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# RESISTANCE OF POTATO TUBERS TO *PHYTOPHTHORA INFESTANS* EVALUATED IN LABORATORY TESTS AND FIELD TRIALS

## ABSTRACT

Thirty potato genotypes of different maturity were evaluated for tuber blight resistance both under laboratory and field conditions. From 2000 to 2003 replicated experiments were conducted with three potato cultivars registered in Russia and 27 tetraploid interspecific hybrids that were generated at VIR. Each genotype was assessed twice using laboratory tests, and its resistance was evaluated every year in a field trial.

The results showed that four hybrid clones: 88–2, 95–23–3, 97–152–6 and 97–162–5 exhibited a superior performance of tuber resistance to *Phytophthora infestans* both in laboratory tests and field trials. Two cultivars and 10 hybrid clones were found susceptible to infection in all tests. Significant differences between the remaining 14 genotypes in tuber slice resistance, whole tuber resistance and the expression of resistance to tuber blight in the field were observed.

*Key words:* advanced potato clone, field trial, *Phytophthora infestans,* tetraploid interspecific hybrid, tuber resistance, tuber slices assay, whole tubers assay

## INTRODUCTION

In our previous investigations, advanced potato clones of different maturity, characterized by a high level of agronomically important traits, were obtained *via* complex interspecific hybridization. These clones express satisfactory foliage resistance to late blight and are able to produce a high quality yield of at least 2.4 kg/m$^2$. Their ratings for resistance are similar or higher than those of standard cultivars (Rogozina and Patrikeyeva 2001). Foliage resistance and tuber resistance are of equal importance for successful protection of potato from late blight. Even though the foliage resistance is high enough to minimalize yield losses during the growing season, considerable post–harvest losses caused by tuber blight may occur during tuber storage unless the pota–

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toes are satisfactorily resistant to the form of late blight that infests tubers.

Several methods have been developed to assess tuber blight resistance. They include the methods to be used in the field or in laboratory conditions, on the day of harvest or after harvest (Lapwood 1967, Bjor 1987, Stewart and Solomon–Blackburn 2002). Significant cultivar–year and year–method interactions have been revealed during the assessment of tuber blight resistance on whole tubers (Darsow 2002).

The objectives of our study were:

— to compare the tuber response of advanced clones to tuber blight both in field and laboratory assays,

— to develop a reliable test that could be used to precise estimation of ratings for tuber resistance to late blight.

Our ultimate goal is to obtain potato clones expressing both foliage and tuber resistance to *Phytophthora infestans,* to be distributed among potato breeders.

## MATERIALS AND METHODS

### Plant material

In total, 27 advanced potato clones and three cultivars were tested. The clones had been selected among the genotypes that originated from crossing of promising tetraploid parents, performed in the years 1990–1997 at the N. I. Vavilov Institute. The parents had been released after a series of hybrid generations and convergent crosses and originated from the combinations of two to six different *Solanum* spp. including *S. stoloniferum, S. demissum, S. vernei, S. chacoense, S. microdontum, S. spegazzinii, S. andigenum*, *S. rybinii* with several Russian and foreign cultivars and breeding clones. The tested material represented three groups of maturity: early – eight clones and cv. Elyzaveta, medium early – ten clones and cv. Nevsky, medium late – nine clones and cv. Petersburgsky.

### Test for tuber resistance

Tuber resistance to late blight was evaluated under laboratory conditions in 2003 using a whole tuber and tuber slices assays. Three whole tubers and three slices per tuber were tested twice for each genotype. Tuber slices were drop–inoculated with inoculum containing 15–20 × $10^3$ sporangia/ml and the whole tubers were sprayed with inoculum containing 30 ×$10^3$ sporangia/ml. The inoculum was prepared as a mixture of *P. infestans* races having all virulence factors: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11. In the whole tuber assay, the size of tuber blight on tuber surface and depth of the pathogen penetration into tuber flesh were assessed 20 days after inoculation, according to 0–4 grade scale. In the tuber slice assay, the expression of necrotic reaction and the area covered with mycelium

were assessed twice, usually 4 and 7 days after drop–inoculation, according to 0–4 grade scale (Table 1).

Table 1

**A scale used to estimate tuber resistance to late blight in laboratory assay**

| Ratings for tuber resistance on 0–4 scale | Whole tuber test | | Slice test | |
|---|---|---|---|---|
| | area of tuber infestation | depth of pathogen penetration into flesh | size of necrosis | area covered with mycelium |
| 0 | tuber blight not detected | tuber blight not detected | no necrosis | no mycelium |
| 1 | 1–2 $cm^2$ of tuber surface | within the tuber peel | small necrotic spot | weak growth of mycelium |
| 2 | about one third of tuber surface | up to 1 cm into flesh | about one third of tuber slice | about one third of tuber slice |
| 3 | above a half of tuber surface | deep penetration into flesh | above a half of tuber slice | above a half of tuber slice |
| 4 | entire surface | | entire surface | entire surface |

Field evaluation for resistance to late blight was conducted each year in trials located at the Pushkin branch of N. I. Vavilov Institute, near St. Petersburg. Environmental conditions of this region (north–western part of Russia) are favourable for late blight development, which results in high natural infection pressure. Local populations of *P. infestans* consist mainly of the races possessing 6 or more virulence genes (80% of identified isolates) (Patrikeyeva 2002).

The clones and cultivars were planted at the end of May in 2–3 replications, 10 plants per plot. Standard methods of potato cultivation were applied. No fungicides were used. In both epidemic disease years (2000 and 2003) the first late blight symptoms on susceptible potato appeared at the end of July, this is between the $60^{th}$ and $65^{th}$ day of potato vegetation period. In moderately disease development years (2001 and 2002) the first late blight symptoms appeared in the middle of August. The tuber resistance to late blight was assessed on the day of harvest and then on the $20^{th}$ day post–harvest by calculating the amount of infected tubers. More than 3% of infected tubers was the threshold of susceptibility/resistance of potato tubers to late blight in the field.

## RESULTS

The results of a laboratory estimation of potato resistance to late blight, related to the maturity of potato clone or cultivar, are shown in Tables 2–4.

Only clone 88–2 from among early ones demonstrated a good level of tuber resistance in both tests (Table 2). The reaction of this clone in a slice assay differed significantly from those of clones 97–152–11 and 97–157–4, and of the vulnerable cv. Elyzaveta. Some clones, e.g.

97–80–1 and 97–162–2, appeared to be not very resistant to late blight, but expressed more effective defense reactions than did cv. Elyzaveta.

Within a group of medium early potatoes two clones: 95–23–3 and

Table 2

**Tuber reactions of early potatoes to *P. infestans* infection in a laboratory assay on a 0–4 scale (see Table 1)**

| Cultivar/Clone | Whole tuber test | | Slice test | | |
|---|---|---|---|---|---|
| | pathogen infestation on tuber surface | pathogen penetration into tuber flesh | size of necrosis | area covered with mycelium | |
| | | | | on 4th day | on 7th day |
| 88–2 * | 0 | 1 | 1 | 1 | 2 |
| 95–25–1 | 0 | 2 | 0 | 3 | 3 |
| 95–29–1 | 1 | 2 | 1 | 1 | 3 |
| 97–80–1 | 1 | 2 | 2 | 2 | 2 |
| 97–152–11 ** | 1 | 2 | 4 | 3 | 3 |
| 97–162–2 | 2 | 2 | 2 | 2 | 2 |
| 97–157–4 ** | 2 | 3 | 1 | 3 | 3 |
| 97–154–2 | 3 | 2 | 4 | 0 | 2 |
| Elyzaveta | 3 | 3 | 0 | 3 | 3 |

Differences between resistant (*) and susceptible (**) clones are significant at $\alpha = 0.05$

Table 3

**Tuber reactions of medium early potatoes to *P. infestans* infection in a laboratory assay on a 0–4 scale (see Table 1)**

| Cultivar/Clone | Whole tuber test | | Slice test | | |
|---|---|---|---|---|---|
| | pathogen infestation on tuber surface | pathogen penetration into tuber flesh | size of necrosis | area covered with mycelium | |
| | | | | on 4th day | on 7th day |
| 95–23–3 * | 0 | 0 | 0 | 0 | 1 |
| 97–152–8 | 1 | 1 | 2 | 0 | 3 |
| 97–81–1 ** | 1 | 1 | 1 | 2 | 3 |
| Nevsky | 2 | 2 | 4 | 1 | 3 |
| 8–86–1 | 2 | 2 | 2 | 1 | 3 |
| 98–38–1 ** | 2 | 2 | 3 | 2 | 3 |
| 97–156–5 ** | 2 | 2 | 3 | 2 | 3 |
| 99–6–10 | 2 | 3 | 4 | 1 | 2 |
| 97–157–1 ** | 2 | 3 | 2 | 2 | 3 |
| 95–26–2 * | 3 | 2 | 4 | 0 | 0 |
| 99–6–1 | 3 | 3 | 1 | 0 | 3 |

Differences between resistant (*) and susceptible (**) clones are significant at $\alpha = 0.05$

95–26–2 were found resistant to late blight in a tuber slice assay, whereas clones 97–81–1, 98–38–1, 97–156–5 and 97–157–1 appeared to be susceptible (Table 3). Clone 95–23–3 expressed the highest resis–

tance. Moreover, it showed strong suppression of pathogen invasion and development. This feature has been confirmed in both tests. The response to infection of the tubers of resistant clone 95–26–2 was unexpected. In a slice test, a large necrosis but no growth of mycelium was observed, whereas the pathogen caused a serious damage to tubers in the whole tuber test. Clone 97–152–8 showed a satisfactory level of tuber resistance after spray–inoculation, and the growth of mycelium on tuber slices was slow.

Table 4

**Tuber reactions of medium late potatoes to *P. infestans* infection in a laboratory assay on a 0–4 scale (see Table 1)**

| Cultivar/Clone | Whole tuber test | | Slice test | | |
|---|---|---|---|---|---|
| | pathogen infestation on tuber surface | pathogen penetration into tuber flesh | size of necrosis | area covered with mycelium | |
| | | | | on 4th day | on 7th day |
| 97–152–6 * | 0 | 0 | 3 | 0 | 2 |
| 93–5–30 * | 1 | 2 | 4 | 0 | 2 |
| 97–155–1 ** | 1 | 2 | 0 | 2 | 3 |
| 95–29–4 * | 2 | 1 | 4 | 0 | 0 |
| 97–162–5 * | 2 | 1 | 4 | 0 | 1 |
| 90–7–7 | 2 | 1 | 4 | 1 | 2 |
| 99–6–4 ** | 2 | 2 | 3 | 1 | 3 |
| 97–159–3 * | 2 | 3 | 4 | 0 | 0 |
| 97–154–6 * | 3 | 2 | 2 | 0 | 1 |
| Petersburgsky ** | 3 | 2 | 2 | 3 | 3 |

Differences between resistant (*) and susceptible (**) clones are significant at $\alpha = 0.05$

Medium late clones differed significantly in their reactions to late blight infection (Table 4). Based on the rate of growth of mycelium on tuber slices six clones: 97–152–6, 93–5–30, 95–29–4, 97–162–5, 97–159–3 and 97–154–6 were characterized as resistant, whereas cv. Petersburgsky and two clones: 97–155–1 and 99–6–4 were found to be susceptible to tuber blight. Very strong defense mechanisms protecting the tubers from pathogen infection and invasion were observed with clone 97–152–6. Clones 97–162–5 and 95–29–4 showed almost identical tuber response to infection in both tests. These only slightly differed in the growth rate of mycelium on tuber slices.

The proportions of tubers infected with late blight in the field trials are presented in Table 5. In 2000 and 2003 the weather conditions were favourable for *P. infestans* development, and heavy rainfalls in 2003 intensified the spread of infection onto tubers. A level of tuber resistance was satisfactorily high in early clones 88–2, 95–25–1 and 97–162–2, medium early clones 95–23–3, 97–152–8 and 99–6–10, and medium late clones 97–152–6, 97–155–1, 97–162–5, 90–7–7, 99–6–4 and 97–159–3. The proportions of infected tubers within the yields produced

by these clones did not exceed 3% throughout the investigations, including the most severe epidemic in the growing season of 2003. Hence, the durable resistance to tuber blight in the field is likely to be a distinctive feature of the clones listed above.

Table 5

**Percentage of tuber infection with *P. infestans* in the field trials**

| Early group | | | Medium early group | | | Medium late group | | |
|---|---|---|---|---|---|---|---|---|
| cultivar/ clone | proportion of infected tubers [%] | | cultivar/ clone | proportion of infected tubers [%] | | cultivar/ clone | proportion of infected tubers [%] | |
| | 2000 | 2003 | | 2000 | 2003 | | 2000 | 2003 |
| 88–2 | 0 | 0 | 95–23–3 | 0 | 0 | 97–152–6 | 0 | 0 |
| 95–25–1 | 3 | 3 | 97–152–8 | 0 | 3 | 93–5–30 | 0 | 7 |
| 95–29–1 | 0 | 18 | 97–81–1 | 3 | 16 | 97–155–1 | 0 | 3 |
| 97–80–1 | 7 | 2 | Nevsky | 5 | 20 | 95–29–4 | 0 | 100 |
| 97–152–11 | 2 | 29 | 8–86–1 | 1 | 20 | 97–162–5 | 0 | 0 |
| 97–162–2 | 0 | 2 | 98–38–1 | 6 | 50 | 90–7–7 | 0 | 1 |
| 97–157–4 | 0 | 24 | 97–156–5 | 6 | 50 | 99–6–4 | 0 | 0 |
| 97–154–2 | 3 | 16 | 99–6–10 | 0 | 0 | 97–159–3 | 0 | 1 |
| Elyzaveta | 2 | 3 | 97–157–1 | 0 | 73 | 97–154–6 | 0 | 7 |
| | | | 95–26–2 | 9 | 64 | Petersburgsky | 15 | 70 |
| | | | 99–6–1 | 0 | 6 | | | |

In general, the rate of tuber infestation was significantly higher in 2003 than in 2000. Comparatively for these years, the extent of tuber infection within early potatoes (0–24% and 0–7%, respectively) was smaller than that within medium early potatoes (0–73% and 0–9%) and medium late potatoes (0–100% and 0–15%).

## DISCUSSION

Management of tuber blight caused by *P. infestans* is vital to ensure cost–effective potato production and to limit disease potential in seed and potato growing areas (Platt 2002). Although tuber reaction to infection is the major factor, differences between genotypes in levels of tuber infection in the field depend on several factors (Lapwood 1977, Bjor 1987, Darsow 2002). For pre–breeding program, both an experimental setup and knowledge of the component that is crucial for selection, are essential. Field testing can only be performed once a year and the results should be examined carefully, as they can be modified to a large extent by environmental conditions. To determine precisely a rating of tuber resistance, field trials are needed to be conducted over a number of seasons and at different locations. For all components of tuber resis–

tance to late blight, the interactions between potato cultivars and pathogen isolates have been revealed in laboratory tests (Flier *et al.* 2001). It is noteworthy that tuber resistance scores given to commonly accepted standards can greatly differ between experiments, most probably because of differences both between and within field and laboratory conditions (Zimnoch–Guzowska and Flis 2002).

In the present study, screening for tuber resistance against late blight was carried out both under natural and laboratory conditions for thirty potato genotypes of different origin. A wide range of variation in the resistance ratings among the tested clones and cultivars was found. Four clones: 88–2, 95–23–3, 97–162–5 and 97–152–6 were distinguished as resistant to infection based on the results obtained in laboratory tests and field trials. Twelve potato genotypes: cultivars Petersburgsky and Nevsky, and clones 8–86–1, 97–81–1, 98–38–1, 97–156–5, 97–157–1, 97–157–4, 95–29–1, 97–80–1, 97–152–11 and 97–154–2 showed susceptibility to infection.

In the case of the remaining genotypes it was not possible to judge the real level of tuber resistance to late blight, as the laboratory estimates (whole tuber test or slice test, or both) did not correspond to the field estimates, the former usually being much sterner. Thus, the laboratory assay is not always a suitable tool to measure the potential of a certain potato genotype to resist late blight attack under natural infection pressure. This conclusion is in agreement with that earlier drawn by Flier and co–workers (Flier *et al.* 2003). The discrepancy between laboratory and field estimates is evident, for example with clone 90–7–7, released in 1990. This clone was assessed as rather susceptible to late blight in laboratory testing, meanwhile it has been successively demonstrating a good level of both foliage and tuber resistance in the field (Rogozina 2003). Another example of durable field resistance is clone 97–155–1, released in 1997.

The findings reported in this paper point out the continuous need for seeking a reliable method to evaluate tuber resistance to late blight as well as to identify the components of resistance, such as resistance to infection and resistance to pathogen invasion.

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