

## The adaptation of MAS for late blight resistance evaluation of potato breeding material

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### SUMMARY

The goal of this study was to determine contribution of race-specific resistance conferred by genes R1 and R3 to general resistance of potato breeding clones to late blight (agent *Phytophthora infestans*). For this purpose the assessment of breeding clones for resistance to late blight of leaf and tubers and field resistance as well their screening for presence of resistant alleles of R1 and R3 genes were performed. No influence of resistance genes on reduction of level of foliar damage in field and leaf resistance was observed. A significant influence of presence of R3 gene on tuber resistance to late blight was found. The significant difference was detected between tuber resistance level of clones with detected presence of resistant allele of R3 gene and clones with detected presence of susceptible alleles of both tested genes and clones with detected resistant allele of R1 gene ( $p < 0.05$ )

### KEYWORDS

*Phytophthora infestans*, MAS, potato breeding, resistance.

### INTRODUCTION

Late blight caused by *Phytophthora infestans* is still challenging potato fields around the globe. Disease mediated by the R genes is one of the cognate effectors that is introduced into the plant cell by the pathogen and induces resistance. R-genes (R1-R11) in potatoes are remaining to be the valuable sources for development of new cultivars resistant to late blight, but potatoes containing these R genes are only effective in preventing the development of late blight. Another application on the resistance is to be the optimal selection of R genes identified by monitoring of *P. infestans* populations in each area where the potatoes are grown (Visser *et al.*, 2008). A marker assisted selection (MAS) is to be the effective tool in use for resistance level improvement in breeding programme. Classical selection of resistant progenies is difficult due to appearance of new pathogenic pathotypes and due to expensive and time consuming field evaluation. Since 1980s molecular markers are being widely used as a principal tool for plant breeding. In several studies molecular markers linked to late blight resistance genes have been found (Leonards-Schippers *et al.*, 1992; Gebhardt and Valkonen, 2001; El-Kharbotly *et al.* 1994; 1996; Li *et al.*, 1998; Ewing *et al.*, 2000; Huang *et al.*, 2004). *P. infestans* genes virulent to R1 and R3 were detected among most common in late blight European populations (Andrivon *et al.*, 1994; Lebreton *et al.*, 1998; Lehtinen *et al.*, 2008; Lebecka *et al.*, 2007) including North-Western Region of Russia and Estonia (Runno-Paurson *et al.*, 2009; Zoteyeva and

Patrikeeva, 2010) neighbouring with Latvia.

The association of resistance genes R1 and R3 presence in genotypes and high late blight resistance was observed in previous research (Khavkin *et al.*, 2010). The R genes contribute some resistance; combining resistance genes with high levels of field resistance would be a desirable goal for breeding programme (Bradshaw, 2009).

The goal of this study was to determine contribution of genes for race-specific resistance to *P. infestans* R1 and R3 to general resistance to late blight of potato genotypes. For this purpose breeding clones were assessed for resistance to late blight of leaf and tubers and field resistance as well as screened for presence of resistant alleles of R1 and R3 genes by molecular markers.

## MATERIAL AND METHODS

### *Description of field growing conditions*

The soil type was sod-podzolic (PVv), loamy sand. Organic matter content in soil was 24 - 27 mg kg<sup>-1</sup>, pHKCl was 5.5 – 5.7, availability of K and P in soil was high. Fertiliser N – 50-60, P – 100, K – 100 kg ha<sup>-1</sup> was used. The fungicides for restriction fungal diseases were used two times in July each year. In 2010 the air temperature in the second part of vegetation was 3-5°C higher than perennial data (PD). In 2011 air temperature was similar to PD. The precipitation exceeded PD by 24 – 31% in 2010. The July was dry in 2011 (precipitation only 85% of PD), but precipitation in second decade of August exceeded PD by 109 %.

### *Plant and infection materials*

Third, fourth and fifth generations of breeding clones (total number 463) were assessed for late blight resistance in field in 2010, for selected clones assessment was continued in 2011. Ten clones involved in field assessment were screened by molecular markers. 100 clones were evaluated for leaf resistance to *P. infestans* and 38 clones were evaluated for tuber resistance. Screening with molecular markers was performed in 69 potato clones undergoing leaf tests and in 28 clones undergoing tuber tests. Complex evaluation of all assessments was performed for 16 potato clones.

The isolates of *P. infestans* were sampled from infected potato plants grown in field. The virulence factors in sampled isolates were studied using a set of Black's differential genotypes R1 – R11 offered by IHAR-Mlochow Research Center, Poland. For inoculum preparation the mixture of two isolates expressing nine and six genes for virulence (1.2.3.4.(5).6.7.10.11 and 1.3.4.7.8.10.11.) were used.

### *Field observation*

Observations were performed from the beginning of July to the end of August once in 7-10 days. The disease development on foliage was assessed as percentage of foliage area damaged by *P. infestans* infection. Diseases damages on foliage for each clone was set to grade scale (assessment key for foliar late blight of the Dutch Plant Protection Service): grade 1 – when 90-100% damaged area out of total foliar area, grade 9 - less than 10 % of total foliar area was damaged (Bus *et al.*, 1995).

### *Marker assisted selection (MAS)*

Clones involved in field observations were tested with molecular markers for presence of resistant alleles of R1 and R3 genes. Resistant allele of R1 gene was detected with marker 76-2S according to protocol developed by Ballvora *et al.* (2002) and resistant allele of R3 was detected with marker RT-R3a\_L01 according to protocol of Huang *et al.* (2005).

### *Leaf and tuber tests*

The leaflets were collected from plants grown in the field in the beginning of flowering. Inoculum

applied comprised 20000 sporangia ml<sup>-1</sup>. Symptoms were observed on 6th day after inoculation using grade scale 1-9, where grade 9 is highest resistance, no disease symptoms observed (Zarzycka, 2001).

Tuber test was performed approximately two months after harvesting. Method of tuber testing described by Zarzycka (2001) was applied using the same *P. infestans* isolates and inoculum concentration as in leaflet tests.

Analysis of variance was done using Minitab 15. The average assessment values were compared using T-test.

## RESULTS AND DISCUSSION

### *Potato clones field (foliar) resistance assessment in field conditions depending on presence of resistance genes*

Percentage of foliage damaged by disease was assessed and ranged from 0% to 70 % in 2010, and from 5% to 100% in 2011. The first symptoms of late blight were recorded in the first dates of July in both years. The intensive disease invasion was observed in mid of August after several rainfalls. The infection of *P. infestans* was higher during 2011 than 2010, when climatic factors of second part of growing season were more favourable for disease development.

*P. infestans* isolates sampled from the field in 2010 were complex and showed large spectrum of genes for virulence on the leaflets of R-1 - R11 Black's differential genotypes. From six (1.3.4.7.10.11.) to ten (1.2.3.4.(5).6.7.(8).10.11.) genes for virulence were detected in tested isolates.

The presence of resistant allele of R1 gene was detected for 8 %, and the presence of resistant allele of R3 gene in 27 % of tested clones. Presence of resistant alleles of both genes was detected in 1.9 % of breeding clones. The source of genes for resistance found in breeding clones were parental varieties, mostly containing resistance genes derived from *Solanum demissum* Lindl.

Presence of resistant allele of R1 gene was almost equal in all groups of clones classified by the level of disease damage (Table 1). In more clones resistant allele of R3 gene was detected compared to resistant allele of R1 gene. In groups of clones characterized by disease development from 0% to 30 % and from 31% to 50% presence of resistant allele of this gene was detected for similar amount of clones as other groups of clones with higher diseases development level. There was not observed dependence of field (foliar) resistance to late blight on detected presence of resistant alleles of tested genes. There was not found significant difference between diseases damages level between groups with detected presence of resistant or susceptible alleles of tested genes ( $p>0.05$ ).

Table 1. Distribution of clones differed by diseases damages level into three groups: with detected presence of resistant to *P. infestans* alleles of R1 or R3 genes and with detected presence of susceptible alleles of genes, Priekuli, 2011

Disease damaged area from total foliage,%	Number of tested clones	Percentage of total number of clones, %		
		Presence of resistant allele of R1 gene	Presence of resistant allele of R3 gene	Presence of susceptible alleles of R1 and R3 genes
0% - 30.0%	20	10	25	65
31% - 50.0%	21	10	29	61
51 % - 70.0 %	39	6	28	66

The analyses of pedigree of tested potato clones revealed that one third of clones with low diseases damage (less than 30 %) were progenies of variety 'Zarevo'. High resistance level to *P. infestans* of variety 'Zarevo' was commonly reported. In late 90's this cultivar was identified as one of the most resistant to *P. infestans* among European cultivars (Douches *et al.*, 1997, Bisognin *et al.* 2002). The variety is an interspecific hybrid of crosses of *S. tuberosum* L. with germplasm of *S. leptophyes* Bitt., *S. demissum* Lindl. and *S. andigenum* Juz. et Buk. (Swiezynski *et al.*, 1997).

#### *Evaluation of leaf and tuber resistance*

High and relatively high leaf resistance to *P. infestans* (resistance score up to 5) was detected for 10 % of tested clones. The presence of one of resistance allele of tested genes were detected for only two out of seven resistant clones, R1 for one and R3 for other. Among clones expressed susceptibility in leaf tests (resistance score less than 5) in about one third of clones' presence of resistant allele of genes have been detected. For one clone presence of resistant alleles of both genes was detected. The presence of resistant allele of R1 gene was detected in 5 clones and presence of resistant allele of R3 gene in 15 ones in this group. There was not found significant difference between leaf resistance assessment between groups with detected presence of resistant or susceptible alleles of tested genes ( $p>0.05$ ).

High tuber resistance (resistance score up to 5 grade on average) was found for 42 % of tested clones. Only in one of 12 clones' resistant alleles of both genes was detected. In group of clones with high tuber resistance the presence of resistant allele of R1 gene was detected in two and presence of resistant allele of R3 gene in seven clones. In clones with low tuber resistance presence of resistant allele of R1 gene was detected in 4 clones and presence of resistant allele in R3 gene for 3 clones. Distribution of clones with resistant or susceptible R1 or R3 genes alleles within whole amount of tested clones is shown in figure 1. The proportions of clones with detected presence of resistant allele of R1 or R3 genes and clones with detected susceptible alleles within the groups of clones with high and low tuber resistance differed. However, within the group of clones with high tuber resistance to *P. infestans* the share of those with detected presence of resistant allele of R3 gene was much higher than in group of clones with low tubers resistance. Share of clones with detected resistant allele of R1 gene was larger in group of clones with low tuber resistance then in group with high tuber resistant. In evaluated clones presence of resistant allele of gene R3 significantly ( $p=0.012$ ) influenced tuber resistance. If the influence of each gene was analysed separately, a significant influence of resistance gene was detected for R3 only ( $p=0.001$ ) and not in case of R1 ( $p>0.05$ ). Comparing clones with presence of resistant allele of R3 gene with clones with presence resistant allele of R1 gene significant difference was found ( $p=0.039$ )

No significant differences between resistance levels of group with detected presence of resistant allele of R1 gene with group of clones with detected presence of susceptible alleles of tested genes ( $p>0.05$ ) were found.

One of breeding clones (nr.322 on Figure 2.) was found to be the most resistant (resistance score 7.8) but neither presence of resistant allele of R1 nor presence of resistant allele of R3 was detected.

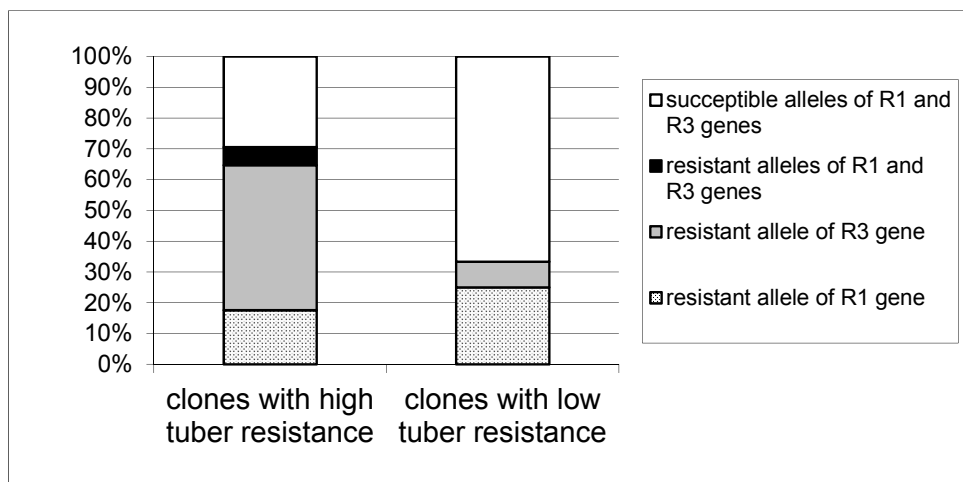


Figure 1. Distribution of clones with detected presence of resistant allele of R1 gene, with detected presence of resistant allele of R3 gene allele and with susceptible those genes alleles, within the group of clones with low tuber resistance and clones with high tuber resistance.



Figure 2. Potato breeding clones with different tuber resistance levels.

#### Complex evaluation of potato clones for tested traits

Comparing groups of potato clones with resistance or susceptibility detected by molecular markers, the slightly increased field resistance (comparing grade of disease damages) in 2011 was noted for groups in which resistance was declared (Table 2.). The average leaf resistance grade was higher for both groups with detected presence of resistant alleles of R1 and R3 genes compared to group with susceptible plants. Comparison of mean values using T-tests showed that the difference was

not significant with confidence level 95 %. The group of plants with resistant allele of R3 gene had higher resistance grade on average than group with susceptibility, but difference was not significant ( $p>0.05$ ). The average tuber resistance grade for potato clones with resistant allele of R1 gene was lower than potato clones with susceptible allele, but difference was not significant, comparing mean values using T-test ( $p>0.05$ ).

Table 2. The evaluation of potato clones general resistance to late blight in field conditions, leaflet and tuber tests (grade scale 1 – 9, where 9 is the most resistant or less damages)

Potato clones	Number of tested clones	Average grade of disease damage		Average grade of leaf resistance	Average grade of tuber resistance
		2010	2011		
Clones with detected presence of susceptible alleles of R1 and R3 genes	6	8.9	6.0	3.4	5.4
Clones with detected presence of resistant allele of R1 gene	4	8.8	7.3	4.1	5.0
Clones with detected presence or resistant allele of R3 gene	6	8.8	6.3	5.2	5.6

$t_{stat} < t_{crit}, p>0.05$

For evaluation of race specific genes R1 and R3 impact on general resistance to late blight more data have to be obtained. In our research influence of R1 and R3 gene resistant alleles' presence on foliage resistance in field and leaf resistance in laboratory was not observed. Data obtained partly confirm the data reported by Umaerus and Umaerus (1994) postulating that the presence of resistance R genes could improve genotypes for tuber resistance to late blight. Presence of resistant allele of gene R3 gene in evaluated clones significantly ( $p<0.05$ ) influenced tuber resistance. If compare with clones with detected presence of susceptible alleles of both genes or resistant allele of R1 gene Stacking of multiple resistance (R) genes is considered to be one of the most promising approaches to provide durable resistance to potato late blight (Li *et al.* 2011) Recent data show that stacking of multiple genes as in variety 'Sarpö Mira' (R3a, R3b, R4, Rpi-smira1 and Rpi-smira2) provide durable resistance (Rietman, 2011). The future work should be focused on stacking additional genes to R1 and R3 to obtain higher resistance level.

As a result of the analysis of the data obtained in 2010 and 2011 seasons, the potato clones combining foliar, leaf and tuber resistance to *P. infestans* were identified.

## CONCLUSIONS

No significant influence of resistant alleles of R1 and R3 gene on foliar (field observations) and leaf (leaflet tests) resistance levels was found.

The significant difference was found between resistance level of clones with detected presence of resistant allele of R3 gene and clones with detected presence of susceptible alleles of both tested genes and clones with detected presence of R1 gene.

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