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## Production of highbush blueberry nuclear stock in Serbia

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### SUMMARY

L.) -  
(*Vaccinium corymbosum*)  
-  
700  
ha  
2015  
2015 2016  
52 - 10  
2016  
ELISA, *Blueberry shock virus*, *Blueberry mottle virus*, *Blueberry scorch virus*, *Blueberry shoestring virus*, *Tomato ringspot virus*, *Tobacco ringspot virus* *Peach rosette mosaic virus* PCR *Blueberry red ringspot virus*,  
-

The highbush blueberry (*Vaccinium corymbosum* L.) is becoming increasingly important small fruit crop in Serbian agriculture. According to some estimations, highbush blueberries are cultivated on approximately 700 ha in Serbia.

In 2015, a program for the production of certified highbush blueberry pathogen-tested material has started in the Fruit Research Institute in a ak. A total of 52 candidate clones of 10 cultivars were selected during 2015 and 2016. Plants were planted in a sterile medium and put in the insect-proof screen-house. Since 2016, plants were regularly visually checked for the presence of bacteria, fungi and other diseases or unknown symptoms. All plants were annually tested by ELISA for the presence of *Blueberry shock virus*, *Blueberry mottle virus*, *Blueberry scorch virus*, *Blueberry shoestring virus*, *Tomato ringspot virus*, *Tobacco ringspot virus* and *Peach rosette mosaic virus*; and by PCR on *Blueberry red ringspot virus*, *Blueberry mosaic-associated virus* and *phytoplasmas*. Samples from candidate clones were also

*Cucumis sativus*.

(*Vaccinium corymbosum* L.)

70-

2005

5 ha.

700 ha.

2015

tested.

EPPO

(OEPP/EPPO, 2008).

14

(Jevremovi and Paunovi, 2010).

"

" (Jevremovi et al., 2016a).

indexed on the herbaceous indicator *Cucumis sativus*.

None of these pathogens have been detected in tested candidate clones so far.

**Key words:** highbush blueberry, planting material, certification, viruses, phytoplasmas

## INTRODUCTION

The highbush blueberry (*Vaccinium corymbosum* L.) is a commercially important berry crop and it is becoming increasingly important small fruit crop in Serbian agriculture. Highbush blueberries were introduced into Serbia in 1970s, but a significant increase of the production started a decade ago. From the first 5 ha in 2005, highbush blueberries are cultivated today on approximately 700 ha.

All highbush blueberry plantations in Serbia were established with the imported planting material, mostly from the Netherlands and Poland.

In 2015, a program for the production of certified highbush blueberry pathogen-tested material has started in the Fruit Research Institute in a ak. The program is in line with domestic low regulations and EPPO recommendations (OEPP/EPPO, 2008).

The main aim of the program is to produce nuclear stock as starting material for further propagation into pre-basic, basic and certified planting material. The realization of the program will reduce large-scale imports in the near future to a significant extent. Similar program that was realized at the Fruit Research Institute has resulted in the production of nuclear stock of 14 plum cultivars (Jevremovi and Paunovi, 2010). The production of red raspberry nuclear stock of cultivars 'Willamette' and 'Meeker' is under way (Jevremovi et al., 2016a).

## MATERIAL AND METHODS

52 - 10  
 ('Bluecrop', 'Bluejay',  
 'Chandler', 'Chanticleer', 'Duke', 'Earlyblue',  
 'Elliot', 'Meader', 'Patriot', 'Spartan') -  
 2015 2016. -  
 ,  
 ,  
 . 2016 . -  
*Armillaria mellea*, *Exobasidium vaccinii* var.  
*vaccinii*, *Godronia cassandrae*, *Diaphorte*  
*vaccinia*, *Agrobacterium tumefaciens*,  
*Cranberry ringspot agent*  
 2016 . 2017 . -  
 ELISA -  
*Blueberry shock virus* (BIShV),  
*Blueberry mottle virus* (BLMoV), *Blueberry*  
*scorch virus* (BIScV), *Blueberry shoestring*  
*virus* (BSSV), *Tomato ringspot virus*,  
*(ToRSV)*, *Tobacco ringspot virus* TRSV)  
*Peach rosette mosaic virus* (PRMV)  
 AGDIA Inc, ( )  
 BIOREBA AG ( )  
 1:10 -  
 405 nm BLM V, BIScV, BIShV,  
 PRMV, TRSV ToRSV; 620 nm  
 BSSV ELISA  
 MULTISKAN MCC/340 (Labsystems,  
 ) 20-120 min.  
 PCR -  
 o *Blueberry*  
*red ringspot virus* (BRRV),  
 (BIMaV) (*Blueberry*  
*stunt phytoplasma*, *Cranberry false*  
*blossom phytoplasma* *Vaccinium*  
*witches' broom*).  
 (TNA)  
 CTAB, Li  
 et al. (2008).

A total of 52 candidate clones of 10 cultivars ('Bluecrop', 'Bluejay', 'Chandler', 'Chanticleer', 'Duke', 'Earlyblue', 'Elliot', 'Meader', 'Patriot', and 'Spartan') were selected during 2015 and 2016. Candidate clones were selected on the basis of trueness-to-type, vigor and absence of pest and disease symptoms. Plants were planted in a sterile medium and put in the insect-proof screen-house. Since 2016, plants were regularly visually checked for the presence of *Armillaria mellea*, *Exobasidium vaccinii* var. *vaccinii*, *Godronia cassandrae*, *Diaphorte vaccinia*, *Agrobacterium tumefaciens*, *Cranberry ringspot agent*, and to other diseases or unknown symptoms.

During 2016 and 2017 all plants were tested by ELISA for the presence of *Blueberry shock virus* (BIShV), *Blueberry mottle virus* (BLMoV), *Blueberry scorch virus* (BIScV), *Blueberry shoestring virus* (BSSV), *Tomato ringspot virus* (ToRSV), *Tobacco ringspot virus* (TRSV) and *Peach rosette mosaic virus* (PRMV) with the reagents of AGDIA Inc, (USA) and BIOREBA AG (Switzerland) according to the producers' recommendations.

Fresh blueberry leaf samples were prepared at 1:10 ratio in the general extraction buffer. Color development was measured at 405 nm for BLM V, BIScV, BIShV, PRMV, TRSV and ToRSV; and 620 nm for BSSV on ELISA reader MULTISKAN MCC/340 (Labsystems, Finland) after 20-120 min.

Molecular PCR test was used for the analysis of candidate clones on the presence of *Blueberry red ringspot virus* (BRRV), *Blueberry mosaic-associated virus* (BIMaV) and phytoplasmas (*Blueberry stunt phytoplasma*, *Cranberry false blossom phytoplasma* and *Vaccinium witches' broom phytoplasma*).

Total nucleic acids (TNA) were extracted from fresh leaves with a modified CTAB method according to Li et al. (2008). Samples were analyzed on the



RT-PCR  
BIMaV, PCR

BRRV.

BRRV BIMaV  
(Jevremovi et al., 2016b).

BRRV BIMaV

BIMaV (1),

BIMaV RT-PCR (Thekke-Veetil et al., 2014; Thekke-Veetil et al., 2015),

country. The planting material was also imported from other countries, as France, Germany, Ukraine, and the USA.

The RT-PCR analysis on BIMaV presence revealed an expected PCR fragment only in positive control, but not in any of the analyzed candidate clones. BRRV was also not detected in any of the analyzed samples. Even those highbush blueberries are cultivated on relatively large area in Serbia, only localized occurrence of BRRV and BIMaV has been confirmed in Serbia (Jevremovi et al., 2016b). The isolated plantation with BRRV infected plants was recently eradicated and BIMaV is the only viral pathogen that is present on blueberries in Serbia. BIMaV is causative agent of the blueberry mosaic disease (Figure 1) whose symptoms may be latent for years. BIMaV may be detected in symptomless plants with RT-PCR test (Thekke-Veetil et al., 2014; Thekke-Veetil et al., 2015) and this test may represent a reliable diagnostic assay for screening blueberry propagation material.



1. BIMaV  
Fig. 1. Blueberry mosaic symptoms caused by BIMaV on highbush blueberry leaves

(Blueberry stunt, Cranberry false blossom  
*Vaccinium* witches' broom ),  
*Vaccinium* spp.  
 (Starovi et al., 2013).  
*C. sativus*  
 3  
 ( )  
 tested.  
 " ),  
 ELISA  
 PCR  
 PCR  
 2008).  
 EPPO 4/18,  
 2008 .,  
 (OEPP/EPPO,  
 PCR

*Stolbur*

No phytoplasmas were detected in analyzed candidate clones. None of the three phytoplasmas (Blueberry stunt phytoplasma, Cranberry false blossom phytoplasma and *Vaccinium* witches' broom phytoplasma) that are listed in the Certification scheme for *Vaccinium* spp. have not been reported in Serbia. Phytoplasmas are common pathogens of fruit species in the country, but only *Stolbur* phytoplasma has been reported on highbush blueberry in Serbia (Starovi et al., 2013).

None of the tested plants was positive in biological testing performed in the glass-house. All inoculated *C. sativus* plants were symptomless. Indicator plants were visually inspected after inoculations in the following 3 weeks, and no symptoms were observed.

The combination of different testing methods (biological, serological and molecular) allows accurate and precise diagnosis of different plant pathogens.

Biological methods are the oldest and irreplaceable testing methods for the production of pathogen-tested plant material. The current unavailability of the some recommended woody indicators (old blueberry cultivars 'Stanley', 'Cabot' and 'Jersey') will extend biological testing in our program.

Serological ELISA test is a rapid and suitable method that allows large-scale testing for numerous viruses.

Molecular PCR test has the greatest sensitivity and is suitable for the detection of known and newly discovered pathogens whose genome is partially or completely characterized. PCR is not yet recommended method in the EPPO 4/18 Certification scheme, that was edited in 2008, but still not updated (OEPP/EPPO, 2008). It can be expected that PCR tests will be incorporated in the revision of the existing scheme.

## CONCLUSIONS

The presented results showed that all selected plants of highbush blueberry cultivars were not infected with analyzed viruses, phytoplasmas and other pathogens. In the following period, all plants will be tested on other preferred indicators, and retested by ELISA and PCR. After the completion of all tests the material will be promoted to nuclear stock and transferred to the separate screen-house.

## ACKNOWLEDGEMENTS

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” ”

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1 , 7007 , 1164 ,  
2 ,

## Study on the influence of "Aminobest" organic fertilizer on the development of the above-ground parts and the root system in the production of vine planting material of cv Misket Rusenski

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### SUMMARY

The objective of the study was to monitor the effect of soil fertility maintaining by modern means, as the influence of the organic fertilizer "Aminobest" on the production of vine plant material to be determined.

The experiment was conducted during the period 2014-2016 at the experimental nursery for grapevine rootings of IASS "Obraztsov Chiflik" on the area of 0,2 da, as cv Misket Rusenski with vines grafted onto SO4 rootstock was put for rooting. The variant treated with "Aminobest", included about 1000 pcs grafted vines in four replications, 250 pcs

2014-2016 .  
" , 0,2 da  
1000 .



250  
(  
(90ml/l) 48  
S04,  
(  
)  
:  
(Kunicki et al., 2010).  
(Ziosi et al., 2013; Bulgari et al., 2014).

each, and was compared with a control (untreated) variant with grafted vines of cv Misket Rusenski, put for rooting in the same number of replications. Treatment with "Aminobest, was made by immersing the section at the base of grafted cuttings in Aminobest aqueous solution (90ml/l) for 48 hours.

Based on some of the parameters, specific for the quality of class vine planting material (number of developed shoots, number of roots) the most efficient variant could be selected.

Average for the period of study, the variant treated with "Aminobest" was found as more efficient for the production of class vines of cv Misket Rusenski, grafted on S04 rootstock. According to the biometric assessment, that variant could be recommended in practice in the production of vine planting material.

**Key words:** organic fertilizers, vine, increment, root formation, vine planting material

## INTRODUCTION

Biostimulants are of interest for the development of sustainable agriculture, as their application activates some physiological processes that increase the efficiency of use of nutrients, stimulate plant growth, allowing reducing the consumption of fertilizers (Kunicki et al., 2010). A lot of biostimulants are able to counteract the effect of biotic and abiotic stresses, such as enhancing the quality and yield of the crops by stimulating the physiological processes of the plants (Ziosi et al., 2013; Bulgari et al., 2014).

In recent years there has been an increased interest in organic farming and its contribution to sustainable and rational land use. This is one of the main reasons for the frequent comparison between organic and conventional farming,

(Popov et al., 2010).

(Shehata and El-Khawas, 2003),

(Wu et al., 2004).

(Alves et al., 2009).

(Vlahova and Popov, 2013).

(Kostadinova and Popov, 2012).

19-

indicating the potential opportunities for organic grape growers (Popov et al., 2010). Biofertilizers have become a promising component of an integrated food supply system in agriculture (Shehata and El-Khawas, 2003), because they were determined as an alternative to chemical fertilizers for increasing soil fertility and yield in the sustainable agriculture (Wu Et al., 2004). The objective of the use of liquid biofertilizers is balanced plant nutrition to be achieved (Alves et al., 2009). The use of such fertilizers is a real opportunity for obtaining quality and healthy foods (Vlahova and Popov, 2013).

For the transition to organic farming, it is necessary to take measures the soil conditions to be improved and humus content to be increased. (Kostadinova and Popov, 2012). In the field of viticulture, the major way to improve the agronomic characteristics of soils, i.e. measures to be taken to restore the quality of agricultural land is by enriching the soil with organic substances and mineral compounds, preserving the active microflora, improving and regulating the nutrition, moisture, air and heat regime of the soil.

The production of vine planting material has been one of the most important trends in the wine-growing practice since the end of the 19th century. The creation of cost-effective vineyards is conditioned to a high extent by the quality of the produced initial planting material. Phytosanitary healthy and long-lasting vineyards are created with high-quality vine planting material. There is a tendency to improvement of the individual technological moments of this production, one of which is the improvement of the soil and the treatment of plant parts with potassium humate, which enriches the soil with humus, improves the moisture retention, improves its structure, the development of the useful microflora and bonds the heavy metals and other



2014-2016 .  
 ” 0,2 da  
 S04.  
 0,60 m  
 0,30 m.  
 (Todorov,  
 2005).  
 ”, 1000 .  
 250 .  
 ( )  
 ” ”  
 (90ml/l) 48 .  
 .  
 ( )  
 .  
 :  
 V0 –  
 V1 –  
 ” ”,  
 (90ml/l) 48 .  
 18 .

The experiment was conducted during the period 2012-2013 at the experimental nursery for grapevine rootings of IASS “Obraztsov Chiflik” on the area of 0,2 da, as cv Misket Rusenski with vines grafted onto SO4 rootstock was put for rooting. The grafted and stratified cuttings were rooted on raised double-row beds with a bed width of 0.60 m and a distance between the rows in the bed - about 0,30 m. . The vines in the nursery for grapevine rootings were grown according to the commonly adopted technology for the production of grafted vine planting material (Todorov, 2005). The variant treated with “Aminobest”, included about 1000 pcs grafted vines in four replications, 250 pcs each, and was compared with a control (untreated) variant with grafted vines of cv Misket Rusenski, put for rooting in the same number of replications.

The treatment with “Aminobest” liquid organic fertilizer was done by soaking the roots of grafted cuttings in Aminobest water solution (90ml/l) at exposure of 48 hours.

The soil type is carbonate chernozem on deep loess. The soil is moderately supplied with nitrogen and phosphorus and well-stocked with potassium.

On the basis of some of the traits, specific for the quality of class vine planting material (number of developed shoots, number of roots), the more effective variant was searched.

To conduct the experiment a comparative study was made in two variants formed:

V0 – grafted and stratified cuttings without treatment

V1 – grafted and stratified cuttings treated with Aminobest, by soaking the petioles of grafted cuttings in Aminobest water solution (90ml/l) at exposure of 48 hours.

Biometric measurements were taken on a sample of 18 class vines of each variant. The number of shoots and

DUNKAN,  
ANOVA (STATGRAPHICS Plus ver. 2.1.)

the number of stepped roots per a vine were recorded.

A mathematical processing of the experimental data was performed by the method of dispersion analysis, and the differences between the variants were determined by the test of DUNKAN, ANOVA, (STATGRAPHICS Plus ver. 2.1).

## RESULTS AND DISCUSSION

The trait characterizing the production of vine planting material is the yield of rooted vines. It is influenced by all changes in the factors determining the normal course of rooting, growth and development of grafted cuttings.

The number of shoots is important and is the basis for determination of first-class grafted and rooted vines. From the data obtained a significant increase was observed in the number of shoots per a vine in the variant, treated with Aminobest, compared with the same trait in the untreated variant (Table 1). In the treated variant, the number of shoots, on average per a vine, was 1,1,61, which exceeded the number of shoots in the control variant by 26,7% (1,1,27 pcs). Data about the obtained differences were statistically proven by the test of Duncan, at P <0.05.

1.  
( 1).  
1,61,  
26,7% (1,27 ).  
Duncan <0,05.  
1.

**Table 1. Influence of Aminobest organic fertilizer on the number of shoots of vines of cv Misket Rusenski**

Variants	Number of shoots per a vine	%	LSD LSD after the method of Duncan	Duncan
/Control	1,27	100,0		a
/Aminobest	1,61*	126,7		b

\* , \*\* , \*\*\* , LSD 0,05; 0,01;0,001.

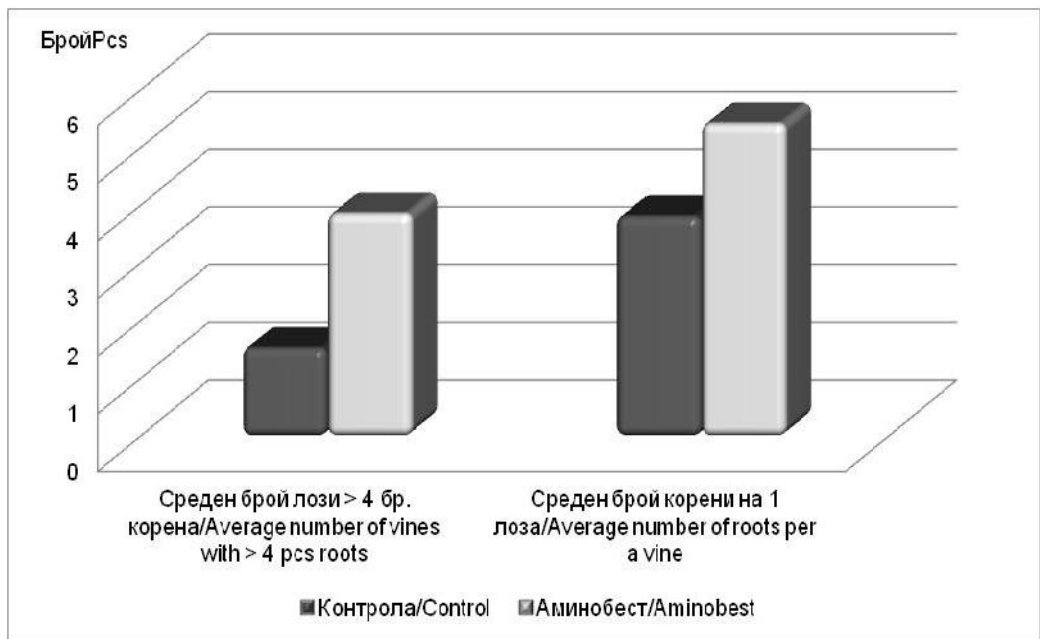
P<0.05

(a,b,c . .),

\* , \*\* , \*\*\* , at LSD <0.05, 0.01; 0.001. All non-star variants have no significant difference with the untreated variant. The values in a column, followed by different letters (a, b, c, etc.), differ significantly in P <0.05

( 1).

Another important trait, determining the quality of the obtained first-class vines, is the number of stepped up roots (Figure 1).



**Fig. 1. Effects of Aminobest organic fertilizer on the average number of vines with > 4 pcs roots, and the average number of roots per a vine of cv Misket Rusenski**

2 ,  
4 . ,  
150,3 %,  
<0,05.  
42%.

It could be seen from Table 2 that in the treated variant, the average number of vines with more than 4 pcs roots exceeded the data for that trait in the control untreated variant.

The action of Aminobest also stimulated the formation of more than 4 stepped up roots per a vine, exceeding the control variant by 150,3%, which was a prerequisite for increasing the quality of grafted vines. The determined differences were found to be significant at  $P < 0,05$ .

The fertilization with Aminobest created conditions for the absorption of the amino acids included in it and influenced on the root system of the grafted cuttings. The average number of stepped up roots per a vine in the variant with applied Aminobest increased, exceeding the control variant by 42%. From the mathematical processing for the

( <0,01).

average number of roots per a vine, a high level of significance was found at P<0,01.

2.

**Table 2. Traits, showing the root formation in the experiment of vines of cv Misket Rusenski, treated by Aminobest organic fertilizer**

/ Variants	>4 Average number of vines with > 4 pcs roots	%	LSD Duncan LSD after the method of Duncan	1 Average number of vines per a vine	%	LSD Duncan LSD after the method of Duncan
/Control	1,55	100,0	a	3,83	100,0	a
/Aminobest	3,88*	250,3	b	5,44**	142,0	b

\*, \*\*, \*\*\*, LSD 0,05; 0,01; 0,001.

(a,b,c . .),

P<0.05

\*, \*\*, \*\*\*, at LSD <0.05, 0.01; 0.001. All non-star variants have no significant difference with the untreated variant. The values in a column, followed by different letters (a, b, c, etc.), differ significantly in P <0.05

## CONCLUSIONS

The development of the root and above-ground parts of the grafts of cv Misket Rusenski showed that in the variant of treating with Aminobest, more highly developed first-class vines were obtained.

On average, over the entire period of study, in the above-shown variant, a higher number of vines with more than 4 roots were registered, and the plants themselves were characterized with a higher average number of stepped up roots and a higher number of shoots.

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## Biometric analysis of some agrobiological and growth characteristics of “Rusensko bez seme” table vine cultivar (*Vitis vinifera* L.), grafted on three vine rootstocks

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### SUMMARY

Using two statistical criteria, the effects of the application of vine rootstocks: Berlandieri Riparia SO4, Rupestris du Lot (with common name Montikola) and Chasselas x Berlandieri 41 B (hereinafter for short Chasselas 41B), on some agrobiological and growth characteristics of Rusensko bez seme table vine cultivar, were analyzed. The study was carried out in the experimental vineyards of the Institute of Agriculture and Seed Science „Obraztsov chiflik”, Rousse in four replications, 11 plants each. First class vines, at one and the same vegetative growth were used.

The objective of the study was the strength of the influence of the relevant rootstock on the most important productive traits to be determined. The

Fisher t-Student.

20%.  
Fisher

(Deidda, 1986; Gorodea et al., 1986; Todorov, 1987; Koblet et al., 1994; Hristov et al., 1998; Arestova et al. 1999; Reynolds et al., 2001; Agut et al., 2003; Bettiga, 2003; Boso et al., 2008 ).

(Parejo et al., 1995; Nuzzo and Matthews, 2006 ).

Mamarov and Dimitrov (1986)

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SO4.

41 , SO4,

F- values of 15 agrobiological and growth traits were reported. Data obtained was statistically analyzed via F-Fisher and t-Student evaluation criteria. The study took place in seven consecutive years, as for one third of the studied traits, the variability was above 20%. The use of the criterion of Fisher in the biometric analysis increased the level of significance of the reported differences between the variants.

**Key words:** rootstocks, Rusensko bez seme seedless table vine cultivar, biometric analysis, agrobiological and growth traits

## INTRODUCTION

The variety with its individual genetic characteristics combined with the rootstock and environmental factors, forms its biological and economic qualities (Deidda, 1986; Gorodea et al., 1986; Todorov, 1987; Koblet et al., 1994; Hristov et al., 1998; Arestova et al. 1999; Reynolds et al., 2001; Agut et al., 2003; Bettiga, 2003; Boso et al., 2008, etc.).

The choice of rootstock is of a great importance in terms of the force that it induces to the grafted variety. Vine rootstocks induce different growth of the grafted vine varieties. Researchers have essentially different views of the relation between the force of growth and fruit-bearing of the grafted varieties (Parejo et al., 1995; Nuzzo and Matthews, 2006, etc.). In that respect, experiments with a wide range of varieties, combinations, and areas have been conducted in traditional wine countries, such as France, Spain, Argentina, etc.

Mamarov and Dimitrov (1986) for a ten-year study period examined cv Bolgar grafted on 14 rootstocks. The variety showed the highest yield when grafted onto Berlandieri x Riparia SO4.

The influence of the rootstocks: Chasselas 41B, SO4, Fercal and III-102 D

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 (Hristov and Popov,  
 2007).  
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 SO4.  
 Sila, Nova Dinka  
 SK-13  
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 Kuljan i et al. (2004).  
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 SO4,  
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 SO4,  
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 (Shanin, 1977).  
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 t F (Student  
 Fisher).

- on the growth and fruit-bearing of the grafted variety - cv Dimiyat was examined and it was found that Chasselas 41B rootstock induced weaker growth power and fruit-bearing of the variety, compared to the other three rootstocks (Hristov and Popov, 2007). The highest annual increment was recorded in the combination with Fercal rootstock, and in terms of the fruit-bearing, the results were the highest with SO4 rootstock.

The potential fertility of Sila, Nova Dinka, and the Riesling Italian branch SK-13, depending on the type of the rootstocks (Kober 5BB, Teleki 5C, SO4, and Chasselas 41B), was followed by Kuljan i et al. (2004). The first two varieties showed best grafting results on SO4, but the authors do not recommend the use of Chasselas 41B for these varieties. The best results in cv Riesling Italian were obtained by grafting onto SO4, while on Chasselas 41B rootstock, the yield was high but the quality of grapes was bad.

The value of data received from field experiments is expressed by their degree of precision. Therefore, an important requirement of these experiments is to ensure the receipt of data with the highest possible accuracy, respectively the smallest possible differences between the tested variants to be identified as statistically proven (Shanin, 1977).

The objective of the study was the character and degree of influence, caused by the rootstocks: Berlandieri x Riparia SO4, Rupestris du Lot and Chasselas x Berlandieri 41 B on a grafted thereon cv Rusensko bez seme to be determined, in regard to a range of economically important, morphological and agrobiological traits and characteristics. Two parametric criteria – t and F (Student and Fisher) were applied in the statistical processing of the results in order that objective to be achieved.



<u>4 (SO4)</u>	
17%.	
25%	14 %
11	2,0 m/1,4 m, 1 km
0,60 m	19 5 2 9
(V1) ( ),	41 (V2) 15 SO4
	SO4.
	:
	( ),

Berlandieri x Riparia SO4 rootstock, Selection Oppenheim 4 (SO4) is mid to fast-growing. It is resistant to drought and to active carbonates in soil up to 17%. It has a good affinity with most of the table and wine vine varieties. The plants grafted onto it distinguished with longevity, abundant fruitfulness and qualitative grapes. It is believed that it improves the maturation of the wood of grafts and makes them more resistant to winter frosts.

Monticola/ Rupestris du Lot rootstock is mid-growing, but gives fast growth to the grafts. Therefore the fast-growing and prone to blossom drops and vergin berries varieties, grafted onto it, enhance the expression of these disadvantages. The rootstock is suitable for warmer areas with deep friable soil with a content of calcium carbonate not more than 25% total and 14% active calcium carbonate. It gives the grafts high productivity, excellent quality of grapes and longevity.

The study was conducted at the Experimental vineyard of IASS „Obraztsov chiflik” - Rousse in four replications, 11 plants in every replication. The vine planting was conducted at the distance of 2,0 m/1,4 m on hilly areas, facing South, at about 1 km from Danube river.

Soil type was carbonate chernozem on deep loess. The formation was half standard Guyot, stem height being 0,60 m and vine loads 19 winter buds, realized in 5 spurs of 2 buds each and 1 fruiting cane of 9 buds.

For cv Rusensko bez seme, grafted onto the three rootstocks — Rupestris du Lot, Chasselas 41B, and SO4 the values of 15 agro-biological and growth traits were registered. In comparisons, the variant, grafted onto SO4 rootstock was accepted as control.

The following agro-biological and growth traits were studied: number of developed shoots per a vine (a), including

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 , kg,  
 , cm,  
 , cm,  
 cm,  
 , cm,  
 cm,  
 , mm,  
 , mm  
 , kg  
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 .  
 Katerov et al. (1990),  
 Lazarevskiy  
 (1959).  
 5 cm  
 .  
 Student (t - test) Fisher (F),  
 SPSS 19  
 .  
 (Zapryanov and Dimova,  
 1995; Mencher and Zemshman, 1986).

fruiting shoots (b); percentage of fruiting shoots, according to the formula  $c = b/a \times 100$ ; number of clusters per a vine, developed on shoots grown from winter buds; fruit-bearing coefficient; grapes yield per a vine, kg; length of mature shoots, cm; length of internode of mature shoot, cm; girth of the rootstock, cm; girth of the graft, cm; length of the cluster, cm; width of the cluster, cm; length of the berry, mm; width of the berry, mm; and mass of the annual increment of shoots per a vine, g.

Traits were determined after the methods adopted in scientific-research practice. The length of internode of mature shoot, the size of cluster and berry were determined after Katerov et al. (1990), and the form of berry – after Lazarevskiy (Lazarevskiy, 1959). Girths of the graft and the rootstock were measured 5 cm below and above the place of the grafting.

Two criteria - Student (t - test) and Fisher (F) were used in the statistical processing, as for the purpose SPSS 19 was used for analysis of the data obtained. Standart formulas were used for calculating the criteria (Zapryanov and Dimova, 1995; Mencher and Zemshman, 1986).

## RESULTS AND DISCUSSION

With the help of two criteria – Student (t) and Fisher (F) the influence of the three rootstocks (SO4, Rupestris du Lot and Chasselas 41B) was statistically evaluated on 15 growth and agro-biological traits, compared to SO4, the control accepted. The three rootstocks have different growth power - SO4 is mid to fast-growing, Rupestris du Lot is mid growing, while Chasselas 41B is with more moderate rate of growth.

- Student (t) Fisher (F)  
 ( SO4,  
 41 ) 15  
 SO4.  
 - SO4  
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 Student

Statistical assessment via Student's criteria is based on the average arithmetical values, and when using the

Fisher – ,  
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 ( ) ,  
 (Lidanski, 1988).  
 Student  
 15-  
 - F (Fisher),  
 ( SO4).  
 41 -  
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 : =1%),  
 (=5%) ( 1).  
 ,  
 SO4, 41  
 =1%.

criterion of Fisher – the comparison is based on variability.

Very often in conducting experiments with different factors of influence, the central (typical) trends of excerpts are retained, but the degree of variation significantly changes (Lidanski, 1988). For that reason, we have carried out parallel biometric analysis.

The mathematical analysis using the criterion of Student showed insignificant influence of the rootstock on the expression of the 15 studied traits, therefore the results were not presented in a table. The reported differences via that criterion could not really determine significance between the individual rootstocks.

After applying the second F (Fisher) criterion, for the variant with varieties grafted on Rupestris du Lot rootstock, a significant difference was observed only for the trait % of fruiting shoots compared to the control variant (grafted on SO4 rootstock). The effect of Chasselas 41B rootstock was considerably more noticeable in one of the most important growth traits: fruit-bearing coefficient (significance at  $p = 1\%$ ), grapes yield and length of the cluster (significance at  $p=5\%$ ) (Table 1).

In that study, as a further analysis, the differences between the both rootstocks - Rupestris du Lot and Chasselas 41B were compared. The results were identical to the comparing of Chasselas 41B with the control SO4, but the trait "length of the berry" with significance at  $p = 1\%$  was also added to the three traits with significant influence on the rootstock.

1.

Fisher (F)

&lt;0.05; 0.01

**Table 1. Comparative evaluation of cv Rusensko bez seme by agrobiological and growth traits via criteria Fisher (F) at appropriate levels of significance <0.05 and 0.01**

/ Traits	/ cv Rusensko bez seme								
	SO4 – o Rootstock SO4 control		Rootstock Rup. du Lot V 1		41 Rootstock Shasla 41B V 2		V 1	V 2	V1-V2
	$\bar{X}$	S%	$\bar{X}$	S%	$\bar{X}$	S%	/ Confidence		
							F	F	F
Number of developed shoots of a vine	17,2	12,6	17,87	12	17	12,8	1,02 ns	1 ns	1,04 ns
including fruiting shoots	9,45	32,8	10,3	34,9	9,85	33,5	0,97 ns	1,2 ns	1,18 ns
% % of fruiting shoots	53,85	21,9	56,65	27,5	56,8	24,6	<b>1,74 +</b>	1,2 ns	1,39 ns
Number of clusters per a vine	12,2	38,5	12,5	40	13	43	1,07 ns	1,1 ns	1,1 ns
Fruit-bearing coefficient	0,71	42,2	0,73	41	1,06	41,5	0,99 ns	<b>23,6 ++</b>	<b>25,2 ++</b>
Grapes yield per a vine, kg	4,5	44,2	4,1	48,7	4,9	51	1,01 ns	<b>1,6 +</b>	<b>1,53 +</b>
Length of mature shoots, cm	176,7	18,7	180,9	17,8	169	19,5	1,02 ns	1,0 ns	1,1 ns
Length of internode of mature shoot, cm	8,2	18,3	8,0	18,7	7,9	18,9	1,1 ns	1,01 ns	1 ns
Girth of the rootstock, cm	14,6	39	16,8	38	15,6	38,5	1,27 ns	1,1 ns	1,1 ns
Girth of the graft, cm	14,18	38,8	15,6	38,7	15,1	39,7	1,22 ns	1,2 ns	1 ns
Length of the cluster, cm	18,4	21,2	18,9	24,8	18,36	15,8	1,46 ns	<b>1,8 +</b>	<b>2,6 +</b>
Width of the cluster, cm	11,12	20,7	11,86	23,6	11,38	22,8	1,51 ns	1,27 ns	1,2 ns
Length of the berry, mm	16,85	17,2	17,45	17,8	17,4	12,07	1,07 ns	1,4 ns	1,5 ns
Width of the berry, mm	14,27	18,2	14,68	23,8	14,26	13,3	1,3 ns	1,42 ns	<b>1,84 ++</b>
Mass of the annual increment of shoots per a vine, g	718	36,9	658,32	33,4	626,5	33,6	1,21 ns	1,26 ns	1,0 ns

At critical values of the criterion:  $F_{p 5\%} = 1,53$   
 $F_{p 1\%} = 1,84$

Fisher

Via Fisher's statistical criterion the presence of significant differences in four of the studied growth traits was proven when Chasselas 41B was used in cv Rusensko bez seme.

These traits have a direct influence on the vine productivity.



## CONCLUSIONS

Based on the results we made the following conclusions:

- The mathematical analysis via the criterion of Student showed insignificant influence of the rootstock on the expression of the 15 studied traits in cv Rusensko bez seme, grafted onto the three rootstocks: Rupestris du Lot and Chasselas 41B and SO4 (variant used as control).
- Via Fisher's statistical criterion the presence of significant differences in four of the studied growth traits was proven when Chasselas 41B rootstock was used in cv Rusensko bez seme. These traits have a direct influence on the vine productivity.
- As a result of the analyzes, we can summarize that for the cultivation of Rusensko bez seme seedless variety in the region of Northeastern Bulgaria, the use of Chasselas 41B rootstock is recommended to ensure higher productivity of the dessert variety.
- In such parallel biometric comparisons via two criteria with identical data, it is more pertinent and adequate to determine the positive effects, in the case of three rootstocks widely used in practice in the cultivation of dessert vine varieties.

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