Influence of plant origin and soil substrate on the behaviour of the MM106 rootstock in stoolbed

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Abstract
The influence of different soil substrate for culturing and plants with different origin are studied during the stoolbed development process in order to identify the behaviour variations of the apple clonal rootstock MM106.

Three types of soil substrate are studied: soil substrate free of woody chips, soil substrate with small fraction of woody chips (1-2 mm) and soil substrate with large fraction of woody chips (8-10 mm).

The origin of the studied plants comes from roots’ adventive buds, clonal micro propagation and from leaf explants.

Best growth indicators values are found on plants derived from leaf explants developed in soil with woody chips with size of 1-2 mm.

Key words: apples clonal rootstocks, MM106, propagation, stoolbed, woody chips

Introduction
The most widely used apple clonal rootstocks propagation method is the vegetative one. The most applied one is through covering of the vertical shoot originating from the adventive buds of the roots in a stoolbed (Trachev, 1973).

The goal of the nursery men is to increase the productivity of first-class root shoots of apple rootstocks. This is the reason why more effective propagation systems are developed. Several decades ago the in vitro propagation technique became popular in apple rootstocks production. There are different methods of micro propagation and the most widely applied method is the clonal one (Ivanova, 1988). Recently, not only in the experimental field (Dobrevska, 2008), but also in practice (Dobrevska, 2011, 2013), somatic organogenesis from leaf implants has been used in the production of apple clonal rootstocks.

Different studies over the years have contributed to better understanding the method of rootstocks production in stoolbed through the traditional method of covering (Trachev, 1973; Trachev et al., 1975; Gryazev, 1979; Koval, 1980; Verobyov, 1985; Karpenchuk, 1993; Pepeyankov and Dobrevska, 1995; Dobrevska and Tabakov, 2002; Lipa and Lipicki, 2006; Dobrevska, 2010; Lipa, 2012). Later, the experimental work for obtaining of apple rootstocks through clonal micro propagation began (Ivanova, 1988; Webster and Jones, 1989; Quamme and Hogue, 1994). The new technology of apple rootstocks micro propagation through somatic organogenesis of leaf implants is still not widely used in practice.

Dobrevska (2008, 2011) develops a technology for micro propagation of apple rootstocks M9 and MM106 from leaf explants and investigates some growth specifics of apple clonal rootstock M9 obtained through the somatic organogenesis method in stoolbed.

The good root development of the shoots is mainly determined by the contents of the covering soil substrate. The change in the soil indicators, when using organic particles from woody fillings, has a beneficial effect for the plants development (Stamatov et al., 1982; Stojanowska, 1987; Szewczuk, 2000; Licznar and Licznar, 2004).
There is no previous research which investigates the role of the soil substrate and the origin of the initial plants for creating a stoolbed. Therefore, the above mentioned two aspects - the different origin of plant when creating a stoolbed plant and the contents of the covering soil layer - are the main subjects of the current study.

**Materials and methods**

The studied plants were monitored in the period between 2011 and 2013 at the experimental field of the Fruit-growing department of the Agricultural University- Plovdiv which is located in the Brestnik village area, near Plovdiv.

The initial plants, which participated in the creation of the stoolbed plant, were developed based on classic technology of clonal micro propagation (Ivanov, 1988), as a result of somatic organogenesis of leaf explants (Dobrevska, 2008) and following the traditional approach through clonal shoots from adventive buds (Mitov et al., 1996).

The covering soil layer at the stoolbed was enriched with: 1. small fraction of woody chips (1-2 mm in size); 2. large fraction of woody chips (8-10 mm in size); 3. no additions of woody chips to the soil.

According to Pepelyankov et al. (1998), the soil in the stoolbed is Chromic cambisols. The Fisher’s block method was applied when launching the experiment (Zapryanov and Marinkov, 1978).

The reported results after recording the growth indicators (average number of shoots per plant, average length of shoots, average length of feathers, average number of feathers per shoots, average thickness of shoots, average number of roots per shoots, average number of nodes per shoots, average length of internodes and leaf area) were analysed using the ANOVA (analysis of variance) with Duncan post hoc test.

**Results and discussion**

When analysing the average number of shoots per plant, the highest results are obtained from the soil combination with small fraction of woody chips (Table 1). When the plant origin is taken into account, the plants produced through somatic organogenesis show the highest results and those produced through adventive buds show the lowest ones.

**Table 1 Average number of shoots per plant, average length of shoots and average number of roots per shoots**

<table>
<thead>
<tr>
<th>Soil substrate</th>
<th>Average number of shoots per plant</th>
<th>Average length of shoots, cm</th>
<th>Average number of roots per shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicator</td>
<td>Composition of soil substrate</td>
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<td>Composition of soil substrate</td>
</tr>
<tr>
<td>Origin</td>
<td>Large fraction</td>
<td>Small fraction</td>
<td>No chips</td>
</tr>
<tr>
<td>Adventive buds</td>
<td>17.7</td>
<td>18.7</td>
<td>15.0</td>
</tr>
<tr>
<td>Clonal micropropag.</td>
<td>29.0</td>
<td>26.7</td>
<td>21.7</td>
</tr>
<tr>
<td>Somatic organogenesis</td>
<td>33.7</td>
<td>34.3</td>
<td>28.3</td>
</tr>
<tr>
<td>Signif. at 5 % (a)</td>
<td>32.0</td>
<td>11.1</td>
<td>14.7</td>
</tr>
<tr>
<td>1 % (b)</td>
<td>53.0</td>
<td>18.3</td>
<td>14.7</td>
</tr>
<tr>
<td>0.1 % (c)</td>
<td>99.1</td>
<td>34.3</td>
<td>14.7</td>
</tr>
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</table>

In accordance to the second indicator - average length of shoots (table 1), the plants produced from leaf explants cultivated in soil substrate with small fraction of woody chips demonstrate the highest number of root shoots. Once again, the same tendency for better results like in the first indicator is repeated. Besides, there are statistically significant differences with all shoots from the soil substrate with no woody chips.

The number of feathers, which increase the over ground vegetative mass, unambiguously demonstrate the improved development of the rootstocks. In our experiment, the differences in all combinations are with
strong and very strong statistical significance; the highest number of feathers is shown in plants with somatic organogenesis origin cultivated in soil with small fraction of woody chips, whereas the smallest number of feathers is related to the plants with adventive buds origin (table 2).

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<tr>
<td>Adventive buds</td>
<td>Large fraction</td>
<td>Small fraction</td>
<td>No chips</td>
</tr>
<tr>
<td>Clonal micropropag.</td>
<td>5.0</td>
<td>7.4</td>
<td>5.6</td>
</tr>
<tr>
<td>Somatic organogenesis</td>
<td>5.0</td>
<td>7.4</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Signif. at 5 % (a) 14.4 11.9 7.1 0.1 0.3 0.3 2.3 0.7 1.0
1 % (b) 23.8 19.7 11.8 0.2 0.5 0.5 3.7 1.1 1.7
0.1 % (c) 44.5 36.9 22.0 0.3 1.0 0.9 7.0 2.1 3.1

In respect to the length of the feathers, there are no statistically significant differences among the different combinations (table 2).

The thickest but still with first-class quality standard are the shoots with somatic organogenesis origin cultivated in soil with small fractions of woody chips (table 2). This development is perhaps due to the higher number of feathers on them which increases the vegetative green over ground mass resulting in stimulated photosynthesis of this combination.

An important indicator which determines the quality of the rootstocks produced in stoolbed is their roothold. The reported data in relation to this indicator are shown in table 1. The plants with somatic organogenesis origin cultivated in soil with small fractions of woody chips demonstrate the best results. These outcomes are perhaps driven not only from the added organic content in the soil which coincides with the results from the previous study in the Plovdiv area, but also due to the different origin of the initial plants used in the stoolbed creation process (Dobrevska, 2013).

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Signif. at 5 % (a) 11.3 24.4 6.1 2.8 3.3 3.0
1 % (b) 18.7 40.5 10.2 4.1 5.3 4.5
0.1 % (c) 34.9 75.6 19.0 6.6 8.5 7.3

The number of nodes of the different combinations do not differ significantly (table 3). When analysing the average length of internodes (table 4) - an indicator which determines the grafting quality- the best results are demonstrated by the combinations with micro propagation origin. The somatic organogenesis leads with 1.9, followed by clonal micro propagation with 1.8 in soil substrate with organic particles followed by soil without particles.
Undoubtedly, the most important physiological process dependent on many factors and determining the good plant development is the photosynthesis. The photosynthesis efficacy depends on the average leaf area of the leathers. In the current experiment, the leaf areas of some plants with small and large fractions of woody chips demonstrate statistically significant differences (table 3). In relation to this, the plants with somatic organogenesis origin cultivated in soil with small fractions of woody chips develop better root shoots. This is partially due to the larger leaf aggregate, which when presented in plants with a higher number of feathers (as it is in this case), result in larger total leaf area (Dobrevska, 2013).

Conclusions

In this experiment, the following conclusions can be drawn: Better first-class root shoots are developed in the stoolbed plants with somatic organogenesis origin cultivated in soil with organic particles of woody chips with 1-2 mm in size.

Because of these results, we can recommend the usage of small fractions of woody chips as an addition of the covering soil layer in the apple clonal rootstock MM106 in stoolbed combined with the plant origin- somatic organogenesis from leaf explants. This method makes the production process more expensive but the extra cost is fully compensated by the higher production of first-class rootstocks.

References


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