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# LABORATORY ASSESSMENT OF SOME POTATO GENOTYPES WITH POTENTIAL RESISTANCE TO LATE BLIGHT DISEASE (*PHYTOPHTHORA INFESTANS*)

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## ABSTRACT

*Late blight (Phytophthora infestans) of potato is considered one of the most devastating plant diseases in the world. Most cultivated potatoes are susceptible to this disease. To NIRDPSB Brasov thirteen potato genotypes (leaves and tubers) were evaluated in laboratory conditions for phenotypic scoring against late blight. Isolates were collected from the greenhouse and inoculated with a complex race of late blight differentials (R1-R11). The best results both at the leaf and tuber level were obtained with the genotypes Sasa (R3) x Rustic (Cl1), Agria x Rustic (Cl 1), Sasa x Fribel and Sasa x Florice which provided a durable source of resistance and can be used in a future breeding program. Instead, the genotypes Sasa (R6) x Orchestra, Sasa (R8) x Orchestra, Sasa (R3) x Orchestra, Sasa (R7) x Orchestra showed sensitivity to the action of the pathogen.*

## INTRODUCTION

Late blight of potato (*Phytophthora infestans* Mont. de Bary) is an important disease of potato which causes heavy yield losses all over the world.

This pathogen is a diploid, heterothallic fungus-like oomycete with two mating types (A1 and A2). Over the past several decades, with the increased incidence of late blight epidemics worldwide, the coexistence of both mating types has been observed throughout the world rather than the confinement inside of Mexico (Goodwin et al. 1994; Jo et al. 2014).

Frequent fungicide spray intervals and rates currently used by growers to control late blight are expensive. Host resistance is an alternative control measure that is more economically and environmentally sustainable (Kuhl et al. 2007). Besides economic costs, frequent fungicide applications have undesirable environmental side effects whereas their mechanical application leads to soil compaction and additional CO2 emission, thus negatively influencing the ecologic footprint of potato production (Haverkort & Hillier 2011).

Wild potato species are a valuable genetic pool for finding late blight resistant genes. The first paradigm came from the hexaploid Mexican wild species *Solanum demissum*. Eleven resistance (R) genes, named *R1* to *R11*, were identified in this wild species and introduced into *S. tuberosum* (Black et al. 1953; Malcolmson

& Black 1966). However, these R genes conferred race-specific resistance and those that were introgressed into potato varieties were quickly overcome by the pathogen because of its high genetic variability (Wastie 1991). Hence, new sources of resistance are required, especially those conferring race non-specific resistance to late blight. The co-evolution of the pathogen and wild species in Central America indicated the possibility of finding resistance in species from Mexico such as *S. bulbocastanum, S. pinnatisectum*, and *S. trifidum*. A set of late blight resistance genes has already been identified in these species. Notably, in *S. bulbocastanum*, four different loci with broad spectrum late blight resistance have been identified, namely *Rpi-Blb1*/*RB* (Helgeson et al. 1998), *Rpi-blb2* (van der Vossen et al., 2005), *Rpi-blb3* (Park et al., 2005a), and *Rpi-apbt* (Park et al. 2005b). Recently, several other wild *Solanum* species have been reported as potential sources of resistance, such as *S. mochiquense* (Jones et al. 2013), *S. chacoense* (Vossen et al. 2011), and *S.* × *edinense* (De Vetten et al. 2014; Yang et al. 2017).

## MATERIAL AND METHODS

Thirteen genotypes: R3SasaxRustic (clone 1), Agria x Rustic (clone1), R6Sasa x Orchestra, R3Sasa x Rustic (clone 2) R3Sasa x Rustic (clone 3), R8Sasa x Orchestra, Sasa x Pamela, R3Sasa x Orchestra, Agria x Rustic (clone 2), R3Sasa x Oceania, R7Sasa x Orchestra, Sasa x Fribel, Sasa x Florice were obtained by crossing Rustic, Orchestra, Pamela, Oceania, Fribel and Florice varieties with some SASA lines with different late blight (*Phytophtora infestans*) race-specific *R* genes (R3,R6, R7, R8).

From these genotypes, two healthy leaves were collected from grenhouse. These leaves of each genotypes, non treated with fungicides, were inoculate on the abaxial side of the superior leaflet with 10 µl droplets (2 droplets per leaflet) of inoculum (50,000 zoospores per ml) with an Eppendorf repeater with a complex race of late blight (R1-R11). The leaves were placed on wet paper and after the tray was keep in laboratory with natural light at a temperature of 21 °C.

The first symptoms were observed 3 days after inoculation as black/brown lesions, sporulation and a water-soaked area at the point of pathogen inoculation. Subsequently, symptoms enlarge and cover the whole leaf in the case of highly susceptible genotypes after 5 days.

The late blight resistance score was determined by visual observation of the spreading lesion size of infected leaves. Scales and ranges of spreading lesions associated with the scale value are as follows: 1 (HR), <3% or no visible infection; 2 (R), 3–10%; 3 (S), 10–30%; 4 (MS), 30–60%; and 5 (HS), >60% (HR=highly resistant; R=resistant; S=sensitive; MS=moderately sensitive; HS=highly sensitive).The method described here is based on the work of Colon et al. (2004).

Also from greenhouse tubers were collected and two slices of 20 mm were cut out from the middle part of the tubers and placed in plastic tray with lid. The plastic tray with the tuber slices were placed at 16°C in the dark. Scoring was performed on a 1-9 scale, where 9 means no disease symptoms or non-sporulating hypersensitivity necroses not exceeding the inoculation droplet/site in size.

## RESULTS AND DISCUSSIONS

The observations made are similar to those of other authors (Brylińska & Śliwka 2017) in what regards the scoring time. This can be adjusted according to the development of the disease symptoms on standard cultivars or the tested

material and it can be done earlier or later than six days after inoculation, when the progress of the disease is faster or slower than expected.

The genotypes were divided into groups, depending on the evolution of the infection on the leaves, as follows: 1) very resistant Sasa (R3) x Rustic (Cl1), Agria x Rustic (Cl2), Sasa x Fribel, Sasa x Florice; 2) resistant Sasa (R3) x Oceania; 3) sensitive Sasa (R6) x Orchestra, Sasa(R3) x Rustic (Cl2), Sasa (R3) x Rustic (Cl3), Sasa (R8) x Orchestra, Sasa (R3) x Orchestra, Agria x Rustic (Cl2), Sasa(R7) x Orchestra and 4) moderately sensitive Sasa x Pamela (table 1).

Table 1

The test for determining resistance to late blight on detached leaves

| No. | Genotype | Affected area (%) | Scale value |
|---|---|---|---|
| 1 | Sasa (R3) x Rustic (Cl1) | 0,1 | 1 HR |
| 2 | Agria x Rustic (Cl 1) | 1 | 1 HR |
| 3 | Sasa (R6) x Orchestra | 20 | 3 S |
| 4 | Sasa (R3) x Rustic (Cl2) | 25 | 3 S |
| 5 | Sasa (R3) x Rustic (Cl3) | 20 | 3 S |
| 6 | Sasa (R8) x Orchestra | 25 | 3 S |
| 7 | Sasa x Pamela | 50 | 4 MS |
| 8 | Sasa (R3) x Orchestra | 25 | 3 S |
| 9 | Agria x Rustic (Cl 2) | 25 | 3 S |
| 10 | Sasa (R3) x Oceania | 10 | 2 R |
| 11 | Sasa (R7)x Orchestra | 25 | 3 S |
| 12 | Sasa x Fribel | 0,1 | 1 HR |
| 13 | Sasa x Florice | 0,1 | 1HR |

Figure 1. Late blight symptoms on leaves five days after inoculation (original)

The resistance response rating of the tubers was grouped into three major categories: susceptible (disease rating 1 to 4), partially resistant (disease rating 5 and 6), and resistant (disease rating 7 to 9). The infected tubers slices showed phenotypes ranging from completely susceptible (Sasa R6 x Orchestra, Sasa R8 x Orchestra, Sasa R3 x Orchestra, Sasa R3 x Oceania, Sasa R7 x Orchestra) to highly resistant (Sasa R3 x Rustic Cl1, Sasa x Fribel) but some showed a partial resistance phenotype (Agria x Rustic Cl 1, Sasa R3 x Rustic Cl2, Sasa R3 x Rustic Cl3, Agria x Rustic Cl 2, Sasa x Florice).

Table 2

Genotypes response to late blight tubers infection

| No. | Potato clones | Marks | Scale value* |
|---|---|---|---|
| 1 | Sasa (R3) x Rustic (Cl1) | 9 | R |
| 2 | Agria x Rustic (Cl 1) | 6 | PR |
| 3 | Sasa (R6) x Orchestra | 1 | S |
| 4 | Sasa (R3) x Rustic (Cl2) | 6 | PR |
| 5 | Sasa (R3) x Rustic (Cl3) | 5 | PR |
| 6 | Sasa (R8) x Orchestra | 2 | S |
| 7 | Sasa x Pamela | 3 | S |
| 8 | Sasa (R3) x Orchestra | 1 | S |
| 9 | Agria x Rustic (Cl 2) | 5 | PR |
| 10 | Sasa (R3) x Oceania | 2 | S |
| 11 | Sasa (R7)x Orchestra | 3 | S |
| 12 | Sasa x Fribel | 8 | R |
| 13 | Sasa x Florice | 5 | PR |

Figure 2. Late blight symptoms on tubers leaves five days after inoculation (original)

The best results both at the leaf and tuber level were obtained with the genotypes Sasa (R3) x Rustic (Cl1), Agria x Rustic (Cl 1), Sasa x Fribel and Sasa x Florice. These genotypes provide a durable source of resistance and can be used in a breeding program. Instead, the genotypes Sasa (R6) x Orchestra, Sasa (R8) x Orchestra, Sasa (R3) x Orchestra, Sasa (R7) x Orchestra showed sensitivity to the action of the pathogen. The Orchestra variety used as a parent shows a low level of resistance that was transmitted to all descendants regardless of the late blight differentials race used (R3, R8 R 6 or R7).

## CONCLUSIONS

Laboratory assessment based on the size of necrotic lesions and the sporulation level measured on detached potato leaves and sliced tubers is an easy method to obtain results regarding the evolution of different genotypes under the late blight attack. Information regarding the behavior of different potato genotypes to late

blight attack allow to develop a protection strategy. Cultivation of resistant genotypes makes possible to reduce the number of treatments. According to current results genotypes Sasa (R3) x Rustic (Cl1), Agria x Rustic (Cl 1), Sasa x Fribel and Sasa x Florice provide a durable source of resistance and can be used in a future breeding program.


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