The adaptation of MAS in late blight resistance evaluation of potato breeding material

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Introduction

Late blight caused by *Phytophthora infestans* is still challenging potato fields around the world. Disease mediated by the R genes is one of the cognate effectors which is introduced into the plant cell by the pathogen and induces resistance. MAS (marker assisted selection) is to be the effective tool in use for resistance level improvement in breeding programme. For this purpose the breeding material evaluation data obtained in molecular screening, field observations and laboratory tests has to be analyzed.

P. infestans genes virulent to *R1* and *R3* were detected between most common of late blight populations in North-western Region of Russia (Zoteyeva and Patrikeeva, 2008). The association of R1 and R3 presence in genotypes and high late blight resistance was observed (Khavkin et al., 2010). The goal of study was to determine genes for race-specific resistance to *P. infestans R1* and *R3* contribution in expression of genotypes general resistance. For this purpose the assessment of resistance to late blight in laboratory tests and field observations was performed for potato clones screened for presence of *R1* and *R3* genes.

Material and methods

The resistance of potato clones from SPPBI breeding programme were investigated in field and laboratory tests during 2010 and 2011.

Field growing conditions. The soil type was sod-podzolic (PVv), loamy sand. Organic matter content in soil – 24 - 27 mg kg^{-1}, pH_{KCl} was 5.5 – 5.7, availability of K and P in soil was high. Fertiliser N – 50- 60, P–100, K–100 kg ha^{-1} was used. The fungicides for restriction fungal diseases was used two times in July. The air temperature in the second part of vegetation was 3-5°C higher than perennial data (PD) in 2010. During 2011 air temperature was similar to PD. The precipitation exceeded PD for 24 – 31% in 2010, but rains were mostly like heavy showers. The July was dry in 2011 (precipitation only 85% of PD), but precipitation in second decade of August exceeded PD for 109 %.

Field observation. Third, fourth and fifth breeding clones generations (total number 463) were assessed for late blight resistance in field in 2010, for selected clones assessment was continued in 2011. Field plot size was 5 – 10 m^2, replications 1 – 4. Observations were performed from the beginning of July to the end of August once in 7-10 days. The disease development on foliage was done in percents of late blight damaged area from total leaf area. Potato clones resistance was set to grade scale where grade 1 – poor resistance, 90-100% damaged area out of total leaf area, grade 9 means an excellent resistance when less than 10 % of total leaf area was damaged.

Marker assisted selection (MAS). Clones involved in field observations were tested with molecular markers for presence of resistant alleles of *R1* and *R3* genes (Figure 1.). 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).

Leaflet and tuber test. Three groups of selected clones, with detected markers for presence of *R1* gene, or presence of *R3* gene, or with absence of R1 and R3 genes, were evaluated for leaf and tuber resistance in laboratory tests. The leaflets were collected from plants grown in the field in the begining of flowering. The inoculum with concentration 20000 sporangia/ml was prepared using the mixture of two *P. infestans* local isolates (1.2.3.4.(5).6.7.10.11+1.3.4.7.8.10.11.). Symptom reading was done on 6th day after inoculation using grade scale 1-9, where grade 9 is excellent resistance, no diseases symptoms. Tuber test was performed approximately two months after harvesting. Method of tuber testing described by Zimnoch-Guzowska (2000) was applied using the same as in leaflet tests *P. infestans* isolates and inoculum concentration.

Table 1. The general resistance of potato clones with detected presence of R1 and R3 genes to late blight, natural infection, 2010.

Potato clones	Number of tested clones	Percentage of clones with relatively high resistance (LB damaged leaf area - 0-5%)	Percentage of clones with relatively low resistance (LB damaged leaf area - >20%)
Absence of R1 and R3 genes	282	51	7
Presence of R1 gene	33	61	3
Presence of R3 gene	139	45	1
Presence of R1 and R3 genes	9	89	11

Results

Late blight resistance in field conditions.

Disease damaged area on foliage of evaluated clones was in range 0 – 28 % in 2010, but assessment in range 5 – 100% was observed in 2011. The expresion of *P. infestans* infection was stronger in 2011 than 2010, when second part of growing season was more favourable for disease development. The late blight assessment was not available for 11 % of clones because of early foliage wilting in 2010.

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. In isolates tested 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.

The presence of late blight reistance *R1* gene was detected for 7.1 % of breeding clones, and of *R3* gene for 30.0 % out of totally tested. Presence of both genes was detected for 1.9 % of breeding clones. The source of genes for resistance found in breeding clones was parental varieties, moustly containing resistance genes derived from *Solanum demissum* Lindl.

The amount of breeding clones with relatively high general resistance levels registered in field observations was not significantly dependent on the amount of clones in which presence of genes for resistance *R1* and *R3a* were detected (Table 1.).

As a result analyses of genealogy of clones shown low and relatively low damaged foliage area has been revealed the cultivar 'Zarevo' to be able to transfer resistance to hybrid progenies. This variety was also found as most frequent parent of 27 breeding clones shown disease development assessment from 30 to 50% (2011).

Comparison of resistance of clones with R1 and R3 genes absence or presence.

Comparing groups of potato clones with detected absence or presence of *R1* and *R3* genes, the slightly increased foliage resistance in 2011 was noted for groups with these gernes presence (Table 2.). The leaflet resistance grade was higher for both groups with presence *R1* and *R3* genes than for group those genes free. The presence of resistance *R* genes could improve genotypes tuber resistance to late blight (Umaerus and Umaerus, 1994). The potato clones group with presence of *R3* gene had higher grade of tuber restance to late blight than gene resistance free group. The average resistance grade for potato clones with presence of *R1* gene was lower than for potato clones genes free.

For detection of race specific genes *R1* and *R3* impact on general resistance to late blight more data have to be obtained. In current research slight influence of gene resistance presence to general resistance level was observed.

As a result of the analysis of the data received in 2010 and 2011seasons the breeding clones combining field, foliar and tuber resistance to *P. infestans* were identified. Potato clones with detected presence of resistance genes *R1* and *R3* shown acceptable general resistance level were selected.

Table 2. Field and laboratory evaluation of potato breeding clones resistance to late blight

Potato clones	Number of tested clones	Average foliage resistance grade in field 1-9 (9-exelent resistance)		Average leaflet resistane grade, 1-9 (9-exelent resistance)	Average tuber resistane grade, 1-9 (9-exelent resistance)
		2010	2011		
Clones with absence of R1 and R3 genes	6	8.9	6.0	3.4	5.4
Clones with presence of R1 gene	4	8.8	7.3	4.1	5.0
Clones with presence of R3 gene	6	8.8	6.3	5.2	5.6

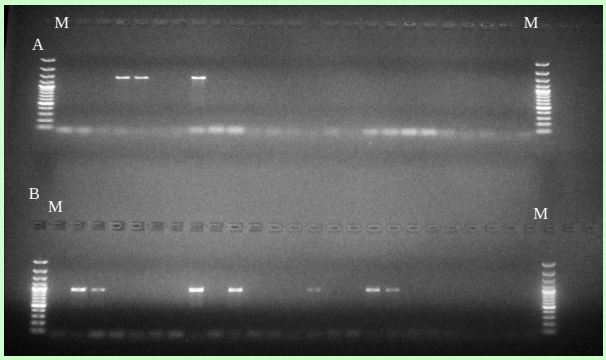

Figure 1. Screening potato clones with SCAR markers A) 76-2sf2 un 76-2SR amplifying 1399 bp fragment linked to R1 gene B) RT-R3a amplifying 981 bp fragment linked to R3 gene. M- marker lane

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