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 gene is one of the protective efficiencies 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 be analyzed. P. infestans genes introgressed in R1 and R3 were detected between most common of late blight populations in North-Eastern region of Russia (Zotevaya and Patrinkov, 2008). The association of R1 and R3 presence in genotypes and high late blight resistance was observed (Kharkin et al., 2010). The goal of study was to determine genes for race-specific resistance to R. 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, pH₅0.1 was 5.5 - 5.7, availability of K and P in soil was high. Fertilizer N – 50 - 60, P - 100, K-100 kg ha⁻¹ was used. The first rains for resistant fungal disease was two to three 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 excessed PD for 24 - 35% 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 m², replication 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 percent of late blight damaged area from total leaf area. Potato clones resistance was set to grade scale where grade 1 – poor resistance, 100% damaged area out of total leaf area, grade 9 mean an excellent resistance when less than 10% of total area was damaged.

Marker assisted selection (MAS). Clones involved in field observations were tested with molecular markers for presence of resistance alleles of R1 and R3 genes (Figure 1.1). Resistant allele of R1 gene was detected with marker 796-26 according to protocol developed by Ballvora et al. (2002) and resistant allele of R3 was detected with marker RTRa1,1 (2002) 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 leaflet and tuber resistance in laboratory tests. The leaflets were collected from plants grown in the beginning of flowering. The inoculum with concentration 20000 sporangia/ml was prepared using the mixture of two P. infestans leaf isolates 2.5-4.5,6.7,10.16,6.4,3,8,7,10.11. Symptoms reading was done six to eight day after inoculation using grade scale 1-9, where grade 9 is excellent, no disease symptoms. Tuber test was performed approximately two months after harvesting. Method of tuber testing described by Zimmnach-Gawszewska was applied using the same as in leaflet test P. infestans isolates and inoculum concentration.

Results

Late blight resistance in field conditions.

Slightly increased foliage indices of selected clones was in range 0 – 28 % in 2010, but assessment in range 5 – 100% was observed in 2011. The expression of P. infestans infection was stronger in 2011 than 2010, when secondarily 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 isolated from the field in 2010 were complex and showed large spectrum of genes for resistance on the leaflets of R1- R3 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 resistance were detected. The presence of late blight resistance R1 gene was detected for 71 % of breeding clones, and of R3 gene for 50 % out of totally tested. Presence of both genes was detected for 1.8 % of breeding clones. The source of genes for resistance found in breeding clones was parental varieties, mostly resistant alleles showed derived from Solanum demissum Linn.L.

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 R3 were detected (Table 1.).

As a result analyses of genotypes of clones showed low and relatively low damaged foliage area has been recorded in the cultivar ‘Zarevo’ to be able to transfer resistance to hybrid progenies. This variety has been revealed the cultivar ‘Zarevo’ to be able to transfer resistance to hybrid progenies. This variety shown acceptable general resistance level were clones with presence R1and R3 genes to late blight, natural infection, 2010.

Comparison of resistance 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 2010 was noted for groups with these genotypes (Table 2.1). The leaflet resistance grade was higher for both groups with R1 and R3 genes than for group those genes. The presence of resistance R3 genes could improve genotypes tuber resistance to late blight (Urmans and Urmaeus, 1994). The potato clones group with presence of R1 gene had higher grade of tuber resistance to late blight than genes resistance free group. The average resistance grade for potato clones with presence of R1 gene was lower than for potato clones genotypes 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.

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

<table>
<thead>
<tr>
<th>Potato clones</th>
<th>Number of tested clones</th>
<th>Percentage of clones with relatively high resistance (LB damaged leaf area ≤ 5%)</th>
<th>Percentage of clones with relatively low resistance (LB damaged leaf area ≥ 20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of R1 and R3 genes</td>
<td>282</td>
<td>51</td>
<td>7</td>
</tr>
<tr>
<td>Presence of R1 gene</td>
<td>33</td>
<td>61</td>
<td>-</td>
</tr>
<tr>
<td>Presence of R3 gene</td>
<td>139</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>R1 and R3 genes</td>
<td>9</td>
<td>89</td>
<td>11</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Potato clones</th>
<th>Number of tested clones</th>
<th>Average foliage resistance grade in field 1-9 (9-excellent resistance)</th>
<th>Average leaflet resistance grade, 1-9 (9-excellent resistance)</th>
<th>Average tuber resistance grade, 1-9 (9-excellent resistance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clones with absence of R1 and R3 genes</td>
<td>6</td>
<td>8.9</td>
<td>6.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Clones with presence of R1 gene</td>
<td>4</td>
<td>8.8</td>
<td>7.3</td>
<td>4.1</td>
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<tr>
<td>Clones with presence of R3 gene</td>
<td>6</td>
<td>6.8</td>
<td>6.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Clones with presence of R1 and R3 genes</td>
<td>8</td>
<td>6.8</td>
<td>6.3</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Figure 1. Scanning potato clones with SCAR markers A) 76-202 uc 76-25B 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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References:


