

LATE BLIGHT ASSESSMENT OF POTATO CULTIVARS USING A NEW EXPRESS METHOD

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Summary

The proposed assessment method makes it possible to evaluate the level of the foliage/tuber susceptibility of potato cultivars to late blight, caused by *Phytophthora infestans* (Mont.) de Bary, under field and laboratory conditions using a mathematical simulation approach.

Key words: *Phytophthora infestans*, late blight, potato resistance

Introduction

The resistance of potato cultivars to *Phytophthora infestans*, a causal agent of the late blight (LB), still plays a key role in the control of this disease. The use of resistant plants requires no any actions from potato growers during the season; it does not harm to the environment and is usually compatible with other disease management techniques; finally, sometimes such approach is sufficient to reduce the disease development to a tolerant level (Fry, 1982). That is why the assessment of potato cultivars for the LB resistance is an important part of the selection process in the breeding of new cultivars.

There are two known resistance types: vertical (absolute) and horizontal (partial). The first one is race-specific, since it is related to dominant genes (*R* genes) observed in wild *Solanum* species (mainly *S. demissum* and *S. stoloniferum*) used in a potato breeding. *R* genes provide a hypersensitive reaction of infected tissues resulting in the localization of the pathogen penetration site by necrotized tissues. As a result, pathogen perishes, leaving only a small necrotic lesion on a leaf.

Numerous attempts to obtain a long-term resistance using the mentioned *R* genes were unsuccessful because of the development of virulent races that always exist in any *P. infestans* population. As a result, breeders started to use another type of resistance, named partial (horizontal) or field resistance (Turkensteen, 1993; Colon et al., 1995). Unlike the race-specific resistance, this type of resistance only controls the development of the disease and does not suppress it completely. It is usually considered that this type of resistance is polygenic, since it is efficient against all *P. infestans* races and, therefore, has a more stable and prolonged effect, than the race-specific resistance. However, the possibility of genetic recombinations, appeared in “new” *P. infestans* populations due to a sexual process, provided the appearance of more aggressive pathogen strains that caused a gradual decrease of this type of resistance. As a result, the partial resistance of some potato cultivars to various *P. infestans* populations can significantly vary. For example, cv. Sante is considered to be moderately susceptible in Netherlands (Baarveld et al., 2003, p. 23), but susceptible in the Moscow region of Russia (Simakov et al., 2010, p. 75). Due to this fact, there should be a permanent control on the level of the LB infection of cultivated potato varieties.

A common method for the field assessment of the LB resistance of potato is based on the scoring of the foliage destruction; this scoring is usually performed at a certain stage of the plant development.

The LB resistance is also scored under laboratory conditions using artificially inoculated detached potato leaves; the assessed parameter is the size of necroses or the level of sporulation. Results of such assessment are presented using a 9-score scale (Colon et al., 1995). We consider that traditional late blight assessment methods can be improved. To do this, one should use the quantitative value of the LB-caused yield loss, calculated from the dynamics of the dying-off of infected tops during the whole vegetation period, considered as the key assessment factor.

This paper outlines procedures required to realize the above-described idea.

Materials and methods

Field tests for foliar blight resistance

The field assessment of the partial LB resistance of tested potato cultivars is carried out against the natural or artificial background via the measurement of the leaf infection level each 10-12 days using a special scale (Table 1).

Table 1. Scale for the assessment of the late blight infection of potato leaves (James, 1971)

Level of infection (%)	Description
0	- No any signs of infection
0,1	- First single spore-bearing spots.
1,0	- Weak level of infection (5-10 lesions per a plant)
5,0	- About 50 lesions per a plant; 1 of 10 leaf lobes is infected
25,0	- Almost all leaves are infected, but plants still keep a normal form; the field looks green.
50	- Each plant is infected; about 50% of the leaf area is dead; the field looks green with brown spots
75	- The infection is spread over 75% of the leaf area; the field looks brown-and-green
95	- Plants have only single leaves, but the stems are green
100	- All leaves died, and stems are died or dry

Based on this assessment data, one can determine the area under the disease progress curve (AUDPC) in the course of the vegetation season, the corresponding yield losses caused by the early destruction of leaves (%) and the LB resistance level (in scores).

The program for such calculation is available at the website of the All-Russian Research Institute of Phytopathology (Rogozhin and Filippov, 2011; http://vniif.ru/index.php?option=com_content&view=article&id=40&Itemid=30&lang=ru).

This program is based on the van der Plank hypothesis (1968), which assumes a direct ratio between the AUDPC for the potato foliage and yield losses. According to our long-term field studies (Gurevich et al., 1977), this dependency can be expressed by the following equation:

$$\tilde{S} = \frac{AUDPC}{q} \cdot 100,$$

where \tilde{S} is a yield loss (%) caused by an early leaf decay and q is the number of days between the bud formation phase and the dying-off of non-infected leaves. The average q value for the early, intermediate, and mid-late potato cultivars is 46, 52, and 84 days, respectively. If the foliage is killed by the frost or desiccant, or the harvesting is carried out before the natural dying-off of the foliage, then q is considered to be the number of days passed between the bud formation phase and the moment of the foliage death (Rogozhin and Filippov, 2012).

The calculated yield losses are then converted to the scores characterizing the level of the LB resistance in accordance with the 9-score scale, where 9 scores represent the highest resistance level.

Detached leaf tests for the foliar blight resistance assessment

The quantitative manifestation of the partial LB resistance within the same potato cultivar depends on the infection load, the level of aggressiveness of *P. infestans* strains and weather conditions. Therefore, an objective assessment can be performed by the arrangement of field trials in regions, which are usually favorable for the LB development (such as the Sakhalin island in Russia or Central Mexico), or under standard laboratory conditions with the use of special tests and the mathematical simulator of the epidemic development (Filippov et.al., 2004).

A laboratory assessment method, developed in the All-Russian Research Institute of Phytopathology (Moscow region, Russia), is based on the joint use of the artificial inoculation of detached potato leaves and the mathematical model, simulating the LB development under standard favorable meteorological conditions and the given primary infection level. This model, based on the measurement of the inoculation efficiency, size of necroses, and sporulation productivity, reproduces the dynamics of the foliage destruction during a vegetation season and calculates the correspondence of this dynamics to the LB-caused yield losses (Gurevich et al., 1979). The method makes it possible to assess a cultivar resistance to the most aggressive *P. infestans* strains, including exotic ones, under isolated laboratory conditions.

The tests are carried out on detached leaves, collected from the tested potato cultivars and inoculated with the studied *P. infestans* isolates, and, in parallel, on detached leaves of the standard potato cultivar, inoculated by the standard *P. infestans* strain.

Plants of the tested cultivars and the standard cultivar (30 plants of each cultivar) are grown under field conditions. During the phase of development of 7-9 leaves, a mid-level leaf is detached from each plant for the testing. Then leaves are transferred to the laboratory facilities and inoculated with the selected pathogen strains. Each tested “cultivar-isolate” pair is compared with the standard pair. The comparison of data, obtained in the course of experiments for each “cultivar-isolate” pair (the number and diameter of necrotic lesions and the sporulation productivity), makes it possible to conclude about any differences in the aggressiveness of isolates from different regions and, therefore, about the level of resistance of tested cultivars.

In the proposed method we used the cv. Sante as a standard cultivar and the N161 *P. infestans* strain, collected in the Moscow region and characterized by a high aggressiveness level and the presence of all 11 basic virulence genes, as a standard strain. The yield losses of the above-mentioned cultivar infected with the chosen strain, make 30% if the weather conditions are favorable for the disease development.

Using the described tests, one can measure the basic parameters of the infection cycle on each tested cultivar as compared to the standard cultivar.

Inoculation efficiency test

Ten leaves of each cultivar are inoculated by spraying with the zoosporangial suspension (30000 spores/m²); the volume of the suspension is 5 ml per a cuvette. After the inoculation, leaves are incubated in a wet chamber for 3 days at 18° ; then the area of leaves is determined using a photoplanimeter, and the number of necrotic lesions per 1 cm² is calculated.

Measurement of the size of necrotic lesions

Potato leaves are inoculated with the zoosporangial suspension (1-2 drops per a leaf) using a microdispenser. The concentration of zoospores is the same as for the previous test. Inoculated leaves are incubated in a wet chamber for 18 h at 20° ; then the drops of suspension are removed by a filter paper and the leaves are placed into a wet chamber for additional 3 days. On the 4th day, the diameter of necrotic lesions is measured.

3. Sporulation productivity measurement

For this measurement, one can use the leaves from the previous test.

The spore formation intensity is assessed using two methods. One of them represents a visual assessment of the sporulation. The more exact way is to calculate the number of conidia per one lesion using a Goryaev's count number. To do this, one should put 10 leaf lobes with necrotic lesions into a glass beaker and to add 15 ml of distilled water (1.5 ml per a lesion). After the shaking of the beaker, leaves should be removed and the remaining water volume should be measured. The number of conidia per a lesion should be calculated using the Goryaev's chamber.

All after-measurement calculations are performed separately for potato cultivars of three maturing groups. The program developed on the basis of the above-mentioned measurements, calculates the AUDPC value, yield losses, and the level of the late blight resistance of the tested cultivar under constant conditions favorable for the disease development. The program can be found on the above-mentioned web site of the All-Russian Research Institute of Phytopathology.

Tuber slice tests for the tuber blight resistance assessment

To assess the LB resistance of potato tubers under laboratory conditions, we propose to use a Lapwood method (Lapwood, 1965, 1967) with some modifications.

Potato tubers are sliced into pieces (7×5×40 mm) in the twenty-fold repeatability. One end of each piece is submerged for 3-5 s into a zoosporangial suspension poured into Petri dishes (2-3-mm layer). After 6 days of incubation, the length of the infected zone is measured by a ruler (mm), and the mycelial covering intensity is determined using a 4-score scale. Tuber slices of the cv. Sante, inoculated with the N161 strain, are used as a standard. According to the expert assessments, the level of the tuber resistance of the cv. Sante to the N161 strain is equal to 5.5 scores of the 9-score scale, where 9 scores correspond to the maximal resistance level.

The cv. Sante and the N161 strain can be replaced by any other “standard” cultivar-isolate pair with the known result of their interaction, expressed in scores. From the practical point of view, it is desirable that the tuber resistance level of the selected “standard” cultivar towards the selected *P. infestans* isolate is scored within the range of 4-7.

Based on the measurements of the size of necrotic lesions and the level of the mycelial covering of tuber slices, the cultivar resistance index is calculated using the following equation:

$$x = \frac{\sum(a \times b)}{n},$$

where x is the resistance index, a is the average size of the lesion of the tested cultivar as compared with the standard one, b is the average mycelial covering intensity as compared with the standard (equal to 1), and n is the number of slices.

The calculated indices are then converted into scores using a chart (Fig. 1). To determine the LB resistance of tested potato cultivars, one can also use a special program (http://vniif.ru/index.php?option=com_content&view=article&id=40&Itemid=30&lang=ru).

Results and discussion

The results, obtained from the laboratory assessment of the LB leaf and tuber resistance of 273 potato cultivars, were processed and added into the corresponding database. According to this study, the majority of cultivars were moderately susceptible and susceptible (93 and 78%, respectively) (Fig. 2). To get an access to the mentioned database, one should use the following link:

<http://vniif.ru/uploads/article/39/> %20 %20 .pdf

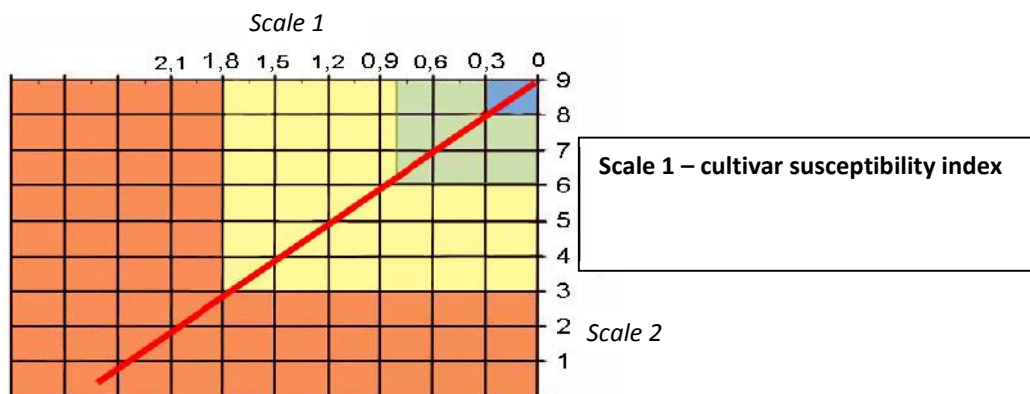


Fig. 1. Chart for the assessment of the LB resistance of potato tubers.

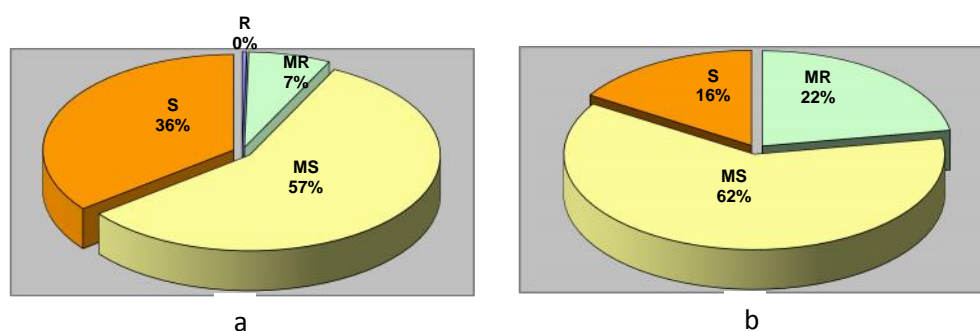


Fig. 2. Results of the laboratory assessment of the LB resistance of (a) potato leaves and (b) potato tubers (2007-2013). Resistance groups: R – resistant (8-9 points), MR – moderately resistant (6-7 points), MS – moderately susceptible (4-5 points), S – susceptible (3 point).

The data on the late blight resistance of potato cultivars added to the above-mentioned database are very important for potato producers. This information allows them to develop the optimal potato protection strategy. For example, the cultivation of the LB-resistant cultivars makes it possible to reduce the number of fungicidal treatments and to reduce the negative influence of the late blight disease on the yield and the quality of tubers; at the same time, the growing of susceptible potato cultivars requires the routine treatment with fungicides to prevent the development of epiphytotes. As we have already mentioned, the common ways to obtain information on the late blight resistance of potato cultivars are the field assessment based on the scoring of a foliage destruction level and laboratory assessment, based on the size of necrotic lesions and the sporulation level measured on detached potato leaves. Both methods have some disadvantages. In the most of regions, weather and phytosanitary conditions, influencing on the results of field trials, vary from year to year, so the field assessment should be repeated for several seasons to obtain reliable results. In the case of laboratory trials, the number of assessed parameters is too small, and their visual estimation is fairly subjective. To overcome these disadvantages, we offer a new method, based on the combination of the artificial inoculation of detached potato leaves and the simulator of a late blight development under standard favorable meteorological conditions and at the given primary infection level. Based on the measurement of the inoculation efficiency, necrotic lesion sizes, and sporulation productivity, the simulator describes a foliage destruction dynamics and calculates its correspondence to yield losses caused by the late blight. The proposed method is able to replace both common methods reducing both cost and time necessary for the cultivar resistance assessment; in addition, it provides the higher assessment accuracy. Now this method is used as a procedure for the state registration of new potato cultivars in Russian Federation.

Conclusion

The use of the new express method made it possible to assess within a short time period the late blight resistance of all potato cultivars, registered on the territory of Russia, using a severe infection background.

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