Late blight resistance in a diploid full-sib potato family

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Abstract

Late blight, caused by Phytophthora infestans (Mont.) de Bary, is the most destructive disease of potato worldwide. As this pathogen can rapidly overcome major race-specific resistance genes, identifying the basis for enhanced quantitative resistance has become a crucial element for implementing advanced breeding strategies. A population of 230 full-sib progeny derived from a cross between two diploid hybrid Solanum phureja × S. stenotomum clones was evaluated for foliage resistance against late blight in replicated trials at multiple locations in Pennsylvania between 1999 and 2002. In field experiments, plants were evaluated visually for per cent defoliation, and area under the disease progress curve (AUDPC) was determined. The two parents and three control cultivars (‘Atlantic’, ‘Kennebec’ and ‘Katahdin’) were included in all trials. In all three experiments, the presence of a significant number of clones exhibiting transgressive segregation were observed. There were significant differences among environments as well as among clones, and the clone × environment interaction was also significant. Stability analysis revealed that 37 clones made a significant contribution to the overall environment × clone interaction. Broad-sense heritability for resistance, measured as AUDPC, was estimated as 0.67. The overall results indicate the presence in this potato family of a high level of field resistance against late blight. This segregating diploid family appears to be a good candidate for quantitative trait loci mapping to identify and characterize the genetic components of partial late blight resistance.

Key words: Phytophthora infestans — Solanum phureja — S. stenotomum — broad-sense heritability — diploid potatoes — stability

Potato late blight, caused by Phytophthora infestans (Mont.) de Bary, is one of the most common and destructive diseases of cultivated potatoes (Solanum tuberosum L.) in the US and worldwide. The oomycete P. infestans can infect foliage, stems and tubers, and cause severe damage during all stages of plant development, which may result in complete loss of the crop. In recent years, late blight has become a reemerging disease worldwide due to the development of resistance to phenylamide fungicides in pathogen populations and the widespread occurrence of new and more aggressive genotypes (Deahl et al. 1991, Drenth et al. 1993, Fry and Goodwin 1997). Moreover, the presence of both the A1 and A2 mating types among this new population of P. infestans provides a ground for a potential increase in the overall genetic variability of this fungus through sexual recombination.

Breeding for resistance to late blight became a major priority after its destructive character was revealed during the epidemic of 1845 that led to the Irish potato famine followed by the mass emigration and death of millions. Due to the tetraploid nature of commercial potatoes, breeding is much more complex than in diploid crop species. Furthermore, the breeding effort has been hampered by the use of closely related germplasm in breeding programmes that resulted in high genetic similarity among the various potato cultivars utilized (Mendoza and Haynes 1974).

In potato, two types of resistance against P. infestans are encountered: race-specific resistance (vertical resistance or hypersensitivity) and quantitative resistance (horizontal or general resistance) (Vanderplank 1968). Hypersensitive genotypes are generally characterized by a rapid necrotic response in the attacked cells. This type of resistance is under direct control of a series of major genes (R genes) that are brought into action by a distinct race of the pathogen. So far, 11 different R genes have been identified all derived from the hexaploid species Solanum demissum (Black et al. 1953, Malcolmson and Black 1966). Several of these 11 R genes have been previously introduced into modern potato varieties (Ross 1986), but compatible races of P. infestans have very rapidly arisen for all of them. Quantitative resistance, on the contrary, usually consists of several components exerting a small effect, resulting in the overall hindrance of the parasitic attack on the host, and is controlled by several interacting genes. Both types of resistance can occur jointly in wild species like S. demissum, S. bulbocastanum, S. polyadenium, S. stoloniferum, S. vernei, S. verrucosum and others (Black 1970, Graham 1963, Toxopeus 1964).

Disease control methods based solely on the introgression of a single or a combination of several race-specific oligogenic resistant genes into tetraploid S. tuberosum had only an initial and limited success (Thurston 1971). Therefore, most potato breeders presently focus their efforts on improving general resistance that is expected to be a more successful and durable strategy. Moreover, breeding for effective forms of durable resistance should also substantially alleviate the economic costs as well as the environmental impacts of frequent fungicide applications currently required to control late blight in commercial production. Besides the difficulties of dealing with more variable and aggressive isolates of the pathogen, breeding for field resistance against P. infestans is complicated by the quantitative nature of this type of resistance.

To expand the present germplasm breeding base of potato, Haynes and Christ (1999) investigated late blight resistance in 281 clones derived from 72 families of a diploid random-mated population, considered devoid of R genes, of S. phureja × S. stenotomum (ph-stdn) population that are short-day species of South American origin. After 2 years of replicated
experiments in Pennsylvania, it was determined that this phu-str population had relatively high levels of field resistance to late blight and that both broad-sense and narrow-sense heritability in it were high.

In the present study, a diploid full-sib potato family has been characterized for the segregation of late blight resistance and the stability of this trait among different environments. This family, constituted in 1997, is the result of a cross between two highly heterozygous diploid clones: BD142-1 (susceptible to late blight) and BD172-1 (partially resistant). The origin of these two individuals can be traced back to the random-mated population constituted from 36 plant introductions each of S. phureja and S. stenotomum (Haynes 1972).

The specific purposes of this study were to evaluate a full-sib family of phu-str that was developed for mapping molecular markers associated with resistance to P. infestans, estimate broad-sense heritability for resistance to late blight in this family; and, determine the stability of resistance to P. infestans in individual clones from this family.

Materials and Methods

**Plant materials:** The full-sib offspring of a cross between the heterozygous diploid hybrid clones BD142-1 and BD127-1 of S. phureja × S. stenotomum were chosen for mapping. The cross of the seed parent BD142-1 (highly susceptible to late blight) with the pollen parent BD172-1 (partially resistant) was made in the spring of 1997. Four plants of each clone were planted in the crossing block, controlled crosses were made, and true seeds were collected and planted. True seeds were planted in individual test tubes containing Murashige and Skoog (1962) media after treatment with 1500 mg/kg gibberellic acid (GA3) for 24 h and subsequent drying for 24 h. A number of plantlets per clone were rapidly micropropagated. The plantlets were transplanted into 9 cm clay pots filled with Jiffy Mix® (Jiffy Products of America, Inc., West Chicago, IL, USA) from 28 January to 17 February and harvested from 30 April to 2 May 1998. Since then, tubers from each clone have been propagated and maintained at Chapman Farm in Presque Isle, Maine, and then shipped to Pennsylvania for disease testing. Both parents were initially selected from the 281 phu-str clones evaluated for resistance to P. infestans by Haynes and Christ (1999). Each parent belonged in the top 5% of those 281 clones for either susceptibility or resistance, respectively. The progeny consisted of approximately 230 individuals and is referred to as the mapping family (BD410).

**Field experiments:** Three separate field experiments were conducted in Pennsylvania between 1999 and 2002. In 1999, the entire diploid potato family was grown in field trials at the Russell E. Larson Agricultural Research Center at Rock Springs, PA. In 2002, the same family was tested twice, once at the Southeastern Research and Extension Center at Landisville, PA, and again at Rock Springs, but plot location within the research farm was, for this year, different than the one used in the 1999 experiment. For brevity, the three field experiments will be hereafter referred to as: 1999 Rock Springs, 2002 Rock Springs and 2002 Landisville.

The entire BD410 family, two parental clones and three standard commercial cultivars 'Atlantic', 'Kennebec' and 'Katahdin' were included in all three experiments and evaluated for reaction to late blight under field conditions. Planting dates for the experimental plots were: 4 June 1999 and 11 June 2002 at Rock Springs, and 27 June 2002 at Landisville. For all experiments, plants were grown in a randomized complete block design with two blocks. Plots consisted of four hills of a clone and were hand planted, spaced 22 cm within and 91 cm between rows with a 1.2 m break between rows and plots.

To ensure uniform disease pressure, each treatment plot had an adjacent row of the susceptible cultivar 'Russet Burbank' acting as a disease spreader row. Standard commercial management practices were followed throughout the season, with no traditional fungicides applied except for one to three early season applications of Foliarz® 3.6 (Tebuconazole) (Bayer Corporation, Crop Protection, Kansas City, MO, USA) specifically targeting Alternaria solani causing early blight. Fertilization was applied in-furrow at an approximate rate of 171 kg/ha of 10-10-10 (N-P-K). If the environmental conditions were less than ideal for disease development, a sprinkler irrigation system was used to create a more conducive environment by extending dew periods, increasing relative humidity and decreasing temperature within the plant canopy.

**Phytophthora infestans isolates and inoculum preparation:** three different isolates of P. infestans were used to produce artificial fungal inoculum. All isolates utilized were US-8 and A2 mating type. They were all originally isolated from naturally infected leaves of potatoes found in various locations in Pennsylvania, and maintained on pea agar media. All isolates were also tested for virulence on an R gene differential set of potato varieties, and comparisons among isolates were made testing them on several common cultivars (B.J.C. Christ and R.V. Valluru, unpublished data). To ensure pathogenicity and virulence of the isolates, these were periodically re-isolated following the protocol described in the International Potato Center training manual (Forbes 1997). In order to obtain a high concentration of virulent sporangia of each isolate, surface disinfected leaflets (2% sodium hypochlorite for 5 min) of ‘Katahdin’ were placed on the surface of 7-day-old V8/Lima bean agar plates and incubated for 7 days at 15–18°C in the dark. After this period, sporangia were removed from the leaflet and agar surface with distilled water. Equal amounts of the three isolates were pooled together to constitute the inoculum and subsequently the sporangial concentration was adjusted, using a haemacytometer, to 1 × 104 sporangia/ml. Inoculum was then chilled for 2–3 h at 4°C prior to inoculation to promote the release of zoospores. As no natural late blight infestation occurred in the experimental fields by early to mid-August in all three experiments, a sporangial suspension of P. infestans was prepared and spreader rows were inoculated with hand held sprayers. The infection spread naturally from those rows into the treatment plots. The ‘Russet Burbank’ spreader rows were inoculated in the evenings of 20 August 1999 and 11 August 2002 at Rock Springs and 23 September 2002 at Landisville.

**Data analysis:** Disease assessment was initiated approximately 14 days after inoculation for all 230 clones, two parental clones plus three standard cultivars, by visually estimating the percentage of necrotic tissue and/or defoliation present in each plot. Three or four assessments were made at 2–4-day intervals. In 1999, at Rock Springs, the experimental plots were assessed four times near the end of the growing season: 9, 13, 17 and 19 September. At the same location, in 2002, four assessments were made 26 and 29 August and 2 and 5 September. The experiment conducted at Landisville in 2002 had only three assessments dates 7, 14 and 17 October. In all three experiments (hereupon referred to as environments), the area under the disease progress curve (AUDPC) was calculated for each individual plot (Shaner and Finney 1977). Values for AUDPC, of the three separate and combined experiments, were analysed using the general linear models procedure in SAS (SAS Institute, Inc. 1987). Residuals were plotted against predicted values and visually examined (Neter and Wasserman 1974). For this study, a square-root transformation to the original trait values was applied to obtain normality.

All effects were considered random. Estimates of the clonal variance (σc²), environment × clone variance (σce²), and the error variance (σ²e) were based upon expected mean squares and calculated by the mixed procedure in SAS (SAS Institute, Inc. 1996). Broad-sense heritability (H) was calculated from these estimates of variance as:

\[ H = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_{ce}^2 + \sigma_e^2/2} \]
where \( r \) is the number of replications and \( E \) is the number of environments (Nyquist 1991), and a 95% confidence interval on \( H \) was calculated from mean squares (Knapp et al. 1985).

The environment \( \times \) clone interaction was partitioned into stability-variance (\( \sigma^2_i \)) components assignable to each clone (Shukla 1972), using PROC IML in SAS (Kang 1989). An environmental index was calculated as the average AUDPC score of all clones in each environment minus the average AUDPC score of all clones over three environments. Heterogeneity, or non-additivity, due to this environmental index was removed from the environment \( \times \) clone interaction, and the remainder of the environment \( \times \) clone interaction was partitioned into \( \sigma^2_i \) components assignable to each clone (Kang 1989).

**Results**

The distribution of the mean AUDPC for the three separate experiments is shown in Fig. 1, while the distribution of the averaged AUDPC across the three environments for the BD410 family is presented in Fig. 2. The frequency distribution of phenotypic classes for foliage resistance to late blight observed in this family was continuous and normal in all three experiments (Fig. 1). Significant skewness of the frequency distributions was present in the 1999 Rock Spring (towards resistance) and 2002 Landisville (towards susceptibility) data. No significant skewness was present in the 2002 Rock Springs data and on the three locations averaged data. In all three experiments, the presence of a significant number of clones exhibiting transgressive segregation were observed. Approximately, 4% of the BD410 clones had mean AUDPC values lower than the moderately resistant parent (BD172-1), but no clones had mean AUDPC values higher than the susceptible parent (BD142-1).

Area under the disease progress curve values for the 235 clones (which included two parental and three standard cultivars) tested were initially analysed separately for each location. Mean AUDPC were 609 in 1999 Rock Springs, 409 in 2002 Rock Springs and 317 in 2002 Landisville. The error variance in the 1999 data set was considerably higher than in either of the two 2002 data sets. Therefore, all AUDPC scores were square root transformed to allow for a combined data set analysis. There were significant differences among environments, and among clones and the clone \( \times \) environment interaction was also significant (Table 1). However, the rep(env) **Table 1: Analysis of variance on area under the disease progress curve for 235 clones, including parental and standard cultivars, from a diploid mapping family (BD410) evaluated for percentage foliar late blight in Pennsylvania, in 1999 and 2002 (two locations)**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean squares</th>
<th>Expected mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Env</td>
<td>2</td>
<td>5513.40**</td>
<td>( \sigma^2_e + 2\sigma^2_{eC} + 6\sigma^2_C )</td>
</tr>
<tr>
<td>Rep (env)</td>
<td>3</td>
<td>10.91</td>
<td>( \sigma^2_e )</td>
</tr>
<tr>
<td>Clone</td>
<td>234</td>
<td>44.24**</td>
<td>( \sigma^2_C )</td>
</tr>
<tr>
<td>Env ( \times ) clone</td>
<td>468</td>
<td>12.41**</td>
<td>( \sigma^2_e + 2\sigma^2_{eC} )</td>
</tr>
<tr>
<td>Error</td>
<td>702</td>
<td>5.70</td>
<td>( \sigma^2_C )</td>
</tr>
<tr>
<td>Total</td>
<td>1409</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significant at \( P = 0.01 \).**

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**Fig. 1:** Distribution by mean area under the disease progress curve (AUDPC) of the 235 potato clones, including parental (BD142-1; BD172-1) and standard cultivars (ATL = ‘Atlantic’; KAT = ‘Katahdin’; KEN = ‘Kennebec’), from a diploid mapping family (BD410) evaluated for percentage leaf necrotic area in Pennsylvania, from three environments (averaged data set). \( \bar{X} \) = average AUDPC value

**Fig. 2:** Distribution by mean area under the disease progress curve (AUDPC) of the 235 potato clones, including parental and standard cultivars, from a diploid mapping family (BD410) evaluated for percentage foliar late blight in Pennsylvania, in 1999 and 2002, in three environments. The parental clone BD172-1 had an AUDPC value of 402.5, 195 and 178.75 for the 1999 Rock Springs, 2002 Rock Springs and 2002 Landisville, respectively. The parental clone BD142-1 had an AUDPC value of 945, 778.75 and 743.75 for the 1999 Rock Springs, 2002 Rock Springs and 2002 Landisville, respectively.
effect was not significant, indicating that disease pressure was uniform across the entire field. Mean AUDPC values ranged from a minimum of 143 (BD410-123, one of the BD410 clones) to a maximum of 823 (BD142-1). The standard commercial varieties ‘Atlantic’, ‘Katahdin’ and ‘Kennebec’ had average AUDPC values of 800, 679 and 596, respectively. The mean AUDPC value across three environments for the female parent (BD142-1) was 823, and 259 for the male parent (BD172-1).

The estimates of $\sigma_G^2$, $\sigma_E^2$, and $\sigma^2$ were 5.30, 3.35 and 5.70, respectively. Broad sense heritability for late blight resistance was estimated at 0.67 with a 95% confidence interval of 0.65–0.78.

From the result of the stability variance test, 37 clones were identified that made a significant contribution to the overall variance (data not shown). Twenty-three clones were significant at a 5% level and 14 at a 1% level.

**Discussion**

In agreement with a previous report (Haynes and Christ 1999), the present study confirms the presence of several clones with relatively high levels of late blight resistance in this segregating family. Although the value of the mean AUDPC for the susceptible parent (BD142-1) was only two times greater than that for the moderately resistant parent, it should be noted that 66 clones (29%) had significantly lower mean AUDPC values ($P = 0.05$) than the best performing commercial cultivar ‘Kennebec’. The estimated broad-sense heritability in the population was 0.67 that fell within the 95% confidence interval from that previously reported (0.79). These estimates provide confidence that the observed phenotypic variation for late blight resistance has a large genetic component and, thus, is readily selectable.

None of the clones, as expected, were immune to infection by *P. infestans*. The frequency distribution of the mean AUDPC values for the BD410 family exhibited a continuous distribution consistent with an additive genetic model for the inheritance of a quantitative trait. Furthermore, the presence of several clones with transgressive segregation suggests that even the susceptible female parent may have some genes to contribute to late blight resistance in the progeny or that the genes governing late blight resistance in the male parent may have existed in a homozygous condition and upon crossing exhibited over-dominance.


Acknowledgements

This research was supported in part by The Agricultural Research Funds administered by The Pennsylvania Department of Agriculture (# ME449305), the USDA (CSREES) Special Research Grants Program Potato Research, the College of Agricultural Sciences, The Pennsylvania State University.

References


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