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Apricot breeding for resistance to Sharka

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Abstract. The Sharka disease caused by Plum pox virus (PPV) was found for the first time in Bulgaria more than 80 years ago and it is an endemic disease for our country, as is for all the East European countries. Like all plant viruses, PPV could not be controlled with treatments and that makes breeding of resistant cultivars and rootstocks a significant tool for limiting its spread. The investigation was carried out in the period 2009–2012 in the collection plantations of the Fruit-Growing Institute, Plovdiv in the frames of FP7 project of the EU „Sharka Containment (SharCo)“. The aim set in one of the work packages of the project was to carry out accelerated breeding activities of apricot cultivars resistant to Sharka by using MAS (marker-assisted selection). The hybridization programme included 24 parental combinations with 12,633 pollinated flowers and as a result 1661 hybrid clones (seeds) were obtained. The molecular marker analysis of the leaf samples from 39 hybrid plants obtained from 7 crossings showed that 8 of the hybrids contained a gene for resistance to PPV. Those were plants of the parental combinations ‘Harcoat’x ‘Lito’, ‘Lito’x ‘SEO’ and ‘Harlayne’x ‘Harcoat’. Data showed that all hybrids obtained from crossing of two resistant cultivars (‘Lito’x ‘SEO’) have a gene for resistance. If only one of the parental cultivars is resistant, part of the hybrids have gene for resistance to PPV and in our case it is 50% of all analyzed hybrids.

Keywords: apricot, breeding, sharka, resistance

Abbreviations: PPV – plum pox virus, MAS – marker-assisted selection

Introduction

The Sharka disease caused by Plum pox virus (PPV) was found for the first time in Bulgaria more than 80 years ago and it is an endemic disease for our country, as is for all the East European countries. In West Europe, still there are regions where the disease is not found, however every year the disease is announced to be spreading to new areas. In practice, Sharka was registered on all six continents (Maejima et al., 2010; Thompson et al., 2006). Losses from the disease are very serious in susceptible cultivars. Cambra et al. (2006) mentioned that worldwide losses associated with the management of Sharka had been estimated at over 10,000 million euros over the last 30 years. Like all the plant viruses, PPV could not be controlled with chemicals and that makes breeding of resistant cultivars and rootstocks a significant tool for limiting its spread. The importance of both elements of the tree – the rootstock and the grafted cultivar – comes from the fact that the infection with the virus could occur at each stage of the tree production and growing: during the two years of growing in the nursery, caused by the aphids; in the mother plantation for the production of cuttings or in the orchard when the aphids infect either the scion or the rootstock through the suckers.

There are no data available in literature about an existing source of resistance in Prunus genus – either resistant or immunological one, which could protect the trees from all the virus strains. According to Kegler et al. (1998) the sources of resistance are polygenic, providing from a medium level of resistance to tolerance towards at least one of the virus strains. It means, when using the existing donors, that the individuals obtained will be of different level of inheritance. According to Cooper and Jones (1983) a genetic type is immunogenic when there is no pathogen-host interaction and the virus can never be found in the latter. The authors suggested that the virus is strongly suppressed in the resistant genotypes (low titre of the virus) compared to the susceptible types and the virus could be sporadically found but not always. Tolerant genotypes are infected by the virus but the symptoms expressed are not apparent. Susceptible genotypes are those which are freely infected and the symptoms in the leaves, fruits, wood and flowers are apparent and specific for the pathogen. Sometimes researchers use their own definitions and scales that differ from the definitions mentioned above, which leads to discrepancy of the information, especially as far as resistant cultivars are concerned.

It was established that there are no immunogenic types among the large number of the studied apricot genotypes originating from Europe, Asia and North America. Using developed markers, carriers of genes for PPV resistance have been found out in a number of cultivars, such as ‘Stark Early Orange’, ‘Harlayne’, ‘Lito’, ‘Goldrich’ and NJA2, which was the reason for including those cultivars in modern breeding programmes. Combining classical and molecular methods and techniques in apricot breeding is at an advanced level and the first breeding results have already been obtained.

Materials and methods

The investigation was carried out in the period 2009–2012 in the collection plantations of the Fruit-Growing Institute, Plovdiv within the frames of FP7 project of the EU „Sharka Containment (SharCo)“. The aim set in one of the work packages of the project was to carry out accelerated breeding activities of apricot cultivars resistant to Sharka by using MAS (marker-assisted selection). The sexual hybridization method was applied for obtaining new hybrids. Flowers were castrated and isolated. The pollen of the selected cultivars was collected at the phenological stage of white button and stored in an excipitor at a temperature of 20°C–22°C. The PPV resistant cultivars

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Results and discussion

In the period 2009–2011, a hybridization programme including 24 parental combinations was implemented, aiming at obtaining apricot hybrid populations with increased resistance to Plum pox virus (PPV) and combining good economic and biological characteristics. A total of 12,633 flowers were pollinated and 1,661 hybrid stones (seeds) were obtained. The percentage of the produced seeds out of all the pollinated flowers was 13.1% on average for all combinations. Those data are consistent with the results of other authors, obtained after investigations on other cultivars, under different climatic conditions. In Hungary, Szalay et al. (2000) reported the percentage of the fruit set after open pollination of 6 Romanian cultivars to be varying between 19.9% and 42.9%, while McLaren et al. (1996) mentioned that the problem of pollination had acquired great importance and they found intersterile groups, in which the percentage of the fruit set was below 2%. After conventional stratification, 620 hybrid plants were grown from the hybrid seeds. They were planted in a breeding orchard for evaluation and selection against natural PPV infection background. 323 of them originated from parental combinations consisting of a cultivar well-adapted to the soil and climatic conditions of Plovdiv region and a cultivar that is a carrier of a gene for resistance to the Sharka virus (Table 1).

Table 1. Number of hybrids obtained involving resistant cultivars

<table>
<thead>
<tr>
<th>Parental combinations</th>
<th>Number of plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Harlayne' x 'Harcot'</td>
<td>225</td>
</tr>
<tr>
<td>'Harcot' x 'Stark early orange' (SEO)</td>
<td>23</td>
</tr>
<tr>
<td>'Hungarian best' x 'Harlayne'</td>
<td>2</td>
</tr>
<tr>
<td>'Krupna Skopjanka' x 'Harlayne'</td>
<td>10</td>
</tr>
<tr>
<td>'Lito' x 'Silistrenska kompotna'</td>
<td>63</td>
</tr>
<tr>
<td>Total</td>
<td>323</td>
</tr>
</tbody>
</table>

The average percentage of the obtained plants out of all the stratified seeds was 37.3%. It is quite low compared to studies by other authors. Polat (2007) achieved values of up to 50–80% under controlled conditions. Similar results were reported by Bassi et al. (1998). The authors underlined that the conditions during stratification were very important for achieving a higher percentage of seed germination. In most cases special treatments with chemical reagents were applied with the aim of increasing that percentage. In the present study such reagents were not used but it was established that germination was different for the different cultivars. The seeds of 'Lito' and 'Harlayne' showed a better germination rate, reaching up to 60% in some years, which makes them suitable to be used as a mother parent.

During the first vegetation period, symptoms of virus infection were not detected in the leaves of the planted hybrids. Observations will continue for selecting elites that would be subjected to biological tests with our isolates of the PPV, strain M. Hybrids showing good results after the biological test will be evaluated for their biological and economic characteristics after reaching the fruit-bearing stage. Thus, the breeding process is expected to be shortened.

The biological test carried out with 38 hybrid plants inoculated with a PPV isolate, showed that during the first vegetation cycle disease symptoms in the leaves were not observed in any of the studied hybrid plants but the virus was detected in 4 plants by the ELISA test (Table 2).

The molecular marker analysis of the leaf samples from 38 hybrid plants obtained from 7 crossings showed that 8 of the hybrids

Table 2. Results from application of phenotyping and MAS (2011–2012)

<table>
<thead>
<tr>
<th>Hybrid combination</th>
<th>PPV symptoms on leaves</th>
<th>Number of tested plants for PPV</th>
<th>Number of positive ELISA samples</th>
<th>Number of tested plants</th>
<th>Number of plants with gene of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Harcot' x 'Roxana'</td>
<td>no</td>
<td>14</td>
<td>1</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>'Harcot' x 'Lito'</td>
<td>no</td>
<td>11</td>
<td>2</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>'Harcot' x 'Krupna Skopjanka'</td>
<td>no</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>'Lito' x 'SEO'</td>
<td>no</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>'Harlayne' x 'Harcot'</td>
<td>no</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>'Hungarian best' x 'Krupna Skopjanka'</td>
<td>no</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>'Krupna Skopjanka' x 'Hungarian best'</td>
<td>no</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>'Krupna Skopjanka' x 'Harcot'</td>
<td>no</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>38</td>
<td>4</td>
<td>53</td>
<td>8</td>
</tr>
</tbody>
</table>
contained a gene for resistance to the Sharka virus. Those were plants of the parental combinations 'Harcot' x 'Lito', 'Lito' x 'SEO' and 'Harlayne' x 'Harcot'. Data showed that all the hybrids obtained from crossing of two resistant cultivars ('Lito' x 'SEO') have a gene for resistance to PPV.

In the other two combinations, in which only one of the parents is resistant, the following results were established: four out of nine hybrid plants from the crossing 'Harcot' x 'Lito' contained a gene for resistance to the Sharka virus and such a gene was detected in two out of the three plants of the crossing 'Harlayne' x 'Harcot'. None of the hybrids obtained from the crossing of two tolerant cultivars ('Harcot' x 'Roxana') contained a gene for resistance.

The eight hybrids with a detected gene of resistance to PPV are going to be propagated in 2013 and in the next 5-6 years their economic and biological characteristics will begin to be evaluated with the aim of identifying an elite or a new cultivar. Thus, applying MAS (marker assisted selection) will enable the shortening of the breeding cycle twice.

The results obtained clearly confirmed that despite cultivar plasticity to the specific soil and climatic conditions and their habits against natural infection background, apricot breeding for resistance to Plum pox virus should continue, using in the parental combination at least one cultivar carrying the gene for resistance.

**Conclusion**

After carrying out molecular studies, it was established that hybrids containing a gene for resistance could be obtained only if at least one of the parental cultivar is a carrier of such a gene. A rich hybrid apricot fund has been developed with the participation of cultivars that are donors of resistance to Sharka, which is a prerequisite for breeding a series of resistant hybrids.

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