Observations on development and incidence of black rot disease with causal agent Phyllosticta ampelicida (asexual phase from Guignardia bidwellii) in organic and conventional agriculture in the conditions of the years 2015 and 2016

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SUMMARY

Two-year observations were held on the development and incidence of black rot disease in the Experimental base of IVE - Pleven.

Two identical plantations with the same varieties located in immediate vicinity were compared. Products for conventional farming were used in one of the plantations while in the other one – products for organic farming.

In 2015 in the organic plantation three varieties showed high susceptibility to the disease, namely: Muscat Vrachanski with index of attack (27.5 %), Muscat Ottonel (7.16 %) and Druzhba (11.5 %).

The highest resistance demonstrated Cabernet Sauvignon (2.5 %) and Muscat
Kaylashki (1.66 %). For the year 2016, except the three susceptible varieties, Naslada variety (organically grown) also showed higher susceptibility as its index of attack for 2015 was (3 %), while in 2016 - (13.3 %).

_key words_: organic, black rot, attack, varieties, susceptibility, plantations, resistance

**INTRODUCTION**

Black rot originated from North America, from where it was transmitted to Europe. In 1885 the disease was observed in France by P. Viala and L. Ravaz, who reported it for the first time in the US phytopathological literature (Vanev, 1963). Later, the disease was found in Italy, Russia, Georgia, Azerbaijan, Ukraine and other countries. According to some authors, black rot could not be related to the regions of Alsace, Champagne and Burgundy, except for Saone and Loire, where the disease was found again. Other areas where the disease did not occur were the regions Languedoc and Roussillon (Besselat, 1983). Black rot was a serious threat to vineyards, particularly in areas with large water sources (Onesti et al., 2016).

In countries where black rot is an economically important disease (USA, Canada, etc.) the pathogen is considered to be the fungus _Guignardia bidwellii_ (Elis) Viala and Ravaz with anamorphic _Phyllosticta ampelicida_ (Engleman) Vander Aa. _Guignardia bidwellii_ is an ascomycetes fungus, forming pseudocetes with cylindrical or club-shaped ascus containing 8 unicellular hyaline ascospores (Vanev, 1963; Harizanov et al., 2009).

The first time the disease in Bulgaria was reported in 1904 by P. Kozarev in the region of Vidin (Raykov and Nachev, 1968; Raykov and Nachev, 1971). In 1961, limited manifestations of black rot were observed in Pleven, Ruse,
The disease resembles the symptoms of grey mould on the leaves and downy mildew on the clusters characterized by irregular spots on the leaves, reddish in colour and surrounded by a brown ring. From the leaves it is transmitted to the clusters and infects the petioles, the rachis and the berries. They turn brownish and are covered by pustules as afterwards wither. On the berries the spots grow quickly and cover the whole berries. Concentric circles are formed on them of relatively large pycnidia (Harizanov, 1994). They have a circular ostiole from which an exudate of picnispores leaks out. In spring, after rain the mature pycnidia release the spores.

The presence of water droplets is an obligatory condition for their germination (Vanev, 1963; Vanev, 1995; Stancheva, 2006). When dried the spores remain viable for more than one year (Raykov and Nachev, 1968; Raykov and Nachev, 1971). Grapevine is susceptible to black rot throughout the whole vegetation period as when rainfall in the spring is heavy the infestation is massive while in the absence of abundant precipitation vines are infected in summer (Besselat and Tisse, 1983).
MATERIAL AND METHODS

In 2015 and 2016 at the Experimental base of IVE - Pleven observations were carried out on the development and incidence of black rot disease. The objective of the investigation was to find out if there was any difference referring this disease by comparing the conventional and organic grown vines as well as some varieties to be established suitable for organic production concerning black rot.

The trial variants were: Muscat Kaylashki, Naslada, Muscat Vrachanski, Muscat Ottonel, Druzhba, Cabernet Sauvignon. Two plantations of equal size were included next to each other as products for organic agriculture were applied in one of them and products for conventional agriculture were used in the other one. The trial also comprised a plantation of the varieties Muscat Kaylashki 1, Muscat Ottonel 1 and Rubin 1, remote from the others, also used for conventional agriculture.

A hundred clusters per variety were assessed by seven-score scale:

(Score 0 – no infestation, score 1 up to 5 %, score 2 from 6 to 15 %, score 3 from 16 to 25 %, score 4 from 26 to 50 %, score 5 from 51 to 75 % and score 6 from 76 to 100 % of the cluster was infected).

The index of attack was calculated by McKinney's formula.

In 2015 in the conventionally grown plot 6 treatments were performed with the following products: 1\textsuperscript{st} treatment (Drago and Systane), 2\textsuperscript{nd} (Ridomil gold and Systane), 3\textsuperscript{rd} (Ridomil gold and Systane), 4\textsuperscript{th} (Drago and Thiovit jet), 5\textsuperscript{th} (Cabrio top and Topaz) and 6\textsuperscript{th} (Corseit and Topaz). For the organic production 7 treatments were applied with the products (Thiovit jet and Funguran).

In 2016 for the conventional production the following products were applied for the 1\textsuperscript{st} treatment (Ridomil gold
RESULTS AND DISCUSSION

When accounting the clusters, three varieties from the organic grown plantation were distinguished clearly, showing high sensitivity to the disease, namely: Muscat Vrachanski with an index of attack (27.5 %), Muscat Ottonel (7.16 %) and Druzhba (11.5 %).

Those with the highest resistance were Cabernet Sauvignon (2.5 %) and Muscat Kaylashki (1.66 %). Also the three varieties (Muscat Kaylashki 1, Muscat Ottonel 1 and Rubin 1) were distinguished compared to the rest as no cluster attack and damages were observed in them.

In 2016 during the routing observation of an area in the base of the Institute of Viticulture and Enology spots of black rot on the leaves were found already in mid-May. Higher intensity of attack was observed in the organic grown plantation varieties Muscat Vrachanski, Muscat Ottonel and Druzhba. Naslada from the organic grown varieties could be referred to them. The index of attack in Naslada variety was 3 in 2015, and in 2016 – (13.3 %). For Cabernet Sauvignon the accounted values in 2015 in the conventional production had an index (0 %) and the organic – (2.5 %)

In 2016, it was respectively (5.8 %) for conventional and (17.6 %) for organic. In the plantation of Muscat Kaylashki 1, Muscat Ottonel 1 and Rubin 1 in 2016 it
Rubin 1 in 2016 showed a manifestation of black rot. Observations shall be carried out over the next years for determining any change in the aggressiveness of the pathogen (Table 1).

### Table 1. Black rot index of attack per clusters

<table>
<thead>
<tr>
<th>Variant</th>
<th>Conventional growing</th>
<th>Organic growing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Index of attack (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infected (No.)</td>
<td></td>
</tr>
<tr>
<td>Muscat Kaylashki</td>
<td>0,66</td>
<td>5,3</td>
</tr>
<tr>
<td>Naslada</td>
<td>1,16</td>
<td>4</td>
</tr>
<tr>
<td>р. искет</td>
<td>4,16</td>
<td>9,1</td>
</tr>
<tr>
<td>Muscat Vrachanski</td>
<td>1,16</td>
<td>2,6</td>
</tr>
<tr>
<td>. отонел</td>
<td>4,16</td>
<td>9,1</td>
</tr>
<tr>
<td>Muscat Ottonel</td>
<td>0,83</td>
<td>17,8</td>
</tr>
<tr>
<td>руж / Druzhba</td>
<td>0,83</td>
<td>17,8</td>
</tr>
<tr>
<td>сувиньон</td>
<td>0,83</td>
<td>17,8</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>0,83</td>
<td>17,8</td>
</tr>
<tr>
<td>К. йълъшки</td>
<td>0,83</td>
<td>17,8</td>
</tr>
<tr>
<td>Muscat Kaylashki 1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>. отонел</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Muscat Ottonel 1</td>
<td>0</td>
<td></td>
</tr>
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</tr>
<tr>
<td>М. отонел</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Druzhba</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>К. совиньон</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Raselt</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>М. кайлъшки 1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>М. отонел</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Rubin 1 / Rubin 1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

In 2015 the precipitation in the region during the month of April and May were significantly less compared to the same period in 2016. As shown in (Figure 1) the humidity was proportional to the amount of rainfall.

The temperature in April of 2016 was higher compared to 2015 while the temperature in May 2016 was lower.

Generally, referring the black rot disease in both years of the investigation there were conditions for the onset and development of the disease, but better conditions were observed in 2016.

The monitoring of the disease shall continue for following its development and incidence in different weather conditions.
1. The organic grapes production has much higher risk of emergence and spread of black rot compared to the conventional growing. The implementation of all methods and agricultural procedures might have a limiting effect on the disease development.

2. It is expected the intensity of the attack to increase each year to follow, and the disease to be manifested in a greater degree by causing more losses in both types of production.

3. The cost for the disease control will increase as additional measures for black rot control will be applied.

4. Muscat Kaylashki variety is suitable for organic farming concerning black rot disease. The susceptible varieties are suitable for control varieties in testing new active substances against black rot on vine.
/ REFERENCES
Impact of weather conditions in the region of Pleven on grapevine damage in organic and conventional grapes production

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SUMMARY

During the period 2014-2016 in the region of Pleven a study was carried out on the impact of the weather conditions during the winter period on the primary and replacement buds of six wine grapevine varieties. It has been found that the climate features during the period 2014-2016 in terms of rainfall during the growing season defined the years as moist (2014 and 2015) and medium-moist (2016) and in terms of average air temperatures as very cool (2014), hot (2015) and average (2016).

Extremely low air temperatures were not recorded during the winter period of 2014, therefore damages to the primary and replacement buds had not been found.

During the winter period of 2015 air
The effectiveness of the organic and conventional grape and wine production depends on many factors, the most important of which are suitably selected terroirs, varietal structure, cultivation technology, technical equipment, etc. The climatic conditions of the micro-region and the characteristics of temperature was registered within the range from -20.0º to -21.9º (January 1st, 2015, 0.00 a.m. to 9.00 a.m.).

In January it was also found significant icing of the vines, longer than 24 hours. In organic production the damages were greater compared to conventional production – the greatest damages to the primary and replacement buds were recorded in Muscat Vrachanski variety, respectively 93.3 and 65.7% in organic and 74.22 and 43.16 % in conventional growing.

The least damages were found in Muscat Kaylashki variety – 46.0 and 22.0% in organic and 5.80 and 1.20% in conventional. In 2016 the maximum low air temperatures were registered on January 20th, ranging from -20.1 to -21.3ºC (lasting for 4 hours). In January 2016 it was also recorded a long period with average temperatures about and below -15.0ºC. In organic growing the damages were again greater than the conventional production - the most severe was the impact on primary buds (32.28%) and replacement buds (14.35%) in organic grown Muscat Vrachanski variety and 24.22 and 20.07% in the conventional production. The least damages had Muscat Kaylashki variety – respectively 8.36 and 5.09% in organic and 3.18 and 0.35% in conventional production.

Key words: grapevine damage, organic, conventional, grapes production, weather conditions
the specific terrain have a decisive impact on vine vitality, growth and fertility as well as on the density and the impact of the economically important diseases.

However, it is significant, especially for the organic production, to be defined the impact of the micro-regions both on vine biological and economic indicators and the efficiency of this production.

The repeated negative results from the organic production of grapes and wine in some regions of the country necessitates further thorough research for identifying and suggesting the practice of rational and effective organizational, technological and technical solutions in this respect.

**MATERIAL AND METHODS**

During the period 2014-2016 in the area of the production-experimental base of the Institute of Viticulture and Enology - Pleven a study was carried out on the impact of the weather conditions during the winter period of these years on the primary and replacement buds and the density and impact of powdery mildew and downy mildew on vines of the varieties Muscat Kaylashki, Naslada, Muscat Vrachanski, Muscat Ottonel, Druzhba and Cabernet Sauvignon.

For the purposes of the study, two separate plots with an area of 0.6 ha each were used (Muscat Kaylashki – 0.2 ha, Naslada – 0.2 ha, Muscat Ottonel – 0.05 ha, Muscat Vrachanski – 0.05 ha, Druzhba – 0.05 ha and Cabernet Sauvignon – 0.05 ha), in a vineyard created in 2006. The planting distances were: 3.20 m between the rows and 1.20 m in the rows, medium-stem training system (2-arm cordon) with a stem height of 1.00 m.

The soil type was slightly leached chernozem formed on clayed loess.
роли с следните показатели:
- средноденонощна температура на въздух в редицата на производствено-експерименталната база на ИЛВ - Плевен, отчитана на всеки час от автоматична стационарна електронна станция, модел IMETOS.
- повреди от ниски зимни температури и обледеняване, определени на основата на степента на загиване на пъпките (главни и заместващи), на тъкани (пълков и дървесин) и органи (пръчки, рамена и кордони – едногодишни и многогодишни) на лозите. За целта се използва методика, разработена в ИЛВ - Плевен.
- повреди на листата и грозде от болестите мана и оидиум - % и индекс на поражение, като отчитането се извършва по утвърдена методика. Оценяването на повредите се извършва по седембална скала, а индексът на поражение е изчислен по формулата на Mc Kinney (Kostadinova, 2012).

РЕЗУЛТАТИ И ОБСЪЖДАНЕ

През зимния период на 2013/2014 г. не са отчетени периоди с критично ниски температури във въздуха – фигура 1, поради което не са констатирани повреди по пъпките (главни и заместващи).

The following indicators were monitored:
- average daily air temperature in the area of the production-experimental base of IVE-Pleven, recorded every hour by an automatic stationary electronic station, IMETOS model.
- damages from low winter temperatures and icing, determined on the basis of the degree of death of the buds (primary and replacement), the tissues (connective tissue and wood), and the organs (canes, arms and cords – the annual and perennial wood) of the vines. A methodology developed at IVE-Pleven was used for this purpose.
- damages to the leaves and the clusters caused by powdery mildew and downy mildew - % and damage index, as the accounting was done in accordance with an approved methodology. The damage evaluation was performed on a seven-score scale, and the damage index was calculated using Mc Kinney’s formula (Kostadinova, 2012).

RESULTS AND DISCUSSION

Extremely low air temperatures were not recorded during the winter period of 2013/2014 – Figure 1, therefore damages to the buds (primary and replacement) had not been found.
During the May-October 2014 vegetation period, the precipitation sum was 511 mm, ensuring 11% of the water supply that determined the period as wet. The average daily air temperature was 17.1°C, ensuring 97%, characterizing the period as very cool while the maximum air temperature was 23.9°C, ensuring 85%, which determined the period as cool (Figure 2).
The typical symptoms of the monitored diseases with high density were found in periods immediately after significant precipitation – in 2014, the rainfall in July reached 122 mm, as only in the period 14–23 July the rainfall in the area of the production-experimental base was 98 l/m² (only on 22 and 23 July – 80 l/m²).

The data on leaf and cluster damage by downy mildew confirmed the above statement – the data, reported on 30 June 2014 for both treatment variants were close, with somewhat higher values of the damage index for most varieties in conventional production (except Muscat Kaylashki variety).

When accounting the damage index rates at the beginning of September, it was found that these values were significantly higher in the organic production, reaching 66.0 for Muscat Vrachanski variety (4.0 for the conventional production), 44.0 for Cabernet Sauvignon variety (2.0 for the conventional production).

A similar significant difference in the rate of this index was also recorded for
The data here were not unidirectional either – for Druzhba variety, the damage index on the clusters was higher in the conventional production – 6.0 compared to 1.0 in the case of the organic one. The powdery mildew damage index on the clusters for most varieties was higher for the conventional production, as the difference in the susceptible varieties was significant – Muscat Vrachanski – 82.0 for the conventional and 21.0 for the organic growing. In the rest of the varieties these differences were smaller. For Cabernet Sauvignon and Naslada varieties, the reported damage index rates were higher for the organic production (Table 2).

A period of critically low air temperatures in 2014/2015 was recorded on 1st January, 2015 – from -20.00°C to -21.9°C, with duration of 9 hours – from 0.00 to 09:00 a.m. On 9th January 2015, over a period of more than 12 hours, it was found icing of the vines, mainly on the one-year-old canes. As a result of the above factors, with a major burden of icing, significant damages to the vines were found, mainly on the primary and replacement buds. The damages were greater for the organic production variants.

The biggest damages were found for Muscat Vrachanski variety – 93.3% in the primary and 65.7% in the replacement buds in the organic production and respectively 74.22% and 43.16% in the conventional growing. The damage ratio was also high for the varieties Muscat Ottonel and Cabernet Sauvignon – Table 1. The smallest were the damages to Muscat Kaylashki variety – 46.0% for the primary and 22.0% for the replacement buds for the organic production and 5.8% and 1.2% respectively for the conventional production.
### Table 1. Damages by low winter temperatures on primary and replacement buds per varieties in organic and conventional production – 2014-2016

<table>
<thead>
<tr>
<th>Variety</th>
<th>Organic production</th>
<th>Organic production</th>
<th>Conventional production</th>
<th>Conventional production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary buds, %</td>
<td>Replacement buds, %</td>
<td>Primary buds, %</td>
<td>Replacement buds, %</td>
</tr>
<tr>
<td>Muscat Kaylasski</td>
<td>46,0</td>
<td>22,0</td>
<td>8,36</td>
<td>5,09</td>
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<tr>
<td>Naslada</td>
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<td>Not found</td>
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<tr>
<td>Muscat Vrachanski</td>
<td>60,0</td>
<td>36,0</td>
<td>9,93</td>
<td>8,22</td>
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<tr>
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<td>Not found</td>
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<td>Not found</td>
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<tr>
<td>Druzhba</td>
<td>72,3</td>
<td>41,9</td>
<td>15,96</td>
<td>6,03</td>
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<tr>
<td>Cabernet Sauvignon</td>
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</table>

<table>
<thead>
<tr>
<th>Variety</th>
<th>Conventional production</th>
<th>Conventional production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary buds, %</td>
<td>Replacement buds, %</td>
</tr>
<tr>
<td>Muscat Kaylasski</td>
<td>5,8</td>
<td>1,2</td>
</tr>
<tr>
<td>Naslada</td>
<td>18,6</td>
<td>10,3</td>
</tr>
<tr>
<td>Muscat Vrachanski</td>
<td>74,22</td>
<td>43,16</td>
</tr>
<tr>
<td>Muscat Ottonel</td>
<td>15,22</td>
<td>4,35</td>
</tr>
<tr>
<td>Druzhba</td>
<td>14,2</td>
<td>8,3</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>64,29</td>
<td>23,21</td>
</tr>
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</table>
Table 2. Impact of the weather conditions during the vegetation period and the products used for downy mildew and powdery mildew on vines in the period 2014-2016

<table>
<thead>
<tr>
<th>Variety</th>
<th>Leaf</th>
<th>Cluster</th>
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<tbody>
<tr>
<td></td>
<td>Conventional</td>
<td>Organic</td>
</tr>
<tr>
<td>2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscat Kaylashki</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Naslada</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Druzhba</td>
<td>4</td>
<td>66</td>
</tr>
<tr>
<td>Muscat Vrachanski</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>Muscat Ottonel</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>44</td>
<td>15</td>
</tr>
<tr>
<td>2015</td>
<td></td>
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<td>Muscat Ottonel</td>
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<tr>
<td>Cabernet Sauvignon</td>
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<td>0,41</td>
</tr>
<tr>
<td>2016</td>
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<td></td>
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<tr>
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</tr>
<tr>
<td>Naslada</td>
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<td>0,33</td>
</tr>
<tr>
<td>Druzhba</td>
<td>1,8</td>
<td>1,83</td>
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<tr>
<td>Muscat Vrachanski</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Muscat Ottonel</td>
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<td>0,33</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>1,6</td>
<td>3,66</td>
</tr>
</tbody>
</table>

During the May-October 2015 vegetation period, the precipitation sum was 491 mm ensuring 14% of the water supply that determined the period as wet. The average daily air temperature was 19.9°C ensuring 30% characterizing the period as hot while the maximum air temperature was 27.7°C, ensuring 7%, which determined the period as very hot (Figure 2). The meteorological conditions in...
месеци юни и септември 2015 г. създадоха благоприятни условия за развитие на проследяваните гъбни болести по лозата и затрудниха извършването на част от технологичните операции, предимно механизирани. Потвърдиха се наблюденията от предходни периоди – появата на значително заразяване става в период непосредствено след значителни валежи – за 2015 г. през месец юни в района на Експерименталната база са паднали валежи от 120.6 mm (за сравнение за 2014 г. такива валежи са констатирани за месец юли – 122 mm).

Данните за повредите по листната маса и гроздовете от болестта манша при първото (23.06.15 г.) и второто отчитане (27.08.15 г.) при двата варианта на третиране са близки, с минимални стойности на индекса на поражение, основно при варианта за биологично производство (най-голям индекс – при сорта Мискет врачански – 1,16, а най-малък – при сортовете Каберне совиньон и Дружба – 0,41).

През 2015 г. е констатирана късна атака от мана (началото на септември), основно на листната маса на страничните леторасти, която не е нанесла повреди по гроздовете (Таблица 2).


Regardless of the fact that in the June and September 2015 created favorable conditions for the development of the monitored fungal vine diseases and made it difficult part of the technological operations to be performed, mainly the mechanized ones. The observations from previous periods were confirmed - the occurrence of significant infestation occurred in a period immediately after significant precipitation – in June 2015, the rainfall in the area of the experimental base was 120.6 mm (compared to 2014 when such precipitation was observed for the month of July – 122 mm).

The findings for the leaf and cluster damages from downy mildew from the first count (23 June 2015) and the second one (27 August 2015) were close for both treatment varieties, with the lowest damage index rates, mainly for the organic production (the highest damage index for Muscat Vrachanski variety – 1.16, and the lowest – for Cabernet Sauvignon and Druzhba varieties – 0.41). In 2015, a late downy mildew attack (early September) was found, mainly on the leaves of the lateral shoots, however it did not cause any damages to the clusters (Table 2).

The powdery mildew damage index on the clusters for all varieties in the organic production variant was higher than the conventional one, as it was the highest for the susceptible varieties – for Muscat Vrachanski the damage index on the clusters in the organic production variant was 77.83, in the conventional variant – 53.83, for Muscat Ottonel 75 and 26.66 respectively, for Cabernet Sauvignon – 52.5 and 32.83, for Druzhba – 34.33 and 15.66. For the other varieties these differences were smaller - for Naslada variety the reported rates of the damage index for the organic production were 16.5 and for the conventional one - 6.5, for Muscat Kaylashki 9.2 and 1.5, respectively (Table 2).

Regardless of the fact that in the
Periods with critically low air temperature during the winter period 2015/2016 (Figure 1) were recorded on:

- 20 January 2016 – from -20.1°C to -21.3°C, with 4 hours duration – from 07.00 a.m. to 10.00 a.m.
- 21 January 2016 – from -19.00°C to -19.5°C, with 3 hours duration – from 04.00 a.m. to 06.00 a.m.

The highest rate of bud damages was found again in Muscat Vrachanski variety – 32.28% of the primary and 14.39% of the replacement buds in the organic variant and respectively 24.22% and 20.07% in the conventional variant (Table 1). The lowest rate of bud damages was recorded for Muscat Kaylashki variety – 8.36% of the primary and 5.09% of the replacement buds in the organic variant and respectively 3.18% and 0.35% in the conventional variant.

During the May-October 2016 vegetation period, the precipitation sum was 384.2 mm ensuring 33% of the water supply that determined the period as average wet. The average daily air temperature was 19.5°C ensuring 50% characterizing the period as average while the maximum temperature was 26.9°C, ensuring 15%, which determined the period as average hot (Figure 2).

The meteorological conditions at
The beginning of the vegetation period of 2016 (Figure 2) created unfavorable conditions for performing part of the technological operations. The occurrence of significant infestation with the monitored diseases was recorded again in a period immediately following significant precipitation – in 2016 the month of May 2016 – 90.8 mm and June - 98 mm. Damages on the leaves and clusters from downy mildew during the first count (4 June 16) for both treatment variants were not found. During the second count (3-5 July 2016) the damage index rates were slightly higher for the organic production variant – 1.8 for Muscat Vrachanski variety (0.66 for the conventional variant), 1.6 for Cabernet Sauvignon variety (0 for the conventional production). The difference in the rate of this index was also insignificant for Muscat Ottonel variety (Table 2).

The damage index by powdery mildew on the clusters was higher for most of the varieties in the organic production variant, as the difference in the susceptible varieties was insignificant – Muscat Ottonel – damage index on clusters 22.5 for the organic and 12.0 for the conventional production, Muscat Vrachanski – damage index 17.3 for the organic and 12.0 for the conventional variant. For the rest of the varieties these differences were smaller. For Cabernet Sauvignon and Druzhba varieties the recorded damage index rates were respectively 11.8 and 10.5 for the organic and 5.8 and 5.6 for the conventional production. For Muscat Kaylashki and Naslada varieties no damages from powdery mildew on the clusters were recorded (Table 2).

CONCLUSIONS

- The significant damages to the vines from downy mildew and powdery mildew affected mainly the vine buds during the
н лозите през последвъщия зимен период. Това в по-голям степен се отнася за биологичното производство.
● ри биологичното производство на грозде влиянието и въздействието на климатичните фактори, върху развитието и плододаването на лозите е по-силно изразено.
● Ъчезното и променливите климатични условия в годините и огрението бор със средствата за борба с вредителите по лозата, създават сериозни трудности за биологичното производство на грозде в района на гр. Плевен.
● Отвърдиха се данните от предходни периоди за негативното влияние на обледеняването на лозите за редици над 24 часа.
● Получените резултати стават ново въпрос и проблем, който трябва да бъде решаван в строга връзка с особените си ситуации от лозовите насаждения, предназначени за биологичното производство на грозде.

subsequent winter period. That referred mostly for the organic production.
● In the organic grapes production the influence and impact of the climatic factors on the vine development and fertility was more pronounced.
● The combination of variable weather conditions throughout the years and the limited means of vine pest control created serious difficulties for the organic production of grapes in the region of Pleven.
● Data for the negative impact of vine icing for over 24 hours from previous periods were confirmed.
● The obtained results have again raised the issue of the proper viticulture zoning in the country. That particularly applies to vineyards intended for organic production of grapes.

/ REFERENCES
Theoretical curves for determining vine leaf surface of Kaylashki ruby variety

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SUMMARY

The investigation was carried out in 2016 with Kaylashki ruby variety for plotting the theoretical curves and finding regression equations by which to determine quickly and accurately the leaf area per leaf, a shoot or a vine.

The theoretical curves were obtained by means of regression analysis based on the correlation between the leaf area (S) on the one hand and the length of the central vein (L₁), the sum of the lengths of the two upper (Σ L₂) and two lower (Σ L₃) lateral veins of vine leaf, on the other hand. The equations describing the regression curves for each of the parameters L₁, Σ L₂ and Σ L₃ were respectively:

\[ y_1 = 1.3208x^2 - 1.5861x + 1.0443; \]
\[ y_2 = 0.4791x^2 - 0.0968x + 2.789; \]
\[ y_3 = 0.8901x^2 - 0.3408x + 6.8914, \]

where \( y \) was the leaf area (in cm²), and \( x \) - the length of the central vein; the sum of the lengths of the two upper lateral veins; the sum of the lengths of the two lower lateral veins of the leaf, respectively (in cm).

In the course of the study it was found that the coefficient values of the definition (R²) for the three parameters were very close, which allowed all three parameters to be used for determining the
яйные листн т повърхност с прибли- 
зительно една съвпада точност. Съвпада то 
отклонената от регресията и при трите 
параметъра с по-малко от 6%, което 
показва, че методът осигурява приемли- 
ви точности.

**Ключови думи:** лоз, листна 
повърхност, сорт йълъшки рубин

Кайлъшки рубин е средно до късо 
зреещ червен винен сорт получен по 
пътя на междувидова хибридиция от 
кръстосването н ( midpoint hybrid V 
2/15) и (памид х Vitis amurensis) в 
- левен, п тентов н през 2010 г. 
(Simeonov et al., 2015; Ivanov, 2016). 
то ср възприема нов сорт, върху него не с 
провеждане изследвания за определяне 
относителна висимост между листн т площ (S) и 
дължината на централна линия нерв (L1), 
сумата на дължините на горните дв (Σ 
L2) или долните дв (Σ L3) странични 
нерви и лозовия лист.

поред Стоев и Добрева (1974), 
всички изследвания върху листн т 
повърхност и продуктивност н лист т 
имат за цел търсене на възможности за 
повишаване н количество и 
кчество лоза от грозде.

олят н лист т к основни 
симилатори органи н лоз т е от 
изключително значение, тъй к ъто н д 90- 
95% от биологичния добив се н трукав в 
процес н фотосинтез т (Nikolov, 
1973). то с що с необходими 
достъпно бързи и точни методи за 
определяне на големината н отделен 
лист, листн т повърхност и цели лози.

елт е д се определят 
висимостите между листн т площ 
(S) и дължината на централна линия нерв 
(L1), сумата на дължините на горните 
нерви дв (Σ L2) или долните дв (Σ L3) 
странични нерви и лозовия лист от 
сорт йълъшки рубин.

зследв нето е проведено през 
2016 г. в експеримент н т б з н 
- левен в опитно н с жажде от

**Key words:** Vitis vinifera L, leaf 
surface, Kaylashki Rubin variety

**INTRODUCTION**

Kaylashki Rubin is medium to late 
ripening red wine variety obtained by 
interspecies hybridization by crossing of 
(Pamid x Hybrid V 2/15) x (Gamey noir x 
Vitis amurensis) at IVE - Pleven, patented 
in 2010 (Simeonov, 2015; Ivanov, 2016). 
Being a relatively new variety it has not 
been studied for determining the 
correlation between the leaf surface (S) 
and the length of the principal vein (L1), 
the sum of the lengths of the two top (Σ 
L2) or two underside (Σ L3) lateral 
veins of the grapevine leaf.

According to Stoev and Dobreva 
(1974), all studies on the leaf surface and 
leaf productivity have been aimed at 
seeking opportunities to enhance the 
quality and quantity of grapes yield.

The role of the leaves as the main 
assimilating organs of vine has been of 
utmost importance, as over 90 – 95% of 
the biological yield was accumulated in 
the process of photosynthesis (Nikolov, 
1973). Therefore fast and accurate 
methods were required for determining 
the size of the individual leaf and the leaf 
surface of whole vines.

The objective was to determine the 
correlation between the leaf surface (S) 
and the length of the principal vein (L1), 
the sum of the lengths of the two top (Σ 
L2) or two underside (Σ L3) lateral 
veins of the grapevine leaf of Kaylashki 
Rubin variety.

**MATERIAL AND METHODS**

The study was carried out in 2016 
at the Experimental base of the Institute of 
Viticulture and Enology - Pleven in a trail
plantation of Kaylashki Rubin variety. The vines were grown on two-sided trellis at planting distance 2.5/1.3 m.

The method proposed by Slavcheva (1983), that improved the one of Carbonneau (1976) was used. It could be considered as a non-destructive method for determining leaf surface (the leaves were not separated from the plant) and utilized when the dynamics of the leaf surface growth over time was monitored (Kerin et al., 2000). It was applied for constructing theoretical curves and finding regression equations for determining, as quickly and accurately as possible the leaf surface per leaf, respectively, per vine of Kaylashki Rubin variety.

For this purpose 100 leaves were selected with the most varied sizes, from different points along the shoot length. The lengths of the leaves were pre-measured along the venation – the principal vein and the lateral ones (cm).

As seen in Figure 1, these were the distances between the basal notch and the apex of each vein. The leaves were scanned. By means of software the leaf surface (cm$^2$) was determined based on the number of pixels. It was also found the sum of the lengths of the two top veins (Σ L$_2$) and the two underside lateral veins (Σ L$_3$).
горните дв стр нични нерв (Σ L₂) и
dолните дв стр нични нерв (Σ L₃).
еоретичните криви с получен
посредством регресионен  н лиз, к то е
използв н пррг мния продукт Microsoft
Excel.

The theoretical curves were
obtained by regression analysis using the
software Microsoft Excel.

RESULTS AND DISCUSSION

Figures 2, 3 and 4 represented the
theoretical curves obtained by non-linear
regression (a second order polynomial)
for the selected indicators of Kaylashki
Rubin variety – L₁, ΣL₂ and ΣL₃, where:

L₁ – the leaf principal vein length;
ΣL₂ – the sum of the two top lateral veins;
ΣL₃ – the sum of the two underside
lateral veins;

The equations describing the
regression curves were respectively: y₁ =
1,3208x² – 1,5861x + 1,0443 (L₁); y₂ =
0,4791x² – 0,0968x +2,789 (Σ L₂); y₃ =
0,8901x² – 0,3408x + 6,8914 (Σ L₃),
kъдето "y" е листн т площ (в cm²), "x" –
дължин т н центр лния нерв; сум т
от дължините н горните дв стр нични
нерв ; сум т от дължините н долните
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L₁ – the leaf principal vein length;
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0,8901x² – 0,3408x + 6,8914 (Σ L₃),
kъдето "y" е листн т площ (в cm²), "x" –
the principal vein length; the sum of the
two top lateral veins; the sum of the two
underside lateral veins, in cm.

![Graph](image-url)
Fig. 3. Theoretical curve for determining leaf surface in accordance with $\Sigma L_2$

There was non-linear correlation between the leaf surface ($S$) and the principal vein length ($L_1$), the sum of the length of the two top ($\Sigma L_2$) or the two underside ($\Sigma L_3$) lateral veins of the vine leaf. Since the correlation ratio could not give an accurate picture of the existing
relationship between the two variables, it was used the determination ratio ($R^2$) (Dimova and Marinkov, 1999). It represented the square of the correlation ratio and justified the assumption that there was a causal relation between the two signs.

The determination ratio value for the indicator $L_1$ was: $R^2 = 0.9443$, meaning that 94.43% of the variation in the leaf surface under the impact of this indicator could be explained by the calculated nonlinear regression. The deviation from the regression $(1 - R^2)$ was 5.57%, indicating the impact rate of the random factors.

For the indicator $\Sigma L_2$ the determination ratio value was slightly higher $R^2 = 0.9829$ or 98.29% of the variation under the impact of this indicator was explained by the curvilinear regression. The deviation from the regression was 1.71%.

The determination ratio for the indicator $\Sigma L_3$ was $R^2 = 0.9742$, therefore 97.42% of the leaf surface variation was explained by the regression and 2.58% was under the impact of random factors.

It could be observed that the determination ratio values for the three indicators were very close. The deviations from the regression for the three indicators were less than 6%, demonstrating that the method ensured reasonable accuracy.

CONCLUSIONS

1. It exists correlation between the leaf surface ($S$) on the one hand and the principal vein length ($L_1$), the sum of the lengths of the two top lateral ($\Sigma L_2$) or the two underside ($\Sigma L_3$) lateral veins of the vine leaf, on the other hand.

2. The deviations from the regression for the three indicators were
less than 6%, demonstrating that the method ensured the determination both of the size of the individual leaves and the leaf surface of whole vines accurately enough.

3. The determination ratios ($R^2$) were very close in value allowing the leaf surface to be determined with approximately equal accuracy by the three indicators.

4. The advantage of this method was that for determining the leaf surface it was not necessary defoliation of the vine, which affected adversely its strength and productive capacity.

/ REFERENCES

Colour parameters of blackberry cultivars after application of fertilizers

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²Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levsky Str., 5600 Troyan, Bulgaria

The study was conducted in 2013 in the collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan. The objects of study are three cultivars of thornless blackberries - 'Hull Thornless', 'Black Satin' and 'Dirksen'. The influence of some fertilizers was followed with conventional and organic application over colour parameters of fresh fruits.

The indicators were given according to the system CIE Lab. At the measurement were taken chromaticity coordinates L, a and b: L – colour brightness; +a – red colour; -a – green colour; +b – yellow; -b – blue.

For all samples, the yellow colour tone was dominant in samples treated by 'Tekamin Brix' 0.2%, 'Tekamin Brix' 0.3% and the control, while in samples with ammonium nitrate the colour indicator got into the blue colour tone. Data are
Differences are statistically differentiable and the fertilization method had an influence over that quantitative indicator (p<0.05).

**Key words:** blackberries, cultivars, fertilizing, colour parameters

**INTRODUCTION**

Blackberries belong to the group of berry crops, characterized by the attractiveness of taste, aroma and rich biochemical composition of their fruits (Halvorsen et al., 2002, De Souza et al., 2014, Skrovankova et al., 2015). Blackberries, including fruit from a number of berry crops, are appealing both for fresh consumption and different products (Yoo et al., 2010; Slatnar et al., 2012).

At present, the interest in them is constantly increasing and requires the introduction of new varieties that are of good economic quality and adaptable to the conditions of cultivation for the respective region (Domozetova, 2012; Stoyanova et al., 2015). Blackberries have relatively good distributional plasticity, but they need certain agro-cultivation activities to realize their reproductive potential.

Fertilization is one of the major factors with a direct impact on the vegetative and reproductive effects of plants. It is essential to observe the impact of fertilizers on the quality of the fruits and the plants respectively.

The purpose of the present study is to investigate the influence of fertilizers with conventional and biological application on the colour parameters of fresh fruit of three thornless cultivars of blackberries, such as ‘Hull Thornless’, 'Black Satin' and 'Dirksen'.

**MATERIAL AND METHODS**

The experiment was set in 2013 in the Research Institute of Mountain Stockbreeding and Agriculture - Troyan. The subject of the study is three cultivars...
The planting is maintained with grassed interrow spacings and black fallow in the intra row spacing. Tekamin Brix, a foliar fertilizer with biological application was used, as it was applied twice in two concentrations during vegetation. Ammonium nitrate was introduced once at the beginning of the vegetation.

The experiment was set in the following variants of fertilization:

- I - Tekamin Brix (foliar application) - in concentration of 0.2%;
- II - Tekamin Brix (foliar application) - in concentration of 0.3%.
- III - Ammonium nitrate at a dose of 0.200 kg/1 linear meter.
- IV - Control

**Method of testing**

The colour characteristics of fresh fruits from the three blackberry cultivars from the variants in the laboratory of Food Research and Development Institute - Plovdiv were reported.

The colour was determined according to Gardner Colour Scale – using the laboratory apparatus ‘GOLORGRAD2000’ of BYK-GARDNER INC. USA. The blackberry samples were grounded in laboratory apparatus MPI –2 with diameter of the holes of 4 mm. The sample was deaerated in a vacuum chamber at vacuum of 0.85 kPa for 10 min.

The indicators were given according to the system CIE Lab. At the measurement were taken chromaticity coordinates L, a and b: L – lightness; + a - red colour; - a green colour; +b - yellow, - b - blue.

The value of the colour tone or the dominant wavelength is represented by the ratio a/b.

**Statistical processing** of the samples was carried out in threefold repetitions, data are presented as average values and processed with the ANOVA program.
RESULTS AND DISCUSSION

The data from the conducted tests are presented in Tables 1, 2, 3 and 4 and Figures 1-9.

The highest value in terms of brightness is found in fruit of 'Dirksen' cultivar from the group of control blackberry plants ('Hull Thornless', 'Black Satin' and 'Dirksen'). Data are statistically distinct, with cultivar diversity influencing the measured value (p<0.05).

<table>
<thead>
<tr>
<th>ОРТОВЕ КЪПИНІ / Blackberry cultivars</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>ул орнлес / Hull Thornless</td>
<td>10,36±0,32</td>
<td>1,65</td>
<td>0,16±0,03</td>
<td>10,31</td>
</tr>
<tr>
<td>лек тин / Black Satin</td>
<td>3,79±0,53</td>
<td>9,97±0,06</td>
<td>1,2±0,32</td>
<td>8,3</td>
</tr>
<tr>
<td>ирксен / Dirksen</td>
<td>12,48</td>
<td>9,21</td>
<td>4,57</td>
<td>2,01</td>
</tr>
</tbody>
</table>

The red and yellow colours have the lowest values in 'Hull Thornless' cultivar compared to the other two samples (p <0.05). In the red colour component for the other two cultivars of 'Black Satin' and 'Dirksen', data are statistically indistinguishable and cultivar difference does not affect the measured indicator (p>0.05).

The highest statistically significant value for the measured yellow colour tone is found in fruit of 'Dirksen' cultivar (p<0.05).

The quality indicator of colour tone has the highest value in fruit of 'Hull Thornless' cultivar.

In the experiments on blackberry cultivars treated with 0.2% of Tekamin Brix, it was found that fertilization manner had an effect on colour characteristics, as the brightness of all cultivars had increased and the highest value was found in 'Black Satin' (p<0.05).
Table 2. Colour characteristics of fresh fruit of 'Hull Thornless', 'Black Satin' and 'Dirksen' fertilized by 'Tekamin Brix' (foliar application) – at a concentration of 0.2%

<table>
<thead>
<tr>
<th>blackberry cultivars</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hull Thornless</td>
<td>7.62±0.44</td>
<td>8.74±0.49</td>
<td>0.92±0.12</td>
<td>9.60</td>
</tr>
<tr>
<td>Black Satin</td>
<td>16.33±2.32</td>
<td>9.31±1.32</td>
<td>1.17±0.15</td>
<td>7.96</td>
</tr>
<tr>
<td>Dirksen</td>
<td>8.14±1.79</td>
<td>12.77±2.53</td>
<td>1.83±0.31</td>
<td>6.75</td>
</tr>
</tbody>
</table>

According red colour tone indicator, the fertilizer 'Tekamin Brix' with this concentration affects the fruits of 'Dirksen' and 'Hull Thornless' cultivars, as it increases its quantitative value compared to the controls (p<0.05). The red colour tone has the highest value for fruits of 'Dirksen' cultivar.

For the indicator of yellow colour tone, 'Tekamin Brix' with a concentration of 0.2% acts by increasing the amount of the value in fruit of 'Hull Thornless' and decreasing it in fruits of 'Black Satin' and 'Dirksen' compared to the controls (p<0.05).

The highest quality evaluation of the colour has 'Hull Thornless' cultivar.

Table 3. Colour characteristics of fresh fruit of 'Hull Thornless', 'Black Satin' and 'Dirksen' treated with 'Tekamin Brix' (foliar application) at a concentration of 0.3%

<table>
<thead>
<tr>
<th>blackberry cultivars</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hull Thornless</td>
<td>10.11±0.56</td>
<td>2.86±0.46</td>
<td>0.08±0.13</td>
<td>35.75</td>
</tr>
<tr>
<td>Black Satin</td>
<td>11.13±1.08</td>
<td>9.93±0.83</td>
<td>2.67±0.73</td>
<td>3.71</td>
</tr>
<tr>
<td>Dirksen</td>
<td>5.19±0.30</td>
<td>7.3±0.40</td>
<td>-0.25±0.13</td>
<td>28.85</td>
</tr>
</tbody>
</table>

In the measurement of red colour tone, the fertilizer 'Tekamin Brix' with a concentration of 0.3%, influences by increasing the quantitative value on all fruit cultivars studied here (p<0.05). The
red colour tone has the highest value in 'Black Satin'.

In the measurement of yellow colour tone, 'Tekamin Brix' with a concentration of 0.3% acts as it quantitatively increases the value of 'Black Satin' fruits and decreases it in 'Hull Thornless' as compared to the control (p<0.05).

In fruit of 'Dirksen', the treatment with 'Tekamin Brix' 0.3% is most effective as the yellow component of the colour changes to a blue colour component.

'Hull Thornless' cultivar has the highest quality evaluation of colour in the application of 'Tekamin Brix' 0.3%.

As a result of fertilizing with ammonium nitrate, the colour brightness indicator increases in fruits of 'Hull Thornless' and 'Black Satin', and decreases in 'Dirksen' cultivar compared to the controls. Data are statistically identifiable, as the fertilizer treatment and the selected concentration, have an impact on the measured parameter (p<0.05).
The manner of treatment with the applied concentration of the solution has a significant influence on the measured values for this parameter (p<0.05). 'Black Satin' fruit has the highest value of the colour tone in the experiment.

With regard to the colour brightness in the studied variants of 'Hull Thornless', the highest percentage impact on the measured values shows the way of treatment with fertilizers 91%, followed by the random factors 6% and the selected variants of the experiment 3%. The lowest colour brightness value was measured for fruit treated with 'Tekamin Brix' 0.2%.

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ервеният цвят тон е с най-висок стойност при плодове третирани с Tekamin брикс 0,2% в сравнение с контрола и останалите варианти. Най-висок процент на върху измеренияте стойности е също на червенияй тон, съотношение 72%, следван от случайни фактори 16% и подбранны варианти 12%.

Фиг. 3. Жълт/син цвят в къпини от сорт Хул Торнлес третирани с торове на конвенционално и биологично приложение

The yellow colour tone has the highest value for fruit treated with Tekamin Brix 0.2% compared to the control and the other selected variants. The highest percentage on the measured values shows the fertilization method 72%, followed by the random factors 16% and the selected variants of the experiment 12%.

Фиг. 4. Яркост на цвят в къпини от сорт Дирксен третирани с торове на конвенционално и биологично приложение

The yellow colour tone has the highest value in blackberries treated with Tekamine brix 0.2% compared to the control and the other selected variants. Ammonium nitrate treatment has a significant impact on the measured indicator of fruit as it changes its colour to a blue colour tone.

Изпълнените резултати показват, че начинът на торене оказва най-голям въздействие върху яркостта на цвята, съотношение 58%. Следващите въздействия са случайни фактори 15% и подбрани варианти 27%.
The brightness of fruit colour in 'Dirksen' decreases in all selected variants compared to the control, as the highest percentage of influence being the fertilization variants of 58%, followed by the selected raw material 27% and the random factors.

Fig. 5. Red colour tone of 'Dirksen' cultivar treated with fertilizers of conventional and biological application.

For selected variants of treatment of fruit of this cultivar, only in fruit treated with Tekamine brix 0.2%, the red colour value increases relative to the control and the other selected variants. The fertilization method has the highest percent influence on the measured values for this indicator, followed by the selected variants and the random factors.

Fig. 6. Yellow/blue colour tone of 'Dirksen' blackberry cultivar treated with fertilizers with conventional and biological application

Influence of way of fertilization, variants of samples and accidental factors on red colour tone in blackberries of 'Dirksen' cultivar.

Red colour tone in blackberries of 'Dirksen' cultivar:

- Variants of fertilization, 58%
- Accidental factors, 15%
- Variants of samples, 27%

Red colour tone in blackberries of 'Dirksen' cultivar:

- Variants of fertilization, 58%
- Accidental factors, 15%
- Variants of samples, 27%

Red colour tone in blackberries of 'Dirksen' cultivar:

- Variants of fertilization, 58%
- Accidental factors, 15%
- Variants of samples, 27%

Fig. 5. Red colour tone of 'Dirksen' cultivar treated with fertilizers of conventional and biological application.

Fig. 6. Yellow/blue colour tone of 'Dirksen' blackberry cultivar treated with fertilizers with conventional and biological application.
For selected fertilization variants, the yellow colour component decreases compared to the control, and for fruit treated with Tekamine Brix 0.3%, the colour tone changes to blue colour.

For all the selected variants of the experiment for this cultivar, the colour brightness increases as a result of applied concentrations and fertilizer selection compared to the control. Fruit treated with Tekamine brix 0.2% has the highest value for this indicator.

The value of the red colour decreases in fruit treated with ammonium.
Фиг. 9. Жълт/син цветови тони на къпини от сорт Лек Тин третирани с торове с конвенционално и биологично приложение

CONCLUSIONS

The influence of some fertilizers with conventional and biological application on the colour parameters of fresh fruit of three cultivars of thornless blackberries was observed, such as 'Hull Thornless', 'Black Satin' and 'Dirksen'.

It was found that fruits of 'Dirksen' and 'Hull Thornless' that were fertilized with 0.2% Tekamin Brix increased their quantitative value in terms of colour brightness, red colour and yellow colour compared to the control.

For fruits of 'Hull Thornless' and 'Black Satin' that were fertilized with ammonium nitrate, the yellow component of the colour changes towards the blue colour tone.

Fruits treated with Tekamin Brix in concentrations of 0.2% and 0.3% reduce the values of the yellow colour tone, and the fruits treated with ammonium nitrate change the colour towards the blue colour tone.
Brix and 0.3% in comparison with the control.

The fertilization method has an impact on the measured values for all studied colour indicators in 'Hull Thornless', 'Black Satin' and 'Dirksen'.

/ REFERENCES


Irrigation scheduling of apple in drip irrigation

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SUMMARY
Establishing irrigation scheduling of apples grown in soil and weather conditions of the Sofia region in the period 2001-2005 research with drip irrigation was conducted. Different regimes of watering to full satisfaction of daily needs of the culture of water to irrigation reduced by 20% and 40% irrigation norms were tested.

Established irrigation scheduling of apples – number irrigations, irrigation depths, irrigation rate and yields of apples of the Sofia plant.

The highest yields are obtained at 100% irrigation rate – 2087 kg/da. The reduced irrigation depths with 20% resulted in rather small drops in yields by 7%. This irrigation scheduling can be applied in conditions of water deficit.

Key words: apple, variety "Florina", drip irrigation, apple crop, productivity irrigation.

INTRODUCTION
Apple ranks first in the country in area and fruit production compared to other fruit crops. Agricultural and economic efficiency of apple production in our climatic conditions largely be
determined by application of rational irrigation regimes and appropriate irrigation techniques.

Microirrigation is one of the most perspective ways for irrigation of fruit crops, mainly due to big water savings and opportunities for full automation of the irrigation process.

Along with all the advantages of drip irrigation in physiological and technological plan, the most significant of which is the preparation of a biologically optimum yield of high quality of the fruits with a considerable saving of water compared to traditional methods of irrigation (Dochev, 1983), it provided best prerequisites for the implementation of the so-called broken irrigation regime by reducing the amount of irrigation norms (Lazarov and Mehandzhieva, 1982).

Studies in Bulgaria on irrigation regime of apple fruit-bearing palmetto plantation near the town. Plovdiv indicate that irrigation with drip irrigation rates ranging from 140 mm to 300,0 mm (Dochev and Gospodinova, 1987).

Doychev (1994) states that intensive apple trees irrigated by dropwise manner must be created by high-yielding and high-quality fruit varieties. It recommends the rootstock MM 106 and applied irrigation regime with irrigation rate of 100% ET, and low water, reduced to 60% of ET.

Applying this method of irrigation at growing apples, which has great economic importance, requires detailed parameters, establishment of irrigation regime in particular conditions in the
The aim of the study is to establish the basic parameters of the irrigation regime of apples grown under drip irrigation.

**MATERIAL AND METHODS**

The studies for the establishment of the irrigation system of fruit-bearing apple variety "Florina" on a rootstock MM 106 in drip irrigation were carried out on an experimental field Chelopechene, during the period 2001-2005.

The following options were tested:
1. Irrigated option;
2. Irrigation with irrigation rate of 100% m;
3. Irrigation with irrigation rate 80% m;
4 Impregnation with irrigation rate 60% m.

The trees were planted approximately 4.5 m spacing and 2.5 m interrow distance or 125 trees per da.

Irrigation is done by superficially dropwise manner with drippers CP - 4.6, a perforated tube through 0.60 m.

Each row of the orchard, consisting of 30 trees represents a variant in four replicates of six trees in the repeat area of each repetition of 67.5 m² (2.5 m x 4.5 m = 11.25 m² area of a tree).

The soil was leached cinnamon forest, slightly sandy loam in the plow layer, formed on a substrate of an old talus cone of alluvial material. It is poorly stocked with nitrogen, an average of phosphorous, and potassium. On average in layers 0-60 cm soil has the following water-physical properties: PPW = 22.1%, humidity reduction – 12.3% by weight of absolutely dry soil, a bulk density in PPW – 1.47 g/cm³. For soil layer 0-100 cm same parameters have the values: PPW – 21.8%, humidity reduction – 12.3% and bulk density – 1.50 cm³.

The soil is suitable for growing apples.
Weather conditions under which the experiments were conducted

Research on irrigation norms suited to water physical properties of soil type and weather conditions over the years, make it possible to establish such an amount of irrigations in which most fully to meet the biological needs of crops without allowing large water losses.

Table 1. Rainfall during apples vegetation period

<table>
<thead>
<tr>
<th>Periods</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>м. IV-IX</td>
<td>358</td>
<td>418</td>
<td>329</td>
<td>258</td>
<td>765</td>
</tr>
<tr>
<td>м. V-V</td>
<td>75</td>
<td>158</td>
<td>104</td>
<td>258</td>
<td>400</td>
</tr>
</tbody>
</table>

Over the years of experiments the rainfall availability in a sequence of 5 years a series characterize the vegetation period of development of culture (April-September) as average – 2001, 2002 and 2003, a damp – 2005 and one – 2004 very dry, as dry 2002 in terms of rainfall for the period VII-VIII, during which culture is the most demanding on the soil moisture. At least rainfall fell in 2004 (258 mm), and most in 2005. (765 mm), while in the remaining three years rainfall of 329 to 418 mm for the period IV-IX (Table 1). Precipitation in the vegetation of culture unevenly distributed, which led to the realization of irrigation in the years of experiments.

RESULTS AND DISCUSSION

The results of the five studies show that the number of irrigations and the amount of irrigation norms are determined by weather conditions (rainfall) over the years.

The number of irrigations per year ranged from 14 to 20, and the amount of irrigation norm of 190 to 360 mm.

On average over the study period (Table 2) during the vegetation period of
Irrigation norms were implemented for the period from early May to late September. Irrigation vegetation periods in culture are different and depend on the amount and distribution of rainfall, and the phases of development of culture. It was found that the requirements of apples to moisture in the soil are larger at the beginning of flowering, fruit formation to harvest the fruit, so that moisture in the soil during this period is necessary to maintain 80-85% of PPW. At the beginning of vegetation of culture, and at the end the irrigations are implemented in 14-15 days and during the active growing season, flowering and fruit formation in 5-6 days (Table 2).

The results yield of apples in different humidity in respect of years demonstrate the influence of drip irrigation on its size. The greatest increase in yield was obtained in 2004 and 2002 (dry), which is a 55%-60% more than the non-irrigated variant (Table 3). At least an increase by 667 kg/ha (25%) was received in the wet in 2005, with the average for the five years studied the increase in yield is 821 kg/ha compared to non-irrigated option.
Realized irrigation regimes over the years have also affected the yields obtained. The highest yields were obtained at variants which are irrigated with 100% irrigation rate 2087 kg/ha, and the lowest in irrigated variants 1266 kg/ha. The decrease of the irrigation rate by 20 and 40% led to a decrease in yields of 7% and 14%. Average for the period reduction in yield is not drastic and irrigation regimes can be used in the case of water deficit (Table 3).

Table 3. Yield of the apples of drip-irrigation in the region near to Sofia, Chelopechene

<table>
<thead>
<tr>
<th>Years</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>Average 2001-2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variant</td>
<td>Yield kg/ha</td>
<td>Relative yield %</td>
<td>Yield kg/ha</td>
<td>Relative yield %</td>
<td>Yield kg/ha</td>
<td>Relative yield %</td>
</tr>
<tr>
<td>100%</td>
<td>1567</td>
<td>100</td>
<td>704</td>
<td>100</td>
<td>1135</td>
<td>100</td>
</tr>
<tr>
<td>80%</td>
<td>2053</td>
<td>131</td>
<td>1603</td>
<td>251</td>
<td>1945</td>
<td>171</td>
</tr>
<tr>
<td>60%</td>
<td>2004</td>
<td>128</td>
<td>1421</td>
<td>202</td>
<td>1712</td>
<td>150</td>
</tr>
</tbody>
</table>

The test irrigation regime had no effect on the productivity of irrigation water. It is highest in the variants irrigated with a lower size irrigation rate. The analysis of the results showed that each cubic meter irrigation water on average in the period of the study were obtained from 7.6 to 9.8 kg of apples (Table 4). The highest productivity of irrigation water in the three experimental years was obtained under the variant of realizing the 60% of the irrigation rate and amounts to 6.6 to 13.4 kg for each cubic meter of water.

The results obtained for the efficiency of irrigation water have shown that with increasing water availability to the plants lowers the productivity of irrigation water, the most pronounced in the variants irrigated with 100% irrigation rate.
Table 4. Irrigation water productivity of apples

<table>
<thead>
<tr>
<th>Variant</th>
<th>2001 средна година</th>
<th>2004 сух година</th>
<th>2005 влажна година</th>
<th>Средно за трите години</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>poitele norm</td>
<td>редуктивн</td>
<td>poitele</td>
<td>редуктивн</td>
</tr>
<tr>
<td></td>
<td>Irrigation rate</td>
<td>вод</td>
<td>irrigation rate</td>
<td>вод</td>
</tr>
<tr>
<td></td>
<td>mm</td>
<td>efficiency</td>
<td>mm</td>
<td>efficiency</td>
</tr>
<tr>
<td>100%</td>
<td>340</td>
<td>6,1</td>
<td>360</td>
<td>5,2</td>
</tr>
<tr>
<td>80%</td>
<td>275</td>
<td>7,5</td>
<td>300</td>
<td>6,1</td>
</tr>
<tr>
<td>60%</td>
<td>217</td>
<td>9,3</td>
<td>220</td>
<td>6,6</td>
</tr>
</tbody>
</table>

CONCLUSIONS

● For the period of active vegetation of culture (April-June) realized 17 the number of irrigations medium irrigation rate – 19,0 mm and total irrigation rate for the period – 323,0 mm.
● Test irrigation regimes biologically most appropriate irrigation mode realization of 100% irrigation rate, which is recommended under good water availability.
● The highest yields were obtained at variants which are irrigated with 100% irrigation rate 2087 kg/ha, and the lowest in irrigated variants 1266 kg/ha. The decrease of the irrigation rate by 20 and 40% led to a decrease in yields of 7% and 14%. Average for the period reduction in yield is not drastic and irrigation regimes can be used in the case of water deficit.
● The greatest increase in yield was obtained in 2004 and 2002 (dry), which is 55%-60% more than the non-irrigated variant. At least increase by 667 kg/ha (25%) was received in the wet in 2005, with the average for the five years studied the increase in yield is 821 kg/ha compared to non-irrigated option.
● The productivity of irrigation water in the drip irrigation of apples is increased by reducing the irrigation norm. The highest values achieved in 40% reduction in irrigation rate.
/ REFERENCES
Ethyl methane sulfonate induced mutation phenotype in M1 generation of Paulownia tomentosa

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SUMMARY

Paulownia is a deciduous, fast-growing, hardwood and multipurpose tree species that is native to China. It exhibits a number of desirable characteristics, such as rot resistance, dimensional stability and a high ignition point. It is a beautiful ornamental tree, which is also suitable for the bioethanol industry and as a source of secondary metabolites. As an economically important species over the past several decades, research on Paulownia has been conducted to develop biotechnological approaches for plant propagation and genetic improvement.
Mutagenesis has been used to increase genetic variability in crop plants through chemical mutagens or irradiation. Mutagen such as ethyl methane sulphonate (EMS) has been widely used to induce a large number of functional variations.

A study has been conducted to enhance genetic variability in Paulownia tomentosa using ethyl methane sulphonate. Exposure to an EMS concentration of 0.6%, v/v for 12h was used to mutagenize 100 seeds for the first generation (M₁). It was observed one phenotypic mutation with altered growth behaviors than in wild type (WT) plants. Produced plant had dwarflike phenotype with broken apical dominance; increased number of branches; smaller leaves and short internodes.

In respond to gibberellins (GA₃) treatment they grow as tall as normal tall varieties but with multiple branched stems and small leaves. Treatment with auxins Indole-3-acetic acid (IAA) and 1-Naphthaleneacetic acid (NAA) had no result on the dwarflike phenotype either. In vitro DWL plants grown on media supplemented with Epibrassinosteroids (EBR) had bigger leaves and unbranched stems but in vivo DWL plants treated with EBR didn’t change their phenotypes. Dwarf phenotypes could be the result of reduced BR biosynthesis or trough up- or down- regulation of other genes.

Key words: Ethyl methane sulphonate (EMS) Paulownia tomentosa, dwarf phenotype, gibberellins (GA₃), Indole-3-acetic acid (IAA), 1-Naphthaleneacetic acid (NAA), Epibrassinosteroids (EBR)

INTRODUCTION

Paulownia is a genus comprised of between 6-17 species in the family Paulowniaceae. They are native to China and have been naturalized in other parts of the world such as Europe and USA.
These deciduous trees are fast growing, and can grow just as quickly on a wide variety of soils and wide range of climatic conditions.

It has been examined the ability of Paulownia to take up nitrates, heavy metals and land contaminants, that make it a useful source for land reclamation and reforestation (Zhang et al., 2007). The leaves and flowers could be used as fodder because of their contents of fats, sugars and proteins. The high level of nitrogen in leaves made them also a good fertilizer.

Paulownia species are rich in antioxidants and secondary metabolites, connected with theirs medicinal properties (Šmejkal et al., 2007; Si et al., 2013).

Paulownia trees become a popular source for cellulosic ethanol as alternative fuels. A risen popularity of Paulownia is due to its many desirable features that determined it as a multipurpose tree with grate economical and practical value.

With the growth of human population, large-scale production systems were developed. This leads to the creation of new plant species, different techniques of cultivation and selection. Conventional selection and breeding has been successful program for genetic improvement but with some limits about woody plants. Reducing the induction time and the subsequent selection of genetic changes could be useful tool for overcoming the problems with selection and species modification of important crops. In Nature, genetic variations are result of keeping of mutations that gave advantages to the plants, for example, greater yield or resistance to adverse factors.

There are three types of mutagenesis – irradiation; treatment with chemical mutagens or site-directed mutagenesis due to DNA insertions, genetic
transformation or activation of transposable elements (Oladosu et al., 2015). Chemical mutagenesis has several benefits, and has been widely used to induce a large number of functional variations in crops. Among chemical mutagens, the alkylating agent, ethyl methane sulfonate (EMS) is the most commonly used in plants as it causes a high frequency of nucleotide substitutions (Talebi et al., 2012).

EMS induces mispairing and base changes, because of guanine alkylation, which cannot pair with cytosine anymore and pairing with thymine (Greene, et al., 2003).

The induced point mutations could lead to loss-of-function or gain-of-function phenotypes with important features to the agriculture. The proper mutations selection is important and after EMS treatment most mutant lines in the M1 generation are removed (Oladosu et al., 2015).

We have used the chemical mutagen EMS to modify the genome and inducing new features in the popular woody species Paulownia tomentosa. In the M1 generation of treated seeds, an altered phenotype was identified whose morphology was compared to that of the parent plants. Visible effect will be produced when the mutagenic factor affects genes associated with plant growth regulators. Here we analyze the responses of produced dwarflike plants to exogenic plant growth regulators as gibberellins (GA$_3$); naphthyl acetic acid (NAA); indolyl acetic acid (IAA) and epibrassinosteroid (EBR), to trace whether the newly acquired phenotype is due to a quantitative shortage or lack of function of some of the listed plant hormones.
MATERIAL AND METHODS

Plant material

The in vitro raised shoots from *Paulownia tomentosa* and dwarflike plants were kindly provided by prof. Alisher Turaev, Faculty of Biology; BIOSS Centre for Biological Signaling Studies; ZBSA Centre for Biological Systems Analysis, University of Freiburg.

EMS mutagenesis (Kim et al., 2006)

100 seeds from *Paulownia tomentosa* were soaked in 100mM phosphate buffer (pH 7.5) overnight at 40 °C. The excess buffer was decanted and fresh buffer was added with EMS to a final concentration 0.6%. Mixture was incubated 12h at room temperature and then seeds were washed 20 times with water.

Culture media and culture conditions

Basal MS (Murashige and Scoog, 1962) media was supplemented with 20 g/l sucrose and 8 g/l agar, the pH of media was adjusted to 5.8.

The cultures for seed germination were incubated at 24 °C in dark for 4 weeks. Aseptic seedlings were used as a source material for micropropagation.

Micropropagated in vitro plants were incubated under 16/8h photoperiod in a growing-room under light intensity 30 Mm⁻²s⁻¹ from cool-white fluorescent lamps at 22°C - 25°C.

The cultures were sub-cultured at four-week intervals. Observation was recorded over 7 days of inoculation and subculture. All experiments were repeated three times with at least ten cultures per treatment.

Acclimatization of plants under in vivo conditions

Two months old *in vitro* plants were adapted to *in vivo* conditions after...
no so počistiv t корените от всички ост тъци, след което се пост вят в пл стм сови контейнер з пълнени със смес от почв и перлит (2:1v/v). стеният се покрив т с прозр чно фолио, което ще з п зи относителн т вл жност от 95% до 98%. клим тизи р ните р стения се отглежд т при фотоперiod 16/8 ч. с интензивност н светлин т 30 μ m²s⁻¹ и темпер тур 22°C - 25°C. planting up gently the roots from media oddments. Plantlets were put into pots containing mixture of peat and perlite (2:1v/v), covered with transparent foil, that would keep 95% - 98% relative humidity. Acclimatized plants were incubated under 16/8h photoperiod in a growing-room under light intensity 30 Mm⁻²s⁻¹ from cool-white fluorescent lamps at 22°C - 25°C.

Фактър на стежните регулатори върху in vitro култивираните dwarfwidni р стения Paulownia. Фактър се изпитв върху регенерация в сред, допълнен с р злични концентрации р стежн регул гори (блиц 1).

Иберелинов т киселин (н ричн още Иберелин или GAs) се р зт вя в 96% ет нол, стерилизира се през б керициден филтър и се приб- вя към основн т сред (MS, 20 g/l з х роз и 8 g/l г р), която предв рително се стерилизира чрез втокл вир не.

фил оцетна киселин (NAA) и ндолип оцетна киселин (IAA) се р зт вя с 1N NaOH и се доб вят към основн т сред преди втокл вир не.

24-епибрасиностероид (EBR) се р зт вя в 96% ет нол, стерилизира се през б керициден филтър и се приб- вя към втокл вир н т основн сред .

Сички култури се инкубир т при фотоперид 16/8 ч. във фитостат под бял флурисцент светодиод с интензивност 30 μ m²s⁻¹ и темпер тур 22°C - 25°C. The effect of growth regulators on dwarfwid Paulownia plants tested by in vitro culture. The effect was tested on regenerating media supplemented with different concentrations of growth regulators (table 1).

Гиберелинова киселина (наричана още Гиберелин или GAs) се разтворява в 96% етанол, стерилизира се през бактерициден филтър и се прибавя към основната среда (MS, 20 g/l сукроза и 8 g/l агар). Гиберелинова киселина (наричана още Гиберелин или GAs) се растворена в 96% этанол, стерилизирована через бактерицидный фильтр и добавляется к основной среде (MS, 20 g/l сахароза и 8 g/l агар).

2-(1-Naphthyl) acetic acid (NAA) and 2-(1H-indol-3-yl) acetic acid (IAA) were dissolved in 1N NaOH and added to the basal media before autoclaving. 2-епибрасинолид (EBR) was dissolved in 96% ethanol, sterilized through bactericidal filter and added to the autoclaved basal media.

All cultures were incubated under 16/8 h photoperiod in plant growth room under cool-white fluorescent lamps at 22°C to 25°C with 65% – 70% humidity. 24-epibrassinolide (EBR) was dissolved in 96% ethanol, sterilized through bactericidal filter and added to the autoclaved basal media.

The effect of growth regulators to in vivo cultured dwarfwid Paulownia plants. The in vivo adapted plants were sprayed with 2ml solutions of plant growth regulators in different concentration. The control plants were sprayed with 2ml distilled water. Treatment was repeated after 7 days. The experiment prolongs 5 to 8 weeks and each experiment was repeated 3 times.
RESULTS AND DISCUSSION

Paulownia tomentosa seeds were EMS treated and produced M1 generation was screened for phenotypic changes. One of the regenerated plants developed bushy phenotype and turns the subject of present study.

The aseptic seedlings were source for micropropagation, using the segments from nodal sections and shoot tips. They were micropropagated on MS media containing 2% sucrose, without any growth regulators, that can affect the phenotype.

Two months old in vitro plants were used for in vivo propagation and were incubated at 24°C under 16/8 h photoperiod and light intensity 30 µm²s⁻¹. The growth pattern and development; height; number of nodes; number and the size of leaves were traced. These criteria were used to compared dwarflike Paulownia (DWL) with the control plants Paulownia tomentosa (PT). The compared two groups of plant propagated and cultivated at the same conditions started with the similar phenotype. The two months old DWL plant could be equal or even slightly taller than PT plant, but generally the whole DWL group is more variable in height.

In their further development DWs get branched, developing all the side buds, while PTs retained their apical dominance (Figure 1). The leaves size followed the same trend; initially plants from both groups had a similar vision. In their further development DWLS leaves were far behind the original size, but numerically get ahead of PTs (Figure 1).

Both analyzed groups were different in their growth and development patterns. PT plants have grown gradually and increased their biomass, gaining each week a new node and two or three new
The Dwarflike plant phenotype could be the result of genetic manipulations, but also could be result of natural changes during acclimatization to stress factors such as soil composition, light (Hutchings et al., 1994), cold (Okamoto at al., 2008) or infection (Scholthof et al., 1995).

Dwarfing in natural condition is obtained not as a result of damage caused by stress factors, but as a result of plant growth regulators produced under stress conditions (Zhang and Turner, 2008).

Searching the connection between dwarflike phenotype and altered levels of growth regulators in analyzed DWL plants we investigated the effect of various concentrations of the major groups of plant growth regulators: gibberellins, auxins and brassinosteroids.

For this purpose were cultured in vitro PT and DWL plants on nutrition media supplemented with growth regulators in various concentrations (Table 1). Simultaneously in vivo cultured PT and DWL plants were sprayed with solutions of the same plant growth regulators in various concentrations.
Табл. 1. Състав на хранителните среди използвани за in vitro култури

Table 1. Composition of in vitro culture media

<table>
<thead>
<tr>
<th>Media</th>
<th>Growth regulators</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MS0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>GA&lt;sub&gt;3&lt;/sub&gt; (mg/l)</td>
<td>IAA (mg/l)</td>
<td>NAA (mg/l)</td>
<td>EBR (μM)</td>
<td></td>
</tr>
<tr>
<td>MSG1</td>
<td>0,1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MSG2</td>
<td>0,5</td>
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</tr>
<tr>
<td>MSG3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>MSG4</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MSA</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MSN</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td></td>
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<tr>
<td>MSE1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MSE2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td></td>
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</table>

MS сред без доб вени р стежки регул тори -MS0<sup>a</sup>. Снови MS сред обог тен с GA<sub>3</sub> - MSG1; MSG2, MSG3 и MSG4. MS среда с доб вени индолил оцетни киселини – MSA. MS сред с доб вени фтил оцетни киселини - MSN. Снови MS сред обог тен EBR - MSE1 и MSE2.

MS media without any regulators -MS0<sup>a</sup>. Basal MS media supplemented with gibberellins - MSG1; MSG2; MSG3 and MSG4. MS media supplemented with indole 3-acetic acid – MSA. MS media supplemented with 1-Naphthaleneacetic acid acetic acid – MSN. MS media supplemented with Epibrassinosteroids (EBR) - MSE1 and MSE2.

The gibberellins (GA<sub>3</sub>) are class of plant hormones that affect several important plant processes, including stem elongation, seed development, and seed germination. Besides GA could stop or reverse the effect of physiological dwarfism and it is one of the specific applications of gibberellins. In this study experiments were conducted with GA<sub>3</sub>, which is known to be the most abounded in the plant kingdom.

Acclimatized plants under in vivo conditions were sprayed with 2 ml solution of GA<sub>3</sub> (10 mg/l). The treatment was repeated every seven days for eight weeks. The control plants were sprayed with 2 ml of distilled water. Treatment with GA<sub>3</sub> induced elongation by an average of 12 cm that was 4 times more compared to controls.

DWLs sprayed with GA<sub>3</sub> kept their “bushy” (branched) phenotype and the small size of the leaf lamina, although there was a prolongation of the petiole (Figure 2).
Fig. 2. DWLs sprayed with distilled water (1) and DWLs sprayed with 10 mg/l GA₃ (2)

The effect of GA₃ was tested by shoot regeneration on media supplemented with different concentration of this hormone (Table 1). After two-months cultivation, under constant regulated conditions, there is no disappearance of the dwarf phenotype. The highest experimental concentration (5mg/l) had inhibitory effect to the tested tissue cultures.

Apical dominance is phenomenon when one meristem prevents or inhibits the growth of other meristems.

During the formation of leaves and elongation of stem some cells are left behind from shoot apical meristem and constitute axillary bud.

If the dominant meristem is cut off, one or more branch tips will assume dominance.

The mechanism of apical dominance is based on the plant hormone auxin (Dun et al., 2006). The absence of apical dominance in DWL plants provoked the experiments with exogenous administration of auxins.

The two-months old in vivo adapted plants were sprayed with solutions of NAA (1mg/l) and IAA (1mg/l) but it didn’t turn dwarf phenotype back to the original morphology. Negative effect was observed when plants were treated with 1mg/l IAA. The sprayed plants show signs of stress associated with yellowing, the leaves curling and necrotic stains.
The shoot regenerates were cultivated on media supplemented with NAA (1 mg/l) and IAA (1 mg/l). The applied concentration had inhibitory effect to the \textit{in vitro} cultured DWL plants (Table 1). Plant steroid hormones called brassinosteroids (BRs) control cell expansion and division, senescence, vascular development, photomorphogenesis and stress responses. BR biosynthesis or perception defective mutants display dwarf phenotypes due to reduced cell elongation. When BR were applied exogenously at nanomolar to micromolar levels, they exhibit a wide spectrum of physiological effects, including promotion of cell elongation and division, enhancement of tracheary element differentiation, enhancement of gravitropic induced bending, promotion of ethylene biosynthesis, and enhancement of stress resistance (Nakamura et al., 2003).

In this study, we were interested in the effect of a brassinosteroid hormone 24-epibrassinolide (EBR) on DWL plants applied by spraying or added into the culture media. The \textit{in vivo} cultivated plants were sprayed with 1 ml solutions of EBR (5 µM and 10 µM). No positive effects, even slightly inhibitory effect were observed. But \textit{in vitro} cultured DWL plants overcome the brunched phenotype and the regenerants had larger leaves and elongated stems (Figure 3).

![Images of DWL plants](image-url)
CONCLUSIONS

Dwarflike plants were produced from *Paulownia tomentosa* seeds using EMS. Morphological characteristic exhibit altered growth behavior than original plants and the conducted hormonal analysis revealed that DWL plants are sensitive to gibberellins, auxins and brassinosteroids.

The lack of apical dominance in DWL plants was not a result of IAA or NAA deficiency because the exogenic allayed hormones didn’t change the bush phenotype.

The changes that were provoked by gibberellins and auxins could not return the original phenotype, therefore dwarfing was not due to shortage of these plant growth regulators.

Only EBR applied to *in vitro* culture resulted in overcoming dwarflike look. Therefore the dwarf phenotypes could be the result of reduced BR biosynthesis or through up- or down- regulation of genes linked to these hormones.

REFERENCES


