This review presents sources of resistance in wild related species of tomato to *A. solani*, progress in their utilization in EB resistance breeding, mapping of the EB resistance genes, and strategies to facilitate and accelerate the transfer of resistance to cultivated tomato.

2. Wild related species of tomato

Wild tomatoes are native to western South America and distributed along the coast and in the Andes from Ecuador through Peru and to northern Chile, and in the Galapagos Islands [12]. Wild tomato species grow in a variety of habitats, from coastal regions to high altitude (over 3,300 m) of mountain regions, near river and creeks, as well as in extreme dry habitats [13]. Taxonomic classification based on morphological data combined with molecular data of chloroplast DNA (cpDNA) restriction fragment length polymorphisms (RFLPs), nuclear microsatellites, isozymes, internal transcribed spacers of nuclear ribosomal DNA (ITS; multiple copy), the single-copy nuclear encoded granule-bound starch synthase gene (GBSS or waxy gene), and amplified fragment length polymorphisms (AFLP) recognized 12 wild related species of tomato [14].

3. Early blight resistance in wild related species of tomato

Extensive screening program in the temperate and tropical areas performed in the field or under controlled environment in glasshouse identified six wild tomato species to have high or useful resistance to *A. solani* (Table 1). These are *S. arcanum* (syn. *L. peruvianum*), *S. chilense* (syn. *L. chilense*), *S. habrochaites* (syn. *L. hirsutum*), *S. neorickii* (syn. *L. parviflorum*), *S. peruvianum* (syn. *L. peruvianum*) and *S. pimpinellifolium* (syn. *L. pimpinellifolium*) (Table 1). *S. lycopersicoides* was also resistant to this fungus in a laboratory test studying host-plant interaction of fungal necrotroph [15]. The resistance of *S. pimpinellifolium*, the most closely related species to cultivated tomato, to *A. solani* is usually less than the other wild species.

Resistance to *A. solani* is characterized by low percentage of disease severity (in the case of natural or artificial infection in field tests and artificial spray inoculation in glasshouse tests) and small lesion size of less than 1 cm² (for droplet inoculation of detached leaves in laboratory or intact leaves on whole plant in glasshouse; Figure 1). Detailed observations on resistant accessions showed that apart from small in size, the frequency of necrotic lesions on resistant species was also lower compared to that on cultivated tomato or susceptible accessions [16].

Fungal growth and sporulation in the lesion of strong resistance source were also limited [15]. Intra-variation of EB resistance occurred within the same species. This was especially observed in *S. habrochaites*, *S. peruvianum* and *S. pimpinellifolium* where different accessions showed a range of disease responses from resistant to highly susceptible [16,17].

Table 1. Wild related species of tomato with resistance to *Alternaria solani*, the fungal agent of early blight disease.

Species and accession	Test method and environment	Disease severity*	Reference
<i>Solanum arcanum</i> LA2157	Droplet inoculation on leaves; glasshouse	1.4 mm ²	[16]
<i>S. chilense</i> G1.1556	Droplet inoculation on leaves; glasshouse	6.7 mm ²	[16]
<i>S. chilense</i> LA3111	Droplet inoculation on leaves; laboratory	Hypersensitive response lesion	[17]
<i>S. habrochaites</i>			
PI126445	Spray inoculation; field	N/A	[18]
PI390513, PI390514, PI390516, PI390658, PI390660, PI390662, PI390663	Spray inoculation; field	N/A	[19]
B6013	Natural infection; field	10.6–10.8%	[20]
LA2100, LA2124, LA2204	Spray inoculation; glasshouse	2.0–2.9 (on a disease scale of 1–9)	[21]
LA2099, LA1777, PI126445, PI390662	Spray inoculation; glasshouse	11.6–21.2%	[22]
<i>S. lycopersicoides</i> LA2951	Droplet inoculation on detached leaflets; laboratory	2 mm ²	[15]
<i>S. neorickii</i> G1.1601	Droplet inoculation on leaves; glasshouse	6.68 mm ²	[16]
<i>S. peruvianum</i>			
LA2192, LA1365, LA1910, LA1983, PI270435, PI365951, PI390665, PE33, PI390671	Spray inoculation; glasshouse	2.0–3.9 (on a disease rating scale of 1–9)	[21]
PE33, PE44, PI390665	Droplet inoculation on leaves; glasshouse	1.46–6.23 mm ²	[16]
<i>S. pimpinellifolium</i>			
PI212408, PI251320, PI365912, PI365928, PI390519, PI303662	Spray inoculation; field	N/A	[19]
EC-65992, EC-65993, EC-85617, EC-96522, EC-121453	Natural infection; field	N/A	[23]
A1921	Natural infection; field	9.0–11.7%	[20]
L4394 (IHR1939)	Spray inoculation; field	38.0%	[24]

*Measured quantitatively based on necrotic lesion size (length × width) in mm², percentage of cumulative disease index or defoliation, or percentage of leaf area infected on a diseases rating scale, or measured qualitatively for the presence of small hypersensitive lesion. N/A = data not available.

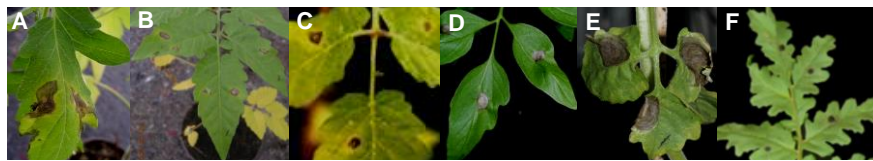


Figure 1. Variation in early blight necrotic lesion sizes among cultivated tomato *Solanum lycopersicum* (A) and its wild relative species *S. arcanum* LA2157 (B), *S. habrochaites* LA2650 (C), *S. neoricki* G1.1601 (D), *S. pennellii* (E), and *S. lycopersicoides* LA2951 obtained after droplet inoculation of *Alternaria solani* spores. Panel A to E were personal documentation, whereas panel F was taken from Smith *et al.* [15].

4. Introgression and marker-assisted breeding for early blight resistance

Introgression breeding often carries negative linkage drag which can persist within a genome despite repeated backcrossing, especially if recombination is suppressed [11]. This phenomenon also occurs in tomato breeding for resistance to EB using wild donor parent. So far, only *S. habrochaites* PI126445 that has been used in the development of resistant tomato and result in several moderately resistant lines [25–28]. EB resistance in these lines is strongly associated with late maturity, low yielding ability, and indeterminate growth habit [22]. Plants with indeterminate/semi-determinate growth habit continue producing younger leaves which are less susceptible to the fungus. Consequently, they appear healthier than determinate plants while they may not possess genetic resistance [9].

Genetic analyses, either classical or via linkage analyses with molecular markers, concluded that EB resistance is under complex genetic control. EB resistance is quantitatively expressed and controlled by additive and non-additive interaction effects of multiple genes and highly influenced by physiological maturity and environmental factors [5,7,24,28,29,30,31,32]. The heritability estimate of EB resistance is low to moderate (0.26–0.72) [5,29,32,34].

The complex and modest heritability of EB inheritance together with the aforementioned confounding factors to EB resistance expression have slowed the breeding process, which relies on phenotypic selection. To speed up breeding process, genotypic selection using closely-linked markers to EB resistance loci is needed. Unfortunately, progress in mapping quantitative trait locus (QTL) with effects on EB resistance in three interspecific crosses, i.e. *S. lycopersicum* NC84173 × *S. habrochaites* PI126445 [26], *S. lycopersicum* cv. Solentos × *S. arcanum* LA2157 [25] and *S. lycopersicum* NCEBR1 × *S. pimpinellifolium* LA2093 [30], has not identified closely-linked markers to EB resistance. Five to 14 QTLs which encompassed a range of marker intervals (1.8 to 73.0 cM) were identified in 7 of 12 tomato chromosomes with a rather low individual effect (3.0% to 25.9%; Table 2). Few QTLs were contributed from the susceptible parent [26]. Some QTLs were species specific, but some were common or in overlapped positions despite identified in different genetic background and environment, indicating their authenticity on EB resistance and deserve further genetic dissection [26].

Within such large intervals, markers are loosely linked with EB resistance and thus are not applicable for marker-assisted breeding because of crossovers between markers and the EB resistance QTLs [2]. Fine mapping to locate the QTLs precisely must be attempted by development of a series of near-isogenic lines (NILs) and sub-NILs consisting of plants each with a different single homozygous introgression containing one target QTL [9]. Marker-assisted selection is applied to speed up the return to recurrent parent type by screening individuals for the presence of the target locus in each generation of backcross and the absence of extraneous donor DNA throughout the rest of the genome. Fine mapping is not only essential for validation the actual effect of individual QTL, but also necessary for reducing the linkage drag associated with introgressed QTL, and to determine whether QTL effects on EB resistance are caused by several tightly linked genes or by one gene with pleiotropic effects [2,4].

Table 2. Quantitative trait loci (QTLs) associated with resistance to *Alternaria solani*, the fungal pathogen of early blight disease of tomato (*Solanum lycopersicum*).

Marker type	Chr ^a	LOD score	PVE ^b or R ² value	Interval (cM)	Reference
<u>Parent; size and type of linkage mapping population; and type of population for QTL analysis</u>					
<i>S. lycopersicum</i> NC84173 × <i>S. habrochaites</i> PI126445; 145 BC ₁ plants; 145 BC ₁ plants and BC ₁ S ₁ families ^{c, d}					
RFLPs (141) and RGAs (23)	1	3.5–7.0	7.5–21.9%	73.0	[33]
	2	2.8–2.9	15.3–15.9%	39.6	
	3	2.9	9.1%	13.1	
	5	2.4–2.6	7.3–7.9%	24.2	
	5	2.4–3.7	7.3–11.3%	27.8	
	8	3.0–3.7	9.0–10.3%	16.3	
	8	5.2–5.4	14.3–21.0%	53.8	
	9	2.8–8.2	7.5–25.9%	51.2	
	9	3.7–5.1	10.1–16.2%	34.7	
	10	4.1–6.8	10.1–20.2%	48.0	
	11	3.2–3.8	11.5–13.2%	15.8	
	11	3.0–3.2	7.1–9.9%	22.2	
	12	2.5–3.1	8.3–10.3%	26.3	
	12	4.1	12.9%	26.7	
<i>S. lycopersicum</i> cv. Solentos × <i>S. arcanum</i> LA2157; 172–6 F ₂ plants; 175 F ₂ plants and 156 F ₃ families ^e					
31 SSRs, SNPs, and 344 AFLPs	1	4.1	6.8%	31.0	[31]
	2	4.2–5.6	7.2–10.3%	42.0	
	2	3.4–9.0 ^f	7.6–16.2%	18.0	
	5	4.0–6.1	8.1–10.5%	36.0–41.0	
	6	3.7–6.3	8.2–10.8%	21.0–36.0	
	7	7.5–8.3 ^f	13.3–16.0%	30.0–33.0	
	9	4.8–5.2	8.2–9.2%	31.0	
	9	4.6–8.7	8.6–15.5%	22.0–23.0	
<i>S. lycopersicum</i> NCEBR1 × <i>S. pimpinellifolium</i> LA2093; 172 RILs (F ₇); 172 F ₇ and 4128 RILs for each F ₈ to F ₁₀ generation ^c					
RFLPs, ESTs, CAPSs, and SSRs (total 294 markers)	2	3.0–3.6	3%	3.5–11.1	[35]
	2	2.5–3.6	8%	13.9–17.2	
	5	3.9–7.1 ^f	11–18%	5.7–12.1	
	6	3.7–4.9	16%	2.5–14.2	
	9	3.0–5.1 ^f	7–14%	1.8–9.0	

^a Chr = chromosome.^b PVE = phenotypic variation explained.^c Identified by linkage analysis of marker with percent defoliation in the field using simple interval mapping (SIM) and composite interval mapping (CIM) approaches.^d Selective genotyping was applied.^e Identified by linkage analysis of marker with lesion size and percentage of small lesions in glasshouse and disease scores in the field using multiple-QTL-model mapping (MQM) procedure.^f Resistance alleles were contributed from the susceptible parent.

Robust marker like single nucleotide polymorphisms (SNPs) which detect minor variation will be useful not only for selection of a resistant genotype at the early seedling stage, but also for dissection of complex quantitative resistance into individual genes, and understanding the genetic basis of correlation between EB resistance and negative horticultural traits [32]. A large number of SNPs obtained from the next generation sequencing (NGS) projects and high throughput genotyping platforms is now available [33,34]. The recent development in genomic research can also aid in QTL dissection. For example the combination of high-throughput transcriptome analyses with a permanent population such as introgressed lines (ILs) [35]. ILs with a differential performance for the trait of interest are comparatively analyzed for transcriptional regulation. Single differential genes identified by microarray analysis are validated using real-time qPCR and then validated for its functionality through mutagenesis or transformation. Closely linked marker can then be used in genomic-assisted breeding [35].

5. Concluding remarks

Seven related wild species of tomato possess medium to strong EB resistance, which is not present in the cultivated tomato. Development of EB resistant tomato lines using wild donor species is still hampered by complex genetic control of resistance and negative linkage drag introgressed from wild donor parent, like late maturity, indeterminism, and reduced yield. Progress in genetic mapping of EB resistance QTL studies has not obtained closely-linked marker to EB resistance genes which can be used in marker-assisted breeding. Development of NILs, sub-NILs and permanent population ILs is necessary to fine-mapping the QTL position, to estimate the actual individual effect of each gene, and to break the linkage between the early blight resistance gene and negative horticultural traits if these traits are caused by several tightly linked genes. The application of recent development in genomic research such as large number of SNPs discovered from the NGS technology and high-throughput transcriptome analyses combined with ILs can aid precise identification of EB resistance genes which are useful for introgression breeding.

6. References

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