Abstract
Comparative study of the technological characteristics of the newly-selected clone 5/76 of Pamid variety and clone Pamid Ruse 1, grown in the soil and climatic conditions of Pleven (Central Northern Bulgaria) was carried out. The study included three consecutive vintages (2006 - 2008). It was found that Pamid clone 5/76 greatly surpassed the productivity and grapes quality of the control - Pamid Ruse 1. The mechanical analysis did not reveal any significant differences in the cluster and berry structure and composition of the investigated clones, as well as in their theoretical yield. Pamid clone 5/76 showed better sugar accumulation capacity. There were no significant differences in the acidity content of the grapes of the two clones. The wines produced from the newly-selected clone Pamid 5/76 had higher alcohol, sugar-free extract, total phenolic compounds and anthocyanins content compared to the control samples from Pamid Ruse 1. In the organoleptic analysis the samples from Pamid 5/76 were assessed the highest. These variants had more intense, vivid color, the soft, elegant tannins dominated in their taste.

Key words: Pamid, clone, grapes, wine, mechanical analysis, chemical composition, organoleptic profile.

Introduction
Pamid is an old local variety grown in Bulgaria since time immemorial, typical of the Black Sea ecologic-geographical region (Constantinescu et al., 1960; Zirojevic, 1974; Kondarev et al., 1976). It is widely distributed in all the Balkan countries under different names: Plovdiv (Macedonia, Serbia), Koplik (Albania), Rishioara (Romania), Sarachibuk (Turkey), Andrionopolitka (Greece). It is also grown in Hungary, Moldova and Ukraine. In the past it was the most widely raised variety in Bulgaria due to its adaptability to soil and climatic conditions and its high yield. Since the middle of the 20th century, the areas planted with Pamid in Bulgaria had declined sharply as in recent years, they were very limited. It is mainly distributed in South and Southwest Bulgaria (Radulov et al., 2004).

Pamid variety has the most recorded and registered mutational changes, mainly due to its long time growing and multiplication and the significant climatic changes over the years. Its great intraspecies diversity created favorable conditions for efficient clonal selection aiming to improve the economic features of the variety (Kondarev et al., 1972). In Bulgaria over the years more than 20 variations have been outlined as based on the positive traits 4 clones were selected and established - Clone 1 with dark red skin (Kondarev et al., 1985), Clone 2 with lighter red skin (Kondarev et al., 1984), Pamid Ruse 1 (Todorov, 1989) and Pamid clone 5/76 (Nakov et al., 2011).

In recent years, in the major grapes and wine regions of Europe there had been an increased interest in the strict differentiation of their wines from those from other regions, and that was carried out primarily through the cultivation of local varieties and clonal selection of clones with better agro-biological and technological indicators (Petrov et al., 2009; Loureiro et al., 2011).

The objective of this study was to make comparative technological characteristics of the newly-selected clone Pamid 5/76 and clone Pamid Ruse 1 grown in the soil and climatic conditions of Pleven, Central Northern Bulgaria.

Material and Methods
The study was carried out in the period 2006-2008 in accordance with the approved methodology for clonal selection (Katerov et al., 1990) in clonal section of the Experimental Base at the Institute of Viticulture and Enology (IVE) – Pleven.
The technological traits of the newly-selected in IVE clone Pamid 5/76 were investigated. Clone Pamid Ruse 1 was used for control. The vines of the studied clones were grafted on rootstock Riparia x Berlandier CO4 at planting distance 2.20/1.30 m. They were grown on ground training system - single Guillot, with equal individual loading per vine – 18 winter eyes (3 x 2 eyes and 1 x 12 eyes).

The vintage of the studied clones of Pamid variety was performed upon the grapes reaching technological maturity in mid September (2006, 2008) and the last week of the month (2007). The grapes were processed in the experimental winery of IVE - Pleven by the classical technology for red wines production (Amerine et al., 1972; Yankov et al., 1992). Each vinificated sample was 30 kg. The sugar content of the experimental variants was proportionally adjusted by adding refined sucrose for obtaining wines with normal alcohol content and chemical composition. After crushing, the grape pulp was sulphited by 50 mg·l\(^{-1}\) SO\(_2\). The alcoholic fermentation was conducted with pure culture dry wine yeast Saccharomyces cerevisiae for red wines, at a dose of 10 g·hl\(^{-1}\) and temperature 25\(^{\circ}\)C. The ongoing process was monitored daily with laboratory hydrometer, reflecting the change in the relative density of the fermenting mass, respectively reduction of the must sugars content. Upon the process completion the young wines were decanted and sulphited to 20 mg·l\(^{-1}\) free SO\(_2\).

The chemical composition of grapes must and the obtained experimental wines were analyzed by the conventional winemaking methods referring the main indicators (Ivanov et al., 1979). The differences in the organoleptic qualities of wines regarding color, flavor, taste and general overview were determined by a 100-point scale (Tsvetanov, 2001) and by the method of the main features through spider diagrams (Prodanova, 2008).

Results and Discussion

It was found that the newly-selected clone 5/76 of Pamid variety greatly surpassed the productivity and grapes quality of the control (Pamid Ruse 1).

The average weight of a cluster of Pamid 5/76 clone was 345.9 g, compared to 256.9 g of Pamid Ruse 1. Over the years, the values ranged from 303.0 g to 401.0 g for the clone and from 210.0 g to 280.8 g for the control. The greater average weight of a cluster and better fertility of the newly-selected clone determined the annual high and stable average yield per vine – 6.238 kg (from 6.010 kg to 6.363 kg). In Pamid Ruse 1 it was 3.608 kg, which during the period of the study was within the range from 3.090 kg to 4.480 kg (Table 1).

The mechanical analysis did not reveal any significant difference in the cluster composition of the studied clones. The ratio of bunches was 3.37% (2.50% - 4.27%) of Pamid Ruse 1 and 3.58% (2.99% - 4.44%) of Pamid 5/76 and of the berries - 96.63% (97.50% - 95.73%) and 96.42% (97.01% - 95.56%), respectively.

Pamid Ruse 1 was distinguished by a greater average weight of 100 berries – 276.67 g, compared to Pamid 5/76 clone – 235.67 g. Depending on the weather conditions over the years, their values varied significantly - in the range of 220.00 g to 335.00 g of the control and 195.00 g to 290.00 g of the clone. The difference in berry weight had an effect on their structure. In Pamid Ruse 1 the share of the solid fraction was smaller - 5.38% (4.78% - 6.36%) skin and 2.88% (2.54% - 3.22%) seeds with higher content of mesocarp respectively - 91.74% (90.42% - 92.46%). The ratio of the skins in the berry of Pamid 5/76 clone was 6.16% (5.17% - 6.82%), of seeds - 3.13% (2.55% - 3.58%), and of mesocarp - 90.71% (89.60% - 92.28%). The lowest values of the berry weight and the greatest share of the solid fraction, both for the clone and the control were reported in 2007 (Table 1).

The difference in the theoretical yield was insignificant in favour of the control and was mainly determined by the mesocarp content in the berries. The theoretical yield of Pamid Ruse 1 was 88.65%, which during the period of the study ranged from 87.41% to 90.15%. Of Pamid 5/76 clone it was 87.46% in the range from 86.64% to 90.15% (Table 1).

Differences were observed when comparing the technological characteristics of the studied clones of Pamid variety. Figure 1 shows the chemical composition of the grapes - the sugars content, titratable acidity and pH of the clones throughout the period of study. The differences in these indicators were due to the agro-biological features and the impact of weather conditions during the year.

Data presented in Figure 1 showed that the most favorable for both studied clones of Pamid variety was the year 2006. Grapes from that vintage had good sugar accumulation and content of titratable acids, due to the favorable weather conditions during the ripening stage. The most unfavorable year was 2007, when sugars concentration in the grapes of both clones was the lowest for the period of study.
Table 1. Mechanical and chemical analysis of cluster and berry of clones of varieties Pamid for 2006-2008 period.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Year</th>
<th>Average weight per cluster</th>
<th>Average yield per vine</th>
<th>Average weight per 100 berries</th>
<th>Mechanical analysis of:</th>
<th>Theoretical yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g</td>
<td>kg</td>
<td>g</td>
<td>Cluster</td>
<td>Berry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bunches</td>
<td>berries</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Pamid Ruse 1</td>
<td>2006</td>
<td>210,0</td>
<td>3,255</td>
<td>335,00</td>
<td>4,27</td>
<td>95,73</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>280,0</td>
<td>4,480</td>
<td>220,00</td>
<td>3,33</td>
<td>96,67</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>280,8</td>
<td>3,090</td>
<td>275,00</td>
<td>2,50</td>
<td>97,50</td>
</tr>
<tr>
<td></td>
<td>average</td>
<td>256,9</td>
<td>3,608</td>
<td>276,67</td>
<td>3,37</td>
<td>96,63</td>
</tr>
<tr>
<td>Clone 5/76</td>
<td>2006</td>
<td>333,8</td>
<td>6,340</td>
<td>290,00</td>
<td>4,44</td>
<td>95,56</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>303,0</td>
<td>6,363</td>
<td>195,00</td>
<td>3,30</td>
<td>96,70</td>
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<tr>
<td></td>
<td>2008</td>
<td>401,0</td>
<td>6,010</td>
<td>222,00</td>
<td>2,99</td>
<td>97,01</td>
</tr>
<tr>
<td></td>
<td>average</td>
<td>345,9</td>
<td>6,238</td>
<td>235,67</td>
<td>3,58</td>
<td>96,42</td>
</tr>
</tbody>
</table>

Table 2. Chemical composition and taste evaluations of experimental wines of clones of varieties Pamid for 2006-2008 period.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Year</th>
<th>Alcohol vol. %</th>
<th>Sugars g.l(^{-1})</th>
<th>Sugar-free extract g.l(^{-1})</th>
<th>Volatile acids g.l(^{-1})</th>
<th>Titratable acids g.l(^{-1})</th>
<th>pH</th>
<th>Anthocyanins mg.l(^{-1})</th>
<th>Total phenolic compounds g.l(^{-1})</th>
<th>Taste evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pamid Ruse 1</td>
<td>2006</td>
<td>12,42</td>
<td>1,33</td>
<td>19,10</td>
<td>0,58</td>
<td>5,43</td>
<td>3,19</td>
<td>126,00</td>
<td>1,12</td>
<td>78,00</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>11,71</td>
<td>1,98</td>
<td>18,22</td>
<td>0,60</td>
<td>4,20</td>
<td>3,31</td>
<td>107,30</td>
<td>0,95</td>
<td>74,72</td>
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<tr>
<td></td>
<td>2008</td>
<td>12,24</td>
<td>1,98</td>
<td>18,42</td>
<td>0,48</td>
<td>4,30</td>
<td>3,28</td>
<td>118,50</td>
<td>1,07</td>
<td>75,90</td>
</tr>
<tr>
<td>Clone 5/76</td>
<td>2006</td>
<td>12,56</td>
<td>1,30</td>
<td>19,47</td>
<td>0,57</td>
<td>5,25</td>
<td>3,20</td>
<td>144,00</td>
<td>1,18</td>
<td>78,83</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>11,92</td>
<td>1,91</td>
<td>18,52</td>
<td>0,47</td>
<td>4,23</td>
<td>3,33</td>
<td>115,60</td>
<td>1,02</td>
<td>75,50</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>12,45</td>
<td>1,78</td>
<td>18,70</td>
<td>0,54</td>
<td>4,00</td>
<td>3,37</td>
<td>128,80</td>
<td>1,13</td>
<td>76,50</td>
</tr>
</tbody>
</table>
It was observed similarity about sugar accumulation capability of both clones. During the study the newly-selected clone 5/76 surpassed the control in sugars content, as the difference was the most significant for vintage 2006 harvest and not so marked in the other years. The concentration of sugars in clone 5/76 ranged from 17.8% (2006) to 19.6% (2006) and in the control clone Ruse 1 - from 17.2% (2007) to 18.6% (2006). Despite the high sugars in vintage 2006, the highest titratable acids for the period were also accounted, respectively 5.63 g.l⁻¹ (Ruse 1) and 5.33 g.l⁻¹ (5/76). In 2007 and 2008, it was observed lower acid content of the grapes, however within the range typical for the variety. No significant differences were found for these vintages between the amount of acids of both clones, although the slightly higher levels reported in the control – 4.90 g.l⁻¹ (2007) and 4.45 g.l⁻¹ (2008), therefore the values of pH were close or equal. The chemical composition and organoleptic profile of wines obtained from the three vintages are presented in Table 2 and Figure 2. The differences in the grapes composition of the investigated clones throughout the period of study, determined the composition and tasting characteristics of the experimental wines. Correlation was found between alcohol content and the sugars amount in the grapes. The lowest rate of alcohol had the wines from vintage 2007. As a result of the complete alcoholic fermentation the concentration of residual sugars in the samples ranged from 1.30 g.l⁻¹ to 1.98 g.l⁻¹. Titratable acidity in the experimental variants was within the range typical for the variety, from 4.00 g.l⁻¹ to 5.43 g.l⁻¹. In conformity with must composition the highest values (over 5.00 g.l⁻¹) had wines, vintage 2006. Acidity determines the freshness of taste, so that samples of this vintage had more harmonious taste indicators. There were no significant differences in the content of titratable acids between samples obtained from the studied clones of the respective vintages. All experimental variants throughout the period of the study had normal volatile acidity ranging from 0.47 g.l⁻¹ to 0.60 g.l⁻¹.
Typical of Pamid wines was that they were low extractive, which was considered their varietal feature. Sugar-free extract (SFE) is an indicator having an effect on their taste characteristics. SFE values of the experimental wines from both investigated clones were within the range from 18.22 g.l\(^{-1}\) to 19.47 g.l\(^{-1}\). No significant differences of SFE were observed in the wines from the experimental variants, vintages 2007 and 2008, however its content was significantly higher in the samples from vintage 2006 (Table 2). Probably that was due to the good weather conditions in the year having favorable impact on grapes ripening and sugar accumulation. During the period of the study wines from clone 5/76 surpassed the control in SFE content, which determined their better dense and extractive taste and higher taste scores respectively (Figure 2). SFE quantity in the samples of the newly-selected clone 5/76 was in the range of 18.52 g.l\(^{-1}\) (2007) to 19.47 g.l\(^{-1}\) (2006), while in the variants of the control clone Ruse 1 – from 18.22 g.l\(^{-1}\) to 19.10 g.l\(^{-1}\).

Anthocyanins and total phenolic compounds (TPC) content is essential for the red wines organoleptic profile. These components of wine composition affect accordingly the color and the taste density. The investigated clones did not reveal significant differences for these indicators both during the different vintages and as a result of their clonal specificity. Data on the composition of the experimental samples showed a correlation in their content. Their values varied within a narrow range, and were typical of the variety (Table 2). The results revealed that the amount of anthocyanins, respectively TPC was the highest in samples, vintage 2006, and the lowest in vintage 2007. That tendency was observed for both studied clones. Wines obtained from clone 5/76 exceeded although insignificantly wines from the control Ruse 1 in anthocyanins and TPC content. Their values in the variants of 5/76 ranged from 115.60 mg.l\(^{-1}\) to 144.00 mg.l\(^{-1}\) and from 1.02 g.l\(^{-1}\) to 1.18 g.l\(^{-1}\), and in the variants of Ruse 1 - from 107.30 mg.l\(^{-1}\) to 126.00 mg.l\(^{-1}\) and from 0.95 g.l\(^{-1}\) to 1.12 g.l\(^{-1}\).

The differences in the composition of wines determine their different tasting characteristics. In the organoleptic analysis the samples, vintage 2006, respectively Pamid 5/76 (78.83 scores) and Pamid Ruse 1 (78.00 scores) were evaluated the highest (Table 2). These wines had more intense, vivid color due to the higher concentration of anthocyanins and better taste indicators for freshness, density and harmony. This was due to the higher content of titratable acids, sugar-free extract and the phenolic compounds in their composition with prevailing of soft, elegant tannins (Figure 2).

Conclusions

1. The newly-selected Pamid clone 5/76 greatly surpassed the productivity and grapes quality of the control - Pamid Ruse 1. The mechanical analysis did not reveal any significant differences in the cluster structure of
the investigated clones. The difference in their theoretical yield is insignificant in favour of the control and it was determined mainly by the mesocarp content in berries.

2. Grown in the soil and climatic conditions of Pleven, Pamid 5/76 clone had better sugar accumulation capability. There were no significant differences in the acid content of the grapes of the investigated clones, which was within the range typical for the variety.

3. The wines produced from the newly-selected clone Pamid 5/76 had higher alcohol, sugar-free extract, total phenolic compounds and anthocyanins content compared to the control samples from Pamid Ruse 1.

4. In the organoleptic analysis the samples from Pamid 5/76 were assessed the highest – the wines had more intense, vivid color, the soft, elegant tannins dominated in their taste.

References