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Genetic Characterization of Late Blight Resistance in *Solanum pimpinellifolium* Accession PI 270442

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Abstract

One of the most destructive foliar diseases of the cultivated tomato (*Solanum lycopersicum*) is late blight (LB), caused by the oomycete *Phytophthora infestans*. Due to the limited number of commercial tomato cultivars with resistance to this disease, control is mainly through cultural practices and heavy use of fungicides. The appearance of fungicide-resistant *P. infestans* genotypes, however, necessitates identification of new genetic sources of host resistance to the disease and subsequent breeding of new resistant cultivars. Several new sources of resistance to tomato LB were identified in a recent screening of the tomato related wild species *S. pimpinellifolium*. In the present study, we examined the genetic basis of LB resistance in accession PI 270442, through parent-offspring correlation analysis, generation means analysis, and analysis of response to selection, using populations derived from crosses with a LB-susceptible tomato breeding line.

Across experiments, estimates of heritability (h^2) for LB resistance ranged from 0.56 – 0.86, suggesting the heritable nature of this resistance and possibility of effectively transferring LB resistance from PI 270442 to the cultivated tomato genetic background through phenotypic selection and traditional backcross breeding. Genetic mapping studies are currently underway to identify the associated molecular markers for LB resistance in PI 270442.

Keywords: disease resistance, generation means analysis, qualitative resistance, resistance breeding, response to selection, *Solanum lycopersicum* L.

1. Introduction

Late blight (LB), caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is one of the most destructive diseases of the cultivated tomato (*Solanum lycopersicum* L.) and potato (*Solanum tuberosum* L.) worldwide [21]. The disease can defoliate an entire susceptible, unprotected crop of tomato or potato within 7 – 10 days [39]. The LB pathogen can reproduce both asexually and sexually, with the more common asexual life cycle generating thousands of asexual zoospores per lesion within 5 – 10 days. Sexual reproduction may occur when both mating types, A1 and A2, are present and interact. From such an interaction, sexual spores, known as oospores, are produced [32], which have the potential to result in new pathogen isolates with resistance to fungicide or the ability to overcome host resistance [13].

Prior to the 1980s, there were few reports of the A2 mating type outside Mexico, limiting sexual reproduction and rapid evolution of the pathogen [20]. Since then, however, A2 mating type has been detected and sexual reproduction reported in several countries around the world [20, 31, 45]. In the United States, oospore production has been limited, though it is thought to have occurred in Washington and Oregon states following detection of A1 and A2 in the Columbia Basin in 1993 [26, 36], and again in central New York state in 2010 and 2011 [12]. The resulting genotypes that have emerged are more aggressive, threatening current disease control measures.

Due to the pathogen's rapid life cycle and the difficulty of detecting pathogen with low inoculum levels in the field, the most common method of controlling LB in tomato is through cultural practices and heavy use of preventative fungicides [16]. The intensive spray programs increase costs for growers and contribute to environmental hazards. Further, they have led to the emergence of new *P. infestans* genotypes and increased prevalence of fungicide resistance in the pathogen. For example, in clonal lineages US-6, 7, 8, 11 and 20, resistance has been observed to metalaxyl or mefenoxam (metalaxyl-M), two of the most effective systemic fungicides against *P. infestans*. Intermediate resistance has also been identified in clonal lineages US-21, 22, 23 (currently the most prevalent in the US) and 24 [9, 27, 33, 44].

The use of host resistance is an alternative and complementary approach to managing LB disease in tomato. A high level of genetic diversity exists in related wild species of tomato, which can be sources of desirable traits such as LB resistance. To date, all commercially available LB resistance genes in tomato (*Ph-1*, *Ph-2*, and *Ph-3*) were derived from *S. pimpinellifolium L.*, the closest wild relative of the cultivated tomato. LB-resistance gene *Ph-1* [4] was shown to be a dominant gene specific to *P. infestans* race T_0 [43]. However, T_0 was soon displaced by T_1, the now dominant race, rendering *Ph-1* ineffective [11, 48]. *Ph-2* offers only partial resistance and incomplete dominance to the pathogen [22]. A high level of resistance against a wide range of *P. infestans* isolates/clonal lineages can be found in *Ph-3* gene [3, 8, 50], which is commercially available in several tomato cultivars such as Plum Regal [25]. Currently, the most effective LB-resistant lines or cultivars of tomato are those with *Ph-2* + *Ph-3* resistance genes combined, which offer a high level of resistance against a broad range of pathogen isolates, including isolates that could overcome either *Ph-2* or *Ph-3* resistance gene [24]. Commercially-available cultivars with both resistance genes include Mountain Magic, Mountain Merit, and Defiant PhR [23, 24, 30, 42]. Another source of LB resistance in *S. pimpinellifolium* was identified in accession PI 270443, which exhibited LB resistance comparable to *Ph-2* and *Ph-3* resistance genes combined [17]. This resistance was determined to be heritable [35] and controlled by two loci on chromosomes 1 and 10 [34]. Efforts are underway to breed resistance from PI 270443 into elite tomato breeding lines and hybrid cultivars (MR Foolad, unpublished).

Additional sources of LB resistance have been identified in tomato wild species *S. habrochaites* S. Knapp & D.M. Spooner and *S. pennellii* Corr. For example, multiple quantitative trait loci (QTLs) conferring race non-specific resistance were reported in different accessions of *S. habrochaites* [1, 2, 5, 6, 14], and a QTL conferring reduced disease symptoms was reported in *S. pennellii* accession LA 716 [46]. However, incorporation of LB resistance from these species into commercial cultivars of tomato has been challenging due to the quantitative nature of resistance, and presence of undesirable horticultural characteristics associated with the resistance (linkage drag) [6, 38].

New *P. infestans* isolates have been reported to overcome *Ph-3* resistance [10, 37], demanding further identification, characterization, and incorporation of new sources of LB resistance in tomato. Recently, 67 accessions of *S. pimpinellifolium* were explored for their response to LB, of which 12 were identified exhibiting strong LB resistance in field, greenhouse and growth chamber (with detached-leaflets) experiments. Accession PI 270442 exhibited LB resistance similar to a commercial breeding line containing *Ph-2* + *Ph-3* resistance genes combined [18, 19]. It also was shown to be highly resistant against *P. infestans* clonal lineages US-13, US-14 (six isolates) and US-23 [19]. In the present study, we characterized the genetic basis of LB resistance in PI 270442 by assessing h^2 of the resistance, determining response to selection, and estimating the number of loci contributing to the resistance.

2. Materials and Methods

Plant materials

Filial and backcross populations were developed by crossing LB-resistant *S. pimpinellifolium* accession PI 270442 (staminate parent) with LB-susceptible *S. lycopersicum* breeding line Fla. 8059. Seeds of PI 270442 and Fla. 8059 were originally obtained from USDA ARS Plant Genetic Resources Unit (PGRU), Geneva, NY, and J.W. Scott, University of Florida, Gulf Coast Research and Education Center, Wimauma, FL, respectively. An F_1 plant was grown in the greenhouse and self-pollinated to produce F_2 progeny, and also backcrossed to Fla. 8059 to produce BC_1P_1 progeny. Selected F_2 plants were grown and self-pollinated to produce F_3 progeny families (as described below). Control genotypes used in the experiments included LB-susceptible inbred cultivar New Yorker (*Ph-1*), and LB-resistant lines NC 63EB (*Ph-2*), NC 870 (*Ph-3*) and NC 03220 (*Ph-2* + *Ph-3*), seeds of which were originally received from R.G. Gardner, North Carolina State University, Mills River, NC.

Pathogen isolates

Two *P. infestans* isolates, PDA-8030275 and RS2009T1 (both race T1), collected from commercial tomato fields at Rock Springs in Centre County, PA, were used in this study because of their high infection rates on tomatoes and predominant natural occurrence in the northeastern U.S. over the past several years [19, 28, 29]. Isolate PDA-8030275 (US-13) was used in screening experiments conducted during 2012, and isolate RS2009T1 (US-23) was used in experiments during 2013 – 2015. All tomato genotypes examined in this and our other studies appeared to respond similarly to both isolates, and thus the data collected using each isolate could be compared.

Inoculum preparation

Pathogen maintenance and inoculum preparation were as described elsewhere [47]. Disease pressure was partially influenced by external conditions, and therefore for each experiment, varying inoculum concentrations were needed to compensate with the average concentration being 6,400 sporangia/ml.

Pathogen inoculation and disease evaluation

In all experiments, similar protocols were used for plant growth, pathogen inoculation, and disease evaluation. Briefly, plants were grown for six weeks under greenhouse conditions and moved to an isolated, environmentally-controlled greenhouse section within 24 hours prior to inoculation. The morning of and prior to inoculation, lights were turned off and blackout curtains lowered along the greenhouse walls. The temperature was reduced to 16 – 18 ^{o}C and overhead sprayers

turned on to increase humidity to > 95%. Clear plastic was hung around the benches to prevent water from directly spraying the plants. Approximately 4 – 6 hours later, the overhead sprayers were turned off, the clear plastic opened, and the plants evenly sprayed with a fine mist of distilled water. One half-hour later, the inoculum suspension was sprayed evenly over the plants using a pressurized spray bottle at a rate of 1 L per 1,000 plants. After one half-hour, the plants were sprayed again at the same rate. The plastic layer was again closed and overhead sprayers turned on to return humidity to > 95%. The following morning, the blackout curtains were raised to allow natural light into the greenhouse. Disease was monitored as it progressed until advanced symptoms were visible on the susceptible control genotypes. Depending on greenhouse conditions and disease progress, most experiments were evaluated 7 – 10 days following inoculation. If sufficient disease was not developed (as determined by disease progress in the susceptible parent and control genotypes), plants were re-inoculated. Plants were scored visually for percent disease severity (% DS) on a scale of 0 – 100%, where 0% indicated no foliar disease symptoms, and 100% indicated complete leaf necrosis or defoliation. The % DS scored in different generations were used for estimation of heritability, as explained below.

Estimation of heritability using parent:offspring correlation analysis

To estimate heritability (h^2) using parent:offspring (P:O) correlation analysis, disease evaluations were performed in F_2:F_3 (P:O) generations. The F_2 individuals (n = 178) were grown along with the parents (Fla. 8059 = P_1 and PI 270442 = P_2) and one LB-susceptible and three LB-resistant control genotypes (n = 6 each). Plants were inoculated using the *P. infestans* US-13 genotype, following the procedures described above. While F_2 plants were scored individually, the six plants of each of the parental and control genotypes were given one overall disease score. Following screening of the F_2 population, 40 resistant plants (% DS $\leq$ 5%) and 20 susceptible plants (% DS $\geq$ 50%) were selected, treated with fungicide Bravo® (Syngenta Crop Protection LLC, Greensboro, NC) to cleanse them of pathogen, and grown to maturity and self-pollinated to produce F_3 progeny seed.

The 60 F_3 progeny families were evaluated in three separate experiments. Experiment I included approximately 12 F_3 plants per family, along with the parental inbred lines (n = 21 – 24 each). Experiments II and III included approximately 6 and 12 F_3 plants per family, respectively, along with the parental lines and three LB-resistant and one LB-susceptible control genotypes (n = 12 each for Experiment II, and n = 22 – 24 each for Experiment III). Across all experiments, 23 – 30 F_3 individuals per family were grown and screened, with the exception of three families with 18 individuals and one family with 20 individuals (due to low germination). When multiple replications were grown within an experiment, each family was divided into replications of 6 plants each and placed on separate benches within the same greenhouse. Two replications of parental and control genotypes were also included. Plants were inoculated using the *P. infestans* US-23 genotype.

Parent:offspring (F_2:F_3) correlation coefficient was calculated as an estimate of h^2, using the following equation:

$$h^2_{F2:F3} = r_{F2:F3} = \frac{Cov_{F3,F2}}{(V_{F3}V_{F2})^{1/2}} = \frac{V_A + 1/2\,V_D}{[(V_A + 1/4\,V_D + 1/n\,V_E)(V_A + V_D + V_E)]^{1/2}}$$

where V_{F2} is the variance of selected F_2 individuals, V_{F3} is the variance of the F_3 progeny families, $Cov_{F3,F2}$ is the covariance between F_2 parental individuals and corresponding F_3 progeny families, V_A, V_D and V_E are the additive, dominance and environmental variances, respectively, and n is the number of individuals in the F_3 progeny families.

Estimation of heritability using generation means analysis

Heritability was estimated by generation means analysis (GMA) using parental, F_1, F_2 and BC_1P_1 generations. Parent (n = 21 – 22), F_1 (n = 24), F_2 (n = 124) and BC_1P_1 (n = 95) plants were evaluated for disease response (% disease severity; % DS), and used to estimate generation means and variances. In the GMA experiments, one LB-susceptible and three LB-resistant control genotypes (n = 23 – 24 plants each) were also included. Plants were divided into two replications of approximately equal numbers per generation, and placed on separate benches in the same greenhouse. The components of total variance were calculated using the following equations:

$$V_E = \frac{V_{P1} + V_{P2} + V_{F1}}{3} \qquad V_A = 2V_{F2} - 2V_{BC1P1} \qquad V_D = V_{F2} - V_A - V_E$$

where V_E, V_A and V_D are environmental, additive and dominance variances, respectively, and V_{P1}, V_{P2}, V_{F1}, V_{F2} and V_{BC1P1} are generation variances. Narrow-sense heritability (h^2_N) was estimated using the formula:

$$h^2_N = \frac{V_A}{V_A + V_D + V_E}.$$

Determination of realized heritability through analysis of response to selection

The F_2:F_3 generations were used to examine the response to selection for LB resistance in two experiments. Realized heritability (h^2_R) was calculated using the response-to-selection equation: $h^2_R = R/S$, where R is the selection response (i.e., the change in the mean % DS from F_2 parent to F_3 progeny generation) and S is the selection differential (i.e., the difference between the mean % DS of selected resistant F_2 individuals and overall mean of the parental F_2 population before selection) [15]. F_2 plants for the first (n = 124) and second experiment (n = 141) were grown alongside the parents, one LB-susceptible and three LB-resistant control genotypes (n = 21 – 24 each). Plants were divided into two replications of

approximately equal numbers per generation, and placed on two separate benches in the same greenhouse. Parental and control genotypes were placed on opposite ends of each bench in four replications. Inoculation took place as previously described, using *P. infestans* clonal lineage US-23. Plants in the first experiment were re-inoculated seven days later due to insufficient disease progression.

In Experiment I, eight resistant F_2 individuals were selected, treated with Bravo®, and advanced to F_3 generation. In Experiment II, 19 resistant F_2 individuals were selected and advanced to F_3 generation. The F_3 progeny for Experiment I (n = 112) included six families with 15 – 18 individuals per family and two families with 4 – 12 individuals per family (due to low seed germination). In Experiment II, F_3 progeny (n = 289) included sixteen families with 14 – 18 individuals and three families with 4 – 13 individuals per family. Parents, one LB-susceptible and three LB-resistant control lines (n = 9 – 12 each) were also included. Each family was divided into three replications of approximately equal numbers, placed at different locations in the same greenhouse. Parents and control lines were divided into two replications (n = 3 – 6 each). Plants were inoculated with *P. infestans* clonal lineage US-23 and evaluated for response to the pathogen, following the same procedures described above.

Estimation of the minimum number of contributing loci

The Castle-Wright equation [7, 15, 49] was used to estimate the minimum number of loci contributing to LB resistance:

$$n = \frac{(m_1 - m_2)^2}{8(V_{F2} - V_{F1})}$$

where n is the minimum number of contributing resistance loci, m_1 and m_2 are the means of % DS of each parent, and V_{F1} and V_{F2} are generation variances. Data used to estimate n were taken from the GMA experiment, described above.

3. Results

Response of parental and control lines, and combined F_2 population

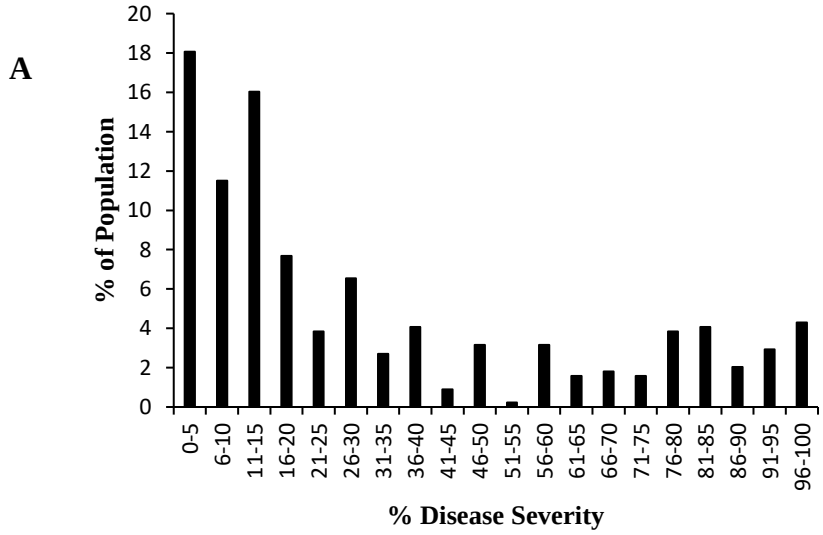
Fla. 8059 was highly susceptible (average % DS = 85.9%) and PI 270442 was highly resistant to LB (% DS = 2.2%) across all experiments. The resistance level in PI 270442 was statistically similar to NC 03220, which has *Ph-2* + *Ph-3* LB-resistance genes (Table 1). Average % DS in the resistant lines NC 63EB, NC 870 and NC 03220 ranged from 1 – 20% and the susceptible line New Yorker ranged from 60 – 91%. The F_2 data from all experiments were pooled (n = 443), and the average F_2 % DS ranged from 26 – 46%. The F_2 disease response distribution was

continuous (Fig. 1A), but not normally distributed as shown by Shapiro-Wilk test ($P < 0.001$). Disease response in the F_2 population was skewed towards resistance (skewness = 0.884).

Table 1. Average late blight (LB) disease severity (% defoliation ± SE) in tomato for resistant (PI 270442) and susceptible (Fla. 8059) parents and control lines across seven experiments, and F_2 population across three experiments

Genotype	Number of plants (n)	Mean % DS[1]	Range of means
P_1 (Fla. 8059)	96	85.9 ± 3.2[a]	76-98
P_2 (PI 270442)	114	2.2 ± 4.2[b]	1-3
New Yorker (*Ph-1*)	96	75.7 ± 12.2[c]	60-91
NC 63EB (*Ph-2*)	91	8.7 ± 3.1[d]	3-20
NC 870 (*Ph-3*)	94	8.9 ± 3.0[d]	4-20
NC 03220 (*Ph-2* + *Ph-3*)	60	2.2 ± 4.3[b]	1-4
F_2	443	35.2 ± 2.5	26-46

[1]Mean comparisons were determined using Tukey's HSD test and are denoted by superscripts (a–d)



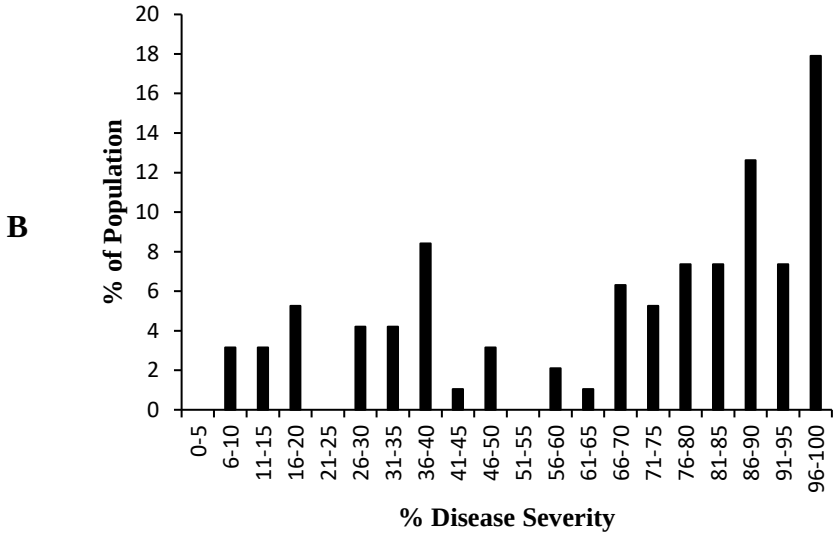


Fig. 1. Frequency distribution of response to late blight disease in tomato for (A) F_2 population (n = 443) and (B) BC_1P_1 population (n = 95). Disease severity (% DS) was measured by % of total leaf area showing necrosis, from 0 – 100% DS.

Parent:offspring correlation analysis

The % DS for the F_2 and F_3 generations and estimates of heritability for the three P:O experiments are shown in Table 2. In the F_2 population used for the P:O correlation analyses (n = 178), the % DS ranged from 0 – 100% with an average of 26.4%. Across the three experiments, the average % DS of the F_2 selected resistant class was ~4%, while that of the F_2 selected susceptible class was ~74% (Table 2). In the F_3 generation, disease response was generally similar across the three experiments: the % DS of the resistant class F_3 progeny averaged 9 – 14%, and that of the susceptible class F_3 progeny averaged 33 – 43% (Table 2). In all three experiments, F_3 progeny families generally resembled their corresponding F_2 parents in % DS, with a few exceptions. Heritability, as estimated based on F_2:F_3 correlation analysis, was similar in all three experiments, ranging 0.56 – 0.69, with an average of 0.63, indicating moderately high heritability for LB resistance in these populations.

Table 2. Late blight (LB) disease severity (% defoliation ± SD) in tomato for F_2:F_3 parent:offspring populations used to estimate h^2 by determining correlation coefficient

Genotype	Number of plants (n)	Mean % DS	Range	Variance	h^2
F_2 population	178	26.4 ± 27.0	0-100	727.1	
F_2:F_3 Experiment I					
F_2 selected individuals (resistant class)	39	4.0 ± 1.6	0-5	2.5	
F_2 selected individuals (susceptible class)	18	72.9 ± 15.5	50-98	241.3	
F_3 progeny families (resistant class)	39	13.7 ± 11.1	2-45	123.6	
F_3 progeny families (susceptible class)	18	41.3 ± 14.9	12-75	221.1	0.69
F_2:F_3 Experiment II					
F_2 selected individuals (resistant class)	39	3.9 ± 1.6	0-5	2.5	
F_2 selected individuals (susceptible class)	20	73.7 ± 15.6	50-98	244.3	
F_3 progeny families (resistant class)	39	9.2 ± 13.8	1-72	189.5	
F_3 progeny families (susceptible class)	20	33.4 ± 21.9	1-73	477.5	0.56
F_2:F_3 Experiment III					
F_2 selected individuals (resistant class)	40	4.0 ± 1.6	0-5	2.5	
F_2 selected individuals (susceptible class)	20	73.7 ± 15.6	50-98	244.3	
F_3 progeny families (resistant class)	40	11.9 ± 13.2	1-60	173.0	
F_3 progeny families (susceptible class)	20	42.9 ± 24.6	3-92	607.0	0.64

Generation means analysis

Generation means and variances, along with the components of the total variance (V_A, V_D and V_E), were used to estimate narrow-sense heritability (h^2_N) for LB resistance in these populations. In the GMA experiments, the susceptible parent (Fla. 8059) showed a high level of disease (% DS = 98.4 %) and PI 270442 exhibited a strong resistance (% DS = 2.3%) (Table 3). The F_1 generation was highly resistant (% DS = 10.9%), though not as resistant as the resistant parent. The average % DS in the F_2 population (n = 124) was 46.3%, similar to the mid-parent value. The distribution of disease response in the F_2 population was similar to the pooled F_2 data (Fig. 1A), with a wide range of response (% DS = 2 – 100%). The disease response was continuous but not normally distributed ($P < 0.001$), and somewhat bimodal with skewness towards resistance (skewness = 0.281). The disease response in the BC_1P_1 population was similar to the F_2 population, showing a wide range of response (% DS = 7 – 100%) with an average of 68.6% DS (Table 3). The disease response in the BC_1P_1 population was continuous but not normally distributed ($P < 0.001$), and significantly skewed towards susceptibility (skewness = -0.661), as expected in this generation (Fig. 1B). The segregating F_2 and BC_1P_1 populations exhibited more phenotypic variances than the non-segregating P_1, P_2 and F_1 generations (Table 3). Based on the components of the total phenotypic variance, the narrow-sense heritability (h^2_N) for LB resistance in PI 270442 was estimated to be 0.60.

Table 3. Late blight (LB) disease severity (% defoliation ± SD) in tomato for resistant (PI 270442) and susceptible (Fla. 8059) parents, F_1 progeny, and F_2 and BC_1P_1 populations used to estimate h^2 by generation means analysis (GMA)

Genotype	Number of plants (n)	Mean % DS	Range	Variance
P_1 (Fla. 8059)	21	98.4 ± 1.9	95-100	3.6
P_2 (PI 270442)	22	2.3 ± 1.2	0-5	1.4
F_1	24	10.9 ± 5.9	5-30	35.2
F_2	124	46.3 ± 34.7	2-100	1206.9
BC_1P_1	95	68.6 ± 29.0	7-100	842.8
V_E = 13.4				
V_A = 728.3				
V_D = 465.2				
h^2 = 0.60				

Response to selection and realized heritability

Realized heritability (h^2_R) was determined in two F_2:F_3 parent:offspring experiments. In Experiment I, the F_2 population (n = 124, average % DS = 46.3%) underwent a selection pressure of p = 6.5% (n = 8, average % DS = 3.9% for the selected F_2 plants), resulting in a selection differential of S = 42.5% DS. The resulting F_3 progeny population (n = 112 plants) exhibited an average % DS of 11.7%, statistically different ($P < 0.001$; *t*-test) from the % DS of the parent F_2 population before selection. The selection response (R) was 34.7% DS, indicating a significant improvement in % DS in the progeny population. Most individuals in the F_3 progeny population (92%) had % DS ≤ 20%. Using the response-to-selection equation ($h^2_R = R/S$), the realized heritability for LB resistance in this experiment was determined to be 0.82 (Table 4).

In Experiment II, the F_2 population (n = 141, % DS = 32.7%) was subjected to a selection pressure of p = 13.5% (n = 19, average % DS = 3.6% for the selected plants), giving a selection differential of S = 29.1% DS. The F_3 progeny (n = 289 plants) of the selected F_2 parents showed improved disease resistance, with an average % DS of 7.7%, statistically different from the parental F_2 population ($P < 0.001$). This indicates effectiveness of selection with a selection response of R = 25.0% DS. In the F_3 progeny population, nearly all (94%) of individuals had % DS ≤ 20%. Realized heritability (h^2_R) for LB resistance in this experiment was determined to be 0.86 (Table 4).

Table 4. Late blight (LB) disease severity (% defoliation ± SD) in tomato for F_2 and F_3 populations used to estimate realized h^2 by response to selection

Genotype	Number of plants (n)	Mean % DS	Range	Variance	h^2
F_2:F_3 Experiment I					
F_2 population	124	46.3 ± 34.7	2-100	1207	
F_2 selected resistant parents	8	3.9 ± 1.2	2-5	1.6	
F_3 progeny of selected F_2	112	11.7 ± 11.1	1-75	122.5	0.82
F_2:F_3 Experiment II					
F_2 population	141	32.7 ± 28.6	1-98	820.8	
F_2 selected resistant parents	19	3.6 ± 1.4	1-5	2	
F_3 progeny of selected F_2	289	7.7 ± 12.1	0-98	145.8	0.86

Estimation of the minimum number of contributing resistance loci

Using the Castle-Wright equation, the minimum number of loci contributing to LB resistance conferred by PI 270442 was estimated as follow:

$$n = \frac{(m_1 - m_2)^2}{8(V_{F2} - V_{F1})} = n = \frac{(98.4 - 2.3)^2}{8(1206.9 - 35.2)} = 1.0$$

suggesting involvement of one or few resistance loci.

4. Discussion

Solanum pimpinellifolium accession PI 270442 displayed strong LB resistance across all experiments, with an average % DS of 2.2%. The disease response of PI 270442 was similar to the LB-resistant control line NC 03220, which contains both *Ph-2* and *Ph-3* LB-resistance genes, and better than NC 63EB (*Ph-2* only) and NC 870 (*Ph-3* only). These results are consistent with previous germplasm screening studies conducted under multiple field and greenhouse conditions [19] as well as in growth chambers using detached leaflets [18]. In a previous study, PI 270442 also exhibited significantly higher LB resistance than the original source of *Ph-3* resistance gene, *S. pimpinellifolium* accession L3708 (a.k.a. LA 1269 and PI 365957) [19], indicating possible presence of new or additional LB-resistance genes in PI 270442. Strong resistance was also observed when PI 270442 was exposed to eight *P. infestans* isolates of three different clonal lineages, US-13, US-14 and a different US-23 isolate than the present study [19].

The presence of stronger LB resistance in PI 270442 compared to commercial and inbred lines containing *Ph-2* and/or *Ph-3* was further confirmed by R.G. Gardner, North Carolina State University, under severe LB field conditions (R.G. Gardner, personal communication). Although it is currently unknown what genes contribute to LB resistance in PI 270442, it was determined in a previous study that while PI 270442 did not have the molecular markers known to be associated with *Ph-3*, it did show markers associated with *Ph-2* resistance gene [19]. While the marker information does not conclusively show the presence of *Ph-2* in this accession, the observation that PI 270442 exhibits a significantly higher level of LB resistance than the *Ph-2* control line suggests the presence of yet unidentified resistance gene(s) or a new *Ph-2* allele. Molecular mapping studies are currently underway to identify and map genes conferring LB resistance in PI 270442. However, the high levels of LB resistance observed in PI 270442 in this and previous studies indicate potential for this accession as a good source of LB resistance in tomato breeding.

Although a high level of LB resistance was observed in the F_1 generation (% DS = 10.9%), it was not as strong as the resistant parent (Table 3). However, presence of strong LB resistance in the F_1 progeny suggests dominant nature of this resistance, which is also consistent with the continuous but rather bimodal disease response distribution in the F_2 generation, where 64% of the population exhibited high resistance (% DS $\leq$ 30%), 21% high susceptibility (% DS $\geq$ 70%), and only 16% intermediate resistance/susceptibility (70% > % DS > 30%) (Fig. 1A). As expected, the response distribution in the BC_1P_1 population was skewed towards susceptibllity (skewness = -0.661), consistent with the genetic make-up of this population; nevertheless, 16% of the population exhibited strong resistance (% DS $\leq$ 30%), suggesting presence of some level of dominance for this resistance (Fig. 1B). Further, the rather bimodal distributions in the F_2 and BC_1P_1 populations indicate involvement of few resistance loci, consistent with the estimate obtained by the Castle-Wright equation. Genetic mapping is required to determine the actual number of resistance loci.

The parental and F_1 generations displayed little variation in disease response, as shown by the narrow range of % DS and small variances in these generations (Table 3). This suggests minimal involvement of environmental variation in these experiments. In comparison, the F_2 and BC_1P_1 populations exhibited wide phenotypic variation (Fig. 1A&B), suggesting greater contribution of genetic differences to the variation observed for LB resistance in these populations. Further, the F_2 population exhibited higher phenotypic variance (V_{F2} = 1206.9) than the BC_1P_1 population (V_{BC1P1} = 842.8), consistent with the greater theoretical genetic variance in the F_2 ($V_{F2} = V_A + V_D + V_E$) than in the BC_1P_1 population ($V_{BC1P1} = \frac{1}{2}V_A + V_D + V_E$). Analysis of the components of phenotypic variance suggested that most variation were due to genetic causes (V_G = 1193.5 *vs.* V_E = 13.4), and most of the genetic variations were due to additive effects (V_A = 728.3, *vs.* V_D = 465.2). Narrow-sense heritability (h^2_N) was estimated to be 0.60, indicating that the LB resistance in accession PI 270442 is moderately heritable and could be bred into other genetic backgrounds via phenotypic selection (see below).

This h^2 estimate is also consistent with previous estimates for LB resistance in other tomato accessions, including PI 163245 [h^2 = 0.63 – 0.94 [40]], PI 224710 [h^2 = 0.87 [41]], and PI 270443 [h^2 = 0.86 [35]].

To determine the realized heritability of LB resistance, directional phenotypic selection for resistance was conducted in F_2 generation in two experiments and the resulting F_3 progeny were examined for resistance. Intense selection pressure (p = 6.5% and 13.5% in the two experiments) resulted in significant ($P < 0.001$) improvement in disease resistance (R = 34.7% and 25.0%) in the resulting F_3 progeny populations (Table 4). These results clearly demonstrate that phenotypic selection for LB resistance was very effective, improving the % DS from 46.3% and 32.7% in the F_2 parental populations to 11.7% and 7.7%, respectively, in the F_3 progeny populations. These analyses indicated realized heritability of h^2_R = 0.82 and 0.86, respectively, in the two experiments (Table 4). These heritability values are somewhat greater than, but comparable with, the h^2 estimates obtained from the F_2:F_3 P:O and generation means analyses (Table 3), confirming the heritable nature of this resistance. Further, these results indicate the feasibility of transferring LB resistance from accession PI 270442 to the cultivated tomato via hybridization and phenotypic selection. The moderately high h^2 values for LB resistance in PI 270442 are consistent with the observed F_2 and BC_1P_1 disease response distributions (Fig. 1A&B), which approached bimodal, and with the Castle-Wright estimate of one or few contributing loci. Small number of genes and high h^2 values generally suggest presence of a rather simple genetic system controlling this resistance. Such a system facilitates rapid transfer of resistance from PI 270442 to the cultivated tomato with reduced chances of linkage drag.

The results of this study suggest that PI 270442 could be utilized effectively as a source of LB resistance in traditional tomato breeding. This accession has shown strong resistance against nine *P. infestans* isolates across three clonal lineages, demonstrating its broad range of resistance. In screenings against all nine isolates, PI 270442 responded similarly to NC 03220, which contains both *Ph-2* and *Ph-3* resistance genes. Because new isolates of *P. infestans* have been identified which overcome the resistance conferred by *Ph-2* and *Ph-3* separately, pyramiding new sources of LB resistance with the known *Ph-2* and *Ph-3* resistance genes would provide stronger and more durable resistance. Genetic mapping studies are underway to determine the nature of resistance loci present in PI 270442 (Sullenberger and Foolad, unpublished).

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