**Genetic authenticity of cherry planting material - the current state in some nurseries as a risk factor for production**

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**Introduction**

Planting material is, for its importance in setting up a high-profile fruit production, an important risk-factor for secure production. One of the critical points in production of cherries is availability of high quality and reliable planting material. Planting material has to meet two important demands which are 'conditio sine qua non' in nursery production:

1. Has to meet sanitary requirements;
2. Has to be genetically authentic.

The first requirement, except absence of plant (quarantine) diseases, implies virus-free status for all planting material placed into trade. The second requirement means that planting material (seedling-trees) has to be authentic to its genetic constitution, i.e. trees for planting have to be in conformity to the declaration. The production and trade of genetically non-authentic plant material - those 'varieties' that do not correspond to its real genetic constitution - results in serious damage consequent not only to the fruit and 'variety' characteristics per se, but also to the genetic constitution of S-alleles which determines the pairs of compatible pollinators included into the orchard. In the latter case, establishing the orchard with genetically non-authentic 'varieties' may be fatal.

Here we will explain the status of genetic authenticity of the sweet cherry nursery mother blocks with the results incidentally revealed during the execution of another research.

**Materials and methods**

The plant material for DNA extraction was taken from mother tree blocks in several nurseries (designated 'BV', 'P', 'M', 'H', 'F' and 'T') or collection orchards (designated 'S', 'B', and 'F') (Table 1). Those material taken from the nursery 'BV' and collection 'S' is considered genetically authentic and reliable. DNA was extracted using Qiagen DNeasy Plant Mini Kit, according to user manual. Microsatellite primers used for DNA analyses are cited in Table 2. The reaction was performed in thermal cycler (Applied BiosystemsVeriti) according to the following PCR protocol:

95°C/5 min. - [94°C/45 s. - 56°C -0.3°C/cycle - 72°C/45 s.]11x - 72°C/5 min.

in the reaction volume of 20 ml consisting of 10 mM Tris-HCl, pH 8.3 and 50mM KCl, 1 U Taq (Sigma), 2.0 mM MgCl₂, 0.2 mM of each dNTP, and 0.4 μM of each primer (forward and reverse). The PCR products were visualized in Genetic Analyzer ABI 3130 (Applied Biosystems). The alleles were analysed using Gene Mapper v.4.0 (Applied Biosystems).

**Results and discussion**

Results show high level of inconsistency for all samples listed in Table 1. The genetic profile of the samples belonging to the same nominal variety, but taken from different location were different, reveals different genotype for samples that should have the same genetic constitution (Table 3). The results determined by molecular analyses pointed out the need of re-evaluation of the mother block trees in commercial nurseries. Those trees are a source of buds for plant propagation, but they do not meet the first of the two demands mentioned in introduction. What is the reason for that? Mother block trees are rather older; they were established more than 20 years, but many of them 40 or even more years ago. They are "inheritance" of the old system of plant material production which lacks an appropriate certification scheme until now one be implemented. Therefore, such 'bad practice' was executed for many years, during the long period when no official institution (ministry of agriculture or relevant agencies) has been recognizing the need of re-examination of the trees. Such mistaken practice, maybe, could have been excusable for the period that preceded to the time when routine use of molecular marker techniques reliable for genetic fingerprinting has become common.

Nowadays, when molecular genetic analyses has become more or less routinized, genetic authenticity of fruit varieties (and other agricultural plants) should not be common problem. But, still, it is a serious production obstacle. Why the practice of non-authenticity genotype was overspread to such big extent, lasting for years? First, different cherry varieties are sometimes hard to differentiate only on the bases of morphological characteristics of the fruit, not to mention other characteristics that are not linked to fruit. It is even harder if taken into consideration the specificity of the technology in nursery production that reduces fruit development on the tree preventing the recognition according to the morphology of the fruit and time of ripening. Second, the demand of high quantities of sweet cherry in Croatia is still rather low, limited to recreational producers who want to have several or only one tree in their backyard. Such producers has never recognized the problem arising from wrong declaration. However, "big" producers in a certain way avoid the problem of genetic non-authenticity by importing certified plant material directly from the producers within the EU countries other than Croatia. The cases of genetically non-authentic varieties are not unknown in the literature. Tobutt et al. (2004) cited two cases found in relevant international collections: 'Black Tartarian' from the collection in East Malling (England) has different S-allele constitution than those from British Colombia (Canada); the variety, and similar case with 'Early Lion's'

**Conclusions**

Inconsistency in several Croatian nurseries demands implementation of detailed certification schemes which should include not only sanitary component, but also genetic re-evaluation of mother block nursery trees using available molecular techniques.

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**Table 1. Varieties and nurseries/collection orchards**

<table>
<thead>
<tr>
<th>Variety</th>
<th>BV</th>
<th>P</th>
<th>M</th>
<th>H</th>
<th>F</th>
<th>T</th>
<th>S</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Bing'</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<tr>
<td>'Gardencroft'</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>'Svibanjska'</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>'Van'</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

**Table 2. Primers used for microsatellite analyses**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Udp96</td>
<td>Forward primer</td>
</tr>
<tr>
<td>Udp97</td>
<td>Reverse primer</td>
</tr>
</tbody>
</table>

**Table 3. Microsatellite profile of nine varieties taken from mother block trees in nurseries (BV', 'P', 'F' and 'M') and collection orchards (S', 'B', and 'F') from where the samples for DNA analysis were taken. Different genetic profiles on eight out of ten SSB loci are designated by Roman numbers I, II, III and IV; the loci where these profiles differ are designated by different variation of grey. The lengths of alleles in bp are not disclosed.**

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**References**


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