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Vegetative and Reproductive Performances of Plum Trees of 'Elena' Cultivar, after Application of Conventional and Biological Fertilizers

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SUMMARY

2016-2018
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-
- 43.80 m (2016 .);
45.53 m (2017 .) 46.88 m (2018 .).
29.32g.
-
(15 kg/).
:
,

In the period 2016-2018 an experiment was conducted at the Research Institute of Mountain Stockbreeding and Agriculture - Troyan on the impact of conventional and biological fertilizers on the vegetative and reproductive characteristics of plum trees of 'Elena' cultivar.

The results of the studies show the most pronounced trunk growth in conventional fertilization – 43.80 cm (2016); 45.53 cm (2017) and 46.88 cm (2018).

It was found that granulated chicken manure increased the average weight of plum fruit up to 29.32 g. The conventional fertilizer variant was characterized by the highest fruit yield (15 kg/tree) over the three-year experimental period.

Key words: plum, cultivar, fertilization, fruit weight, yield

INTRODUCTION

Plum is most adaptive in regions with temperate climate (Ertekin et al., 2006; Blazek, 2007; Kumar et al., 2018), but widely spread throughout the world (Son, 2010).

The production of annual high quality fruit harvest depends on the cultivar, growth power and age of the orchard trees (Lichev et al., 2004; Dzhuvinov et al., 2014; Miletovic et al., 2015).

Cultivated plants have particularly high nutrient needs in their growth phases. The balanced fertilization, in optimal form and concentration (Todorova and Boteva, 2015), consistent with soil characteristics and cultivar needs, is also a prerequisite for obtaining high yields of high quality (Bozhinova, 2017).

The aim of the study is to investigate the impact of different types of fertilization – (biological, conventional and organic) on the vegetative and reproductive performance of plum tree cultivars of 'Elena'.

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MATERIAL AND METHODS

The plantation with the German introduced cultivar 'Elena' was created in the spring of 2001 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture. The fruit trees are planted in trenches with a planting pattern of 4.0/2.5 m.

The row spacing is grassed with tall fescue and the intra row spacing is kept in black fallow. The crop is grown according to its agro-technical requirements.

In the spring of 2016, the following fertilization variants were set in the plum plantation:

- I variant – Bio-fertilization – including fertilizers: Agriful (soil) – 5 l/da, Tecamin Flower (leaf) – 0.3%, Tecnocel

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<p>Ca () – 0,4% ;</p> <p>• II –</p> <p>Yara Mila Complex () – 0.500 kg/ , YaraVita Frutrel () – 0.500 ml/da, Yara Vita Universal Bio () – 0.500 ml/da</p> <p>• III –</p> <p>– 0.500 kg/</p> <p>• IV –</p> <p>:</p> <p>• –</p> <p>15-20 ;</p> <p>• –</p> <p>;</p> <p>:</p> <p>• –</p> <p>,</p> <p>;</p> <p>• (g) – , -</p> <p>25 -</p> <p>;</p> <p>• ;</p> <p>kg/ ;</p> <p>• Ca –</p> <p>;</p> <p>• Yara Mila Complex – 2016 2018 .;</p> <p>• –</p> <p>2017 . – 0.220</p> <p>kg/ ;</p> <p>• YaraVita Frutrel –</p> <p>,</p> <p>;</p> <p>• Yara Vita Universal Bio –</p> <p>,</p> <p>;</p> <p>•</p> <p>2016 2018 .</p> <p>-</p> <p>(Nedev et</p>	<p>Amino Ca (leaf) – 0.4%;</p> <p>• II variant – Conventional – Yara Mila Complex (soil) – 0.500 kg/tree, YaraVita Frutrel (leaf) – 0.500 ml/da, Yara Vita Universal Bio (leaf) – 0.500 ml/da</p> <p>• III variant – Granulated Chicken Manure – 0.500 kg/tree</p> <p>• IV variant – Control</p> <p>Fertilization periods:</p> <p>• Agriful – applied five times from the beginning of the vegetation for a period of 15-20 days;</p> <p>• Tecamin Flower – 2 applications. Applied before blossoming and during the formation of a fruit-set;</p> <p>Reproductive manifestation:</p> <p>• useful fruit-set share – reported thirty days after mass flowering, on a marked branch;</p> <p>• average weight per fruit and stone (g) – determined by measuring 25 randomly selected fruits and their stones, a technical scale was used;</p> <p>• average fruit yield kg/tree;</p> <p>• Tecnocel Amino Ca – 2 applications. Applied after blossoming and a month before harvesting;</p> <p>• Yara Mila Complex – 1 application in 2016 and 2018;</p> <p>• Ammonium nitrate – 1 application in 2017 – 0.220 kg/tree;</p> <p>• YaraVita Frutrel – 4 applications. The first application was at the phase of winter buds, at the phase of white button, at the fruit-set formation and a month before the harvest;</p> <p>• Yara Vita Universal Bio – 3 applications. Applied before and after bloom and after harvest;</p> <p>• Granulated chicken manure – one application in 2016 and 2018.</p> <p>Each fertilizer variant includes four trees.</p> <p>According to the methodology for study of plant resources (Nedev et al.,</p>
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al., 1979)

• (cm) –

• (cm) –

• (m³) –

$V = \frac{\pi d^2 h}{12}$, m –

• (cm) –

40 m

• (cm) –

5 cm

1979) the vegetative and reproductive indicators of the fruit trees are taken into account over a three-year period:

Vegetative indicators:

- crown height (cm) – measured from the first skeleton branch to the top,
- crown width (cm) – recorded in two directions – to the row spacing and along the row;
- crown volume (m³) – calculated by the formula $V = \frac{\pi d^2 h}{12}$, m³, including d - diameter, m – average of the two mutually perpendicular directions; h – crown height, m;
- trunk circumference (cm) – measured on a marked ring at a height of 40 cm from the soil surface after the end of the vegetation;
- Annual shoot length growth – the indicator includes an average length (cm) of shoots longer than 5 cm and an annual shoot length over marked branches after the end of vegetation.

RESULTS AND DISCUSSION

The vegetative indicators are related to the growth potential of the cultivar and rootstock combination, the planting density, pruning, harvesting and soil surface maintenance (Dzhuvinov et al., 2012). Climatic conditions (sunlight, temperature, humidity, precipitation) (Legave et al., 2006) also have an impact.

The reported vegetative indicators for the period of 2016-2018 are presented in Table 1. The experiment showed a dynamics in the annual growth of the trunk circumference, most pronounced in the conventional fertilization variant - 43.80 cm (2016); 45.53 cm (2017) and 46.88 cm (2018), and during the three-year period, the indicator had a low coefficient of variation (CV, %).

(Dzhuvinov et al., 2012).

() (Legave et al., 2006).

2016-2018

1.

– 43.80 m

(2016 .); 45.53 m (2017 .) 46.88 m

(2018 .),

e

(CV, %).

Table 1. Vegetative indicators for 'Elena' cultivar for the period 2016-2018

/Indicators /Variant	Trunk diameter cm	Crown height m	Crown width in the row m	Crown width in the row spacing m	Crown volume m ³	Annual shoot length growth cm	Total annual shoot length growth cm
2016							
I Bio-fertilization	42.63	4.72	4.26	4.08	21.48	9.88	39.52
II Conventional	43.80	4.87	3.67	4.11	19.28	11.12	44.50
III Chicken manure	43.28	4.24	4.20	4.27	19.90	8.74	34.96
IV /Control	45.50	5.39	3.89	4.12	22.62	9.90	39.62
St error	0.61	0.24	0.14	0.04	0.76	0.48	1.95
St Dev	1.23	0.47	0.27	0.08	1.51	0.97	3.89
CV %	2.80	9.86	6.89	2.05	7.28	9.81	9.83
LSD _{0.05}	ns	ns	ns	ns	ns	ns	-
2017							
I Bio-fertilization	43.63	4.72	3.86	4.31	20.61	9.07	36.28
II Conventional	45.53	5.79	4.02	4.44	27.11	10.53	42.12
III Chicken manure	43.95	5.24	4.11	4.30	24.24	9.54	38.16
IV /Control	46.58	6.37	3.95	4.32	28.50	7.58	30.30
St error	0.69	0.35	0.05	0.03	1.74	0.61	2.46
St Dev	1.38	0.71	0.10	0.06	3.49	1.23	4.92
CV %	3.08	12.84	2.66	1.51	13.89	13.38	13.40
LSD _{0.05}	ns	ns	ns	ns	ns	ns	-
2018							
I Bio-fertilization	44.23	4.33	2.99	4.20	14.88	8.68	34.74
II Conventional	46.88	4.25	3.28	3.56	14.61	9.96	39.83
III Chicken manure	44.83	4.50	3.10	3.46	14.42	12.28	49.10
IV /Control	47.18	4.69	3.26	3.43	16.89	13.08	52.32
St error	0.73	0.09	0.06	0.18	0.57	1.01	4.06
St Dev	1.47	0.19	0.13	0.36	1.14	2.03	8.13
CV %	3.21	4.27	4.12	9.83	7.5	18.45	18.48
LSD _{0.05}	ns	ns	ns	ns	ns	ns	-

(2004)

Costes et al.

With respect to the vegetative indicators of crown height and width along the row and towards the row spacing during the experimental period, there were no significant differences between the fertilization variants and the control. According to Costes et al. (2004), the final size of trees is a cumulative

2016 2017 . -
 (11.12 cm; 44.50 cm 10.53 cm;
 42.12 cm), 2018 . -
 : 9.81 18.48%.
 (p>0.05)
 (kg)
 (1). 2016 .
 22.85 kg, 2017 . – 13.98 kg 2018 . –
 8.45 kg.
 2018 (-
)

, variable, depending on the annual shoot
 , length growth, which is formed each year.
 In 2016 and 2017, the highest annual and
 - total growth rates were found in
 - conventional fertilization (11.12 cm, 44.50
 - cm and 10.53 cm, 42.12 cm), whereas in
 2018 higher values were recorded in the
 control variant.

The average annual and total growth over
 the three-year period was characterized
 by a high variation coefficient ranging
 from 9.81 to 18.48%.

All the vegetative indicators studied
 had a statistically unproved difference (p>
 0.05) between the experimental variants.

The highest average yield per tree
 (kg) for 'Elena' cultivar over the three
 experimental years was found in the
 conventional fertilization variant (Figure
 1). In 2016, the average quantity of fruit
 obtained from that variant was 22.85 kg,
 2017 – 13.98 kg and 2018 – 8.45 kg. The
 climate changes in 2018 (the higher
 humidity in February, March, June and
 July, and the subsequent drought in
 August) had a negative impact and were
 the cause of lower yields of plum
 plantations.

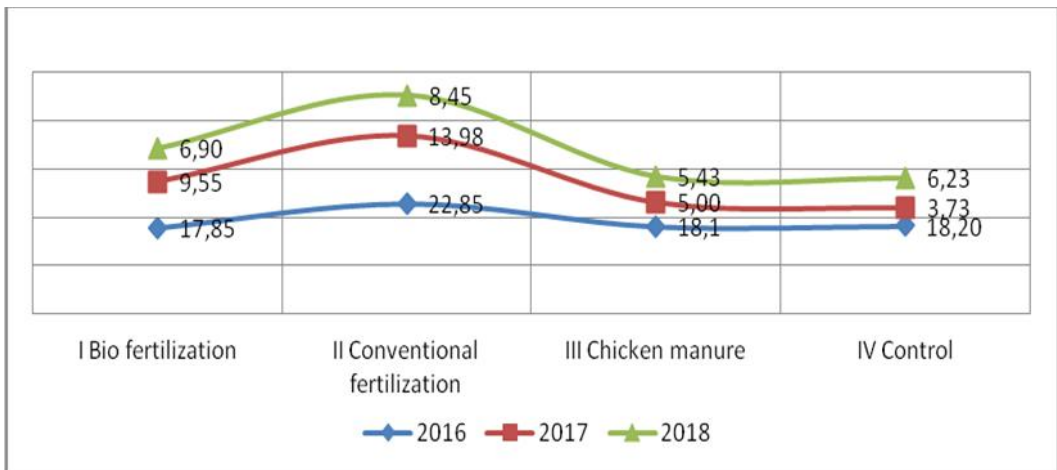


Fig. 1. Average yield of fruit, kg/tree of 'Elena' cultivar

(Lace et al., 2015), (Gitea et al., 2019) (Guerra and Casquero, 2009).

The average fruit weight is an important quality indicator of the yield. It is a cultivar characteristic (Lace et al., 2015), influenced by climatic (Gitea et al., 2019) and agro-technical conditions (Guerra and Casquero, 2009). Figure 2 presents the results of the analyzed reproductive performance average fruit weight (g).

(g).

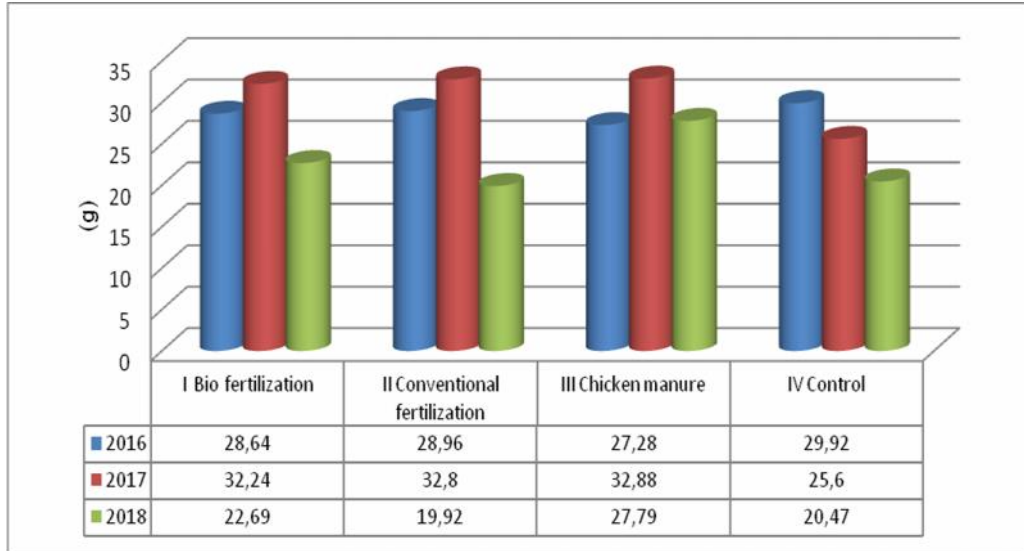


Fig. 2. Average fruit weight (g) of 'Elena' cultivar

g.

32.88g (P<0.05) 27.79g (P>0.05).

(2009), (2008) 38.76g. Blazek and Pistekova Walkowiak-Tomczak

The highest average fruit weight in the first year of the experiment was found in the control variant – 29.92 g. In the next two years, the largest fruit size was found in the variant with application of granulated chicken fertilizer – 32.88g (P <0.05) and 27.79g (P> 0.05). Similar results for the fruit weight are reported by Blazek and Pistekova (2009), but according to Walkowiak-Tomczak et al. (2008) the weight can reach 38.76g.

CONCLUSIONS

The data of this investigation show that conventional fertilization had a greater effect on the growth rate and higher values of the tree trunk

(kg/),
(29.32 g).

circumference for the plum cultivar 'Elena'.

The highest average fruit weight (29.32 g) was found in the variant with granulated chicken manure.

The highest fruit yield per tree (kg/tree), over the three-year experimental period was found in the variants with conventional and organic fertilizers.

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CaCl₂

(*Malus domestica* Borkh.)

Effect of CaCl₂ Application on Yield and Quality of Economically Important Apple Cultivars (*Malus domestica* Borkh.)

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SUMMARY

2018 .
(CaCl₂)
(„Gloster 69 , Golden Reinders , Granny Smith , Morrens Jonagored , and Red Chief).
l₂ (21
)
„Red Chief
„Granny
” (73.50 t/ha⁻¹),

During 2018 at Fruit Research Institute in a ak, a research on sodium chloride (CaCl₂) application on the yield and quality of fruit of five introduced apple cultivars (Gloster 69 , Golden Reinders , Granny Smith , Morrens Jonagored , and Red Chief) was conducted. Treatments were performed with CaCl₂ solution in four times (each 21 day from the beginning of June until the mid of August).

In harvest period of investigated cultivars, morphometric characteristics of fruit, firmness and soluble solids content in the fruit as well as yield per unit area were determined. The highest yield per unit area was found in cultivar Red Chief (73.50 t ha⁻¹), nd the lowest in Granny Smith (45.83 t ha⁻¹).

Smith" (45.83 t/ha⁻¹).
 Morrens Jonagored (218.92 g;
 70.64 mm 80.97 mm),
 "Golden Reinders"
 (147.25 g) (70.55 mm).
 -
 CaCl₂.
 Morrens Jonagored
 Golden Reinders
 (12.91°Brix) -
 (12.61°Brix),
 (8.93°Brix)
 „Gloster 69 ”.
 CaCl₂, :

- Based on morphometric characteristics,
 - cultivar Morrens Jonagored (fruit weight – 218.92 g; height and width – 70.64 mm and 80.97 mm, respectively) singled out as an apple cultivar with large fruits.

On the other hand, cultivar Golden Reinders had the lowest weight (147.25 g) and fruit dimensions (height – 63.94 mm and width – 70.55 mm). In all examined cultivars, application of CaCl₂ resulted in greater flesh firmness. In respect of the soluble solids content, cultivars Morrens Jonagored (12.91 ° Brix) and Golden Reinders (12.61 ° Brix) singled out, whereas the lowest soluble solids content (9.05 ° Brix) was determined in the fruit of the cultivar Gloster 69 .

Key words: apple, cultivar, CaCl₂, yield, fruit quality

INTRODUCTION

Total annual production of apple in the world amounts about 83.139.326 t, of which in the Republic of Serbia is produced about 378.644 t on approximately 25.134 ha under apple (Faostat, 2017). In the structure of fruit production in our country, apple is second, right behind plum, while in European production, it takes 12th place.

Good market supply with diverse apple fruits demands an offer of fruits with high external and internal quality characteristics (O'Rourke, 2003). Sams et al. (2008) states that many physiological and pathological disturbances of apple fruits are related to the content of calcium (Ca²⁺) in the tissue of the fruit.

With this regard, concentration of calcium in plant tissue has an extremely important role in maintaining the quality of fruits after harvest.

Application of foliar fertilizer based on calcium positively affects cell membrane

83.139.326 t,
 378.644 t,
 25.134 ha (Faostat,
 2017).
 -
 12-
 (O'Rourke, 2003). Sams et al.
 (2008)
 (Ca²⁺)

(Pervaiz et al., 2002; Hossain et al., 2005; Abdi et al., 2006; Misra and Gupta, 2006; Naeem et al., 2009),

("bitter pit")

(Raese and Drake, 2002; Dierend Rieken, 2007).

(Lester and Grusak, 2004).
Gvozdrenovi (1998)

("bitter pit") (Fellahi et al., 2010). Zavalloni et al. (2001)

Stopit,

2006
233 m (43°89'40 "N
20°43'42" E),

stabilization and delays senescence (Pervaiz et al., 2002; Hossain et al., 2005; Abdi et al., 2006; Misra and Gupta, 2006; Naeem et al., 2009), maintains fruit firmness, decreases occurrence of the so called 'bitter pit' and internal rot in fruits (Raese and Drake, 2002; Dierend and Rieken, 2007).

There are numerous foliar fertilizers containing calcium that are used before and after harvest in order to delay senescence with no harmful effect on the consumers (Lester and Grusak, 2004). According to Gvozdrenovi (1998), need of apple fruit for calcium is very low and in the lack of it all aging processes in the fruit flow much faster and fruits have lower storage capacity.

Calcium application efficiency is determined by the application time in a way that earlier application during vegetative period is far more efficient than the later in terms of reduction of 'bitter pit' (Fellahi et al., 2010). Zavalloni et al. (2001) emphasize that foliar application of a composition containing calcium in thick apple plantations resulted in a slower leaf-to-fruit mobility of calcium.

The aim of this study was to determine the effect of foliar fertilizer Stopit application on yield and qualitative traits of apple fruit, which would significantly improve the quality of fruit during storage.

MATERIAL AND METHODS

The study was conducted in the production-experimental apple orchard at site Donja Trep a, of the Fruit Research Institute in a ak. Apple orchard was set up in 2006 and located at an altitude of 233 m (43° 89' 40" N and 20° 43' 42" E). The growing shape is slender spindle. During the study, standard agro-technical and plantation maintenance measures were applied.

: 'Gloster 69', 'Golden Reinders', 'Granny Smith', 'Morrens Jonagored' and 'Red Chief'.

9, 4 m x 1.25 m (2000 h⁻¹), „Red Chief“, 4 x 1 m (2500 ha⁻¹).

2018 . Stopit, (160 g L⁻¹).

Yara

(5-10 L ha⁻¹)

7 20 , (80 Stopit 7.5 L ha⁻¹ (150 mL

10 L). Stopit 21

7 , 2 , 20 15

SR 420 (STIHL International GmbH Waiblingen,) 1000 L ha⁻¹.

Stopit (kg ha⁻¹),

: „Gloster 69“ 10 ; „Morrens Jonagored“ 11 ; Red Chief”

The study included five apple cultivars: 'Gloster 69', 'Golden Reinders', 'Granny Smith', 'Morrens Jonagored' and 'Red Chief'. All cultivars were grafted on the rootstock M9, and the tree spacing was 4 m x 1, 25 m (2,000 trees ha⁻¹), apart from the cultivar 'Red Chief' which was planted at a distance of 4 x 1 m (2,500 trees ha⁻¹).

The study was conducted in 2018 by applying a liquid foliar fertilizer 'Stopit', based on calcium chloride with high concentration of calcium (160 g L⁻¹).

The above mentioned fertilizer is produced in the corporation 'Yara' in Great Britain and it can be applied together with pesticides. It is used from the beginning of flowering until the end of fruit maturation phenophase (the phase of pigmentation until harvest). The recommended amount is 5-10 L ha⁻¹ and the last treatment is to be done at least 7 days before harvest.

Treatments were applied on 20 trees in four replications (total 80 trees per treatment). 'Stopit' was used in the amount of 7.5 L ha⁻¹ (150 mL on 10 L of water).

Treatments with foliar fertilizer 'Stopit' were applied four times on each 21 day during the period from early June to mid-August. The first treatment was conducted on June 7th, second treatment on July 2nd, third on July 20th and fourth on August 15th, by using motor sprayer SR 420 (STIHL International GmbH Waiblingen, Germany) with a consumption of 1,000 L ha⁻¹.

The influence of application of foliar fertilizer 'Stopit' were analysed on the basis of yield per unit area (kg ha⁻¹), morphometric properties of the fruit, as well as the fruit firmness and the soluble solids content. Yield of the tested cultivars was measured at harvest, in cultivars 'Gloster 69' and 'Morrens Jonagored' on 10th of September, in cultivar 'Red Chief' on 11th of September, in cultivar 'Golden Reinders' on 26th of September, and in

„Golden Reinders” 26 ;
 „Granny Smith” 9 .
 (g), -
 (mm), -
 (Pa), (°
 Brix) 80
 (20).
 (Adventurer
 Pro AV812M,),
 (Carl Roth, Germany). -
 (FHT-803, -
) Pa. -
 (Carl Zeiss, Jena) °Brix.
 (20 °C) (SE).
 ±
 LSD ,
 (ANOVA),
 MSTAT-C (Michigan State University,
 East Lansing, MI, USA).
 0.05 .

cultivar ‘Granny Smith’ on 9th of October.
 Testing of mass (g), height and width (mm), fruit firmness (Pa), as well as the soluble solids content in the fruit (° Brix) was carried out by conventional morphometric methods on a sample of 80 fruits (four replications of 20 fruits). Fruit weight was determined by measuring on a technical scale (Adventurer Pro AV812M, Switzerland), and the length and width of fruit by digital calliper (Carl Roth, Germany).
 Fruit firmness was determined by digital penetrometer (Model FHT-803, Italy) and the obtained values were expressed in Pa. Soluble solids content was determined using a binocular refractometer (Carl Zeiss, Jena) at room temperature (20° C) and the values were given in ° Brix.

The results are presented as mean ± standard error of mean (SE). Differences between mean values were compared by LSD test in two-way analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). Differences with *p* values of 0.05 were considered insignificant.

RESULTS AND DISCUSSION

Study results related to the apple fruit morphometric properties depending on the application of foliar fertilizer ‘Stopit’ are shown in Table 1.

Morphometric properties of apple fruit were statistically significantly influenced by cultivar and variability factor interaction determined by the analysis of variance.

'Gloster 69' (231.78 g), „Golden Reinders“ Stopit (145,66 g). Miši (1994), 500 g, 98% „ „ (120-200 g), „ „ (180-250g) (Miši , 2004). Asgharzade et al. (2012) , , , Ashour (2000), 0.5% 70.47 82.42 mm. Stopit, Morrens Jonagored (80.97 mm), „Golden Reinders” (70.55 mm). Stopit Morrens Jonagored (82.42 mm), „Golden Reinders“ (70.47 mm). 75 mm, 63.43 75.18 mm.

value of the respective parameter is registered in cultivar 'Gloster 69' with application of foliar fertilizer (231.78 g), and the lowest in cultivar 'Golden Reinders' in the treatment without 'Stopit application (145.66 g). According to Miši (1994), fruit mass of domesticated apple cultivars range from 70 to 500 g, of which 98% is edible. Fruits of the cultivar 'Golden Delicious' belong to a group of mid-large to large (120-200 g), and 'Gloster' in the group of large to very large (180-250 g) (Miši , 2004). Asgharzade et al. (2012) indicate that by application of calcium chloride solution during vegetation a higher apple fruit mass is obtained. The results obtained in these studies are consistent with the statements of Ashour (2000) who found that spraying with 0.5% calcium chloride positively affects fruit mass.

Average width values of the investigated apple fruit ranged from 70.47 to 82.42 mm. Higher width values of apple fruit were found in foliar treatment with 'Stopit though differences were not statistically significant.

Observed by cultivars, the greatest value of the above mentioned parameter was recorded in cultivar Morrens Jonagored (80.97 mm) being significantly higher in comparison with other studied apple cultivars. The smallest fruit width was found in cultivar Golden Reinders (70.55 mm). In interaction effect of variability factor, the greatest fruit width was recorded in treatment without application of foliar fertilizer 'Stopit in cultivar Morrens Jonagored (82.42 mm), and the smallest in the same treatment in cultivar Golden Reinders (70.47 mm).

According to Gvozdenovi (1998), dimensions of fruits of attractive apple cultivars should be from 65 to 75 mm, which has been confirmed in our studies, accordingly.

The average height of apples varied in the range 63.43 to 75.18 mm.

Stopit, , -
 . -
 „Gloster 69“ (71.51 mm),
 „Golden Reinders“ „Granny
 Smith“, -
 (63.94 69.73 mm,). -
 "Stopit" , -
 (75.18 mm), 'Gloster
 69' "Stopit" -
 -
 'Golden Reinders' (63.43 mm) (
 1). -
 , -
 (Krgovi , 1990; Krpina et al., 2004). -
 , Amiri et al. (2008) -
 , -
 , -
 2 .
 Stopit

Stopit

Higher values of the mentioned parameter were determined in the treatment with foliar application of fertilizer 'Stopit , in relation to the values recorded in the treatment without application. By analyzing values of the investigated apple fruit height, the greatest value is registered in cultivar 'Gloster 69' (71.51 mm) which significantly differed in comparison with cultivars 'Golden Reinders' and 'Granny Smith', which had the lowest values (63.94 and 69.73 mm, respectively). In interactive effect of application 'Stopit / cultivar, the highest average value of apple fruit height was found in treatment with foliar fertilizer application 'Stopit in cultivar 'Gloster 69' (75.18 mm), whereas the lowest value in the same treatment in cultivar 'Golden Reinders' (63.43 mm) (Table 1). Morphometric properties of apple fruit are genetically conditioned although their variation to a considerable extent can be conditioned by environmental factors (Krgovi , 1990; Krpina et al., 2004). On the other hand, Amiri et al. (2008) point out that foliar application of some fertilizers can be more efficient in terms of morphometric properties of a fruit compared to standard fertilization method.

In Table 2 are shown the results of studying foliar fertilizer 'Stopit effect on fruit firmness and soluble solids content of investigated apple cultivars.

According to the variance analysis, statistically significant effect of foliar fertilizer 'Stopit application and variability factor interaction on fruit firmness and soluble solids content in apple fruit was determined.

Table 2. Effect of application of Stopit on fruit firmness and soluble solids content of investigated apple cultivars

/Treatment		/Fruit firmness (Pa)	/Soluble solids content (°Brix)
Stopit (A)/Application Stopit (A)			
/Treatment		14.41±0.22 a	11.58±0.49 a
/Control		13.08± 0.29 b	10.77±0.24 b
/Cultivar (B)			
Gloster 69		13.68±0.35 ab	9.05±0.12 d
Golden Reinders		12.86±0.48 b	12.61±0.38 a
Granny Smith		14.54±0.42 a	11.50±0.11 b
Morrens Jonagored		13.10±0.32 b	12.91±0.52 a
Red Chief		14.53±0.49 a	9.81±0.24 c
Stopit x (x B) / Application Stopit x Cultivar (A ∩ B)			
/Treatment	Gloster 69	14.34±0.44 abc	9.18±0.21 e
	Golden Reinders	13.96±0.28 bcd	13.59±0.12 b
	Granny Smith	15.39±0.48 a	11.64±0.19 c
	Morrens Jonagored	13.49±0.44 bcd	14.23±0.28 a
	Red Chief	14.86±0.30 ab	9.28±0.24 e
/Control	Gloster 69	13.02±0.28 cde	8.93±0.09 f
	Golden Reinders	11.76±0.46 e	11.63±0.19 c
	Granny Smith	13.69±0.33 bce	11.36±0.10 c
	Morrens Jonagored	12.72±0.44 de	11.59±0.18 c
	Red Chief	14.20±0.98 abc	10.34±0.12 d
ANOVA			
A		*	*
B		*	*
A ∩ B		*	*

p 0.05 , *LSD* , *ns* –
 Values within each column followed by the same small letter are not significantly different at *p* 0.05 by *LSD* test; *ns* - non-significant differences.

Stopit (14.41 Pa) and soluble solids content (11.58°Brix) were found in apple fruit compared to the treatments without the fertilizer (13.08 Pa and 10.77 °Brix). Observed by cultivars, the greatest average value of fruit firmness was found in cultivar "Granny Smith" (14.54 Pa), and the highest soluble solids content in cultivar "Morrens Jonagored" (12.91°Brix).
 The lowest average values of fruit firmness were recorded in cultivar "Golden Reinders" (12.86 Pa) and of soluble solids content in cultivar "Gloster 69" (9.05°Brix).

In application with foliar fertilizer 'Stopit', greater fruit firmness (14.41 Pa) and soluble solids content were found in apple fruit (11.58 ° Brix) compared to the treatments without the fertilizer (13.08 Pa and 10.77 ° Brix). Observed by cultivars, the greatest average value of fruit firmness was found in cultivar Granny Smith (14.54 Pa), and the highest soluble solids content in cultivar Morrens Jonagored (12.91 ° Brix).

The lowest average values of fruit firmness were recorded in cultivar Golden Reinders (12.86 Pa) and of soluble solids content in cultivar 'Gloster 69' (9.05 ° Brix).

In interaction variable factor effect

<p>„Stopit“/), - (application ‘Stopit’/Cultivar), the greatest average values of apple fruit firmness were recorded in treatment with foliar fertilizer application in cultivar Granny Smith (15.39 Pa), whereas soluble solids content was highest in the same treatment in cultivar Morrens Jonagored (14.23 ° Brix). In treatments without foliar fertilizer ‘Stopit’ application, the lowest values were found in both studied parameters, for apple fruit firmness in cultivar Golden Reinders (11.76 Pa), and for soluble solids content in cultivar ‘Gloster 69’ (8.93 ° Brix).</p> <p>Asgharzade et al. (2012),</p> <p>Siddiqui Bangerth (1995)</p> <p>Benavides et al. (2002) Casero et al. (2004),</p> <p>(Saure, 2005).</p> <p>11.0%,</p> <p>;</p> <p>(- 13.5%),</p>	<p>(application ‘Stopit’/Cultivar), the greatest average values of apple fruit firmness were recorded in treatment with foliar fertilizer application in cultivar Granny Smith (15.39 Pa), whereas soluble solids content was highest in the same treatment in cultivar Morrens Jonagored (14.23 ° Brix). In treatments without foliar fertilizer ‘Stopit’ application, the lowest values were found in both studied parameters, for apple fruit firmness in cultivar Golden Reinders (11.76 Pa), and for soluble solids content in cultivar ‘Gloster 69’ (8.93 ° Brix).</p> <p>Study results obtained in this work are in compliance with the reports of Asgharzade et al. (2012) that preharvest calcium chloride treatment significantly affect flesh firmness.</p> <p>Siddiqui and Bangerth (1995) report that positive effects of calcium chloride application on fruit firmness increase is connected with the calcium content in fractions of covalently linked pectin.</p> <p>Similar results were reached by Benavides et al. (2002) and Casero et al. (2004) who point to the positive correlation between fruit firmness and calcium content in apple fruit. Moreover, calcium affects cell membrane stabilization and may accordingly prevent physiological disorders (Saure, 2005).</p> <p>Soluble solids content represents one of the key parameters determining quality and consumer acceptability of fruits.</p> <p>Soluble solids content is increased by fruit maturity and it is a basic indicator of quality and ripeness. If it is greater than 11.0%, it is considered acceptable in fruit production; therefore the higher values of the mentioned fruit quality indicator (greater than 13.5%), the better consumer acceptability.</p>
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Yield of investigated apple cultivars depending on foliar fertilizer Stopit application is shown in Figure 1.

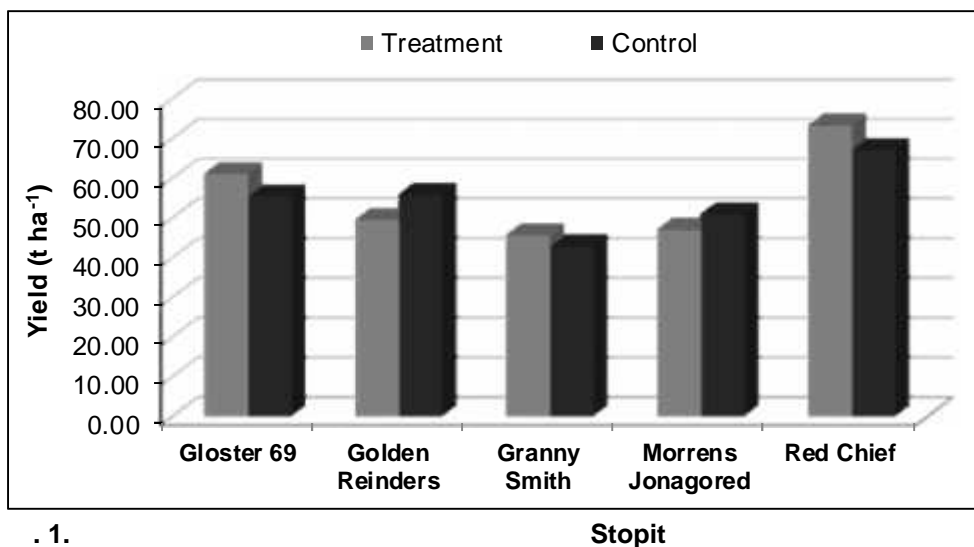


Fig. 1. Effect of application of Stopit on yield of investigated apple cultivars

Comparing treatments with application of foliar fertilizer 'Stopit', higher yield per unit area is found in application, and the highest yields in both treatments are recorded in cultivars 'Red Chief' (73.50 t ha⁻¹ with 'Stopit' application; 67.37 t ha⁻¹ without Stopit application). The same trend in terms of yield per unit area is also observed in cultivar 'Gloster 69' (61.25 t ha⁻¹ with 'Stopit' application; 55.65 t ha⁻¹ without Stopit application). On the other hand, in cultivars 'Golden Reinders' and 'Morrens Jonagored' a lower yield in treatment without application of foliar fertilizer 'Stopit' is registered (56.00 t ha⁻¹, 51.10 t ha⁻¹, respectively), in relation to the treatment with application of fertilizer (49.70 t ha⁻¹, 47.25 t ha⁻¹ respectively). By analyzing results in all treatments, the lowest yield per unit area is found in cultivar 'Granny Smith', both in treatment with application of foliar fertilizer 'Stopit' (45.83 t ha⁻¹), as well as in treatment with no fertilizer application (43.05 t ha⁻¹).

Comparing treatments with application of foliar fertilizer 'Stopit', higher yield per unit area is found in application, and the highest yields in both treatments are recorded in cultivars 'Red Chief' (73.50 t ha⁻¹ with 'Stopit' application; 67.37 t ha⁻¹ without Stopit application). The same trend in terms of yield per unit area is also observed in cultivar 'Gloster 69' (61.25 t ha⁻¹ with 'Stopit' application; 55.65 t ha⁻¹ without Stopit application). On the other hand, in cultivars 'Golden Reinders' and 'Morrens Jonagored' a lower yield in treatment without application of foliar fertilizer 'Stopit' is registered (56.00 t ha⁻¹, 51.10 t ha⁻¹, respectively), in relation to the treatment with application of fertilizer (49.70 t ha⁻¹, 47.25 t ha⁻¹ respectively). By analyzing results in all treatments, the lowest yield per unit area is found in cultivar 'Granny Smith', both in treatment with application of foliar fertilizer 'Stopit' (45.83 t ha⁻¹), as well as in treatment with no fertilizer application (43.05 t ha⁻¹).

(Amiri et al., 2008).

Jafarpour and Poursakhi (2011) report that application of foliar fertilizer based on calcium has positive effect on increasing fruit yield; a positive correlation between the number of applications and the yield was found. The best results of the application with calcium chloride are obtained by combination of foliar application and standard fertilization method (Amiri et al., 2008).

CONCLUSIONS

One of the ways for improvement of apple fruit quality is treatment with foliar fertilizer 'Stopit' containing calcium chloride. Foliar application of the respective fertilizer positively affected the most important fruit quality indicators of investigated apple cultivars.

According to the confirmed positive effect of calcium chloride application on certain qualitative apple fruit properties, apple producers can be given some guidelines for 'Stopit' use in cultivation in order to improve quality and storage potential of apple fruits.

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Micropropagation of Cherry Rootstock Gisela 5

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SUMMARY

- The paper presents a protocol for micropropagation of cherry rootstock Gisela 5 (*Prunus cerasus* x *Prunus canescens*).
- ,
- ,
- (PGRs)
-
- With standard method of surface sterilization of initial explants, aseptic culture was established on Murashige and Skoog (1962) medium (MS) with 2.0 mg l⁻¹ BA, 0.5 mg l⁻¹ IBA and 0.1 mg l⁻¹ GA₃.
- After leaf rosettes initiation, shoots were multiplied in five successive subcultures on MS medium containing 1.0 mg l⁻¹ BA in combination with IBA i GA₃ each applied at 0.1 mg l⁻¹.
-
- Decline in shoot formation capacity over repeated subcultures was observed, so as the highest value of multiplication index (3.2) was noticed in first subculture after initiation. However the subculturing improved the shoot elongation, and the

highest value of shoots length, especially in axial shoots (1.32 cm), was observed in fifth subculture.

Change in BA concentration in combination with NAA instead of IBA in fifth subculture did not influence multiplication index, as well as most other multiplication parameters. Gisela 5 displayed similar rooting ability on media containing 1.0 mg l⁻¹ IBA or NAA (65% and 70%, respectively). 1-min dip treatment in NAA dissolved in sterile water (500 mg l⁻¹) followed by growing on hormone free (HF) medium significantly decreased rooting rate (50%), while shoots grown just on HF medium did not root at all. The percentage of acclimatization was significantly higher in *in vitro* rooted (61.8%) than in unrooted shoots (33,3%).

Key words: cherry, aseptic culture, multiplication, subculturing, rooting, acclimatization

INTRODUCTION

In Serbia, cherry is most often cultivated on generative rootstocks (Mahaleb cherry and wild cherry). Growing cherries on new fully- or semi-dwarfing rootstocks enables establishment of high-density orchards that afford high early yields and easier management. Over the last decades much cherry rootstock breeding and research have been done in a number of research centers in Europe and the worldwide.

New rootstocks should be long-lived, low to moderate vigorous, well adapted to the environmental conditions, well anchored with few root suckers, have good affinity with grafted cultivars, provide precocity, regular and abundant yields, as well as high-quality fruit (Ruzic et al., 2007 b).

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Vegetative rootstocks of Gisela

5
Prunus cerasus
 "Schattenmorelle" × *P. canescens*
 (Clapa et al., 2013).

(Šiško, 2011). 5
 (Exadaktylou et al., 2009),
 (Ruži et al., 2000;
 Nacheva and Gercheva, 2009; Bošnjak et al., 2012; Clapa et al., 2013; Fallahpour et al., 2015).

in vitro

70- 20-
 20

in vitro

(Cossio et al., 1981), (Ruži and Cerovi, 1987), PH-L (Dziedzic and Malodobry, 2006; Sedlak et al., 2008), 5 14 (Hepaksoy, 2004; Buyukdemirci, 2008), Damil GM 61/1 (Ruži et al., 2007 b).

series gained important place in many countries with intense fruit production. Gisela 5 is a dwarfing rootstock for sweet cherry that was developed from the cross *Prunus cerasus* 'Schattenmorelle' × *P. canescens* at the University of Giessen (Clapa et al., 2013). It is considered as very useful and economically important dwarfing rootstock for intensive sweet cherry growing in the temperate conditions (Šiško, 2011). Gisela 5 can be propagated with greenwood, soft or hardwood cuttings (Exadaktylou et al., 2009), as well as by micropropagation (Ruži et al., 2000; Nacheva and Gercheva, 2009; Bošnjak et al., 2012; Clapa et al., 2013; Fallahpour et al., 2015).

Micropropagation is a method of rapid vegetative propagation of plants under *in vitro* conditions of controlled light intensity, temperature and a defined nutrient medium. The technique has been applied to a substantial number of commercial vegetatively propagated herbaceous and woody plant species.

The application of micropropagation for vegetative propagation of rootstocks started in the mid-70s of the 20th century, so considerable number of improved protocols for different temperate fruit tree rootstocks were developed in the next 20 years.

Thus, effective protocols for *in vitro* propagation of vegetative rootstock for sweet and sour cherry were established by optimizing the hormonal composition of the nutrient medium in the multiplication and rooting phase: Vladimir (Cossio et al., 1981), Colt (Ruži and Cerovi, 1987), rootstock PH-L series (Dziedzic and Malodobry, 2006; Sedlak et al., 2008), Gisela 5 and Maxma 14 (Hepaksoy, 2004; Buyukdemirci, 2008), Damil GM 61/1 (Ruži et al., 2007 b).

Considering that micropropagation is usually aimed at achievement of high multiplication rate and its maintenance

in vitro ,
 ,
 (PGRs) 5.
 5
 (1.5-2
 (1
 70% (/)
 20) 10% (/)
 (12 .),
 (0,3-0,8 cm)
 Murashige Skoog (MS)
 (Murashige Skoog, 1962),
 2,0 mg l⁻¹ N6- (BA), 0,5
 mg l⁻¹ -3- (IBA)
 0.1 mg l⁻¹
 (GA₃).
 7 20 g l⁻¹ ().
 MS 1 mg l⁻¹ BA
 IBA GA₃
 0.1 mg l⁻¹.

during *in vitro* growth, the objective of this study was to assess the influence of repeated subculturing on proliferation capacity of shoots as well as the effect of type and concentration of plant growth regulators (PGRs) on multiplication and rooting parameters of Gisela 5 rootstock.

MATERIAL AND METHODS

Following serological testing for the possible presence of viruses, the virus-free plants of cherry rootstock Gisela 5 were individually planted in a sterile substrate and kept in greenhouse conditions. Actively growing leaf buds taken directly from these plants were used as initial explants.

Standard surface sterilization method involved: washing explants under running tap water (1.5-2 h), sterilisation in 70% ethanol (1 min and 20 sec) and 10% (v/v) commercial bleach solution (12 min) followed by triple rinsing with sterile water.

Buds (0.3-0.8 cm long) were isolated under a stereomicroscope and placed on the Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 2.0 mg l⁻¹ N6-benzyl-adenine (BA), 0.5 mg l⁻¹ indole-3-butyric acid (IBA) and 0.1 mg l⁻¹ gyberellic acid (GA₃). The medium contained agar and sucrose at 7 to 20 g l⁻¹ (respectively). The following parameters were monitored: percentage of contamination, percentage of necrotic explants and percentage of explants initiated leaf rosettes.

After the establishment of aseptic culture, shoots were multiplied on the MS medium with 1 mg l⁻¹ BA in combination with IBA and GA₃ both applied at 0.1 mg l⁻¹. Medium of constant PGR composition was used up to the fifth subculture, and multiplication parameters, i.e. multiplication index and length of axial and lateral shoots, were determined upon each subculture.

(IBA NAA) BA
 7 g l⁻¹ 1. 20 g l⁻¹
 5.7 0.1 N KOH.
 121°
 20
 :
 (FW) (DW)
 48 65-70
 48

- In order to optimize multiplication, the influence of BA concentration and type of auxins (IBA or NAA) on the multiplication capacity and shoot quality were examined in the fifth subculture. The combinations of PGRs used in this stage of micro-propagation are presented in Table 1.

- All media contained 7 g l⁻¹ agar and 20 g l⁻¹ sucrose, and pH of media was adjusted to 5.7 with 0.1 N KOH. Sterilization of the media was carried out in autoclave, 20 minutes at 121 °C. Shoots were twice subcultured at 20 day-interval on the medium of the same PGR composition, so that all the parameters monitored were determined in the second subculture.

- The following multiplication parameters were monitored: multiplication index, the length and number of leaves of axial and lateral shoots, as well as specific phenomena such as consistency and color of the callus, coloration and position of the leaves, the occurrence of chlorosis, necrosis, etc.

- Also, fresh (FW) and dry (DW) shoot weight was further determined on the analytical balance. Upon removal from the medium shoots were washed in distilled water, dried with filter paper and their FW was determined.

- For the DW, shoots were dried in oven at 65-70°C for 48 h. Each treatment included 48 uniform shoots.

1.
5

Table 1. PGR composition of the media used in the 5th subculture

Medium	Mineral composition	Organic complex	/Hormonal composition (mg l ⁻¹)			
			BA	IBA	NAA	GA ₃
1	MS	MS	0.5	0.1	-	0.1
2	MS	MS	0.5	-	0.1	0.1
3	MS	MS	1.0	0.1	-	0.1
4	MS	MS	1.0	-	0.1	0.1

PGR – ; MS – *Murashige Skoog (1962)*

PGR – plant growth regulator; MS – composition corresponds to the *Murashige and Skoog medium (1962)*

MS , | In the rooting phase, MS medium with mineral salts reduced to ½- strength

2).

28

40

2.

- and unchanged organic complex was used. Rooting treatments included variation of PGR composition of medium or different pretreatments of the shoots before their placing on the rooting (Table 2).

-

-

The subculture lasted for 28 days, and the following parameters were monitored: the percentage of rooting, the average number and length of the roots and the length of the rooted plants. Each rooting treatment included 40 uniform shoots.

5

Table 2. PGR composition of the medium and pretreatment type in the rooting phase of cherry rootstocks Gisela 5

Medium	Pretreatment	IBA (mg l ⁻¹)	NAA (mg l ⁻¹)	GA ₃ (mg l ⁻¹)
1	-	1.0	-	0.1
2	-	-	1.0	0.1
3	NAA (500 mg l ⁻¹), HF / The basal parts of the shoots were plunge (1 min) into NAA solution (500 mg l ⁻¹) and then the shoots were placed on the HF medium	-	-	-
4	-	-	-	-

PGR – ; HF – („hormon free medium“)
 PGR – plant growth regulator; HF medium – medium without hormones („hormon free medium“)

in vitro,

7

ex vitro.

16 h,

41 μmol

m⁻² s⁻¹,

(40 W,

6,500°K).

23 ± 1 °

ANOVA,

(<0.05).

" , "

In vitro rooted as well as non-rooted shoots were planted in a sterile soil substrate and acclimatized under a misting system in greenhouse. Percentage of acclimatized plants was determined 7 days after planting, i.e. removing plants in *ex vitro* conditions.

The cultures were grown in growth room under a 16 h photoperiod, with a light intensity of 41 μmol m⁻² s⁻¹ provided with cool white fluorescent tubes (40 W, 6,500°K). The temperature was 23 ± 1° C.

All data were analyzed by ANOVA, followed by the Duncan's Multiple Range Test for mean separation (p <0.05). Data presented in the form of percentage were subjected to arcsine transformation.

RESULTS AND DISCUSSION

in vitro

()

(Stanisavljevi et al., 2017),

and Kester, 1983).

5,

44.19%.

55.81%,

(Hartman

(1).

One of the first critical steps in *in vitro* propagation is successful establishment of aseptic culture. The efficiency of this step is determined by a large number of parameters, among which the key role is played by the time (season) and the way of taking initial explants, the type of explants, the health and physiological status of the donor plants, manipulation skills, the procedure of surface sterilization (Stanisavljevi et al., 2017), PGR composition of medium for rosette initiation, growing conditions of initial explants (Hartman and Kester, 1983).

In the case of explants that were taken directly from the greenhouse-grown plants of Gisela 5, the initiation rate was 55.81%, while the percentage of infected cultures was 44.19%. Occurrence of necrosis of initial explants was not observed (Figure 1).



. 1.

5

Fig. 1. Induction of the leaf rosettes for cherry rootstocks Gisela 5

5

MS

After the establishment of aseptic culture, shoots of Gisela 5 were multiplied on MS medium of constant PGR composition, which was previously found to be most appropriate for the examined genotype.

Monitoring of regeneration ability of shoots in five successive subcultures, expressed

through the multiplication index, the length of the axial and lateral shoots, revealed the decline in shoot formation capacity over repeated subcultures (Table 3). Non significant difference in the multiplication index was observed between first and second subculture – 3.2 and 3.1, respectively (Table 3).

Significant decline in shoot number formed occurred after the second subculture and remained constant afterwards. Similarly, sharp decline in shoot formation capacity after the second subculture was also noticed in *Sterculia urens* Roxb. (Hussain et al., 2007) and after the first subculture in Gisela 6 and Fereley Jaspi (Vujovi et al., 2012).

Contrary to multiplication index, the highest values of both axial and lateral shoots were observed in the latest subculture. Hamad and Taha (2008) also reported that the subcultures improved shoot elongation at short-lasting incubation (30 or 45 days).

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Table 3. Multiplication parameters for cherry rootstocks Gisela 5 in consecutive subcultures on the MS medium with 1 mg l⁻¹ BA, 0.1 mg l⁻¹ IBA and 0.1 mg l⁻¹ GA₃

Number of subculture	Multiplication index	Axial shoots length (cm)	Lateral shoots length (cm)
1	3.2 a ¹	1.05 b	0.59 a
2	3.1 a	0.95 b	0.57 a
3	2.6 a	0.92 b	0.60 a
4	2.6 a	1.00 b	0.60 a
5	2.6 b	1.32 a	0.61 a

¹Average values for examined multiplication parameters in the same column marked with different letters are significantly different (Duncan Multiple Range Test, p < 0.05)

According to Hussain et al. (2007) and Varjda and Varjda (2001), decline in shoot multiplication capacity of *in vitro* shoots during repeated subculturing may be overcome or delayed by reducing the cytokinin concentration and/or employing HF medium in later subcultures.

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5,	HF -	-	-	In the case of cherry rootstock Gisela 5, reducing BA concentration to 0.5 mg l ⁻¹ did not improve multiplication capacity of shoots in fifth subculture (Table 4a).
(4).	0.5 mg l ⁻¹	(2.6)	-	The highest average values for this parameter (2.6) were obtained on the media with 1.0 mg l ⁻¹ BA, regardless of the type of auxin used (media 3 and 4). Shoots were uniform, with large, wide, dark green leaves, and maximum multiplication index being 3 (Figures 2c and 2d).
(3 4).	1.0 mg l ⁻¹ BA,	3 (2 2d).	-	Callus was medium-sized, sturdy, nodular, basically dark brown with tiny nodules of light green colour. Large number of small buds was observed in more than 90% of axial shoots grown on the medium 3. The length of the axial shoots significantly differed in media with 1 mg l ⁻¹ BA, and had a higher value (1.32 cm) on that containing IBA (medium 3).
90%	1 mg l ⁻¹ BA (1.32 cm)	3.	-	On the media with 0.5 mg l ⁻¹ BA, type of auxin used did not significantly affect the length of axial shoots, although a slightly higher average value (1.31 cm) was determined in combination with 0.1 mg l ⁻¹ NAA (Table 4a, Figure 2b).
IBA (mg l ⁻¹ BA, 3).	0.5	(1.31 cm)	-	The type of auxin used significantly affected the length of lateral shoots, too. Higher values of this parameter were obtained on IBA-containing media (media 1 and 3) compared to the corresponding media with NAA (media 2 and 4).
4 , 2b).	0.1 mg l ⁻¹ NAA (IBA (1 3)	-	The number of leaves of the shoots did not significantly vary depending on the hormonal composition of the medium, while the number of leaves in lateral shoots had the highest value on the medium 3 (BA 1, IBA 0,1 and GA ₃ 0,1 mg l ⁻¹). The fresh and dry weight of callus had higher values on medium 1 (BA 0.5, IBA 0.1 and GA ₃ 0.1 mg l ⁻¹) compared to all other investigated media (Table 4b).
NAA (2 4).	IBA (1 3)	1 (BA 0.5, IBA 0.1 GA ₃ 0.1 mg l ⁻¹),	-	The shoots were uniform, with a long
3 (1, IBA 0,1 GA ₃ 0,1 mg l ⁻¹).	1 (BA 0.5, IBA 0.1 GA ₃ 0.1 mg l ⁻¹),		-	
IBA 0.1 GA ₃ 0.1 mg l ⁻¹),			-	
(4b).			-	

IBA, (1 3),
 NAA (2 4).

1,
 (4b).

stems and wide, intense green leaves. Callus was large, sturdy, nodular, with a large number of small light green nodules (Figure 2a).

DW of axial and lateral shoots had significantly higher values on IBA-containing media regardless of the BA concentration (media 1 and 3) compared to the media with NAA (media 2 and 4). DW of axial and lateral shoots had higher values on the medium 1 compared to all other media (Table 4b).



A



B



C



D

Fig. 2. The shoots for cherry rootstocks Gisela 5 on mediums with a different hormone composition: BA 0.5, IBA 0.1 and GA₃ 0.1 mg l⁻¹ (a); BA 0.5, NAA 0.1 and GA₃ 0.1 mg l⁻¹ (b); BA 1, IBA 0.1 and GA₃ 0.1 mg l⁻¹ (c); BA 1, NAA 0.1 and GA₃ 0.1 mg l⁻¹ (d)

Table 4a. Multiplication parameters for cherry rootstock Gisela 5 in fifth subculture

Medium number	Multiplication index	Axial shoot length (cm)	Lateral shoots length (cm)	No. of leaves of axial shoots	No. of leaves of lateral shoots
1	2.4 a ¹	1.21 ab	0.65 a	14.0 a	5.2 b
2	2.2 a	1.31 a	0.58 bc	13.2 a	4.0 c
3	2.6 a	1.32 a	0.61 ab	13.9 a	6.2 a
4	2.6 a	1.17 b	0.52 c	13.8 a	5.3 b

(, p 0.05)
¹Average values for examined multiplication parameters in the same column marked with different letters are significantly different (Duncan Multiple Range Test, p 0.05)

4b.**5****Table 4b. Fresh and dry weight of callus and shoots of cherry rootstocks Gisela 5**

	Fresh weight of shoots (mg)			Dry weight of shoots (mg)		
	Callus	Axial shoots	Lateral shoots	Callus	Axial shoots	Lateral shoots
1	109.0 a ¹	231.6 a	32.6 a	21.3 a	54.4 a	7.0 a
2	48.6 c	193.9 b	22.2 b	10.2 c	46.0 b	5.1 b
3	59.7 bc	219.8 a	31.4 a	11.3 bc	45.0 b	5.4 b
4	77.0 b	177.1 b	23.8 b	15.0 b	41.1 b	4.6 b

(, p 0.05)
¹Average values for examined multiplication parameters in the same column marked with different letters are significantly different (Duncan Multiple Range Test, p 0.05)

5,

: BA 1 mg l⁻¹,
 IBA 0,1 mg l⁻¹ GA₃ 0,1 mg l⁻¹.

F12 / 1 (Grant
 Hammat, 1999),
 -14 (Muna et al.,
 1999),

5.

Although change in PGR composition of multiplication medium did not significantly affect multiplication index of Gisela 5 shoots, based on other indicators of regenerative capacity and shoots quality, it can be concluded that, in the multiplication phase the most optimal PGR combination is: BA 1 mg l⁻¹, IBA 0,1 mg l⁻¹ and GA₃ 0.1 mg l⁻¹.

This PGR composition proved to be the most suitable for micropropagation of some vegetative cherry rootstock such as F12/1 (Grant and Hammat, 1999), Maxma-14 (Muna et al., 1999), but the multiplication indexes obtained had notably higher values in relation to Gisela 5.

The value of the multiplication index obtained on media containing 1 mg l⁻¹ BA (2.6) may be sufficient to maintain

(2.6),	1 mg l ⁻¹ BA	cultures, but not for highly productive <i>in vitro</i> propagation for commercial purposes.
	<i>in vitro</i>	
	Ruži (1998)	Ruži (1998) found that this genotype performed the best growth and development on MS medium with double strength of macro salts (MS 2x). However multiplication index, length of lateral shoots were significantly lower compared with those grown on MS medium.
	MS (MS 2x).	
Nacheva et al. (2009)	MS	Nacheva et al. (2009) have found that low multiplication index of Gisela 5 shoots on BA- and IBA-containing medium can be markedly increased if sucrose and sorbitol (ratio 2:1) are used instead of sucrose.
	5	
	BA IBA	
	(2:1).	The ultimate success of commercial micropropagation depends largely on successful rooting and acclimatization of <i>in vitro</i> derived plantlets.
	<i>in vitro</i>	
a,	:	Apart from genotype dependence, rooting process is influenced by many factors: explants age, i.e. total time spent in <i>in vitro</i> culture (Grant and Hammat, 1999), type and concentration of cytokinins and cytokinin/auxin ratio used in multiplication phase (Muna et al., 1999), as well as the level of cytokinins absorbed by tissues during multiplication <i>in vitro</i> (George, 1993).
Hammat, 1999),	(Grant and	
/	(Muna et al ,	
1999),	<i>in vitro</i> (George, 1993).	
MS,	HF	In many plant species rooting can be induced by cultivation on HF MS medium with the concentration of macronutrients reduced to half-strength. However it is not applicable for woody tree species and presence of auxins is essential for root induction.
	HF	
(5, 3d).	That is confirmed in our experiment since no rooting was observed on HF medium (Table 5, Figure 3d). The auxins, most commonly used for <i>in vitro</i> root formation in woody plant species are IBA and NAA. IBA proved to be a very effective auxin in the rooting phase of different cherry
	<i>in vitro</i>	
	IBA NAA. IBA	

– Inmil GM 9, Camil GM 79, Tabel Edabriz, Maxma 14 (Ruži Cerovi , 2001; Fidanc et al., 2008; Buyukdemirci, 2008)

rootstocks – Inmil GM 9, Camil GM 79, Tabel Edabriz, Maxma 14 (Ruži and Cerovi , 2001; Fidanc et al., 2008; Buyukdemirci, 2008).

In the present study, no significant difference is found in the rooting percentages between shoots rooted on the medium containing 1 mg l⁻¹ IBA (65%) and 1 mg l⁻¹ NAA (70%) both in combination with 0.1 mg l⁻¹ GA₃. However, number of roots, as well as the length of the rooted shoots, had significantly higher values (3.9 and 1.54 cm, respectively) on the medium containing IBA. The rooted shoots had a similar morphology to those rooted on medium with NAA, but they were longer with broad dark green leaves (Figure 3a and 3b). The highest root length (3.08 cm) was obtained on medium with NAA, which was significantly higher comparing to the same parameter measured on other media (Table 5).

Shoots on this medium were long, well developed. Callus was small, solid, nodular, green color. The roots were thick, light red, long, airy and without secondary roots (Figure 3b). Poor quality of rooted plants as well as low rooting rate were observed with 1-min dip treatment in NAA followed by growing on HF medium (treatment 3, Figure 3c).

5. Table 5. Rooting parameters of cherry rootstocks Gisela 5

Medium number	Percentage of rooting	Number of roots	Root length (cm)	Plant length (cm)
1	65.0 a ¹	3.9 a	1.82 b	1.54 a
2	70.0 a	2.8 b	3.08 a	1.31 b
3	50.0 b	2.2 c	2.13 b	1.15 b
4	0 c	-	-	-

¹Average values for examined rooting parameters in the same column marked with different letters are significantly different (Duncan Multiple Range Test, p = 0.05)



A



B



C



D

3. IBA 1 GA_3 0.1 mg l⁻¹ (a); NAA 1 GA_3 0.1 mg l⁻¹ (b);
 5 :
 1 500 mg l⁻¹ NAA
 (HF) (c); (d)

Fig. 3. The shoots for cherry rootstocks Gisela 5 in rooting phase: medium with IBA 1 and GA_3 0.1 mg l⁻¹ (a); medium with NAA 1 and GA_3 0.1 mg l⁻¹ (b); immersion of the basal portion of the shoots 1 min in 500 mg l⁻¹ NAA, and then placing on the HF medium (c); HF medium (d)

5 - Gisela 5 shoots exhibited relative
 - low potential to acclimatize under the
 - misting conditions in greenhouse. The
 - percentage of acclimatization of rooted
 (61,8%) shoots (61.8%) was significantly higher in
 - comparison with those failed to root *in*
vitro (33,3%). As regards rooted shoots
 - indirect *in vitro* rhizogenesis through
 - callus formation can be one of the
 reasons for low percentage of
 acclimatization in Gisela 5, as it was
 shown in pear cultivar Bartlett
 (Bommineni et al., 2001).
 5,
 (Bommineni et al., 2001).

CONCLUSIONS

The results obtained in this paper completely justify the application of micropropagation for the rapid and effective propagation of healthy planting material of Gisela 5 rootstock, that ensures plant availability throughout the year accordingly.

However decline in shoot multiplication ability over repeated subculturing implies necessity for further investigation in order to find the proper protocol to reestablish proliferation capacity of *in vitro* shoots and/or delay the decline.

The research should aim at finding adequate hormonal composition of the nutrient medium that will provide the maintenance of high multiplication rate of *in vitro* shoots.

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Effect of Fertilization on Biochemical Composition of Fruits of Black Currant and Red Currant

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SUMMARY

2015 .

Tekamin Brix
(0,2% 0,3% –
).

6.2%)

The experiment was conducted in 2015 in the collection plantation at the Research Institute of Mountain Stockbreeding and Agriculture in Troyan, Bulgaria. The study included the following black currant cultivars: „Ometa:”, „Hedda”, ‘Silvergitte schwartze’, ‘Titania’ and the red currant cultivar ‘Rovada’. Conventional and organic fertilizations were applied. As conventional fertilizer was used ammonium nitrate (soil application), and as organic fertilizer – Tekamin Brix at two concentrations (0.2% and 0.3% – twice foliar application). The indicators of biochemical composition of fruits and their average weight in the variants with fertilization, compared to control were investigated. The reported results indicate the effects of fertilization on the following indicators: anthocyanins, inverted sugar and total sugars. Higher values of total and inverted sugar (7.04% and 6.2%, respectively) in the application of Tekamin Brix (0.2%). The highest

(0.2%). - (156.838 mg/%)
 (0.3%). -
 - 1.24 g (
 - 0.2 %) - 1.22 g
 (- 0.3 %).
 :
 ,
 ,
 ,

values of anthocyanins (156.838 mg) were reported in the variant with Tekamin Brix (0.3%). The highest average fruit weight was recorded for Silvergitte schwartze'– 1.24 g (Tekamine Brix – 0.2%) and Ometa' – 1.22 g (Tekabin Brix – 0.3%).

Key words: total sugar, inverted sugar, anthocyanins, average weight of the fruit, organic fertilizer

INTRODUCTION

The fertilization is one of the main agrotechnical measures in the cultivation of fruit crops. It directly affects the vegetative, reproductive and other manifestations of the plants. Proper and accurate application of the fertilizer is a guarantee of high yields. The study of the properties of fertilizers and new ones and their impact on the respective plant manifestations is an important stage in their agrotechnology.

The growing interest in blackcurrant fruit is probably due not only to their large amount of ascorbic acid in their buds, leaves and blossoms but also because of the extremely rich biochemical composition of fruits including common anthocyanins, common phenols, flavonols, phenol acids etc. (Nikolov, 1971; Zotova, 1982; Borcheva, 1984, Burmistrov, 1984; Stoyanov and Hristov, 1983; Hristov, 1988; Stoyanova, 2005; Miladinovi, 2015; Paunovi, 2015).

Fruit of berry crops is a valuable source of biochemical substances (Stoyanova et al., 2015). They attract the attention of consumers because of their inviting taste, aroma and appealing appearance. Their economic qualities also determine their wide application (Domozetova, 2012).

The aim of the present study is to trace the impact of conventional and organic fertilization on the biochemical composition of black currant and red currant.

(Nikolov, 1971; Zotova, 1982; Borcheva, 1984, Burmistrov, 1984; Stoyanov and Hristov, 1983; Hristov, 1988; Stoyanova, 2005; Miladinovi, 2015; Paunovi, 2015).

(Stoyanova et al., 2015).

(Domozetova, 2012).

MATERIAL AND METHODS

2015 .

kg/ –

0.2 % 0.3 %, ,

(Nedev et al., 1979).

Re (%), (mg %),

(%) – 0,1n NaOH; (mg %)

Fuleki Franciss, (mg %)

(%) –

(Lidanski, 1988).

The experiment was set up in 2015 in a collection plantation of the Institute of Research Institute of Mountain Stock-breeding and Agriculture in Troyan. The subject of the survey are the following cultivars of currants: 'Ometa', 'Hedda', 'Silvergitte schwartze', 'Titania' and the red currant cultivar of 'Rovada'. Ammonium nitrate was applied as a conventional fertilizer at a dose of 0.200 kg/shrub once, and the organic fertilizer Tekamin Brix in two concentrations: 0.2% and 0.3% foliar application, imported twice during the vegetation period.

The experiment was set up according to the Methods for studying plant resources (Nedev et al., 1979). The following biochemical composition parameters of fruits are defined: dry matter in Re (%), dry weight (%), sugars (%) (total, inverted and sucrose) – according to Schoorl and Regenbogen method, acids (%) – by means of titration with 0.1n NaOH; ascorbic acid (mg %) – according to Fialkov method; anthocyanins (mg %) – according to Fuleki and Franciss method; pectin (%) – according to Melitz method, tanning substances (%) – according to Levental method.

Data were processed by two-factor analysis of variance (Lidanski, 1988).

RESULTS AND DISCUSSION

1.

() 23.32% ()

(P<0.05).

15-17%.

20.59%

The results of the biochemical composition of black currant and red currant berries are presented in Table 1. Dry weight matter in fruits of blackcurrant for both types of fertilization is within the range from 20.59 % ('Hedda') to 23.32% (for the control cultivar 'Ometa'). The differences between variants of fertilization and the cultivars have been mathematically proven (P<0.05).. In red currant, the same is lower in the order of 15-17%. Perhaps the diversity observed is due to the type of culture. Perhaps the diversity observed is due to the type of crops. Differences in soluble dry matter of cultivars and variants are more significant. Higher values were reported for 'Ometa'

18-20%,
20%.
17.2%.
-
(P<0.05),
10-11%

cultivar, where the variants of Tekamin Brix were 18-20%, and for conventional fertilization were 20%. For comparison for the control was 17.2%. The lowest amount was found in the variant of 'Hedda' and 'Silvergitte schwartze'. The differences among the different cultivars were proven (P <0.05), but not those in the type of fertilization. Values of 'Rovada' cultivar are 10-11 % in the experiment.

1.

Table 1. Effects of conventional and biological fertilization on the biochemical composition of fruits of different cultivars of currants and red currants

Cultivars	Fertilization variants	Dry weight %	KE DM in RE %	Total sugars %	Inverted sugar %	Sucrose %	Acid %	Ascorbic acid mg/%	Tannin %	Anthocyanins mg/%	Pectin %
Rovada	Tekamin brix 0.2%	15.67	10	8.05	8.05	0	1.08	8.8	0.229	14.19	1.14
	Tekamin brix 0.3%	15.45	10	8.05	8.05	0	1.08	8.8	0.229	6.61	1.13
	Ammonium nitrate	16.34	11	6.65	6.65	0	0.96	7.04	0.166	8.39	0.58
	Control	16.5	11	6.65	6.65	0	0.96	7.04	0.166	15.81	0.43
Hedda	Tekamin brix 0.2%	20.59	16	5.35	4.2	1.09	1.08	10.56	0.27	28.55	0.14
	Tekamin brix 0.3%	20.59	15	4.2	4.2	0	0.96	12.32	0.145	78.87	0.32
	Ammonium nitrate	20.72	14.5	7.35	4.05	3.14	0.96	11.44	0.125	67.58	0.66
	Control	23.02	15	6.5	4.5	1.9	0.96	8.8	0.145	45.48	1.36
Silvergitte schwartze	Tekamin brix 0.2%	21.45	14.5	4.85	4.85	0	1.15	17.6	0.166	31.29	0.67
	Tekamin brix 0.3%	22.17	16	5.35	5.35	0	0.89	27.28	0.166	22.42	0.73
	Ammonium nitrate	21.95	14	4.05	2.85	1.14	0.96	22	0.145	16.45	0.31
	Control	22.35	14.5	3.05	2.04	0.62	0.83	17.6	0.104	8.06	1.16
Omota	Tekamin brix 0.2%	21.88	18	8.05	6.5	1.47	1.4	17.6	0.166	304.03	0.84
	Tekamin brix 0.3%	22.73	20	8.05	6.5	1.47	1.08	17.6	0.187	332.42	1.1
	Ammonium nitrate	22.94	20	8.05	6.65	1.33	1.15	17.6	0.104	315.16	1.24
	Control	23.32	17.2	8.05	6.0	1.95	1.21	17.6	0.27	314.84	1.19
Titania	Tekamin brix 0.2%	21.37	18	8.9	6.5	2.28	1.28	26.4	243.23	243.13	1.21
	Tekamin brix 0.3%	22.14	19.7	7.85	5.35	2.38	1.28	26.4	343.87	343.87	1.27
	Ammonium nitrate	21.93	18.2	7.85	5.7	2.04	1.28	26.4	212.9	212.9	0.86
	Control	22.29	18.5	8.9	6.5	2.28	1.21	26.4	196.61	196.61	0.48

(P<0.05).
 -
 - 7.35%.
 4.85% (0.2%) 5.35% (0.3%).
 3.05%.
 8.05%.
 (P<0.05),
 ú
 4.2-4.5%,
 6.5%.
 -
 - 8.05%.
 (P<0.05).
 3.14%,
 -
 -
 (P<0.001)
 (P<0.01)
 -
 -
 - 0.2%.
 -
 -
 - 1.08%.

The influence of the cultivar on the indicator of total sugars (P <0.05) was mathematically proven. For example, the highest values for 'Hedda' cultivar were obtained in the conventional fertilization option – 7.35%. For the other cultivar of Silvergitte schwartze, they are higher for organic fertilizer variants – 4.85% (0.2%) and 5.35% (0.3%). For comparison, they are 3.05% in the control. The other two cultivars of black currant do not show signs for the influence of fertilization. For 'Roda', sugars are more than the fertilization with Tekamin Brix – 8.05%. The cultivar identity has a credible effect on the invert sugar content (P <0.05), and in the case of fertilization, the differences are unproved. Its values vary considerably among cultivars and fertilization variants. Disaccharides are in the range of 4.2% – 4.5% for 'Hedda' cultivar. The highest values are found in 'Ometa' cultivar, as they are almost the same for all variants – about 6.5%. The highest content of invert sugar in red currant is found in case of organic fertilizer – 8.05%.

The differences in sucrose content in fruits (P <0.05) among cultivars of black currant are reliable. The highest content is found in fruit of 'Hedda' cultivar in the conventional fertilizer – 3.14%, and the most constant and in relatively high amounts are those of 'Titania' cultivar - more than two percent. No sucrose is registered in fruit of 'Rovada' variants.

Very good provability (P<0.001) is found in the values of organic acids among the cultivars and good one (P<0.01) from the influence of fertilization. The highest content is found in fruit of 'Ometa' with the lowest concentration of Tekamin Brix – 0.2%. The values for 'Titania' are almost equal among the variants. The organic acids for 'Rovada' are higher in the variants with Tekamin Brix – 1.08%.

Differences in content of ascorbic

(P<0.05).

-

-0.3% – 27.28 mg%

-

-

8.8 mg%.

8.8 mg%,
– 7.04 mg%.

-

243.23%,

- 343.87%,
– 212.9%
– 196.61 %.

-

0.270%.

0.229%,

- 0.166 %.

(P<0.05),

343.87 mg% (
0.3%).

- 332.42 mg%.

- 15.81 mg%.

0.14% (I variant for 'Hedda')

acid in relation to cultivar are proven (P<0.01). The effect of fertilization on that indicator has not been proven. The highest values were recorded in fruits of Silvergitte schwartze in the fertilization with Tekamin Brix – 0.3% – 27.28 mg%, and the results for 'Titania' and 'Ometa' were slightly lower.

The lowest values are for 'Hedda', where the control reaches up to 8.8 mg%. For 'Rovada', the ascorbic acid of the first two variants is 8.8 mg% and for the other two – 7.04 mg%.

The cultivar specificity has been proved to affect the quantities of tanning substances. The highest values of that indicator for all the variants are found in 'Titania'. They are 243.23% in the first one, in the second – 343.87%, for the conventional fertilization – 212.9% and the lowest for the control – 196.61%. Generally, the amount of tanning substances is considerably smaller in the variants of other cultivars, with the exception of the first one of 'Hedda', where the values of the index reach 0.270%.

Tanning substances for red currant are in the range of black currant cultivars. In organic fertilization they reach 0.229% and in conventional and the control they are less – 0.166%.

The differences in anthocyanin content among cultivars (P <0.05) have been statistically proven, but not the type of fertilization, The distinction between 'Ometa' and 'Titania' is very significant and distinct, compared to the other two black currant cultivars. They reach values up to 343.87 mg% for 'Titania' (Tekamin Brix – 0.3%). It is similar for the same variant of 'Ometa' – 332.42 mg%. The highest amount is found in 'Rovada' in comparison with the control – 15.81 mg%.

There are no statistically proven differences among cultivars and fertilization for pectin values. Pectin levels range from 0.14% (I variant for 'Hedda')

) 1.36%
 -
 -
 -
 -
 -
 (P>0.05)
 -
 -
 -
 -
 -
 -
 2 1, 2, 3, 4, 5.

to 1.36% in comparison with the control of the same cultivar. For Rovada, pectin values are up to twice as high in the variants with Tekamin Brix.

The effect of conventional and biological fertilization on the size of the fruits of the studied varieties was investigated and no proven differences (P> 0.05) between the type of fertilization and the cultivar identity were found. Indicator data are presented in Table 2 and Figures 1, 2, 3, 4, 5.

Table 2. (SD) (CV)

Table 2. Standard deviation (SD) and coefficient of variation (CV) of fruit weight in different fertilizing variants of black currant and red currant.

/ Cultivars	/ Fertilization variants	SD	CV
/Rovada	-0.2%/Tekamine Brix 0.2%	0.19	47.50
	-0.3%/Tekamine Brix 0.3%	0.27	56.69
	/Ammonium nitrate	0.27	56.27
	/Control	0.22	51.27
/Hedda	-0.2%/Tekamine Brix 0.2%	0.37	54.01
	-0.3%/Tekamine Brix 0.3%	0.35	66.03
	/Ammonium nitrate	0.29	61.12
	/Control	0.29	57.01
/Silvergitte schwartze	-0.2%/Tekamine Brix 0.2%	0.47	79.27
	-0.3%/Tekamine Brix 0.3%	0.35	52.43
	/Ammonium nitrate	0.42	75.56
	/Control	0.36	64.12
/Ometa	-0.2%/Tekamine Brix 0.2%	0.37	53.88
	-0.3%/Tekamine Brix 0.3%	0.41	55.83
	/Ammonium nitrate	0.40	58.51
	/Control	0.29	51.26
/Titania	-0.2%/Tekamine Brix 0.2%	0.45	66.48
	-0.3%/Tekamine Brix 0.3%	0.38	62.31
	/Ammonium nitrate	0.45	80.68
	/Control	0.38	63.02

-
 - 0,69 g
 () 0,73 g (- 0.3%).
 -
 - 1,24 g
 (- 0.2%) - 1,22
 g (- 0.3%). Stoyanova et al. (2015)

The highest values for this indicator were measured for 'Ometa' cultivar – 0.69 g (Ammonium nitrate) and 0.73 g (Tekamin brix – 0.3%). The highest maximum weight was found in 'Silvergitte schwartze' – 1.24 g (Tekamin Brix – 0.2%) and 'Ometa' – 1.22 g (Tekamin Brix – 0.3%). Stoyanova et al. (2015) show similar results with respect to the average weight of the fruit in blackcurrant cultivars, in the

0.65-0.95 g

0.78 g

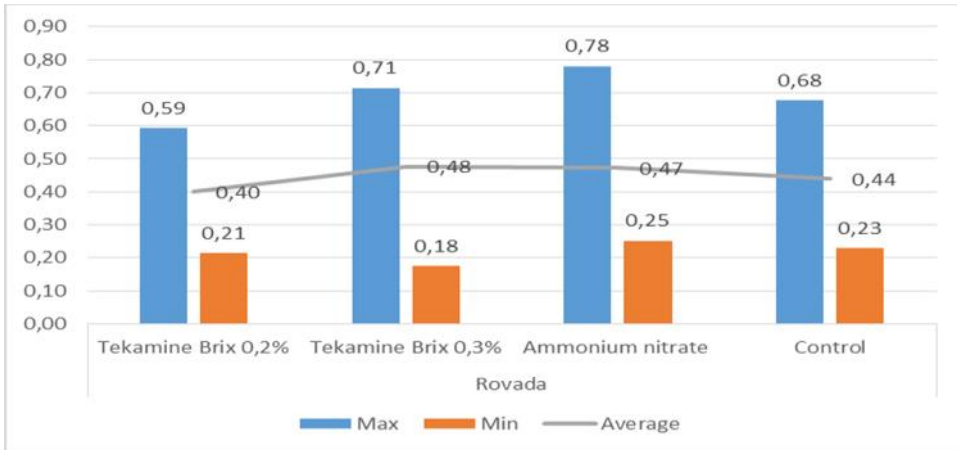
(0.47 g)

(0.48 g). Yakovenko and Lapshin (2015)

0.30-0.85 g.

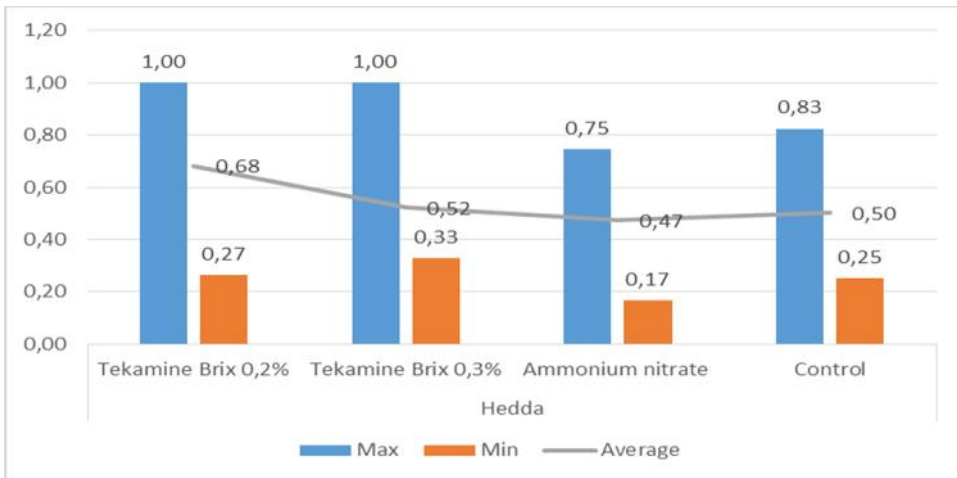
range 0.65-0.95 g. For red currant, the maximum fruit weight reaches 0.78 g in the ammonium nitrate variant.

The values for the average fruit weight is the highest in the same variant (0.47 g) and that of the organic fertilizer Tekamin Brix-0.3% (0.48 g). Yakovenko and Lapshin (2015), classify the cultivars of red currant according to the average fruit weight in the range of 0.30=0.85 g.



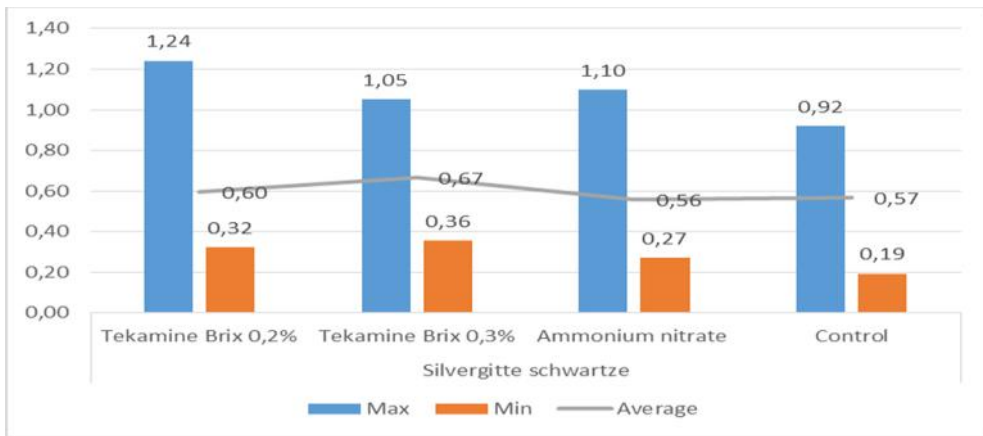
. 1. (g)

Fig. 1. Average weight (g) of the fruits of variants of the cv. Rovada



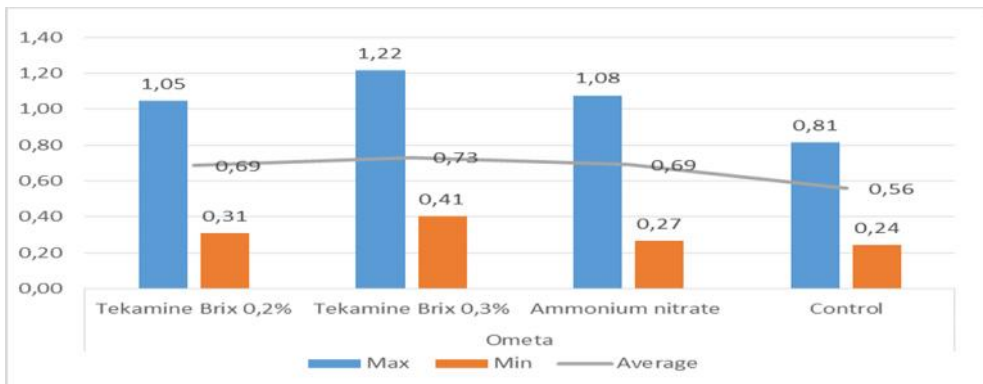
. 2. (g)

Fig. 2. Average weight (g) of the fruits of variants of the cv. Hedda



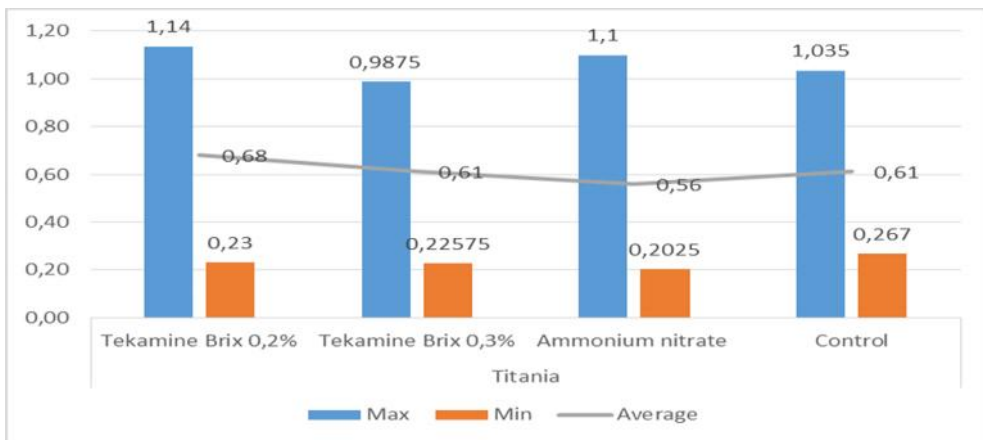
. 3. (g)

Fig. 3. Average weight (g) of the fruits of variants of the cv Silvergitte schwarzte



. 4. (g)

Fig. 4. Average weight (g) of the fruits of variants of the cv. Ometa



. 5. (g)

Fig. 5. Average weight (g) of the fruits of variants of the cv. Titania

CONCLUSIONS

- The studied influence of conventional and organic fertilizer on the biochemical composition of fruit of blackcurrant and red currant.
- The impact of fertilization on dry weight and organic acids has been proven.
- The influence of the cultivar has been statistically proven in all biochemical composition indicators of the fruits, except for pectin.
- The complex assessment of the weight of the fruit under the influence of fertilization is most pronounced in the cultivars of black currant cultivar of 'Omega' and Silvergitte schwartze.

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Traditional Bulgarian Plant Species

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SUMMARY

The development of science and food technology in modern conditions is the reason for the wide variety of plant species and foods. Traditional Bulgarian plant species have specific composition and properties for the area in which they are grown.

The purpose of this study is to present some terms and definitions related to the term "traditional", as well as some of our traditional plant species, their properties and nutritional value.

Key words: traditional plant species, properties, nutritional value

INTRODUCTION

The production of traditional Bulgarian foods dates back to ancient times, and has consistently evolved, expanded and enriched over the years. The traditions of the production of plant species and foods in the neighbouring Balkan countries have been influenced.

- manufacturing conditions described in the relevant documentation requesting protection in Brussels.

- The purpose of this study is to present the achievements in the scientific publications and the existing legal framework to highlight the specific terms associated with the term "traditional" as well as to study and present some traditional Bulgarian plant species and foods of plant origin, their properties, nutritional value and application.

MATERIAL AND METHODS

- The objects of discussion are some of the traditional Bulgarian plant species and foods of plant origin, their properties, nutritional value and application. In order to achieve the research aim, scientific publications and normative basis (national and Union legislation).have been studied and subjected to systematic analysis.

RESULTS AND DISCUSSION

- 1. Terms and definitions related to traditional plant species and food of plant origin**

- Traditional raw materials: Traditional raw materials are the raw materials used in the past and presently alone or combined as components of identified geographical origin that have properties and composition that meet the requirements of the European (Alliance) and the national legislation (Ribarova, 2007). The ingredients in their composition must meet the requirements of quality, safety (chemical, microbiological and physical)

¹ 16 14.09.2007 .

² 6 5 2011 .

³ OB L 93, 31.3.2006 .

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 (Boyadzhiev and Markova,
 1987).

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(Ribarova et al., 2005).
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(Hristov,
 2017).

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()³ (Irechek, 1974;
 Ribarova et al., 2005; Hristov, 2017). -

and comply with the hygienic rates and rules of processing and production (Boyadzhiev and Markova, 1987).

Traditional recipe: The uniqueness of the traditional recipe is in the detailed description of the ingredients and the way they are processed, arranged in order of their use and passed down from generation to generation over the years (Ribarova et al., 2005).

Method of production: The production and/or processing of plant products must fully correspond to those used in the past, without the inclusion of modern processing techniques.

Legislation on traditional foods and the production of plant species is dynamic, but needs to be adapted with appropriate modifications and additions, specific to the particular species^{4,5}.

The preservation of national traditional plant species aims to protect them from imitations and this necessitates the creation of a system of registration at national and Union level (Hristov, 2017).

Traditional specific foods: There are the following three regulated categories of foods of a specific nature: Protected Designation of Origin (PDO); Protected Geographical Indications (PGI); Traditional Specialties Guaranteed (TSG)⁶ (Irechek, 1974; Ribarova et al., 2005; Hristov, 2017).

A better interpretation and clarification of the terminology presented in line with the current regulatory framework is needed. In the European Commission's Traditional Food Register, very few products are classified under the

⁴ Ordinance No 16 of 14 September 2007 on the preparation and submission of applications to the European Commission on agricultural products and foodstuffs with protected geographical indications.

⁵ Ordinance No 6 of 5 May 2011 on the specific requirements for the official control of the use of protected geographical indications and food with traditional specific character.

⁶ OB L 93, 31.3.2006 .

(Ribarova, 2007, Hristov, 2017).

name of GTS, and most of the food is registered under the PGI and PDO brand (Ribarova, 2007, Hristov, 2017).

- Labelling rules make it easier for consumers to identify quality food produced by a particular technology. A comprehensive Quality Policy – Protected Designations including Protected Designations of Origin, Protected Geographical Indications and Traditional Specific Foods has been developed in the EU.

The main objective is to highlight the specific properties of certain typical products on the market and to distinguish them from the others in the market by signifying them with the symbols of the European Community. Protected Geographical Indications have protective intellectual property rights (Pashova, 2015).

(Pashova, 2015).



1. Fig. 1. Special EU logos, designations of the quality scheme⁸

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A special EU logo (Figure 1) shall be affixed to the packaging of products of a given geographical origin: Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and those prepared or produced by traditional way:

⁷ () 668/2014 13 2014 () 1151/2012

⁸ COMMISSION IMPLEMENTING REGULATION (EU) No 668/2014 of 13 June 2014 laying down rules for the implementation of Regulation (EU) No 1151/2012 of the European Parliament and of the Council on agricultural product quality schemes. Published L OB. No. 179 of 19 June 2014.

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- food with Traditional specialty guaranteed (TSG)²⁴.
 - Traditional Specific Food (TSG) describes a specific product or food that: (a) results from a method of production, processing or composition that conforms

⁹ ()

¹⁰ () . 2008, London Economics.

¹¹ () 1151/2012

¹² Divino - <https://www.divino.bg/encyclopedia/>

¹³ - <https://festivalnaslivata.com/bg/za-slivata/about-plum/61-slivata.html>

¹⁴ , 2016 - <http://www.mnekov.eu/bg-taste/da-zashtitim-bulgarskiia-vkus-reseleshki-voden-luk.html>

¹⁵ - <http://www.menumag.bg/za-hranata/statii/kultura-na-vodniya-luk-vtora-chast>

¹⁶ , 2016 - <http://www.mnekov.eu/bg-taste/da-zashtitim-bulgarskiia-vkus-kurtovski-rozov-domat.html>

¹⁷ , 2016 - <http://www.mnekov.eu/bg-taste/da-zashtitim-bulgarskiia-vkus-kurtovska-kapiia.html>

¹⁸ - <https://trud.bg/> - - - - - /

¹⁹ - <https://lechenie.bg/fastatsi>

²⁰ | () -

²¹ <http://www.strandja.iag.bg/protected/lang/1/id/108/species>

²² , 2017 - <http://pchelendar.com/-1290.html>

²³ - <http://apteka-optima.com/185/718/> - - - - - /

() 510/2006 (2) „ “

(BULGARSKO ROZOVO MASLO) E : BG-PGI-0005-01050 – 26.10.2012 . (X)

²⁴ Evaluation of the Common Agricultural Policy on Protected Designations of Origin (PDO) and Protected Geographical Indications (PGI). 2008, London Economics.

²⁵ Treaty on the Functioning of the European Union (TFEU)

²⁶ Regulation (EU) No 1151/2012 of the European Parliament and of the Council on schemes for the quality of agricultural product and foods.

²⁷ Divino - <https://www.divino.bg/encyclopedia/>

²⁸ Bulgarian Plum Festival - <https://festivalnaslivata.com/bg/za-slivata/about-plum/61-slivata.html>

²⁹ Protecting the Bulgarian taste: Resell Water Onions, 2016 - <http://www.mnekov.eu/bg-taste/da-zashtitim-bulgarskiia-vkus-reseleshki-voden-luk.html>

³⁰ Water Onion Culture - Part Two - <http://www.menumag.bg/za-hranata/statii/kultura-na-vodniya-luk-vtora-chast>

³¹ To protect the Bulgarian taste: Kurtovski pink tomato, 2016 - <http://www.mnekov.eu/bg-taste/da-zashtitim-bulgarskiia-vkus-kurtovski-rozov-domat.html>

³² To protect the Bulgarian taste: Kurtovska kapia, 2016 - <http://www.mnekov.eu/bg-taste/da-zashtitim-bulgarskiia-vkus-kurtovska-kapiia.html>

³³ How to distinguish the real Smolyan beans - <https://trud.bg/> - - - - - /

³⁴ Peanuts - <https://lechenie.bg/fastatsi>

³⁵ Directorate of Nature Park Strandzha | Protected varieties Strandzha (Crimean) Tea - <http://www.strandja.iag.bg/protected/lang/1/id/108/species>

³⁶ Herbs in Strandzha – Kaluna, Strandzha Tea, Long-leaved Mint, 2017 - <http://pchelendar.com/-1290.html>

³⁷ The Bulgarian oil rose, a valuable ingredient for health and beauty - <http://apteka-optima.com/185/718/> - - - - - /

³⁸ COUNCIL REGULATION (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs (2) BULGARIAN ROZOVO MASLO EC No: BG-PGI-0005-01050 - 26.10.2012 PGI (X) PDO.

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Vitis.

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17 23,

24°, 10,7 g/dm³.

(*Prunus domestica*)

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(Iliev et al., 1985).

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87 %, (9 %),

(0,8 %),

(0,8 %), (0,5

%)

(1,3 %),

2, (10 mg %)

2. Traditional Bulgarian plant species

Grape variety “Mavrud”. Grapes are called fruits of plants of the genus *Vitis*. Mavrud is a traditional Bulgarian red wine grape variety, one of the oldest and considered one of the most valuable and high quality local varieties. It is an old local variety that is still found in Greece and probably in Macedonia and Bessarabia. It is distributed mainly in the Thracian Plain – Plovdiv (mostly around Asenovgrad) and less in the regions of Pazardzhik and Stara Zagora, and in the South-Bulgarian wine region. It occupies an insignificant share of the area of red wine vineyards. It is encountered, though limited, along the southern Bulgarian Black Sea coast - the region of Burgas and Pomorie²⁷.

The fruits are used to produce high-quality wine. Mavrud is one of the most valued local varieties for the production of red wines. Grain sugar is typically between 17 to 23, sometimes 24°, and the titratable acidity is from 6.1 to 10.7 g/dm³.

Kyustendilska blue plum. Plum (*Prunus domestica*) is a fruit culture. The Kyustendilska blue plum is a major variety in our country. Known as the Winter Plum or the Madjarqinia. It has been grown since the 17th-18th centuries, along with a large number of local rakia varieties. With the significant increase in the plum areas after the First World War, the variety of Kyustendilska Blue Plum is even more widely distributed (Iliev et al., 1985).

In 100 grams of plum fruit are found about 30 calories. The water content is 87%, sugar (9%), pectin (0.8%), protein (0.8%), cellulose (0.5%). The predominate organic acid is the apple acid (1.3%), vitamins B₁, B₂, PP, C (10 mg%) and provitamin A. Mineral substances contain salts of sodium,

2,1 mg %.

(*Allium cepa* L.)

potassium, magnesium, phosphorus and iron with a total content of about 2,1 mg%.

The fruits of Kyustendilska blue plum are very valuable both for fresh consumption and for processing (for drying, for production of fruit and fruit-sugar cans). A significant drawback of this variety is the high susceptibility to disease and air drought, as well as the unsatisfactory size of the fruit²⁸.

Resell water onion. The onion (*Allium cepa* L.) is grown in and around the village of Reselet, which is located in the Pleven region. The quality and typical taste of Resell's onion is determined by the abundant watering of the crops.

Resell water onions have rounded heads that vary in shape. The colour is yellow-brown or pink-purple. The onion has tender, juicy fleshy scales. The taste is fresh, dazzling and sweet. It is suitable for direct consumption in fresh state (leaves and bulbs), preparation of salads, dishes, etc. Because of its taste properties, local people have once called it "*Resellers' cheese*". Resell water onion is known for their curative properties for the heart, arteries, and with the traditional general-acting on the immune system²⁹.

Preservation of local varieties is an important task, which is to ensure food independence, opportunities for adaptation of agriculture to climate change, cost-effective approaches to pest control and soil quality, preservation of local culture and traditional knowledge. Resell water onions are extremely associated with the soil's and climate conditions of the region.

This is the main reason because of which this vegetable has been identified as a suitable candidate for a protected designation of origin (PDO). This status will protect it from possible counterfeits

(Paskova, 2017).
(Capsicum annuum L.)
 (17.
(Phaseolus vulgaris)
(Fabaceae). (

30

Kurtovo Konare is registered in the World Treasure of Flavors. At present, the producers of this tomatoes variety are also struggling for recognition by the European Union. This will ensure its origin in the European Register of Protected Designations (Paskova, 2017).

Kurtovska kapia. Traditional variety (*Capsicum annuum L.*) type Capia is suitable for open-air cultivation, for medium early and late production. The production is intended for fresh consumption and processing (mainly for baking, marinats, production of canned vegetables – puree, lutenitsa, etc.).

The variety is traditionally grown in Plovdiv – Kurtovo Konare, Novo Selo, Trivoditsi and others, in some villages in Pazardzhik region, in villages around Kardzhali, Haskovo and others³².

Smilyan beans. The bean (*Phaseolus vulgaris*) is a plant species of the *Fabaceae* family. Smilyan beans are called the bean seed.

It is one of the few Bulgarian foods protected by a patent for a word mark concerning its cultivation in the area of the upper valley of the river Arda. The locals call it with the name of *fasuluvitsa*.

Bean breeding traditions in the area date back more than 250 years. The specific soil conditions, the high humidity due to the proximity of the Arda river, the temperature limits and the quality of the water typical of the Smilyan region are suitable for bean cultivation.

Smilyan beans have a unique taste and are famous both in Bulgaria and abroad.

The method of cultivation has traditionally been maintained for generations. Plantations are manually handled with natural manure. Annually, around 30 tons of Smilyan beans are grown in the region.

Smilyan beans grow in the region of the village of Smilyan, as well as the neighboring Mogilitsa and Arda, situated in the area of the upper reaches of the river Arda. The area of cultivation is characterized by a mountainous climate where the autumn-winter season is relatively mild and the summer is cool. Other factors that determine the good quality of Smilian beans are altitude and humidity of the air. The area where the plant grows is at an altitude of 820 to 875 m. Temperatures are moderate and cool in the summer. At more than 33 °C the bean blossoms burn and fall off.

There are two main varieties of Smilyan beans:

- Large (Fasuluvitsa-Smilyan big). The color of the beans is white and colorful, ranging from black to light-purple. Traditionally, this form of Smilian beans is mainly used for salads and for panning;
- Small (Smilyan bean-small). Grains are considerably smaller than the previous one, with light brown beans dominated by dark brown and almost black strips (Stoyanova, 2012).

Both types of beans are distinguished from the rest of the unique patterns. The traditional way of growing beans guarantees specific flavour properties – density, fat and high protein content.

True Smolyan beans have a protected trademark and geographical origin since 2007 and with extended protection until 2024, the two varieties of beans are offered with specially developed labels. Within the scope of registration are six varieties – three of the small and three of the big salad beans³³.

Sadovski peanut. Peanut (*Arachis hypogaea*) is a type of annual herbaceous plant of the same name peanut (*Arachis*) of the bean family. In 1926 the peanuts were first registered as a culture in Bulgaria. In the

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80- 65%
 80%
 1991 150 000 da
 (Zdravkov, 2011).

(1, 2, PP, B₃, B.
 E 375),
 ()

- 1980s, 65% of the peanuts in Europe were cultivated in Bulgaria, and production was up to 80% of the European one. In 1991, the peanuts in Bulgaria reach 150000 da and are on second place only after the sunflower. Currently, the main areas with peanuts are in Sadovo, there are small once in the regions of Southwestern Bulgaria (Zdravkov, 2011).

- Nutritional value of peanuts is determined by their content of protein substances, fats and carbohydrates.

- They also contain vitamins B₁, B₂, niacin (also known as vitamin PP, nicotinic acid, vitamin B₃, nicotinamide is a water-soluble vitamin of group B. It is synthesized by the alkaloid nicotine. In the human body, niacin is imported from outside with food or is synthesized endogenously by the amino acid tryptophan. In the food industry it is used as a food additive E 375), vitamin C as well as salts of potassium, calcium, magnesium, iron, sodium and phosphorus. They contain antioxidants, which are mainly represented by polyphenols.

- Because of this, they are used as a prophylactic in cardiovascular diseases, to stop early aging processes and develop malignant tumors. Scientific studies put peanuts (also called walnuts) on the same level as strawberries and blackberries, and with antioxidant effects yield only to pomegranate.

- A major drawback of frequent peanut consumption is their allergenic effect. The allergic reaction occurs as reddening of the skin, itching, vomiting, stomach acids and even as a laryngeal discharge and anaphylactic shock.

- On the other hand, the high content of protein substances in peanuts can harm people with osteoarthritis, arthritis and

5,0 24,0 35,0%,
 12,0%, 13,0
 22,0%, 1,5 %,
 2,5%,
 2,0%, - 1,4%,
 (17 ()) 1,0
 2,5%, 19 () 8,0
 15,0%, 19 () 2,0 5,0%,
 21 () 3,0 5,5%, 23
 () 0,5 1,5%)²³.

(*Lavandula*)

(*Lamiaceae*).

1 da
 10-18 kg

5.0 to 12.0%, geraniol from 13.0 to 22.0%, geranyl acetate to 1.5%, eugenol to 2.5%, methyl eugenol to 2.0%, farnesol at least 1.4%, hydrocarbons (C₁₇ (heptadecane) from 1.0 to 2.5%, C₁₉ (nonadecane) from 8.0 to 15.0%, C₁₉ (nonadecane) from 2.0 to 5.0%, C₂₁ (heneicosane) from 3.0 to 5.5%, C₂₃ (tricosan) from 0.5 to 1.5%)³⁸.

Lavender. *Lavandula* (*Lavandula*)

is a plant of the Orniformes family (*Lamiaceae*). The name is most commonly used for herbaceous species. The lavender has been known as an aromatic-oil plant since ancient times. Lavender originates from the Mediterranean region. As a wild plant, it occurs in southern France, the Alps, Eastern Spain, Italy and North Africa. With the development of the perfumery industry, the area of cultivation is expanding, including the Balkan countries, the countries of the Black Sea Basin, the United States, Great Britain, Australia and others.

It is widely used in native medicine, but is also used as herb or ornament. Lavender flower is an insecticide resource, most often used to combat the moth and in the civilized world shifted the use of naphthalene. Lavender is a valuable honey plant. It is found that from 1 da, bees collect 10-18 kg honey. Lavender is also used as a spice. It is the base of the famous herb mixture **erb de provans** – fragrant mixture which contains in its composition - savory, oregano, rosemary, thyme, major and lavender leaves.

Lavender is mainly grown because of the flowers from which is produced the essential oil. It is used in perfumery, cosmetics, paints and ceramics industry.

Lavender oil has a yellow-green colour, a strong, saturated aroma that is slightly different from the aroma of the flowers.

- The subtle scent of lavender oil is emphasized if it is dissolved with alcohol.

- Lavender essential oil contains esters of linalol and especially linalyl acetate (depending on the conditions of cultivation, this ester ranges from 30 to 60%). They give the specific pleasant aroma. Geraniol, borneol, tsineol, pinen, camphor, coumarin and others are also found in the composition of the oil. Along with the essential oil in the composition of lavender are found up to 12% of sugars, tannins, organic acids, mineral salts, anthocyanins and other substances (Trifonova, 2014).

- It is believed that the highest quality French lavender oil is obtained from alpine lavender fields. Bulgarian lavender oil is characterized with its long lasting aroma, although the content of linalyl acetate is low, it is proved that the composition and aroma of Bulgarian lavender oil does not yield to the quality of world equivalents.

- Presented traditional plant species are typical of our country and have long-standing traditions in their production. Fresh fruits and vegetables obtained from them are distinguished with specific composition and nutritional value, and this also determines their use for – fresh consumption, processing in fruit and vegetable cans, production of wine, alcoholic beverages and others.

- The flowers of oil-bearing rose and lavender are the main raw material for the production of essential oils, toilet waters, etc., and they are used in perfumery and cosmetics industry.

CONCLUSIONS

In conclusion, after the study, it can be summed up that traditional plant species are a tremendous treasure for our country. They have been grown since ancient times in our country and the

<p>2014</p> <p>(</p> <p>2007</p> <p>2024</p> <p>–</p>	<p>In 2014, <i>the pink tomato from Kurtovo Konare</i> is registered in the World Treasure of Flavors. At present, the producers of this variety of tomatoes are also struggling for its recognition by the European Union. This will ensure its origin in the European Register of Protected Designations.</p> <p><i>Smilyan bean (Smilyanski fasul)</i> is one of the few Bulgarian plant species (food from plant origin), protected by a patent for a word mark and geographical origin since 2007 and with extended protection until 2024. It is offered with specially developed labels. There are six varieties in the scope of registration – three varieties small beans and three varieties large salad beans.</p>
<p>2014</p> <p>25.4.2014</p>	<p><i>Rose oil</i> has a protected geographical indication and a designation of origin for an agricultural product from 2014 and is listed in the Official Journal of the European Union from 25.4.2014. This gives rose oil a protected status.</p> <p>The described traditional plant species are known only for the regions where the most favorable soil and climate conditions for their production exist and are unknown to consumers in other EU countries.</p> <p>In this connection, a recommendation could be made to develop a national policy and procedures to closely monitor the proposals and the registration for the protection of traditional plant species and foodstuffs of plant origin in the European Union's list.</p>

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