

Influence of Blueberry Mosaic Associated Virus on Some Fruit Traits of Highbush Blueberry ‘Duke’

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Original scientific paper

SUMMARY

- Blueberry mosaic has been described several decades ago, but the viral nature of the disease was confirmed recently. The causal agent of the disease is blueberry mosaic associated virus (BIMaV), genus *Ophiovirus*. BIMaV induce leaf mottling and bright yellow, green, and pink to red mosaic patterns in infected bushes.

- Symptoms usually appear on several leaves or cover the entire bush, but the infection may be latent for several years.

- BIMaV was confirmed in two locations in Serbia in varieties ‘Duke’, ‘Bluecrop’ and ‘Goldtraub’. The aim of this study was to evaluate the influence of BIMaV on some fruit traits of highbush blueberry ‘Duke’.

- The trial was conducted in 2019 in locality Budoželja, West Serbia in the orchard with the confirmed BIMaV presence.

- Based on the results of RT-PCR analysis,

Ophiovirus. BIMaV

(BIMaV),

BIMaV

BIMaV

BIMaV.

RT-PCR

BIMaV, BIMaV
BIMaV
BIMaV
BIMaV
BIMaV

RT-PCR, BIMaV,

Varney (1957),

(Ramsdell and Stretch, 1987). Thekke-
Veetil et al. (2014)

(BIMaV).
BIMaV

BIMaV
BIMaV

(Jevremovi et al., 2015; Jevremovi et
al., 2016).

three groups of blueberry 'Duke' bushes were selected: BIMaV-free, BIMaV infected – mosaic symptoms present on only several leaves, and BIMaV infected – mosaic symptoms on entire bush. The influence of BIMaV on pomological variables (fruit length, width and height, shape, fruit weight, and soluble solids content) was examined.

The results showed that BIMaV did not have statistically significant impact on any of the examined fruit traits. The evaluation of virus influence on the composition of nutritive and bioactive compounds is underway.

Key words: Blueberry, BIMaV, RT-PCR, pomological traits, statistical analysis

INTRODUCTION

Blueberry mosaic disease was thought to be of genetic origin until the report of Varney (1957) that showed its virus etiology. For a decades, there were several attempts to purify virus particles from infected tissue and to examine them using electronic microscopy (Ramsdell and Stretch, 1987). Combining traditional methods and next generation sequencing Thekke-Veetil et al. (2014) detected a new virus in symptomatic plants tentatively named blueberry mosaic associated virus (BIMaV) in blueberry plants with mosaic symptoms. In infected plants BIMaV induces leaf mottle and light green, yellow, pink and dark red mosaic patterns. Also, the infection may be latent for several years.

BIMaV presence was officially confirmed in the United States, Canada, Japan, Slovenia and Turkey, but the blueberry mosaic symptoms were reported in many other countries. BIMaV was detected in Serbia in two distinct locations in varieties 'Bluecrop', 'Goldtraub' and 'Duke' (Jevremovi et al., 2015; Jevremovi et al., 2016).

(*Vaccinium corymbosum* L.)

70%

70-

15

1000

15

1986

(Retamales and Hancock, 2018).

" "

2019

(43° 32.262'N 20°16.568'E)
652 m.

BIMaV

(Jevremovi et al., 2015, Jevremovi et al., 2016).

2,5 1,5 m

Highbush blueberry (*Vaccinium corymbosum* L.) is one of the most valued small fruit crop in the world. Because of the nutritional value of the fruits, the production of blueberries is in the constant increase. More than 70% of highbush blueberries are produced in the United States, Canada and Chile. Sustained growth of production is evident in Asia (namely China), Europe and South America. The first introduction of highbush blueberries in Serbia was during 1970s, but the production was unnoticeable.

The second, more successful introduction started 15 years ago. Today, blueberries are grown on at least 1,000 hectares across the country.

Orchards are planted with the imported planting material. More than 15 varieties are present in the orchards, from standard to the most recently released. Blueberry 'Duke', that is one of the most economically important variety in the world, is the most prevalent variety in the orchards in Serbia. It was bred in the United States and introduced into the production in 1986 (Retamales and Hancock, 2018). Fruits are medium to large sized, uniform, medium colored, firm, of sweet-acid aroma and taste.

The aim of our study was to evaluate the influence of blueberry mosaic associated virus on some fruit traits of highbush blueberry 'Duke' in a commercial orchard.

MATERIAL AND METHODS

Material

The trial was carried out in 2019 in an orchard in locality Budoželja, Ivanjica (43° 32.262'N 20° 16.568'E) at the altitude 652 m above the sea level. The presence of BIMaV in this orchard was reported in earlier studies (Jevremovi et al., 2015, Jevremovi et al., 2016). Blueberries are grown at 2.5 x 1.5 m spacing according to the integrate pest and disease

15

3 / : 5

(1-); 5

(2-); 5

(3-).

BIMaV

(RT-PCR).
(TNA)
CTAB

(Li et al., 2008).

Maxima
Reverse Transcriptase (Thermo Scientific,
) . PCR

BIMaV
RNA3 BIMaV
(Thekke-Veetil et al., 2014). PCR
1,5%

Doc EZ System (Biorad laboratories,
). Gel

blueberry shock virus (BIShV), blueberry
mottle virus (BIMoV), blueberry scorch
virus (BIScV), blueberry shoestring virus
(BSSV), tomato ringspot virus (ToRSV),
tobacco ringspot virus (TRSV) and peach
rosette mosaic virus (PRMV)

ELISA
(Agdia
Inc., BIOREBA AG,).

PCR
blueberry red ringspot
virus (BRRV).

management system.

The trial included 15 plants that were randomly selected in the orchard. All plants were divided into 3 groups/variants: 5 plants with mosaic symptoms on entire bush (1-infected); 5 plants with slight symptoms on several leaves (2-infected); and 5 plants without symptoms (3-healthy).

Reverse transcription and polymerase chain reaction

The presence of BIMaV in the selected bushes was investigated using reverse transcription and polymerase chain reaction (RT-PCR). Total nucleic acids (TNA) were extracted with a modified CTAB method (Li et al., 2008). Reverse transcription was performed in two steps with random hexamers and Maxima Reverse Transcriptase (Thermo Scientific, USA). PCR was done with specific BIMaV primers amplifying the fragment from RNA3 of the BIMaV genome (Thekke-Veetil et al., 2014). PCR products were analyzed by 1.5% agarose gel electrophoresis, ethidium bromide staining and visualized in Gel Doc EZ System (Biorad laboratories, USA).

To exclude the possible effect of other viruses on examined traits, all plants were also tested on the presence on other economically important viruses. For the presence of blueberry shock virus (BIShV), blueberry mottle virus (BIMoV), blueberry scorch virus (BIScV), blueberry shoestring virus (BSSV), tomato ringspot virus (ToRSV), tobacco ringspot virus (TRSV) and peach rosette mosaic virus (PRMV) plants were tested by ELISA test according to manufacturers' protocols (Agdia Inc., USA and BIOREBA AG, Switzerland). PCR assay was performed to test the plants on the presence of blueberry red ringspot virus (BRRV).

(5 plants per each variant; 20 fruits per each plant). In total, 300 fruits were picked and measured. Fruit dimensions (fruit length, width and height) were determined with digital caliper (Carl Roth, Germany).

XL-1810 (Denver instruments, USA).
 (SSC, °Brix)
 871 (Milwaukee, USA)
 20° . SCC 25

(ANOVA)
 CoStat, 6.311 (CoHort Software, Monterey, CA, USA).
 $p < 0.01$.

RT-PCR
 BIMaV 10
 (1 2)
 5

Fruit traits

Fruits from selected plants were hand-picked during middle July in the optimal maturity stage for this variety. Fruits were picked from 15 plants (5 plants per each variant; 20 fruits per each plant). In total, 300 fruits were picked and measured. Fruit dimensions (fruit length, width and height) were determined with digital caliper (Carl Roth, Germany).

Fruit shape index was given as the ratio of mean height to mean width. Fruit weight was measured with a digital balance XL-1810 (Denver instruments, USA).

The soluble solids content (SSC, °Brix) of the fruits was determined in a sample of fruit juice with a digital brix MA871 refractometer (Milwaukee, USA) at 20°C. SCC was determined in 25 fruits from each variant. Distilled water was used for refractometer calibration.

Statistical analysis

Obtained data was analyzed by one-way analysis of variance (ANOVA) with CoStat software program, version 6.311 (CoHort Software, Monterey, CA, USA). Differences between variants were tested for significance by means of Duncan's multi-range tests. Tests were considered significant at $p < 0.01$.

RESULTS

Results of the RT-PCR analysis confirmed the BIMaV presence in 10 symptomatic plants (Figures 1 and 2), and the absence of the virus in 5 plants without symptoms. Other viruses were not detected in the analyzed plants.



. 1.

BIMaV (1)

Fig. 1. Fruits and leaves from the plants with severe mosaic symptoms induced by BIMaV (variant 1)



. 2.

BIMaV (2)

Fig. 2. Fruits and leaves from the plants with slight mosaic symptoms induced by BIMaV (variant 2)

100

- A total number of 100 fruits per variant were hand-picked in optimal maturity and measured in the laboratory.
- Results of the investigated physical properties (fruit length, width, and height

) and fruit shape index) of the fruits are given in Table 1.

1.

BIMaV

Table 1. Fruit length, width, height, fruit shape index, and soluble solids content in BIMaV infected and healthy plants

/Trait	/Variants					
	1-	/1-Infected	2-	/2-Infected	3-	/3-Healthy
/Fruit length (mm)	16.69±0.54	a	16.67±0.17	a	16.59±0.38	a
/Fruit width (mm)	16.28±0.53	a	16.30±0.14	a	16.23±0.49	a
/Fruit height (mm)	12.26±0.15	a	12.09±0.24	a	12.24±0.17	a
Fruit shape index	1.02±0.003	a	1.02±0.007	a	1.02±0.01	a
/Fruit weight (g)	2.02±0.17	a	2.05±0.06	a	2.05±0.14	a
Soluble solids content (°Brix)	12.90±1.23	a	12.16±0.87	a	13.02±0.34	a

$p < 0.01$.

No statistically significant differences between averages denoted with the same letter in variants by Duncan's multi-range test at $p < 0.01$.

BIMaV

ANOVA

" " ($p < 0.01$).

SSC BIMaV

0,93–6,60%

(1).

($p < 0.01$).

Statistical evaluation of the obtained results in ANOVA showed that BIMaV had no significant effect on the physical properties of 'Duke' fruits ($p < 0.01$).

The analysis confirmed that BIMaV infection decreased SSC in fruits on infected plants with mosaic symptoms from 0.93–6.60% (Table 1). This increase was with no significant effect ($p < 0.01$).

DISCUSSION

The investigation on blueberry virus diseases in Serbia has begun 15 years ago at the Fruit Research Institute in a ak. Blueberry mosaic associated virus was detected in Serbia on two distant locations in three varieties. BRRV was found in one location and afterwards eradicated (Jevremovi et al., 2018). Other viruses infecting blueberries are not present in Serbia. Due to the fact that variety 'Duke' has a largest share in the orchards and the highest number of infected plants, the aim of our work was to examine the impact of the BIMaV on its fruits.

Depending on the intensity of

BRRV

2018).

" "

15

(Jevremovi et al.,

BIMaV

infection, mosaic may be present on entire bush or localized on few leaves or branches.

BIMaV (Thekke-Veetil et al., 2014).

(Ramsdell and Stretch, 1987). Caruso and Ramsdell (1995) reported 15% crop losses in variety 'Bluecrop'. After BIMaV detection in the orchard where we conducted our study, during visual inspections we did not observed changes in the ripening time of the fruits from infected and healthy plants (unpublished results).

BIMaV

BIMaV

().

BIMaV

2,02-2,05 g,

(12,90 12,16 ° Brix) (13,02 °Brix).

SSC ($p < 0.01$).

infection, mosaic may be present on entire bush or localized on few leaves or branches.

BIMaV was also detected in asymptomatic plants (Thekke-Veetil et al., 2014). The information on the influence of the blueberry mosaic infection on the fruit properties and yield is scarce. Reports from the literature suggested that blueberry mosaic disease reduced yield, fruit quality and influenced on the ripening time of the fruits (Ramsdell and Stretch, 1987). Caruso and Ramsdell (1995) reported 15% crop losses in variety 'Bluecrop'. After BIMaV detection in the orchard where we conducted our study, during visual inspections we did not observed changes in the ripening time of the fruits from infected and healthy plants (unpublished results).

Results obtained in our study showed that BIMaV did not have any impact on the fruit size. Fruits on infected bushes, both on the plants with severe mosaic and on the plants with symptoms on several leaves, were normally developed.

There were no differences in any of the three measured fruit dimensions between the examined variants. Fruit weight was within the range 2.02–2.05 g confirming no difference between variants.

Soluble solids content in the infected fruits was slight lower (12.90 and 12.16 °Brix) compared to the healthy fruits (13.02 °Brix). This decrease was without statistical effect ($p < 0.01$). SSC content is an important quality parameter of fresh fruit and determines the moment of maturity for harvest.

CONCLUSIONS

The results of our study showed that BIMaV did not have statistically significant impact on the fruit length, width, height, fruit shape index and

BIMaV

soluble solids content in 'Duke' fruits.

The slight decrease of soluble solids content in BIMaV infected fruits was with no statistically significant effect.

The influence of the virus on the composition of nutritive and bioactive compounds is underway.

ACKNOWLEDGEMENTS

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451-03-68/2020-14/200215

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/zeatin 2-iP/ in vitro

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1 , , ,
2 , , , ,
3 , , , ,
4 , , , ,

Impact of Different Concentrations of Cytokinins /Zeatin and 2-iP/ on *in vitro* Propagation of Wild Species of Genus *Vaccinium*

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Original scientific paper

SUMMARY

The present study is focused on the optimization of some of the parameters of the micropropagation process in lingonberry and bilberry, originating in the Troyan Balkan, Bekleme area. 17 variants of modified WPM medium were tested (McCown and Lloyd, 1981), with different concentrations of zeatin, 2-iP, casein hydrolysis and pH (zeatin 2-3.5 mg/l; 2-iP 1.5-3.5 mg/l; casein hydrolysis 150-250 mg/l and pH 4.3-4.8).

The highest values of the micropropagation coefficient in lingonberry -

- 4,4 ./
 4,
 3,5 mg/l zeatin, 1,5 mg/l 2-iP
 250 mg/l casein hydrolysisate,
 - 2,8 ./ 5
 (2,0 mg/l zeatin,
 3,5 mg/l 2-iP 250 mg/l casein
 hydrolysisate).
 -
 17 - 4,2 cm (2,75
 mg/l zeatin, 2,5 mg/l 2-iP 200 mg/l
 casein hydrolysisate). - -
 - 1,6 cm
 4 (3,5 mg/l
 zeatin, 1,5 mg/l 2-iP 250 mg/l casein
 hydrolysisate).
 9 -
 IAA /0,5
 mg/l; 0,75 mg/l; 1,0 mg/l/
 (WPM). -
 () -
 0,5-1,0 mg/l myo-inositol
 250-500 mg/l.
 :
 , ,
 , ,
 2-iP, zeatin, IAA, casein hydrolysisate
 : WPM, zeatin -
 , 2-iP - , IAA -
 , IBA -
 , CH -
 , MI -

4.4 pcs./explant were put on nutrient medium M4, enriched with 3.5 mg/l zeatin, 1.5 mg/l 2-iP and 250 mg/l casein hydrolysisate, and for bilberry - 2.8 pcs./explant of M5 modified medium (2.0 mg/l zeatin, 3.5 mg/l 2-iP and 250 mg/l casein hydrolysisate).

The largest average length of shoots in lingonberry was reported on medium M17 - 4.2 cm (2.75 mg/l zeatin, 2.5 mg/l 2-iP and 200 mg/l casein hydrolysisate). The best results regarding the length of shoots in bilberry - 1.6 cm was observed in variant M4 (3.5 mg/l zeatin, 1.5 mg/l 2-iP and 250 mg/l casein hydrolysisate).

Nine variants with varying concentrations of auxin IAA /0.5 mg/l were tested; 0.75 mg/l; 1.0 mg/l/ and reduced by half the basic medium salt concentration (WPM). Wild blueberry species (lingonberry and bilberry) were successfully rooted at a concentration of auxin 0.5-1.0 mg/l and myo-inositol 250-500 mg/l.

Key words: lingonberry, bilberry, micropropagation, tissue culture, 2-iP, zeatin, IAA, casein hydrolysisate

Abbreviation: Woody Plant Medium (WPM), zeatin (4-Hydroxy -3-methylbut-2-enylamino) purine, 2iP (N⁶ - isopentenyladenine), IAA (Indole-3-acetic acid), IBA (indole-3-butyric acid), CH - casein hydrolysisate, MI - myo-inositol

INTRODUCTION

Lingonberry and bilberry, as representatives of the flora of Bulgaria, are of interest to science because of their extremely valuable biochemical composition and because of their dietary and healing properties, which have been known to humans since ancient times.

There are numerous reports on the health benefits of bioactive components, such as total phenols, flavonoids, anthocyanins etc. in their fruits and

(Jaakola et al. 2001; Wang et al., 2005; Battino et al. 2009; Lätti et al., 2011).

“ ”
“
(Kalf and Dufour, 1997; Nikolaeva-Glomb, 2014).

(Mazza and Miniati, 1993; Stothers, 2002).

- 2-iP, zeatin, IAA,
- casein hydrolyisate, myo-inositol
pH *in vitro*
(
),

leaves, which are responsible for their anti-cancer, anti-inflammatory and anti-diabetic properties (Jaakola et al. 2001; Wang et al., 2005; Battino et al. 2009; Lätti et al., 2011). Blueberries are also known as "super fruit" all over the world.

Plant cell, tissue and organ cultures have been used as an alternative tool for studying the biological and physiological characteristics of wild representatives, with a view to their application in breeding programs and food industry (Kalf and Dufour, 1997; Nikolaeva-Glomb, 2014).

In addition, the application of the tissue culture method to wild blueberries would be useful for their characterization and selection of genotypes with increased content of biologically active substances, for the purposes of the pharmaceutical and medical industry (Mazza and Miniati, 1993; Stothers, 2002).

The aim of the present study is to optimize the growth regulators - 2-iP, zeatin, IAA, organic additives - casein hydrolyisate, myo-inositol and pH used in *in vitro* propagation of wild blueberries (lingonberry and bilberry) originating from the Troyan Balkans.

MATERIAL AND METHODS

Plant material

The natural populations of wild blueberries in the Troyan Balkan, Bekleme area are located at an altitude of 1440 m.

The scientific experiment was conducted in the laboratory for micropropagation of small fruit species at RIMSA - Troyan

Sterilization of plant material and introduction into *in vitro* culture

Green cuttings with two buds and length 1.5-2 cm from *in vivo* plants were used as starting explants. The micro cuttings were isolated from the middle

1440 m.

in vitro

1,5-2 cm *in vivo*

and Lloyd 1981),
().

WPM (McCown
3 mg/l 2-iP
4,2 4,8.

0,1 % HgCl₂
(3 . 3,5).

➤ :
➤ :

- 70 % C₂H₅OH 30 .
- 0,1% HgCl₂
-

in vitro

Vaccinium

16/8 /
2000-3000 lx.
30 .

Vaccinium

in vitro

WPM

(1-2).

part of annual shoots.

The nutrient medium for the introduction of blueberries is WPM (McCown and Lloyd 1981), enriched with 3 mg/l 2-iP (E). Blueberry is an acidophilic species and the acidity of the medium is 4.2 and 4.8.

In the present experimental protocol, 0.1% HgCl₂ with different exposures (3 min and 3.5 min) was used as a sterilizing agent.

Sterilization of plant material includes:

- Flushing on running water for one hour:
- Surface sterilization in laminar box conditions:
 - 70% C₂H₅OH for 30 sec.
 - 0.1% HgCl₂
 - Rinse three times with sterile distilled water.

Introduced in *in vitro* culture micro cuttings from members of genus *Vaccinium* are grown in growth chambers, under controlled conditions. The maintained temperature is 22 ± 25 °C, the photoperiod 16/8 day/night and the illumination 2000-3000 lx. The duration of sub-cultivation is 30 days. The development of a successful sterilization protocol for genus *Vaccinium* will find application in the selection programs of small fruit.

In the process of *in vitro* propagation and rooting, we used a basic WPM nutrient medium with varying concentrations of growth regulators, organic additives and pH values (Table 1-2). Explants separated from pre-selected wild blueberries branches were cultivated.

1.

Table 1. Hormonal balance variants in the multiplication phase

Variants of nutrient medium	Basic medium	Zeatin mg/l	2-iP mg/l	Casein hydrolysate mg/l	pH
M1	WPM	3,5	3,5	250	4,8
M2	WPM	3,5	3,5	250	4,3
M3	WPM	3,5	3,5	150	4,8
M4	WPM	3,5	1,5	250	4,8
M5	WPM	2,0	3,5	250	4,8
M6	WPM	2,0	1,5	150	4,3
M7	WPM	2,0	1,5	150	4,8
M8	WPM	2,0	1,5	250	4,3
M9	WPM	2,0	3,5	150	4,3
M10	WPM	3,5	1,5	150	4,3
M11	WPM	3,5	3,5	150	4,3
M12	WPM	3,5	1,5	250	4,3
M13	WPM	3,5	1,5	150	4,8
M14	WPM	2,0	1,5	250	4,8
M15	WPM	2,0	3,5	150	4,8
M16	WPM	2,0	3,5	250	4,3
M17	WPM	2,75	2,5	200	4,55

2.

The rhizogenesis of wild blueberries was performed on the media listed in Table 2.

2.

Table 2. Composition of nutrient media tested during the rooting phase of wild blueberries

Variants of nutrient medium	1/2WPM	IAA mg/l	Myo-inositol	pH
R1	1/2WPM	1,0	500	4,8
R2	1/2WPM	0,5	500	4,8
R3	1/2WPM	1,0	250	4,8
R4	1/2WPM	1,0	500	4,3
R5	1/2WPM	0,5	250	4,3
R6	1/2WPM	0,5	250	4,8
R7	1/2WPM	0,5	500	4,3
R8	1/2WPM	1,0	250	4,3
R9	1/2WPM	0,75	375	4,55

- **Influence of nutrient medium on the growth and proliferation of wild blueberries**

The absolute dry weight of the studied blueberries was reported during the stage of multiplication of the different nutrient media.

The total amount of fresh biomass was weighted at the beginning and end of the experiment.

0,5-1,0 g

° 24

%
in vitro

(ADB)

:

60

Dry biomass is defined as 0.5-1.0 g of fresh biomass, which was dried in a weight glass at a temperature of 60 °C for 24 hours in a ventilated dryer. % Dry weight was measured.

The development of *in vitro* plants was monitored by determining the accumulated dry biomass (ADB) according to the following equation:

$$ADB=FDW-IDW, g,$$

➤

ADB -

, g;

➤

FDW -

, g; -

*

IDW -

, g -

*

where:

➤ ADB - accumulated dry biomass, g;

➤ FDW - final amount of dry biomass of the suspension, g; - this is the amount of fresh biomass at the end of cultivation * determined dry

➤ IDW - initial amount of dry biomass of the suspension used for seeding material, g - this is the amount of fresh biomass at the beginning of cultivation * determined dry.

RESULTS AND DISCUSSION

- As a result of the experiment, the protocols for sterilization, multiplication and rooting of bilberries and lingonberries originating in the Bekleme area were optimized.

- The choice of HgCl₂ as a sterilizing agent has been determined to be suitable for wild blueberries, as a result of numerous of our studies in which testing of various sterilizers has not yielded reliable results (unpublished data).

A relatively high % of sterile cultures was reported for lingonberries - 59.5%, and 46.7% for bilberries with the application of 0.1% HgCl₂ and exposure of 3 min. A slight increase in sterile cultures - 48.2% (Table 3) was a result of the increase of the exposure to 3.5 min for bilberries.

(

3

%

46.7%.

3.5

).

0,1 % HgCl₂

- 59.5%,

- 48.2%

(

3).

3. *(Vaccinium myrtillus L.)* *(Vaccinium vitis-idaea L.)*

(Vaccinium

Table 3. Sterilization efficiency in bilberries (*Vaccinium myrtillus L.*) and lingonberries (*Vaccinium vitis-idaea L.*)

Cultivar	Exposition with HgCl ₂	Nutrient medium	Number of introduced	Number of infected	% infected	% sterile crops
lingonberries	3 min.		252	102	40,5 %	59,5 %
bilberries	3 min		351	187	53,3 %	46,7 %
bilberries	3,5 min		83	36	48,2 %	51,8 %

c

(Georgieva et al., 2016).

1,3 ./ (16 17) 4,4
./ (4) (1, 3).

- 1,2 ./
(11) 2,8 ./ (5).

zeatina : 2-iP
3,5 mg : 1,5 mg
zeatina,
- 2 mg : 3,5 mg
2-iP.

in vitro

In our previous experiments, we found that the behavior of lingonberries and bilberries is different depending on the nutrient medium, the growth regulators involved and the interactions among them (Georgieva et al., 2016).

The analysis of the results shows that the multiplication coefficient depends on the concentration of the applied growth regulator and organic additives. For example, it is in the range of 1.3 pcs/explant (M16 and M17) to 4.4 pcs/explant (M4) for lingonberries (Figure 1, 3). This coefficient for bilberries is in a lower range from 1.2 pcs/explant (M11) to 2.8 pcs./explant (M5).

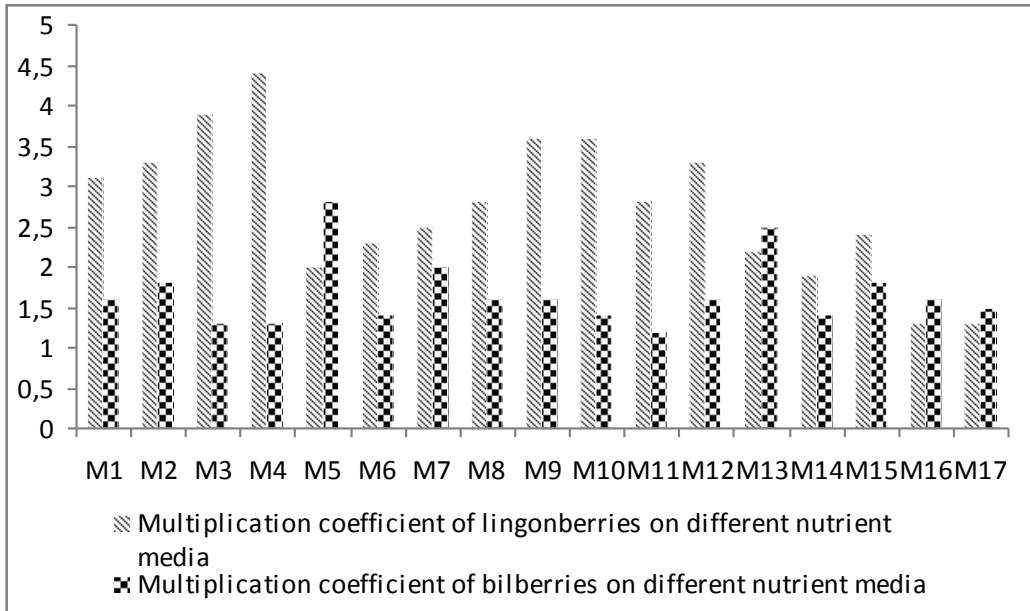
In general, the induction of a regenerative process in wild species is associated with the use of higher doses of growth regulators, which differ even within the same species. The results show that the ratio of cytokinins zeatin: 2-iP in lingonberries is 3.5 mg: 1.5 mg in favour of zeatin, while in bilberries it is the opposite - 2 mg: 3.5 mg in favour of 2-iP.

The benefit of accumulation of high doses of cytokinins in the initial phase of their development is manifested in a strong effect after the release of *in vitro* regenerants from hormonal pressure.

Jaakola et al. (2001; 2002) -
 2-iP -
 c
 - casein hydrolysat
 myo-inositol.

Jaakola et al. (2001; 2002) report that high concentrations of 2-iP in the nutrient medium lead to increased browning of lingonberries and bilberries.

There wasn't such browning in the present experiment, which in our opinion is due to the enrichment of the media with organic additives - casein hydrolysat and myo-inositol.



. 1.

Fig. 1. Multiplication coefficient of bilberries and lingonberries on different nutrient media

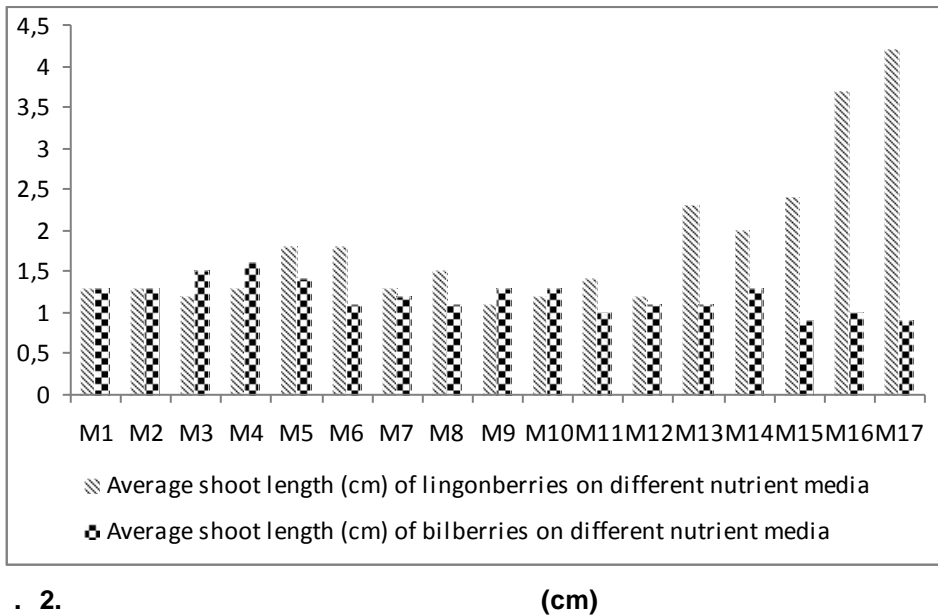


Fig. 2. Average shoot length (cm) of bilberries and lingonberries on different nutrient media

1,1 cm
 9 4,2 cm (17),
 cm (11) 1,6 cm (4) (2).

(4).

R6 R8 – 11,1 %.

16,7 % (R7) 57 % (R4) (5).
 Jaakola et al. (2001) 81,3 %

0,49 μ IBA

Shibli and Smith (1996) (40 %)

The average length of shoots in lingonberries varied from 1.1 cm on nutrient medium M9 to 4.2 cm (M17), and in bilberries from 1 cm (M11) to 1.6 cm (M4) (Figure 2). No definite correlation was observed between length of shoots and their number.

Rooting was registered only in some of the nine variants of rooting medium (Table 4). For example, in the case of lingonberries, rooting was reported only at R6 and R8 - 11.1%.

Root formation in bilberries was observed in only four variants and ranged from 16.7% (R7) to 57% (R4) (Table 5). Jaakola et al. (2001) reported 81.3% rooting in bilberries, with the addition of 0.49 μ M IBA in the nutrient medium.

Researchers such as Shibli and Smith (1996) successfully rooted bilberries (40%) from leaf explants in a nutrient medium without growth regulators after eight weeks.

-

- 3 (R6
0,5 mg/l IAA 250 mg/l myo-inositol, pH
4,8) 2,8 (R3 1 mg/l
IAA 250 mg/l myo-inositol, pH 4,8).

-

2

cm

R6, - 1,8 cm R7.

The data for the largest average number of roots in both types of berries are similar - 3 in lingonberries (R6 0.5 mg/l IAA and 250 mg/l myo-inositol, pH 4.8) and 2.8 in bilberries (R3 1 mg/l IAA and 250 mg/l myo-inositol, pH 4.8).

The maximum average length of the roots in lingonberries is 2 cm on nutrient medium R6, and in bilberries - 1.8 cm on R7.

4.

Table 4. Rooting of lingonberries in different nutrient media

Variants of nutrient medium	Rooting %	Average number of roots	Average length of roots (cm)
R1	-	-	-
R2	-	-	-
R3	-	-	-
R4	-	-	-
R5	-	-	-
R6	11,1 %	3	2
R7	-	-	-
R8	11,1 %	1	1
R9	-	-	-

5.

Table 5. Rooting of bilberries in different nutrient media

Variants of nutrient medium	Rooting (%)	Average number of roots	Average length of roots (cm)
R1	-	-	-
R2	-	-	-
R3	33,3 %	2,8	1,4
R4	57 %	2	1,3
R5	50 %	0,8	0,8
R6	-	-	-
R7	16,7 %	1,5	1,8
R8	-	-	-
R9	-	-	-

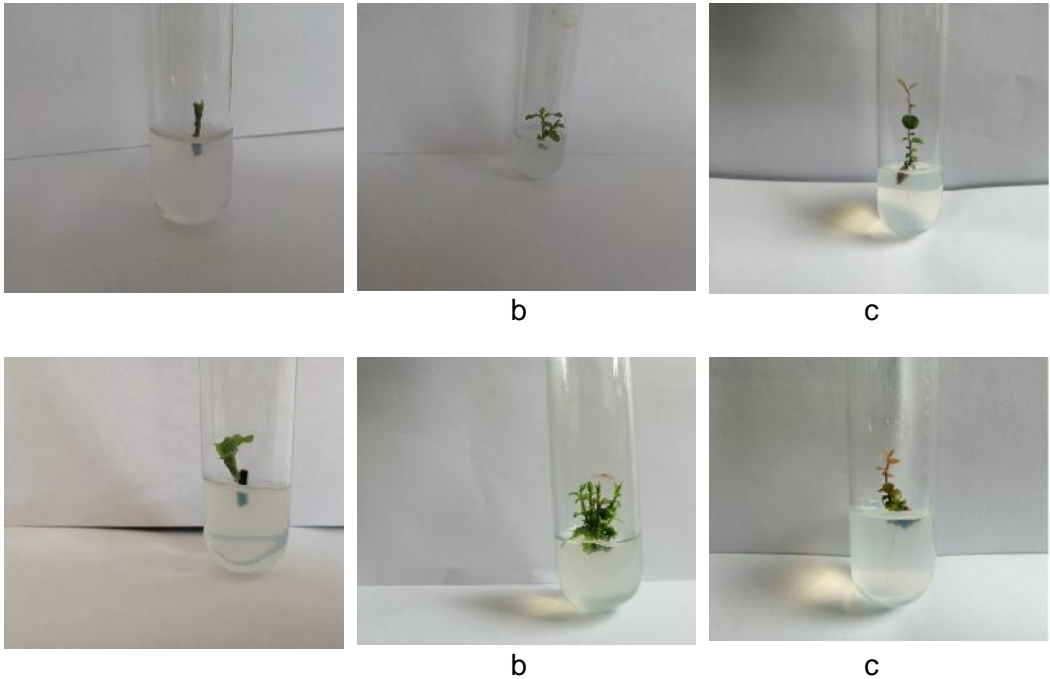
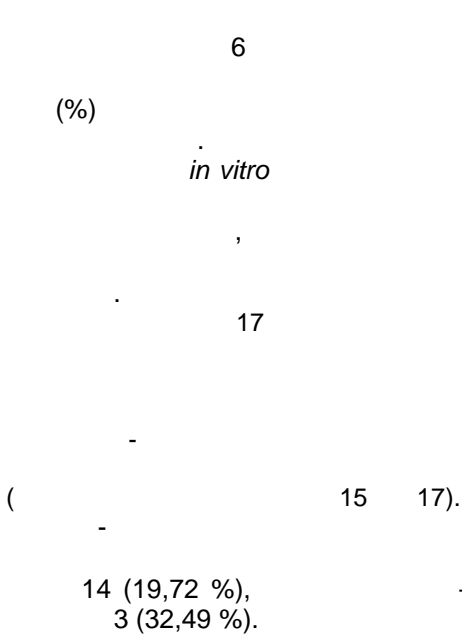


Fig. 3 Scheme of *in vitro* propagation (introduction (a), multiplication (b), rooting (c)) of lingonberries and bilberries



Absolutely dry weight

Table 6 presents the results regarding the absolutely dry weight (%) of the studied lingonberries and bilberries. This is an indicator characterizing *in vitro* growth and gives an idea of the degree of development of the introduced explant, depending on the exogenous application of growth regulators.

Of the 17 nutrient media tested on the increase in fresh biomass of bilberries and lingonberries, there was a tendency to establish higher values of absolutely dry weight in bilberries (except for variant M15 and M17).

The strongest growth in lingonberries was reported on nutrient medium M14 (19.72%), and in bilberries - on medium M3 (32.49%).

6.

(%)

Table 6. Absolute dry weight (%) of lingonberries and bilberries from the region of origin Bekleme area

Variants of culture medium	Absolute dry weight % of lingonberries (average)	Absolute dry weight % of bilberries (average)
M1	14,68	32,49
M2	11,19	25,70
M3	11,25	31,00
M4	11,77	26,79
M5	14,87	25,77
M6	13,01	23,84
M7	12,76	19,00
M8	13,04	18,17
M9	10,71	24,21
M10	13,12	19,06
M11	13,47	20,06
M12	13,67	19,06
M13	13,38	19,96
M14	19,72	23,93
M15	17,22	15,69
M16	17,60	24,84
M17	18,80	15,10

(
vivo 4).

in vitro

in vitro
(90 %).

in vitro
40

Adaptation

The adaptation of *in vitro* plants from bilberries and lingonberries under laboratory conditions is trouble-free (Figure 4). Transfer from *in vitro* to *in vivo* conditions was successful (90%). No morphological changes were observed and the plants developed normally. The duration of adaptation was 40 days. Optimized container growing conditions lead to the production of viable plants with balanced growth and a well-developed root system.



4. () ()
Fig. 4. Adaptation of bilberries (A) and lingonberries (B)

in vitro ,
Vaccinium ,
In vitro -
 (1,5 -3,5 mg/l/ 2-iP). (zeatin
 Jaakola et al. (2001; 2002),
 myo-inositol). (casein hydrolysat
in vivo .
in vitro
 ()

CONCLUSIONS

The development of a model system for *in vitro* propagation, rooting and adaptation of wild species belonging to genus *Vaccinium* is of great importance for future research aimed at determining their biological, physiological and health status. The results so far prove their pharmacological value and their importance for the food, pharmaceutical and cosmetic industries.

In vitro propagation of wild species is associated with greater difficulties compared to their selected cultivars. The results show that higher doses of 1.5-3.5 mg/l of two cytokinins (zeatin and 2-iP) are used to induce organogenesis. The ratios between them differ in both types - lingonberries and bilberries.

Overcoming physiological abnormalities, which are commented by Jaakola et al. (2001; 2002), has been successful with the inclusion of organic food additives (casein hydrolysate and myo-inositol).

The post- cumulative effect of the use of higher doses of cytokinins is manifested in the processes of rooting and *in vivo* adaptation.

The developed effective protocol for *in vitro* cultivation of wild berries (lingonberries and bilberries) makes it

possible to create plantations of them in suitable habitats, which will allow the conservation of these valuable species.

ACKNOWLEDGEMENTS

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Assessment on the Preservation of Fresh Fruit Quality of 'Elena' Plum Cultivar under Refrigeration after Application of Biological and Conventional Fertilizers

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Original scientific paper

SUMMARY

2018 - 2016-2018

The present study was conducted in 2016-2018 at RIMSA-Troyan. Its purpose was to monitor the effect of fertilizers for conventional and biological fruit production on the quality and refrigeration of fresh fruit of 'Elena' plum cultivar. The best sensory characteristics and quality for consumption were found in the variants with chicken manure and bio fertilization. Fruit was kept from 0 to 4 °C up not more than 35 days.

0 4 °C 35

After staying in refrigerated conditions, the amount of biochemical indicators, such as total, invert sugar and sucrose in fruit, decreased.

An increase was found in the percentage of tannins and pectin, most pronounced in the variants with chicken manure (0.167%; 1.11%) and biofertilizer (0.173%; 1.03%). The results obtained

(0.167 %; 1.11%)
(0.173%; 1.03%).

Ca.

(Auger et al. 2004; Cevallos-Casals et al., 2006; Milošević and Milošević, 2012).

(Radev, 2017).

(Cautín and Agustí, 2005; Barbosa-Canovas et al., 2006; Dimitrova and Borisova, 2007; Rato et al., 2008; Gitea et al., 2019).

(Crisosto and Mitchell, 2002).

(Brizzolara et al., 2020).

are likely influenced by the composition of the applied organic chicken manure and bio fertilizer Tecnokel Amino Ca.

Key words: plum, fertilization, storage, biochemical composition, total polyphenols

INTRODUCTION

Fresh fruits are an important source of vitamins, minerals, antioxidants, flavonoids and dietary fiber (Auger et al. 2004; Cevallos-Casals et al., 2006; Milošević and Milošević, 2012). After harvesting, as a result of the effects of microorganisms, breathing and loss of moisture they quickly reduce their durability and nutritional value (Radev, 2017).

Soil characteristics, seasonal and climatic changes during vegetation have an impact on the quantity and quality of the production (Cautín and Agustí, 2005; Barbosa-Canovas et al., 2006; Dimitrova and Borisova, 2007; Rato et al., 2008; Gitea et al., 2019).

The storage and consumption of plum fruits varies considerably, depending on the cultivar, care, harvesting and conditions provided after that (Crisosto and Mitchell, 2002). Temperature is an important technology for storing fruits, influencing the changes in their physiology and biochemistry (Brizzolara et al., 2020).

The desire to consume fresh plum fruit depends on their sensory characteristics, such as appearance, colour, firmness, taste and aroma. The sugar/acid ratio determines their taste qualities and the content of certain volatile aromatic components, their odour. In addition to giving colour, flavour and astringency, the polyphenols in fruits act as protection against adverse environmental conditions, destroy free radicals, destroy the way of oxidation

(Robbins, 2003; Simon-Graoa et al., 2014).

(Ariona et al., 2014).

Moyer et al., (2002)

(Guerra et al., 2009; Popović et al., 2018).

- formed by them as lipid peroxidation and DNA damage in the human body (Robbins, 2003; Simon-Graoa et al., 2014). Studies have found that autumn plum cultivars have a higher antioxidant capacity than summer (Ariona et al., 2014).

- According to Moyer et al., (2002), the antioxidant effect of bioactive substances and their beneficial effect on human health correlates with their content.

- Therefore, it is important to follow the biochemical analysis for the quantification of separate indicators and after storage (Guerra et al., 2009; Popović et al., 2018).

- The purpose of the present study is to monitor the influence of some fertilizers intended for conventional and organic fruit production on the biochemical composition of fresh fruit of 'Elena' plum cultivar, before and after their storage.

MATERIAL AND METHODS

The experiment was conducted in the period 2016-2018 in the collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture - Troyan. The objective of the present study is fruit of the German cultivar of 'Elena', a selection of Dr. Hartmann created as a crossing between Fellenberg x Stanley.

The scheme of planting fruit trees is 4/2.5 m. The harvest was carried out during the following periods: in 2016 - 26.08; 2017- 04. 09; 2018-21.08, immediately after which they were placed for storage.

2016-2018

Fellenberg x Stanley.

4/2.5 m.

: 2016 . - 26.08.; 2017 . - 04.09.; 2018 . - 21.08,

I

Ca () - 0,4%,

II

Mila Complex () - 0.500 kg/ , YaraVita Frutrel () - 0.500 ml/da, Yara Vita Universal Bio

Fertilization variants:

I variant - Organic fertilization - including fertilizers: Agriful (soil) - 5 l/da, Tecamin Flower (foliar) - 0.3%, Tecnocel Amino Ca (foliar) - 0.4%;

II variant - Conventional - Yara Mila Complex (soil) - 0.500 kg/tree, YaraVita Frutrel (foliar) - 0.500 ml/da, Yara Vita Universal Bio (foliar) - 0.500 ml/da,

() - 0.500 ml/da,
- 0.220 g/

III -
0.500 kg/ ;

IV -

A -
-306g/l; -306g/l;
(N)-55g/l; (P₂O₅)-13g/l;
(K₂O)-13g/l;
-551g/l; pH-4.7

-51g/l; "L"
-38 g/l; (N)-38 g/l;
(P₂O₅)-127g/l; ()-13g/l;
(Mo)- 6.5 g/l; pH-2

Ca -
-148 g/l;

() - 3 g/l; "L"
-89 g/l; pH-4.0-4.5

Yara Mila Complex (N)-12%;
(K)-18%; (MgO)-2.7%;
()-0.015%; (Mn)- 0.02%;
(P)-11%; (SO₃)- 20%;
(Fe)- 0.2%; (Zn)- 0.02%.

YaraVita Frutrel -

()- 280 g/l; (P)- 104 g/l;
(N)- 69 g/l; (MgO)- 100 g/l;
(Zn)- 40 g/l; ()-20 g/l

YaraVita Universal Bio - (N)-
100 g/l; (P₂O₅)-40 g/l;
(K₂O)- 70 g/l; (Mn)-1.3 g/l;
(Cu)- 1.0 g/l; (Zn)- 0.7 g/l; (B)-
0.2 g/l; (Mo)- 0.03 g/l

Organic - (N)- 1.2%; (P)-
1.99%; (K)-2.5%; (Ca)-
10.85%; (Mg)- 0.75%;
(Zn)-350 mg/kg; (Cu)-50 mg/kg;
(Mn)- 443 mg/kg; (Fe)-
3450 mg/kg.

:

15-20 ;

Ammonium nitrate - 0.220 g/tree;

III variant - Granulated chicken manure -
0.500 kg/tree;

IV variant - Untreated control

Fertilizer composition:

Agriful - Total humic extract-306g/l;
Fulvic acid - 306g/l; Nitrogen (N) - 55g/l;
Phosphorus (P₂O₅) -13g / l; Potassium
(K₂O) -13g/l; Total organic matter - 551g/l;
pH-4.7

Tecamin Flower - Seaweed
extract-51g/l; Free "L" amino acids -38 g/l;
Nitrogen (N)-38 g/l; Phosphorus (P₂O₅)-
127g/l; Boron ()-13g/l; Molybdenum
(Mo)- 6.5 g/l; pH-2

Tecnocel Amino Ca - Calcium
oxide (CaO) water-soluble -148 g/l; Boron
(B) water-soluble - 3 g/l; Free "L" amino
acids - 89 g/l; pH-4.0-4.5

Yara Mila Complex Nitrogen (N) -
12%; Potassium (K) -18%; Magnesium
(MgO) -2.7%; Boron (B) -0.015%;
Manganese (Mn) - 0.02%; Phosphorus
(P) -11%; Sulphur (SO₃) - 20%; Iron (Fe) -
0.2%; Zinc (Zn) - 0.02%.

YaraVita Frutrel - Calcium oxide
(CaO) - 280 g/l; Phosphorus (P) - 104 g/l;
Nitrogen (N) - 69 g/l; Magnesium (MgO) -
100 g/l; Zinc (Zn) - 40 g/l; Boron (B) - 20 g/l

YaraVita Universal Bio - Nitrogen
(N) - 100 g/l; Phosphorus (P₂O₅) - 40 g/l;
Potassium (K₂O) - 70 g/l; Manganese
(Mn) - 1.3 g/l; Copper (Cu) - 1.0 g/l; Zinc
(Zn) - 0.7 g/ l; Boron (B) - 0.2 g/l;
Molybdenum (Mo) - 0.03 g/l

Granulated chicken manure Vita
Organic - Nitrogen (N) - 1.2%;
Phosphorus (P) - 1.99%; Potassium (K) -
2.5%; Calcium (Ca) - 10.85%; Magnesium
(Mg) - 0.75%; Zinc (Zn) - 350 mg/kg;
Copper (Cu) - 50 mg/kg; Manganese (Mn) -
443 mg/kg; Iron (Fe) - 3450 mg/kg.

Fertilizer application periods:

Agriful - applied five times at the
beginning of vegetation for a period of 15-
20 days;

Tekamin Flower _ 2 applications.
Applied before blossoming and during

Ca - ;

Yara Mila Complex - ;

2016 2018 ;

2017 ;

YaraVita Frutrel - ;

Yara Vita Universal Bio - ;

2016 2018 .

Re (%), (%) , (%) -

(%) - 0,1n

NaOH; (mgL⁻¹) -

(mgL⁻¹) -

Fuleki Franciss,

(%) - ,

(%) - ,

Singleton and Rossi (mgGAE/100gFW).

4 °C

0-4 . 7

E

5 kg

2017

fruit-set formation;

Tecnocel Amino Ca - 2 applications. Applied after blossoming and a month before harvest;

Yara Mila Complex - 1 application in 2016 and 2018;

Ammonium nitrate- 1 application in 2017;

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.

Yara Vita Universal Bio - 3 applications. Applied before and after blossoming and after harvest;

Granulated chicken manure - one application in 2016 and 2018.

The biochemical composition of fruit was studied at the chemical laboratory of RIMSA - Troyan. The following indicators were monitored: dry matter according to Re (%), dry mass (%), sugars (%) (total, invert and sucrose) according to the method of Shoorl and Regenbogen, acids (%) - by titration with 0.1 n NaOH; ascorbic acid (mgL⁻¹) - according to the method of Fialkov; anthocyanins (mgL⁻¹) - according to the method of Fuleki and Franciss, pectin (%) - according to the method of Melitz, tannins (%) - according to the method of Leventhal, total polyphenols (mgGAE/100g)- according to Singleton and Rossi.

Storage of fruits at a temperature of 4 °C

The fruits were stored in plastic boxes under refrigeration conditions at temperatures in the range 0-4 °C. Every 7 days the rotten and unfit for consumption fruits were reported. The biochemical composition after storage of good fruit quality was analyzed.

In the first experimental year, after the harvest of 'Elena', 5 kg of fresh and visibly healthy plums were taken from each variant.

In the next 2017, due to their insufficient

1.500 kg.

2018 .

0.500 kg

1

2016 2017 ., 29

35 , 2018 15 ,

Abdi et al., (1997) Crisosto et al., (2004),

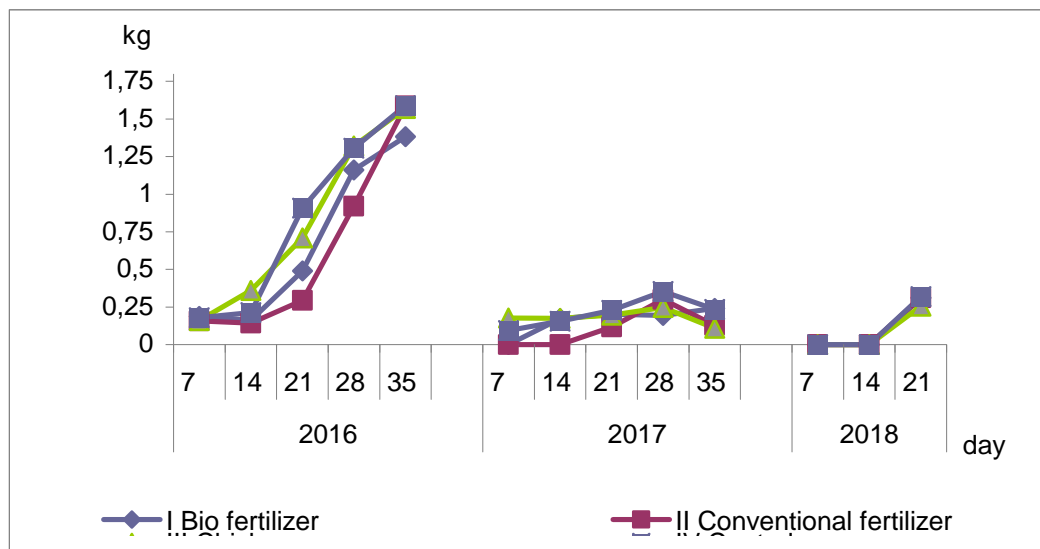
quantity, their storage was monitored for 1,500 kg. The unfavorable climatic conditions during the vegetation period of 2018 and the lower yield for storage were tested with 0.500 kg of plums from each variant.

Statistic analysis

For data processing the following statistical analyses were used: variation, correlation and regression analysis.

RESULTS AND DISCUSSION

Based on the results presented in Figure 1 it was found that the duration of storage of fruit of 'Elena' plum cultivar in 2016 and 2017 was of 35 days, and in 2018 up to 21 days. These data corresponding to those indicated by Abdi et al., (1997) and Crisosto et al., (2004), according to which, after harvesting, depending on the cultivar, plums can be stored in refrigerated conditions for two to six weeks.



1. (kg)

2016-2018 .

Fig. 1. Dynamics of the quantity (kg) of fresh plums of Elena removed, unfit for consumption, stored under refrigerated conditions for the period 2016-2018

(5;1.5 0.5 kg), 7 14

2016 2018 .

0.215 kg, 2017 .

0.240 kg (2).

Ca.

It was reported that during the three-year experimental period for the conventional fertilization variant, regardless of the amount of stored fruit (5; 1.5 or 0.5 kg), after 7 and 14 days of storage, a minimum number of unfit fruits were missing or removed after which their quantity increased.

The test shows that most fruits that retained their quality characteristics for a longer period during storage in 2016 and 2018 were in the variant with chicken manure, respectively - 0.309 kg and 0.215 kg, and in 2017 in the organic fertilization variant - 0.240 kg (Figure 2). The results are likely influenced by the composition of the organic chicken manure and biofertilizer Tecnocel Amino Ca.

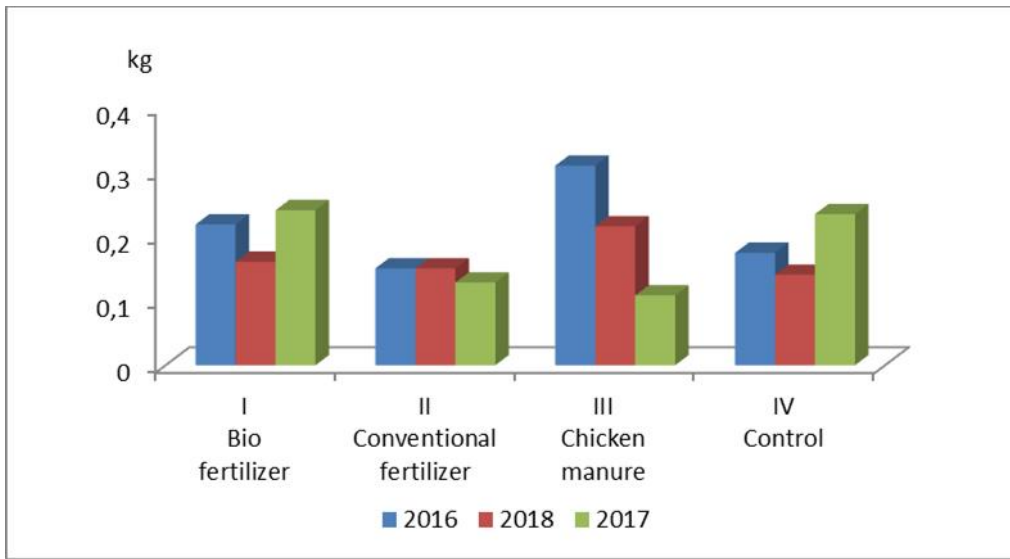


Fig. 2. Quantity (kg) of fresh plums of Elena suitable for consumption, after storage under refrigerated conditions for the period 2016-2018

2016-2018 .

1 2.

The analyzed biochemical indicators, before and after storage of fresh plums of 'Elena' cultivar by years and on average for the period 2016-2018, are presented in Tables 1 and 2.

2016-2018 .

Table 1. Biochemical composition of fresh plums of 'Elena' cultivar, before storage by years and on average for the period 2016-2018

Indicators Variants	Dry mass, %	Dry in Re, %	Total sugars, %	Invert sugar, %	Sucrose, %	Acids as malic, %	Ascorbic acid, mgL ⁻¹	Tannins, %	Anthocyanin, mgL ⁻¹	Pectin, %	Acidimetric coefficient	
2016	I Biofertilizer	20.46	21.00	14.30	6.00	7.89	0.45	5.28	0.145	13.39	0.91	31.77
	II Conv. fertilizer	18.70	18.00	10.05	5.35	4.47	0.32	5.28	0.145	18.23	0.86	32.81
	III Chicken manure	19.89	20.25	10.40	5.70	4.47	0.45	5.28	0.233	34.52	0.42	23.11
	IV Control	21.02	19.00	10.90	5.35	5.27	0.45	5.28	0.187	18.23	0.94	24.22
	CV %	4.95	6.80	17.17	5.53	29.35	15.57	-	22.22	43.81	30.77	17.94
2017	I Biofertilizer	17.08	22.00	9.90	7.00	2.76	0.48	8.80	0.169	29.03	1.49	20.62
	II Conv. fertilizer	16.68	21.00	12.95	6.35	6.27	0.41	5.28	0.056	6.45	1.19	31.58
	III Chicken manure	18.22	20.00	13.15	7.00	5.84	0.41	7.04	0.094	13.23	1.34	32.07
	IV Control	18.55	19.50	11.10	7.00	3.90	0.48	7.04	0.131	22.26	0.92	23.12
	CV %	5.07	5.37	13.19	4.75	35.16	9.08	20.41	43.15	55.96	19.68	21.76
2018	I Biofertilizer	17.25	18.50	12.95	8.70	4.04	0.45	8.80	0.181	9.84	0.06	28.77
	II	19.05	19.00	13.25	8.20	4.80	0.38	10.56	0.145	8.06	0.06	34.86
	III Chicken manure	16.23	17.00	13.15	8.05	4.85	0.38	12.32	0.072	6.13	0.02	34.60
	IV Control	17.18	18.00	13.65	8.55	4.85	0.51	8.80	0.163	13.06	0.13	26.76
	CV %	8.18	4.69	2.18	3.58	8.42	13.95	16.60	28.57	31.71	66.66	13.12
average	I Biofertilizer	18.26	20.50	12.38	7.23	4.90	0.46	7.63	0.165	17.42	0.82	27.05
	II Conv. fertilizer	18.14	19.33	12.08	6.63	5.18	0.37	7.04	0.115	10.91	0.70	33.08
	III Chicken manure	18.11	19.08	12.23	6.92	5.05	0.41	8.21	0.133	17.96	0.59	29.93
	IV Control	18.92	18.83	11.88	6.97	4.67	0.48	7.04	0.160	17.85	0.66	24.70
	CV %	2.07	3.75	1.72	3.46	4.24	9.3	7.48	14.28	21.33	13.04	12.61

CV - coefficient of variation

16.23
21.02 %
% (2018 .)
(2016 .)
18-19%,
19-20%.

Based on the results, it was found that the values of dry mass before storage of fruits were in the range from 16.23% (chicken manure 2018) to 21.02% (control 2016). The average value of the indicator for the studied three-year period between the experimental variants was 18-19%, and after storage in the refrigerator was 19-20%. That is, there are no significant differences in the values of the indicator.

2016-2018 .

Table 2. Biochemical composition of fresh plums of 'Elena' cultivar, after storage by years and on average for the period 2016-2018.

Indicators Variants	Dry mass, %	Dry in Re, %	Total sugars, %	Invert sugar, %	Sucrose, %	Acids as malic %	Ascorbic acid, mgL ⁻¹	Tannins, %	Anthocyanins, mgL ⁻¹	Pectin, %	Acidimetric coefficient	
2016	I Biofertilizer	22.87	21.50	11.45	8.55	2.76	0.32	3.52	0.262	11.93	0.95	35.78
	II Conv. fertilizer	18.75	17.00	12.45	8.35	3.89	0.32	3.52	0.150	1.77	0.71	38.91
	III Chicken manure	22.49	20.00	13.15	8.20	4.70	0.32	5.28	0.206	5.81	0.59	41.09
	IV Control	21.09	19.00	12.30	8.35	3.75	0.39	7.04	0.243	9.84	0.52	31.54
	CV %	8.73	9.70	5.59	1.67	20.95	10.60	34.71	19.04	61.25	27.24	11.24
2017	I Biofertilizer	20.73	17.50	8.20	4.85	3.18	0.55	7.04	0.131	11.94	1.04	14.91
	II Conv. fertilizer	22.50	19.00	9.05	4.70	4.13	0.48	8.80	0.169	11.94	1.60	18.85
	III Chicken manure	19.69	16.00	8.70	4.05	4.42	0.48	7.04	0.150	8.87	1.94	18.13
	IV Control	20.76	17.50	5.70	3.35	2.23	0.48	5.28	0.131	8.39	1.06	11.88
	CV %	5.54	6.97	19.08	16.07	28.36	7.14	20.31	12.41	18.67	31.06	20.07
2018	I Biofertilizer	17.06	19.00	8.20	4.70	3.33	0.51	14.08	0.127	11.77	1.10	16.08
	II Conv. fertilizer	15.69	17.00	8.20	5.85	2.23	0.51	17.60	0.127	12.90	0.60	16.08
	III Chicken manure	17.06	19.00	9.55	7.35	2.09	0.45	8.80	0.145	7.48	0.80	21.22
	IV Control	16.51	18.50	9.05	7.20	1.76	0.51	17.60	0.145	4.52	0.83	17.75
	CV %	3.90	5.14	7.62	19.77	28.93	6.06	28.63	7.35	42.24	24.69	13.61
average	I Biofertilizer	20.22	19.33	9.28	6.03	3.09	0.46	8.21	0.173	8.44	1.03	20.18
	II Conv. fertilizer	18.98	17.67	9.90	6.30	3.42	0.44	9.97	0.149	8.87	0.97	22.67
	III Chicken manure	19.75	18.33	10.47	6.53	3.74	0.42	7.04	0.167	7.38	1.11	25.12
	IV Control	19.45	18.33	9.02	6.30	2.58	0.46	9.97	0.173	7.58	0.80	19.60
	CV %	2.65	3.69	6.62	3.17	15.31	4.26	16.26	6.64	8.68	13.40	11.55

CV - coefficient of variation

Manganaris et al., (2008)

With regard to the dry matter content of refractometric substances, the effect of lower storage temperatures was not taken into account.

According to Manganaris et al., (2008), the most relevant factor determining the degree of ripeness in plums is their quantitative sugar content. In the present study, the analyzed values of total sugars were diverse.

It is reported that as a result of the low

(12.23%),
 (10.47%).
 Kaulmann et al. (2014),
 8.5
 19.6g/100g.
 -
 -
 -
 -
 () – 2016 . –
 6.00%; 2017 . – 7.00% 2018 . - 8.70%.
 -
 2016-2018 -
 - 5.18%.
 -
 ,
 10.47%; 6.53% 3.74%.
 et al. (2013),
 Valero
 -
 -
 ,
 0.40%.
 Lee and Kader (2000),
 -

- temperature during storage of plums, their quantity decreased. On average for the experimental period with a high percentage of total sugars, the variant with granulated chicken manure is distinguished, both before (12.23%) and after refrigerated storage (10.47%). According to Kaulmann et al. (2014), the total sugar content in fresh plums varies between 8.5 and 19.6 g/100g. The range indicated by the author also includes the results analyzed in the present study, reported on average for the entire experimental period, before and after storage.

- From the three-year biochemical analysis was found that with each subsequent experimental year, the content of the analyzed invert sugar increased in all variants. The highest values were reported in the first variant (bio fertilizer) - in 2016 - 6.00%; 2017 - 7.00% and 2018. - 8.70%.

- Regarding the sucrose, on average for the time interval 2016-2018, the highest percentage was found in the variant of conventional fertilization - 5.18%. After staying in the refrigerator, lower results were reported in terms of total, invert sugar and sucrose, but the highest average values were reported in the third variant with chicken manure, respectively 10.47%; 6.53% and 3.74%.

- Despite the characteristic decrease in total acidity during storage in fleshy fruits Valero et al. (2013), the average percentage of organic acids reported for the test period before and after storage of the fruit in all variants was approximately 0.40%.

- The quantified ascorbic acid in changes over the years and experimental variants, which confirms the report from Lee and Kader (2000), according to which the indicator is sensitive to the application of nitrogen fertilizers and storage

2017 . 2018 .
 2018
 ()
 7.04mgL⁻¹.
 2016-2018
 ()
 - 0.173%.
 0.165%
 6.45mgL⁻¹ (2017 .)
 34.52mgL⁻¹ (2016 .),
 5.81mgL⁻¹ (2016 .)
 12.90mgL⁻¹ (2018 .).
 1.11%
 0 4 °C (3).
 - 318,68 mg/100gFW.

conditions after harvest.

During the three-year experimental period, after storage, an increase in ascorbic acid was registered in 2017 and 2018 with the conventional fertilizer and in 2018 in the biofertilizer variant. With a higher average value of the indicator for the period are distinguished all experimental variants, except for the third (with the application of chicken manure) in which the content of 7.04 mgL⁻¹ was analyzed.

In all three years after storage, the percentage of tannins increased. For the period 2016-2018 in the first variant (bio fertilizer) the highest average value was reported, as before the storage of the fruit was 0.165%, and after it - 0.173%.

As a result of the analysis it was found that after storage of fresh plums the amount of anthocyanins decreased. Their average content for the studied period before their placement in the refrigerator was in the range from 6.45 mg% (second variant 2017) to 34.52 mgL⁻¹ (third variant 2016), and after storage it was in the range of 5.81 mg% (third variant 2016) up to 12.90 mgL⁻¹ (second variant in 2018).

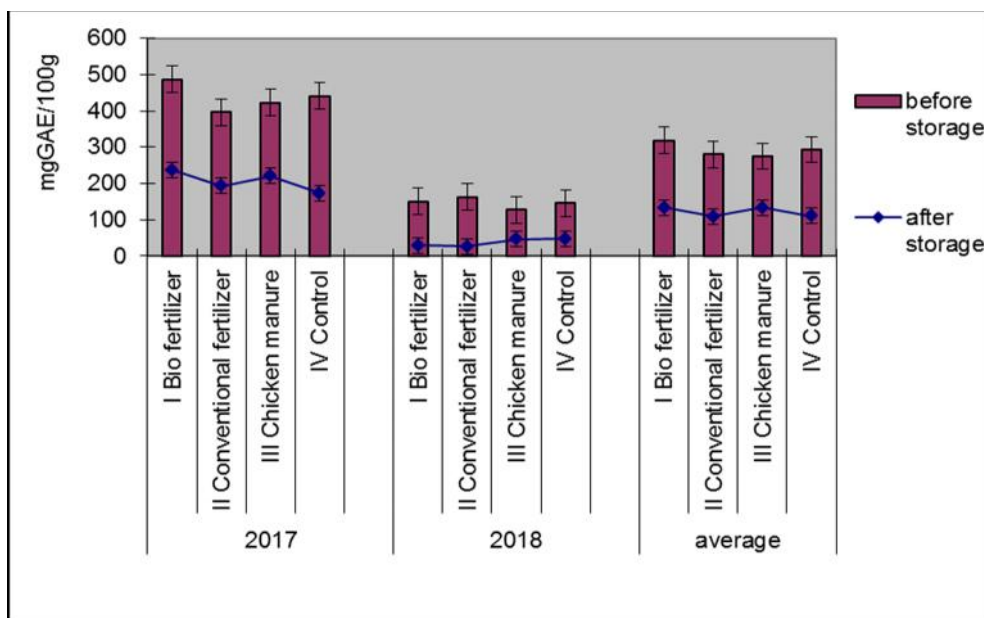
Regarding the percentage content of pectin in plums, an increase in its values was found after their stay in the refrigerator. With the highest average result of 1.11% for the test period, a third variant of fertilization, with chicken manure, is distinguished.

Total polyphenols content

The total polyphenols content in fresh plums of 'Elena' plum cultivar decreased after storage in refrigerated conditions at a temperature of 0 to 4 °C (Figure 3). On average for the test period, the highest result of the indicator was found before storage in the bio fertilizer variant - 318.68 mgGAE/100gFW.

- After the storage period, the amount of total polyphenols decreased in all experimental variants, being most pronounced in conventional fertilization and the control. The lower content of total phenols may be due to the faster respiration of fruits, leading to their degradation (Niara et al., 2018).

(Niara et al., 2018).



. 3.

(mg/100gFW)
E

Fig. 3. Impact of different fertilization variants on the content of total polyphenols (mgGAE/100gFW) before and after storage of fresh plums of 'Elena' cultivar

3

Correlation dependencies between basic biochemical parameters before and after storage of fresh fruit of 'Elena' plum cultivar

The correlation analysis presented in Table 3, describing the strength and direction of dependence between the main biochemical parameters of fresh plums of 'Elena' cultivar, shows that the percentage of dry mass correlates positively before storage with organic acids ($r = 0.76$) and after storage with tannins ($r = 0.79$).

($r=0.76$)
($r=0.79$).

Table 3. Correlational dependencies between basic biochemical parameters before and after storage of fresh fruit of 'Elena' plum cultivar

	Dry weight, %	Dry in Re, %	Total sugars, %	Invert sugar, %	Sucrose, %	Acids as malic, %	Ascorbic acid, mgL ⁻¹	Tannins, %	Anthocyanins mgL ⁻¹	Pectin, %	Acidimetric coefficient
Before storage											
Dry mass, %	1,00										
Dry in Re, %	-0,40	1,00									
Total sugars, %	-0,73	0,82	1,00								
Invert sugar, %	0,22	0,61	0,50	1,00							
Sucrose, %	-0,91	0,12	0,40	-0,60	1,00						
Acids as malic, %	0,76	0,14	-0,13	0,80	-0,96	1,00					
Ascorbic acid, mgL ⁻¹	-0,54	0,17	0,68	0,37	0,24	-0,07	1,00				
Tannins, %	0,60	0,38	0,11	0,91	-0,86	0,97	0,02	1,00			
Anthocyanins, mgL ⁻¹	0,39	0,03	0,16	0,79	-0,70	0,79	0,53	0,77	1,00		
Pectin, %	-0,06	0,89	0,48	0,50	-0,09	0,26	-0,28	0,45	-0,12	1,00	
Acidimetric coefficient	-0,81	-0,03	0,21	-0,74	0,98	-0,99	0,08	-0,94	-0,80	-0,17	1,00
After storage											
Dry weight, %	1,00										
Dry in Re, %	0,96	1,00									
Total sugars, %	-0,16	-0,41	1,00								
Invert sugar, %	-0,42	-0,63	0,73	1,00							
Sucrose, %	-0,04	-0,25	0,96	0,51	1,00						
Acids as malic, %	0,21	0,47	-0,98	-0,83	-0,90	1,00					
Ascorbic acid, mgL ⁻¹	-0,68	-0,46	-0,60	-0,28	-0,63	0,57	1,00				
Tannins, %	0,79	0,79	-0,47	-0,24	-0,49	0,41	-0,36	1,00			
Anthocyanins, mgL ⁻¹	-0,27	-0,11	-0,14	-0,60	0,06	0,28	0,45	-0,63	1,00		
Pectin, %	0,41	0,21	0,78	0,20	0,89	-0,68	-0,85	-0,14	0,03	1,00	
Acidimetric coefficient	-0,18	-0,43	1,00	0,78	0,94	-1,00	-0,59	-0,44	-0,21	0,74	1,00

0.05,

(r=0.96).

- Highly positive and statistically significant for a significance level of 0.05, a correlation was reported after storage of fruit in the refrigerator between the amount of total sugars and sucrose (r = 0.96).
- The invert sugar before storage correlated with organic acids (r = 0.80), tannins (r = 0.91) and anthocyanins

(r=0.79).
 (r=0.91)
 (r=0.80),
 (r=0.98; r=0.94)
 (r=-0.96; r=-0.90).
 (r=0.79)
 4
 0.0537,
 $R^2=0.94$.

(r = 0.79).

- Before and after fruit storage, sucrose correlated strongly positively with acidimetric factor ($r = 0.98$; $r = 0.94$) and strongly negatively with acids ($r = -0.96$; $r = -0.90$).

- The determined high correlation between the percentage of tannins and acids in fresh plums of 'Elena', before storage under refrigeration conditions ($r = 0.79$) allows to deduce the regression equation presented in Figure 4 $y = 0.4581x - 0.0537$, with a coefficient of determination $R^2 = 0.93$.

$$y = 0.4581x - 0.0537$$

CONCLUSIONS

The impact of some biological and conventional fertilizers on the biochemical composition of plum fruits, as well as the changes of the indicators and their quality after storage have been observed.

It is reported that the maximum storage of fruits ranged from 0 to 4 °C was up to 35 days.

With the best sensory characteristics and quality for consumption, during the studied period are fresh plums treated with chicken manure and organic fertilization.

After staying in refrigerated conditions, the amount of biochemical indicators decreased, such as total, invert sugar and sucrose in fruit.

An increase was found in the percentage of tannins and pectin, most pronounced in the variants with chicken manure (0.167%; 1.11%) and bio fertilizer (0.173%; 1.03%).

(0.167 %; 1.11%)
 (0.173%; 1.03%).

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