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## **SAPS EFFECT ON FARM INCOME DISTRIBUTION AND INEQUALITY IN MOUNTAINOUS AREAS IN BULGARIA**

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### **SUMMARY**

The article focuses on analysis of inequality in the distribution of the farm business income of agricultural producers in mountainous areas in Bulgaria, by way of the assessment of the impact of subsidies from the single area payment scheme on the said inequality.

The main methodological tool used is the quantification of the Gini coefficient and its elasticity. The main aim of the article is to investigate the impact the SAPS had on the farm income inequality in the chosen area.

Two scenarios for the development of the value of the coefficient have been considered - with and without subsidies. It was discovered that the inequality in the distribution of farm incomes would be a little higher if the farms does not receive subsidies.

The data used in the analysis is from the national statistics as well as the FADN, collected by the Ministry of Agriculture

Gini,

2007-2013.

and Food.

- The definition of mountainous area used in the article corresponds to the definition used in the Bulgarian Rural Development programme 2007-2013.

**Key words:** farm income, inequality, Gini coefficient, mountainous areas, SAPS

## INTRODUCTION

- Ensuring a fair standard of living for the people employed in agriculture is one of the main objectives of the EU Common Agricultural Policy. The specifics of agriculture and agricultural production as a whole are a prerequisite for the manifestation of the so-called. "farm problem" (Gardner, 1992).

" 1992).

" (Gardner,

The narrow definition of the "farm problem" is most often associated with low and unstable incomes in agriculture compared to the incomes in other sectors of the economy.

- Policy measures aimed at income support are trying to solve exactly this problem.

During the long years of its evolution until reaching its present form, increasing the incomes of those employed in agriculture is among the main goals of the agricultural policy of the European Union.

There have been several studies on the impact of subsidies on agriculture and farm activities, both in Europe and in Bulgaria.

20%

80%

( , 2011;  
, 2012).

El Benni & Finger, 2013

1990-2009

FADN

Gini

(Payt at al. 1980 Lerman and  
Yitzhaki, 1985).

There are not many studies in our country, however, that focus on the impact of subsidies on inequality in the distribution of farm incomes.

Some studies reported uneven distribution of these payments and the fact that around 20% of the farms receive 80% of the subsidies (Popov, 2011; Popov and Ivanov, 2012).

But how does this affect the overall distribution of farm incomes and whether it contributes to some extent to overcome inequality has been insufficiently researched problem.

The results of the impact evaluation of the agricultural policy's various elements on farm income inequality are somewhat mixed. El Benni & Finger, 2013 in a study of the effects of the reform of agricultural policy in Switzerland reached the conclusion that direct payments lead to a reduction in inequality of incomes of agricultural households.

Their analysis is based on data from the FADN for the period 1990-2009 year, using a methodology based on the adjusted Gini coefficient (Payt at al. 1980 and Lerman and Yitzhaki, 1985). They also reported that the inequality in household income in the period 1990-2009 increased

1990-2009	marginally, but inequality in farm incomes rose strongly.
	The impact of the transition from coupled to decoupled direct payments was also researched.
(Deppermann, Grethe, & Offermann, 2011; Schmid, Sinabell, & Hofreither, 2006; Severini & Tantari, 2015; Trnková & Malá, 2013).	There are also studies on income inequality of farm households incomes and farm incomes, and the impact of the measures of agricultural policy on them in EU countries - Ireland, Italy, Greece, Czech Republic and other (Deppermann, Grethe, & Offermann, 2011; Schmid, Sinabell, & Hofreither, 2006; Severini & Tantari, 2015; Trnková & Malá, 2013).
	The obtained results are not always similar. Overall income inequality from farming is high, but at the same time direct payments are not always confirmed as a key limiting factor to this inequality.
(2011) . Deppermann et al.	Deppermann et al. (2011) in his study of northern Germany concluded that the implemented policy measures to support agricultural income did not contribute to overcoming inequality.
(2006) . Schmid et al. -15,	Schmid et al. (2006) presented results for the EU-15, indicating that despite the stated objectives, the direct decoupled payments are not a suitable instrument for

	dealing with inequality in income distribution to farmers.
Severini & Tantari (2013, 2015)	Severini & Tantari (2013, 2015) on the other hand conclude that the direct payments are working instrument to reduce the variability in income distribution in Italy.
Trnková & Malá (2013)	In a study on income inequality of Czech farmers Trnková & Malá (2013) also noted that the direct support part of the CAP has a positive effect on reducing inequality in income distribution.
Mishra, El-Osta, & Gillespie (2009)	In a study Mishra, El-Osta, & Gillespie (2009) reported similar results for the United States.
2007	Since its accession to the EU in 2007, Bulgaria started to implement key CAP policy measures.
	The decoupled direct payments decoupled in our country are applied through the single area payment scheme. This in itself is a prerequisite that large farms with more arable land, which are generally more cost effective, receive more support.
	Much of the country is in mountainous areas where the specifics of relief and climate determine the possibility of growing

specific crops and livestock.

The purpose of this study was to determine the income inequality of farm incomes in mountain and normally developed areas of the country and the impact that the SAPS payments have on it.

To achieve this, a methodology based on calculation of inequality through the Gini coefficient is used, and FADN data for 2007 and 2011.

Gini  
2011.

2007

The dynamics in the development of farm income in the period was also indicated. Of interest for further study is the inequality in other subgroups of farms – based on specialization and farm size.

The limitations of the analysis are associated with the data we have for the farms in the mountainous areas of the country and the fact that the only policy tool analyzed is the SAPS payments.

## **MATERIAL AND METHODS**

The main methodological approach used in this study is the calculation of the Gini coefficient for income generated by farms.

Gini

Gini (G) (Stuart, 1954), Y ; μ ; F(Y) (cumulative distribution function) cov.

This tool is widely used in the literature to help determine the extent of inequality in the society.

Its application is common also in research and analysis related to agricultural incomes.

Gini coefficient (G) could be calculated by equation 1 (Stuart, 1995), where Y is the net income received by individual farm households in the sample, arranged in ascending order of income; μ is the average income received by the concerned households;

F (Y) is the cumulative distribution function of the income received and cov. is the covariance between the income and the cumulative distribution function.

$$G = \frac{2cov[y, F(Y)]}{\mu} \quad (1)$$

0 1, 0 . . . 1 - 1 .

The obtained values are in the range of 0 to 1, with 0 indicating complete equality, i.e. all units have equal income, 1 - complete inequality where 1 unit receives all the income.

Due to the specifics of the agricultural income and the calculation of net income per farm, there are a large number of farms that have negative income over the

, respective calendar years.  
 This presents difficulties in applying the methodology for calculating the Gini coefficient and the interpretation of results as the value obtained is higher than 1. In this way the inequality in income received is overestimated (Chen at al., 1982).  
 To overcome this drawback Chen at al. (1982) developed an approach that allowed the calculation of the so-called adjusted Gini coefficient ( $G^*$ ) (equations 2, 2a and 2b). These equations were modified further by Berrebi and Silber (1985).  
 With their application, the result is within standard again as the values obtained are in the range  $<0; 1>$ .

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$$G^* = \frac{\left(\frac{2}{n}\right) \sum_{j=1}^n j y_j \frac{n+1}{n}}{\left[1 + \left(\frac{2}{n}\right) \sum_{j=1}^m j y_j\right] + \left(\frac{1}{n}\right) \sum_{j=1}^m y_j \left[\frac{\sum_{j=1}^m y_j}{y_{m+1}} - (1+2m)\right]} \quad (2)$$

$$y_j = \frac{Y_j}{n \bar{Y}} \quad (2)$$

$$\bar{Y} = \frac{\sum_{j=1}^n Y_j}{n} \quad (2)$$

(2, 2 2 ) n  
 ; j  
 j-  
 ; Y<sub>j</sub>  
 ; y<sub>j</sub>

Where n is the total number of farms in the sample;  
 j marks the position of the j-th farm in the sample, arranged in ascending order, based on the income therefrom;  
 Y<sub>j</sub> is the income of the concerned farm; y<sub>j</sub> - the concerned farm's



$G = G^*$ .  
 (Mishra et al., 2009).

Gini  
 (Mishra et al., 2009),

Boisvert Ranney (1990)

Gini

1%.

3

$$E = \frac{G_1^* - G_0^*}{G_0^*}$$

(3)

income share of the total income generated by all farms;

$m$  - the number of farms, the sum of whose income is a negative number;  $m + 1$  is the first farm, the addition of which results in a positive sum.

In case that there are no negative values for the income  $G = G^*$ . Otherwise  $G^* < G$  (Mishra et al., 2009).

Although the application of this equation does not allow the use of the method of decomposing the Gini coefficient (Mishra et al., 2009), i.e. determining the actual impact of different income sources on the overall inequality, still there is an approach that allows the determination of anticipated variations of some components of income.

Using the approach of Boisvert and Ranney (1990) it is possible to determine the so-called elasticity of the Gini coefficient by simulating a change in a component of income by 1%.

Then with applying equation 3, the impact of the component under consideration on overall income inequality can be determined.

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2007 , 2011

Specific subject of analysis in this study is the net income (NI) of farms determined with the methodology of the FADN.

Generally the way of forming this net income can be represented as follows:

$$NI = \text{Net value added (NVA)} - \text{Costs for land, labor and capital} - \text{Balance of subsidies and taxes on production}$$

Where

$$NVA = \text{Gross output} - \text{Intermediate consumption} + \text{Subsidies} - \text{Taxes} - \text{Depreciation}$$

In this study we assume that the net income consists of a market-specified component and subsidies, which are respectively decoupled, on production and for investments. In order to determine the impact of SAPS payments, we construct the following indicator:

$$\text{Corrected Net Income} = \text{Net income} - \text{SAPS payments}$$

According to the assumption we made, the corrected net income includes all revenues and expenditures of the farm and all other subsidies, excluding subsidies received under the single area payment scheme.

The analyses and the calculations are based on balanced panel of 896 farms covered by the FADN for the period 2007 - 2011. For each one of the reference years, farms with

896

highest and the lowest income are eliminated because they represent outliers and distort the statistical analysis.

From the resulting sample (which because of the large number of its subjects, even though unrepresentative, can be considered as valid for credible conclusions) are separated two groups of farms - those in mountainous disadvantaged areas and ones in other parts of the country. The classification of specific farms is done as it is assumed that the farms that are included into one group in 2011 continue to figure in the same group for the remaining years, even if they did not receive support under the RDP 2007-2013 measures to assist farms in less favored areas.

The methodology under which FADN defines these farms is based on the "Regulation for determining the criteria for disadvantaged areas and their territorial scope", adopted by Decree 30 of 15.02.2008 Within the scope of designated areas according to the ordinance, through the whole country there are 1714 towns and villages, and their adjacent lands. They represent a total of 38.20% of the country's territory and 16.60% of the UAA.

On the basis of the designated objects of observation, calculations

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Gini,

Excel.

2007

1.

were conducted and the adjusted Gini coefficient was quantified, as a comparison between the two groups has been made. The influence of SAPS payments was also defined. All calculations are made with the EXCEL program.

## RESULTS AND DISCUSSION

Bulgaria's accession to the European Union in 2007 was associated with the synchronization of our legislation and policies with the European ones.

- For the agriculture, the Common Agricultural Policy is the basic framework defining the development of the sector and the implementation in practice of the political decisions related to
- ensuring a sustainable and effective development of the industry. As stated above the income policy is an integral part of the CAP.

In Bulgaria it is executed with the implementation of the Single Area Payment Scheme.

- The main data source used to perform the analysis is the database of the Farm Accountancy Data Network. The main income indicators and the subsidies farmers receive are presented in Table 1.

- The farms that fall in the general

1  
 ( 2000 )  
 2007  
 146 010, 2011 115 270,  
 20%  
 9 . . 15,7 .  
 2011 14,9 . .  
 19% 53%  
 1).  
 2009

population of market-oriented farms annually, are those with economic size is over 1 ESU (SO €2,000).

Their number in 2007 was 146,010 and in 2011 was 115 270, which represents a decrease of about 20% due to restructuring of the sector.

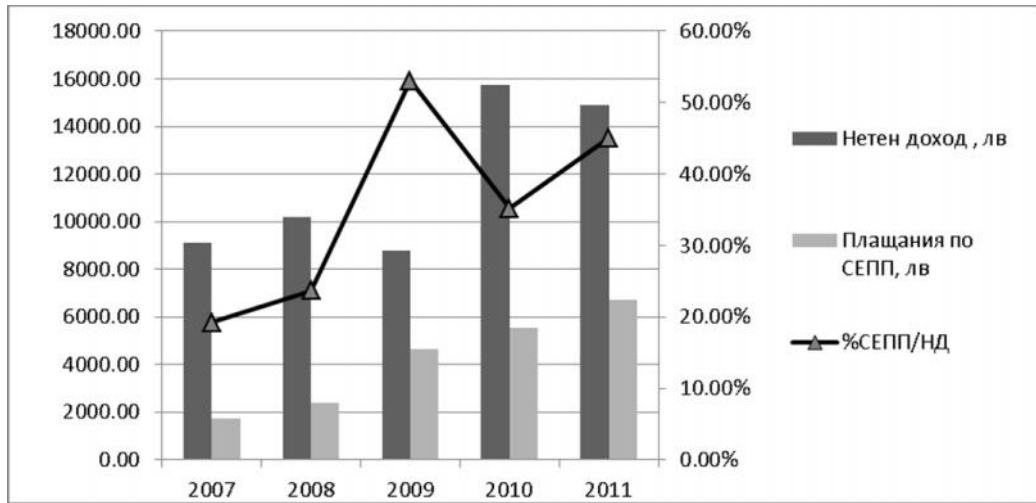
The average net income in the beginning of the period was just over 9 thousand BGN per farm and reached its peak of 15.7 thousand BGN per farm in 2010, and in 2011 was 14.9 thousand BGN. The value of average SAPS payments per farm moves within the 19% to 53% scope of the Net income (Fig. 1). Payments and subsidies part of other policy measures and schemes are significantly less.

Payments for disadvantaged areas, average per farm in the country, accounted for only about 2.6%, which compared to other funds received by the farmers, is minimal.

2009 is indicative of the importance of income from farming for the development and the levels of net farm income.

Poor production results, the low value of total output, incurred costs cannot be offset by the subsidies received and as a result the average farm income was the

lowest over the period.



**Fig. 1. Average net income, average SAPS payments per farm, and share of SAPS payments in Net income**

(FADN, Source: DG "Agriculture", FADN, own calculations)

/ Net value added / annual work unit, which besides indicator of labor productivity is often taken as a proxy indicator for income of those employed in agriculture, also shows a slight tendency to increase during the period (Table 1).

1).

1.  
, 2007-2011  
**Table 1. Average indicators for the incomes of market-oriented agricultural holdings in Bulgaria, 2007-2011**

/ Indicators	2007	2008	2009	2010	2011
Agricultural holding, number	146010	146000	115080	115270	115270
Net Income, lv	9123.95	10199.65	8779.72	15744.43	14891.69
NVA/AWU, lv	6625.70	7636.19	8002.71	12220.81	13322.16
Total subsidies, excl. subsidies for investments, lv	3154.75	6252.79	11150.19	12122.23	10494.98
RDP payments, lv	0.00	117.35	1865.86	2307.88	1191.10
SAPS payments, lv	1750.47	2413.49	4658.79	5542.82	6712.41
Less-favored areas payments, lv	0.00	17.60	9.78	101.70	393.12
Other subsidies, lv	694.32	1994.95	3033.49	3407.06	1770.03

: FADN  
Source: FADN

- The determination of inequality in the distribution of farm income is based on the presented above methodology and calculation of the adjusted Gini coefficient.

- Gini.

- 2007

2011. 2007 ,

- 2007

25%

2008 – 30%,

2009 – 35%, 2010 – 40%, 2011 – 50%. 2016

- The analysis was made for data for 2007 and 2011. 2007 was the first year in which the direct area payments in Bulgaria were applied. As agreed for the accession of the country, farmers will receive 25% of payments in 2007, 30% in 2008, 35% in 2009, 40% in 2010; 50% in 2011.

- In 2016 they will reach the





total number of monitored units.

2.

2007 2011

**Table 2. Major descriptive statistics characteristics for the Net Income of the analyzed farms in 2007 and 2011**

Statistics characteristics	Farms in mountainous areas	Farms in normally developed areas	Farms in mountainous areas	Farms in normally developed areas
	2007		2011	
Mean net income	14160.53	74527.54	13712.33	102745.89
Mediana	10271.00	11877.00	8866.00	12640.00
Mode		280.00		-3510.00
Standard deviation	42820.84	302398.69	27386.80	335763.29
Kurtosis	13.56	48.52	6.30	14.94
Skewness	-2.10	3.19	1.42	3.01
Range	340025.00	6310913.00	176400.00	3861493.00
Minimum	-213371.00	-2761530.00	-46928.00	-1029675.00
Maximum	126654.00	3549383.00	129472.00	2831818.00
Sum	991237.00	55224905.00	959863.00	76134705.00
Count	70	741	70	741

Source: FADN, own calculations

According to the data for net income of farms it is clear that there is an uneven distribution, since the parameters skewness and kurtosis are positive for almost all examined groups of farms.

The values of these indicators

show that the number of farms with lower income is significantly higher than those with higher.

3.

2007 2011

**Table 3. Major descriptive statistics characteristics for the Corrected Net Income of the analyzed farms in 2007 and 2011**

Statistics characteristics	Farms in mountainous areas	Farms in normally developed areas	Farms in mountainous areas	Farms in normally developed areas
	2007		2011	
Mean corrected net income	9395.37	50625.17	5257.83	42951.96
Mediana	8286.00	7720.00	7690.00	4659.00
Mode		6346.00		2109.00
Standard deviation	39663.58	285027.67	27334.54	271188.24
Kurtosis	17.99	61.28	7.56	16.83
Skewness	-2.93	3.50	0.13	2.47
Range	331425.00	6310913.00	209555.00	3277591.00
Minimum	-217371.00	-2761530.00	-80083.00	-1067448.00
Maximum	114054.00	3549383.00	129472.00	2210143.00
Sum	657676.00	37513251.00	368048.00	31827403.00
Count	70	741	70	741

Source: FADN, own calculations

Gini.

( 4)

To determine the value of this inequality we used the Gini coefficient.

On the basis of the calculations (Table 4) the value for the relevant holdings in mountain areas for 2007 is 0.87.

		2007	
	0,87.	0,6,	The value of the coefficient is over 0.6, which means that there is a strong inequality.
	,	,	
	.	,	This means that a large number of farms receive negative or low incomes and fewer farms - very high ones.
	,	-	
	,	,	
	.	-	The most of the farms in our sample have a negative net income.
	,	-	
	.		
	,	2011	This applies to the same farms and in 2011, although there was a decrease in the degree of inequality.
	,	,	
	.		However, it continues to be strong
0,79	,	-	- 0.79 is the value of the
.	,	-	coefficient. A similar study of farms raising field crops in the Czech Republic determined that the average Gini coefficient for the period 2005 -2010 was 0,84 (Trnková & Malá, 2013).
,	,		
		Gini	
	2005 -2010		
0,84	(Trnková & Malá, 2013).		
,	,	-	In Switzerland, where income inequality is low, the Gini coefficient for the distribution of farm income is higher than that of farm household income for the period 1995 - 2009.
		Gini	
-			
	1995 – 2009.		
	0,27	1990	0,38
	2009 (El Benni & Finger, 2013). Severini & Tantari (2013)		It runs from 0.27 in 1990 to 0.38 in 2009 (El Benni & Finger, 2013). Severini & Tantari (2013) in their study calculated the value of the Gini coefficient for different types of farms for two periods 2003-

		2003-2004	2006-	2004 and 2006-2007.
2007.				Their results also suggested that the business incomes of agricultural holdings are very unevenly distributed and also that market incomes are the main sources of this inequality.
			0,65	The values of coefficients are from 0.65 to 0.79 for the three types of analysed farms.
0,79		2007	2011	In 2007 and in 2011 the value of the Gini coefficient for corrected net income of farms is higher than the value of the Gini coefficient of net income, including SAPS subsidies.
		Gini		This allows us to confirm that even minor SAPS payments contribute to reducing inequality and without them it would be higher.
				For the holdings in normally developed regions in 2007 and 2011 the observed values of the Gini index are higher than those of the farms in mountain areas.
		2007	2011	The values of the coefficient are 0.94 for 2007 and 0.93 for 2011.
		Gini		
G	0,94	2007	0,93	These figures show very high inequality of income received that is most probably due to the greater diversity in the type, structure and organization of the holdings within the sample.
2011.				



4. Gini  
, 2007 2011

**Table 4. Gini coefficient of the chosen income indicators. Elasticity of Gini coefficient. 2007 and 2011**

/ Years	Farms in mountainous areas		Farms in normally developed areas	
	2007	2011	2007	2011
G*	0.867	0.78674	0.93844	0.93127
G* <sub>NI</sub>	0.920	0.963	0.972	0.986
G* <sub>CNI</sub>	0.866	0.78530	0.93813	0.93071
G* <sub>NI+1%SAPS</sub>	-0.001	-0.002	0.000	-0.001

Source: Own calculations

**CONCLUSIONS**

1. Farm income inequality in our country is very high. Many farms, especially smaller ones, have a negative economic result of their activities.

2. Net income of the market-oriented farms during the period 2007-2011 shows a slight tendency to increase. The value of SAPS payments per farm moves within the 19% to 53% of their Net income, and this share increases annually by an increase in total payments.

3. The income of the farms in mountain areas shows a high degree of inequality both in 2007, 2011

1. Farm income inequality in our country is very high. Many farms, especially smaller ones, have a negative economic result of their activities.

2. Net income of the market-oriented farms during the period 2007-2011 shows a slight tendency to increase. The value of SAPS payments per farm moves within the 19% to 53% of their Net income, and this share increases annually by an increase in total payments.

3. The income of the farms in mountain areas shows a high degree of inequality both in 2007 and in 2011.

	<p>The importance of SAPS payments to overcome inequality, though minimum is stronger pronounced at farms in mountainous areas of the country.</p>
4.	<p>This is probably due to the higher share of SAPS payments in the formation of net income.</p>
4.	<p>4. The equality in normally developed areas is higher compared to those of the farms in mountainous areas, due to the greater homogeneity of farms in mountain areas in our sample.</p>
5.	<p>5. SAPS payments do not contribute enough to overcome the inequality in income distribution, both in mountainous and in normally developed areas.</p> <p>This is due to the way they are paid – based on the arable land the holding has.</p>
6.	<p>6. On the basis of the analyses we can assume that inequality largely depends also on the economic size and specialization of holdings in the sample, and their market returns, which need to be studied further.</p>

**/ REFERENCES**

1. . 2011,





**(PELARGONIUM GRANDIFLORUM WILLD.)  
(PELARGONIUM ZONALE (L.)  
L'HÉR. EX AITON)**

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**INVESTIGATING THE POSSIBILITIES FOR INITIATING IN VITRO  
CULTURE AND MICROPROPAGATION OF PELARGONIUM  
GRANDIFLORUM WILLD. AND PELARGONIUM ZONALE (L.)  
L'HÉR. EX AITON**

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**SUMMARY**

Pelargonium species are one of the most common container plants for decoration of windows and balconies. They are also often used in the interior or as annual plants in gardens and parks.

Due to the great interest towards these plants it is essential to explore possibilities for their micropropagation.

In this study, a research was carried out to identify the most suitable explants and procedure for initiation of in vitro culture of royal and zonal pelargoniums.

The effects of some auxins and cytokinins, and two basal salt media - Murashige & Skoog 1962 (MS) and McCown Woody Plant Medium (WPM) in different stages of micropropagation were also studied.

The two species of pelargonium

Murashige & Skoog  
1962 (MS)      McCown Woody Plant  
Medium (WPM)





	<p>multiplication and propagation of various pelargonium species are</p> <ul style="list-style-type: none"> <li>- focused on the development of protocols for their industrial production and to application of modern biotechnological methods for selection.</li> </ul>
<p>20-60- (Dunbar &amp; Stephens, 1989; Hassanein &amp; Dorion, 2005; Saxenaa et al., 2000).</p>	<p>The first experiments for in vitro cultivation of different species of pelargonium began in the late 60s of the 20th century. Most authors reported successfully cultivation of pelargonium in vitro on basal salt medium MS (Dunbar &amp; Stephens, 1989; Hassanein &amp; Dorion, 2005; Saxenaa et al., 2000).</p>
<p>(Nasser, 2013).</p>	<p>Reports for using other nutrient media are significantly limited (Nasser, 2013).</p> <p>There are different parts of the plants that are appropriate for introducing in in-vitro culture: apical buds, nodal explants, segments of stems, petioles, pedicels and peduncles, leaf blades and others.</p>
<p>Cassells (1998)</p> <p>Xanthomonas pelargonii,</p>	<p>The effectiveness of the different types of explants depends on the availability of various endogenous bacterial infections. Cassells et al. (1998) reported a research on endogenous microflora of royal pelargonium that established that the basic and ubiquitous contaminant is Xanthomonas pelargonii.</p>

<p>Xanthomonas pelargonii</p>	<p>The other identified bacteria are not specific. They also established that the presence of Xanthomonas pelargonii in plant tissue depends largely on the history of the plant, and that the youngest parts are usually free from it.</p>
<p>Dunbar &amp; Stephens (1989)</p>	<p>Researchers report different types and concentrations of auxins and cytokinins as the most suitable, which is probably due to the significant differences in the reactions of the different genotypes to the growth regulators. Dunbar &amp; Stephens (1989) for example, report that zeatin is more suitable for obtaining regeneration for zonal pelargonium, while the royal pelargonium achieved satisfactory regeneration on medium containing only 2 mg/l<sup>-1</sup> BAP.</p>
<p>BAP. Brown &amp; Charlwood (1986)</p> <p>2 mg/l<sup>-1</sup></p> <p>0.50 mg/l<sup>-1</sup></p> <p>0.05 mg/l<sup>-1</sup> NAA</p> <p>2,4 – D P. australe, P. citriodorum, P. crispum, P. graveolens, P. filifolium, P. quercifolium, P. tomentosum</p> <p>'Duke of York', 'Miss Australia', 'Prince of Orange'. Saxenaa (2000)</p> <p>P.graveolens</p> <p>0,5 mg/l<sup>-1</sup></p> <p>1mg/l<sup>-1</sup> NAA.</p> <p>MS</p>	<p>Brown &amp; Charlwood (1986) managed to achieve regeneration on a medium containing 0.50 mg/l<sup>-1</sup> BAP and 0.05 mg/l<sup>-1</sup> NAA from callus culture induced and maintained on medium with 2,4 – D, from P. australe, P. citriodorum, P. crispum, P. graveolens, P. filifolium, P. quercifolium, P. tomentosum and the varieties 'Duke of York', 'Miss Australia' and 'Prince of Orange'. Saxenaa at al. (2000) reported the successful regeneration of P. graveolens on medium containing 0.5 mg/l<sup>-1</sup> kinetin and 1 mg/l<sup>-1</sup> NAA. They also reported that MS supplemented with 8 mg/l<sup>-1</sup> kinetin and 1 mg/l<sup>-1</sup></p>

1	1 mg/l <sup>-1</sup> NAA,	NAA, as a suitable medium for multiplication of nodal explants.
Hassanein & Dorion (2005)	0.5 mg/l <sup>-1</sup> NAA 1 mg/l <sup>-1</sup>	Hassanein & Dorion (2005) achieved a high degree of regeneration of scented-leaved pelargonium plants on a medium containing 0.5 mg/l <sup>-1</sup> NAA in combination with 1 mg/l <sup>-1</sup> BAP and 1 mg/l <sup>-1</sup> zeatin, while the zonal pelargonium's maximum regeneration has been achieved on medium containing 0.2 mg/l <sup>-1</sup> NAA in combination with 0.5 mg/l <sup>-1</sup> BAP. Wojtania (2010) reported that in vitro cultures of varieties of zonal pelargonium and cascade pelargonium show greater productivity, stability and vitality on media containing meta-Topolin compared to the ones with BAP.
Wojtania (2010)	0.2 mg/l <sup>-1</sup> NAA 0.5 mg/l <sup>-1</sup>	In addition, some authors have examined other factors influencing the regeneration of callus.
Galston (1967)	Chen &	Chen & Galston (1967) reported that regeneration from a callus culture of pelargonium species depends on the length of callus cultivation. They observe regeneration on medium without growth regulators when the culture is old.
Kuchtová-Jadrná (2009)	Vejsadová &	Vejsadová & Kuchtová-Jadrná (2009) indicate positive influence of darkness on the process of regeneration.
		All authors are united around the conclusion that rooting pelargonium micro-plants is easy and fast. There are reports of

1/2 MS, (1987)	MS, Aldrufeu	-	successful rooting on basal salt medium MS and 1/2 MS supplemented with different amounts of auxins. Aldrufeu (1987) compared several different substrates for rooting pelargonium in in-vitro conditions and set the most appropriate material to be perlite.
(1983)	Cassells & Minas	,	Another important question is that of the genetic stability of the in vitro produced plants and their varietal identity. Cassells & Minas (1983) compared plants propagated in vitro, initiated from apical buds, with such propagated in vivo and found only minor deviations.
Saxenaa	(2000)	-	Saxenaa at al. (2000) found a significant somaclonal variation in P. Graveolens propagated in vitro from callus.
P. Graveolens,		-	They found differences between the different clones in their habit, bloom and in the chemical content of their essential oil.
		-	The main goal of this study is to identify the critical stages of the in vitro cultivation of royal and zonal pelargoniums, and to distinguish the objective possibilities for commercial production of in vitro plants from them. In order to reach this goal the possibility for isolating sterile plant material, and the most appropriate type of explants for

each of the two studied genotypes should be determined.

- The most suitable nutrient media for introduction, multiplication and rooting should also be established, as well as the need of growth regulators and their optimal concentration in the different stages of in-vitro culture.

### MATERIAL AND METHODS

Two basal salt media - Murashige and Skoog 1962 (MS) and McCown Woody Plant Medium 1980 (WPM), were tested in this study.

Each of the nutrient media was supplemented with vitamins MS and 30 g/l sucrose, and solidified with 7 g/l<sup>-1</sup> agar. 6-Benzylaminopurine (BAP) - DUCHEFA; 2,4-Dichlorophenoxyacetic Acid (2,4-D) - FLUKA and -Naphthylacetic Acid (NAA) - FLUKA were also used.

The explants were put into individual tubes with length of 150 mm, 20 mm in diameter and capacity of 45 ml, which were poured with 10 ml nutrient medium and sealed with aluminium foil lids. The sterile explants, callus and micro plants were cultured in glass jars, 10 in each jar.

The jars were 80 mm high, 95 mm in diameter and a capacity of 400 ml. 50 ml of nutrient media was

Murashige and Skoog, 1962 (MS) McCown Woody Plant Medium, 1980 (WPM).

MS 30 g/l<sup>-1</sup> 7 g/l<sup>-1</sup>  
 6-Benzylaminopurine (BAP) - DUCHEFA;  
 2,4-Dichlorophenoxyacetic Acid (2,4-D) - FLUKA  
 -Naphthylacetic Acid (NAA) - FLUKA.

150 mm, 20 mm  
 45 ml, 20 mm  
 10 ml

10 mm 80 mm, 95 mm  
 400 ml, 400 ml  
 50 ml



24±2 °C			poured in each. The jars were closed with metal caps. The introduced explants, callus and micro plants were grown in greenhouse premises at 24±2°C and white artificial lighting intensity 30 μmol m <sup>-2</sup> s <sup>-1</sup> and photoperiod of 16 hours day and 8 hours night.
30 μmol m <sup>-2</sup> s <sup>-1</sup>		30	
16		16	
8			
Philips 40 W (Bulgaria).	Philips 40 W (Bulgaria).	Philips 40 W (Bulgaria).	Fluorescent lamps Philips 40 W (supplied by Philips - Bulgaria) were used.
3		3	The material for introduction in in-vitro culture was taken from three varieties of royal pelargonium and 3 zonal pelargonium varieties. All plants were propagated vegetatively from top cuttings in September 2013.
2013			
30		30	The plant material needed for introduction in in-vitro culture was taken in the second half of April 2014 from actively growing plants. 30 leaf petiole explants from every variety were introduced. The petioles of the youngest 3-4 fully developed leaves on the stems were used as explants. All explants were washed to remove the surface pollution and were then sterilized for 10 minutes in 0.1% solution of mercuric chloride (HgCl <sub>2</sub> ).
0,1%		0,1%	
(HgCl <sub>2</sub> ).	(HgCl <sub>2</sub> ).	(HgCl <sub>2</sub> ).	
10		10	After sterilization, the explants were washed three times by soaking for 10 minutes in sterile distilled water. Sterilized explants

50

MS

were introduced in individual tubes on basal MS medium without growth regulators.

The percent of sterility was recorded repeatedly for 50 days until new unsterile explants ceased to appear.

The one variety showing highest sterility from royal and the one from zonal pelargonium were used for further research. The research included determining the most suitable type of explants and their requirements for growth regulators during the introduction phase and organogenesis, the process of multiplication and rooting.

5

The sterility of 5 types of explants was compared – lateral and apical buds, leaf petioles, pedicels and peduncles.

1-1,5 cm.

The explants of apical and lateral buds were from 1 to 1.5 cm long. After they were sterilized under aseptic conditions, the buds were removed carefully with a scalpel.

The segments of the peduncles were prepared from peduncles of young inflorescences not yet in bloom.

10-15 2-4 mm

They were cut into 2-4 parts 10-15 mm each after sterilization. The pedicels were taken from not-blooming but fully developed

30

MS

BAP, 2,4-D

20

:

MS

; MS,

0,25 mg/l<sup>-1</sup> BAP; MS,

0,5 mg/l<sup>-1</sup> BAP; MS,

0,1 mg/l<sup>-1</sup> 2,4-D 0,25

mg/l<sup>-1</sup> BAP;

WPM

; WPM,

0,25 mg/l<sup>-1</sup> BAP; WPM,

0,5 mg/l<sup>-1</sup> BAP WPM,

0,1 mg/l<sup>-1</sup> 2,4-D 0,25

mg/l<sup>-1</sup> BAP.

,

MS, 0,1 mg/l<sup>-1</sup> 2,4-D

0,25 mg/l<sup>-1</sup> BAP

6

inflorescences.

30 explants of each type of both varieties of pelargonium were sterilized following the already described procedure and put into individual tubes on basal MS medium without growth regulators.

To establish the effects of BAP, 2,4-D and the main nutrient medium on the formation and growth of the callus and the emergence of organogenesis, their effect on the explants from the pedicels of the two species examined was studied. 20 explants of both varieties were set with the following variants: basal MS medium without growth regulators; MS supplemented with 0.25 mg/l<sup>-1</sup> BAP; MS supplemented with 0.5 mg/l<sup>-1</sup> BAP; MS supplemented with 0.1 mg/l<sup>-1</sup>, 2,4-D and 0.25 mg/l<sup>-1</sup> BAP; basic WPM medium without growth regulators; WPM supplemented with 0.25 mg/l<sup>-1</sup> BAP; WPM supplemented with 0.5 mg/l<sup>-1</sup> BAP; WPM supplemented with 0.1 mg/l<sup>-1</sup> 2,4-D and 0.25 mg/l<sup>-1</sup> BAP.

Callus culture induced from pedicels on basal MS medium with 0,1 mg/l<sup>-1</sup> 2,4-D 0,25 mg/l<sup>-1</sup> BAP was transferred on 6 combinations of basal nutrient medium with growth regulators in order to test the best possible combination for plant regeneration from callus.

:  
 MS  
 ; MS,  
 mg/l<sup>-1</sup> BAP; MS,  
 mg/l<sup>-1</sup> BAP;  
 WPM  
 ; WPM,  
 0,25 mg/l<sup>-1</sup> BAP; WPM,  
 0,5 mg/l<sup>-1</sup> BAP.

30

WPM                      BAP  
 30                      - 0,25    5 mg/l<sup>-1</sup>.

:  
 MS  
 ; MS,  
 0,25 mg/l<sup>-1</sup> BAP; MS,  
 0,5 mg/l<sup>-1</sup> BAP;  
 WPM  
 ; WPM,  
 0,25 mg/l<sup>-1</sup> BAP;  
 WPM  
 0,5 mg/l<sup>-1</sup> BAP.

MS

10

NAA

:

The following combinations were tested: MS without growth regulators; MS supplemented with 0.25 mg/l<sup>-1</sup> BAP; MS supplemented with 0.5 mg/l<sup>-1</sup> BAP; WPM without growth regulators; WPM supplemented with 0.25 mg/l<sup>-1</sup> BAP; WPM supplemented with 0.5 mg/l<sup>-1</sup> BAP; WPM supplemented with 0.1 mg/l<sup>-1</sup> 2,4-D and 0.5 mg/l<sup>-1</sup> BAP.

30 callus lumps were used on each of the media types.

The effect of two main nutrient media – MS and WPM and the nutrient media with BAP in two concentrations of 0.25 and 5 mg/l<sup>-1</sup> – on the development of explants of apical buds was studied. 30 sterile explants of each of the two varieties of pelargonium studied were set on the following nutrient media: MS basal medium without vitamins; MS supplemented with 0.25 mg/l<sup>-1</sup> BAP; MS supplemented with 0.5 mg/l<sup>-1</sup> BAP; basal WPM medium without growth regulators; WPM supplemented with 0.25 mg/l<sup>-1</sup> BAP; WPM supplemented with 0.5 mg/l<sup>-1</sup> BAP.

10 combinations of two basal nutrient media, activated carbon and two concentrations of NAA were tested to establish which one is most suitable for rooting of each of the two species examined.

The following options were tested: Basic MS medium without growth

MS  
 ; MS  
 + 2 g/l<sup>-1</sup>  
 ; MS,  
 1 NAA; MS, 0,2 mg/l<sup>-1</sup>  
 NAA; 0,5 mg/l<sup>-1</sup>  
 WPM ;  
 WPM +  
 2 g/l<sup>-1</sup> ; WPM,  
 0,2 mg/l<sup>-1</sup> NAA; WPM,  
 0,5 mg/l<sup>-1</sup> NAA.

ml , 90  
 50 ml  
 - MS,  
 - WPM.  
 , 30 g/l<sup>-1</sup>  
 .  
 (5 50  
 10  
 ).  
 (ANOVA)  
 p < 0.05.

regulators; MS without growth regulators + 2 g/l<sup>-1</sup> activated carbon; MS supplemented with 0.2 mg/l<sup>-1</sup> NAA; MS supplemented with 0.5 mg/l<sup>-1</sup> NAA; basic nutrient medium without growth regulators; WPM; WPM without growth regulators +2 g/l<sup>-1</sup> activated carbon; WPM supplemented with 0.2 mg/l<sup>-1</sup> NAA; WPM supplemented with 0.5 mg/l<sup>-1</sup> NAA. Two variants of the nutrient medium solidified with perlite were also tested. The jars contained 90 ml perlite and 50 ml liquid medium – MS or WPM. Both media were without vitamins and supplemented with 30 g/l<sup>-1</sup> sucrose, and without growth regulators.

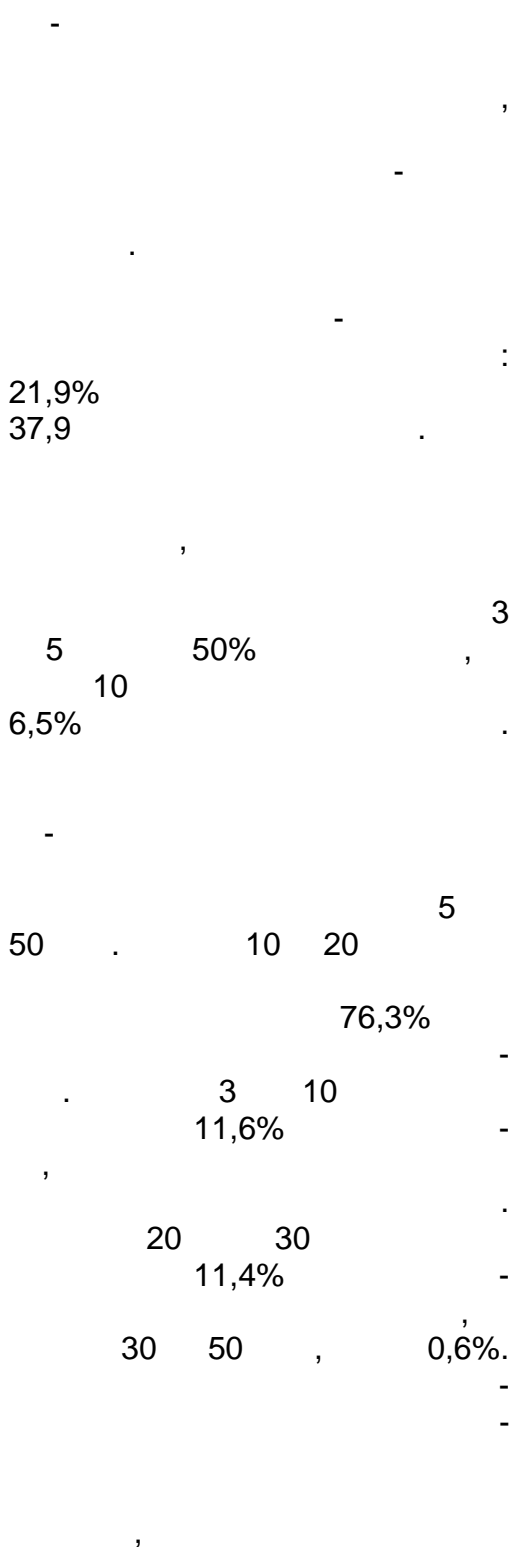
50 explants were tested on each variant (5 jars with 10 plants each).

Data were subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range test at probability level  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Introduction in in-vitro culture

The comparison of sterility in explants from leaf petioles taken from 3 different varieties from royal pelargonium and 3 varieties of zonal pelargonium showed that there is a statistically significant difference between the varieties (Table 1).



A higher overall percentage of sterility showed the varieties of zonal pelargonium, while the varieties of royal pelargonium showed significant difference between the varieties.

The contamination with fungal organisms in both species is less compared with the bacterial one: 21.9% in the royal pelargonium and 37.9 in zonal pelargonium plants. From the observations made on the introduced explants it was found that the contamination of the explants with various fungal micro organisms occur between 3<sup>rd</sup> and 5<sup>th</sup> day in 50% of cases, and after 10 days in only 6.5%.

Symptoms of bacterial infection were discovered from 5<sup>th</sup> to 50<sup>th</sup> day. 76.3% of the cases of bacterial infection were detected between the 10<sup>th</sup> and the 20<sup>th</sup> day, following the introduction in in-vitro culture.

11.6% of the explants in which was detected bacterial contamination were discovered between 3<sup>rd</sup> and 10<sup>th</sup> day. 11.4% of explants with bacterial infection were detected between 20<sup>th</sup> and 30<sup>th</sup> day and only 0.6% between 30<sup>th</sup> and 50<sup>th</sup>.

Since the main microbiological contamination is bacterial and its development is delayed and stretched in time we have reason to assume that a significant part of



2. ( )

Explants from pedicels showed the highest percent of sterility that is probably due to the fact that they are younger; they are located away from the stems on peduncles that probably delays the reaching of endogenous bacterial infections.

Since the explants from apical and lateral buds develop directly into plants, while the other three types of explants have direct or indirect organogenesis, important for the industrial production from in vitro propagation is also the genetic stability that the new plants show (further research is needed).

**Table 2. Percentage of sterility of 5 types of explants from one genotype royal and one genotype zonal pelargonium**

Type of explants	<i>P. grandiflorum</i>	<i>P. zonale</i>
	%	%
	% sterile explants	
/ Apical buds	46.66 <sup>b</sup>	53.33 <sup>b</sup>
/ Lateral buds	40 <sup>a</sup>	36.66 <sup>a</sup>
/ Leaf petioles	43.33 <sup>a</sup>	50 <sup>b</sup>
/ Peducles	60 <sup>d</sup>	66.66 <sup>c</sup>
/ Pedicels	66.66 <sup>d</sup>	80 <sup>d</sup>

<0.05.

Values with different letters in the same column are significantly different at p < 0.05.

The implemented research on the influence of the basal salt media and the growth regulators



( 3, . 1 (1; 2; 3)).

2,4-D

95%  
100%

BAP

( 3).

WPM

MS,

BAP.

MS.

- 94%,

on the regeneration of plants from sterile pedicels showed that both factors are essential for the successful regeneration (Table 3, Fig. 1 (1; 2; 3)).

The explants on media containing 2,4-D didn't showed any regeneration, but 95% of royal pelargonium explants and up to 100% of the explants from zonal pelargonium formed abundant soft callus.

The correlation between the percentage of regeneration and the concentration of BAP was statistically significant and by increasing the ratio of cytokinin in the media the regeneration in both species increased (Table 3).

The basal salt media also show influence on the process of regeneration. Royal pelargonium explants showed better regeneration (two times) on WPM medium compared to MS medium with similar BAP concentration. Higher regeneration of explants from zonal pelargonium was discovered on MS basal medium.

We recorded that the examined genotypes of royal pelargonium showed a high propensity for regeneration to plants on the tested media (94%) while the highest regeneration obtained for zonal pelargonium is only 25% on basal medium MS supplemented

25%,  
MS  
30 g/l<sup>-1</sup> 7 g/l<sup>-1</sup> 0,5 mg/l<sup>-1</sup> BAP,  
with vitamins 30 g/l<sup>-1</sup> sucrose and  
0.5 mg/l<sup>-1</sup> BAP, solidified with 7 g/l<sup>-1</sup>  
agar.

3. 2,4-D BAP

**Table 3. Influence of basal salt medium, concentration of BAP and the presence of 2,4-D on the formation of callus, direct or indirect organogenesis in explants of pedicels from royal and zonal pelargonium**

Nutrient medium / growth regulators	<i>P. grandiflorum</i>			<i>P. zonale</i>		
	%	%	%	%	%	%
MS	0 <sup>a</sup>	5 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
MS + 0,25 mg/l <sup>-1</sup> BAP	25 <sup>c</sup>	10 <sup>b</sup>	15 <sup>b</sup>	15 <sup>b</sup>	0 <sup>a</sup>	10 <sup>b</sup>
MS + 0,5 mg/l <sup>-1</sup> BAP	65 <sup>e</sup>	25 <sup>c</sup>	40 <sup>c</sup>	30 <sup>c</sup>	10 <sup>b</sup>	15 <sup>b</sup>
MS + 0,1 mg/l <sup>-1</sup> 2,4-D	95 <sup>f</sup>	0 <sup>a</sup>	0 <sup>a</sup>	100 <sup>d</sup>	0 <sup>a</sup>	0 <sup>a</sup>
0,25 mg/l <sup>-1</sup> BAP						
WPM	0 <sup>a</sup>	10 <sup>b</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
WPM + 0,25 mg/l <sup>-1</sup> BAP	15 <sup>b</sup>	25 <sup>c</sup>	25 <sup>b</sup>	10 <sup>b</sup>	5 <sup>b</sup>	5 <sup>b</sup>
WPM + 0,5 mg/l <sup>-1</sup> BAP	45 <sup>d</sup>	49 <sup>d</sup>	45 <sup>c</sup>	35 <sup>c</sup>	5 <sup>b</sup>	5 <sup>b</sup>
WPM + 0,1 mg/l <sup>-1</sup> 2,4-D	90 <sup>f</sup>	0 <sup>a</sup>	0 <sup>a</sup>	90 <sup>d</sup>	0 <sup>a</sup>	0 <sup>a</sup>
0,25 mg/l <sup>-1</sup> BAP						

<0.05.

Values with different letters in the same column are significantly different at p < 0.05.

2,4-D

2,4-D

( 4.).

The potential shown by the callus of the two species on medium containing 2,4-D has been satisfactory.

After the transfer of the callus on different nutrient media that did not contain 2,4-D a growth of callus was observed and regeneration of plants depending on the number of set callus lumps (Table 4.).

Callus growth was observed in all

WPM,  
0,25 mg/l<sup>-1</sup> BAP.

MS

30

WPM,  
0,5 mg/l<sup>-1</sup> BAP.

MS,  
0,5 mg/l<sup>-1</sup> BAP,

– 23,3%

variants containing BAP except WPM enriched with 0.25 mg/l<sup>-1</sup> BAP.

The callus grown on MS showed faster growth and larger total volume in 30 days. The callus from both species reacts in a similar manner to the salt concentration and the content of growth regulators.

Callus of royal pelargonium showed a higher propensity to regeneration of plants in all tested variants (Table 4).

The highest rate of regeneration was obtained on a WPM basal salt medium supplemented with 0.5 mg/l<sup>-1</sup> BAP. The regeneration from callus of royal pelargonium directly from the sterile explants or from callus culture is very similar and not statistically different.

Zonal pelargonium showed the highest regeneration (23.3%) on MS basal salt medium supplemented with 0.5 mg/l<sup>-1</sup> BAP, which is significantly lower compared to the royal. The regeneration from callus of zonal pelargonium directly from the sterile explants or from callus culture is very similar and not statistically different as well.

4.

**BAP**

**Table 4. Effect of basal salt medium and two concentrations of BAP on growth and regeneration of the callus from the peticels**

Basal salt medium / Growth regulators	<i>P. grandiflorum</i>		<i>P. zonale</i>	
	Callus growth	% of regenerated explants	Callus growth	% of regenerated explants
MS	-	3,3 <sup>a</sup>	-	0 <sup>a</sup>
MS + 0,25 mg/l-1 BAP	+	23.3 <sup>c</sup>	+	13.3 <sup>c</sup>
MS + 0,5 mg/l-1 BAP	++	43.3 <sup>d</sup>	++	23.3 <sup>d</sup>
WPM	-	6.6 <sup>b</sup>	-	0 <sup>a</sup>
WPM + 0,25 mg/l-1 BAP	-	46.6 <sup>d</sup>	-	6,6 <sup>a</sup>
WPM + 0,5 mg/l-1 BAP	+	63.3 <sup>e</sup>	+	13.3 <sup>b</sup>

<0.05.

Values with different letters in the same column are significantly different at p < 0.05.

**Multiplication**

Explants from apical buds from both species showed satisfactory growth and with few exceptions, all developed in-vitro plants (Fig. 1 (4; 5)).

The rapid growth of the explants is a prerequisite for short subculture (25-35 days). This is a guarantee of greater efficiency of the in-vitro production.

Depending on what type of explants were used, on the best option for royal pelargonium (WPM with 0.25 mg/l<sup>-1</sup> BAP) a propagation coefficient of 15 was obtained (one-node explants). Zonal pelargonium showed even more opportunities for multiplication with over 20 new plants of any successful micro plant cultivated on MS with 0,25 mg/l<sup>-1</sup> BAP.

1 (4; 5)).

(25-35 )).

(WPM + 0,25 mg/l<sup>-1</sup> BAP)

15 ( ),

20

0,25 mg/l<sup>-1</sup> BAP.

MS +

The formation of lateral shoots

BAP,			depends on the content of BAP and the number of shoots decreases either when the optimum dosage is increased or decreased (Table 5).
3,62,		( 5). -	The largest number of lateral shoots (3.62) was recorded in plants cultivated on WPM medium supplemented with 0.25 mg/l <sup>-1</sup> BAP for the royal pelargonium.
WPM, BAP.	0,25 mg/l <sup>-1</sup>	-	The largest number of lateral shoots for the zonal pelargonium. (3.25) was reported for plants grown on MS medium supplemented with 0.25 mg / l <sup>-1</sup> BAP.
-	- 3,25,	MS, 0,25 mg/l <sup>-1</sup> BAP.	-
MS	0,5 mg/l <sup>-1</sup> BAP	-	The combination of MS basal salt medium and 0.5 mg/l <sup>-1</sup> BAP led to callus formation of the royal pelargonium plants and on a later stage - development of many micro plants.
WPM, BAP.	0,5 mg/l <sup>-1</sup>	-	The same combination, as well as the combination of WPM medium supplemented with 0.5 mg/l <sup>-1</sup> BAP have similar effect for the zonal pelargonium explants. Unlike royal pelargonium, only single micro-plants were observed on the callus-formed explants from zonal pelargonium.
		( 5). 5).	The greatest height for both species was recorded in the explants cultured on medium without growth regulators (Table 5). The height of plants with more

lateral shoots was lower while the height of explants that had formed callus was practically equal to that of the initial explant.

Most of the micro plants grown on medium without growth regulators formed roots and were fully prepared to adapt to conditions in vitro.

## 5.

### BAP

**Table 5. Influence of basal salt medium and two concentrations of BAP on growth and productivity of explants of apical buds**

Basal salt medium / Growth regulators	<i>P. grandiflorum</i>		<i>P. zonale</i>	
	Number of stems	Height (mm)	Number of stems	Height (mm)
MS	1.26 <sup>a</sup>	23.62 <sup>c</sup>	1.02 <sup>a</sup>	18.67 <sup>b</sup>
MS + 0,25 mg/l-1 <sup>-1</sup> BAP	1.19 <sup>a</sup>	19.28 <sup>bc</sup>	3.25 <sup>c</sup>	14.83 <sup>a</sup>
MS + 0,5 mg/l-1 <sup>-1</sup> BAP	-	-	-	-
WPM	1.09 <sup>a</sup>	24.35 <sup>c</sup>	1.06 <sup>a</sup>	19.44 <sup>b</sup>
WPM + 0,25 mg/l-1 <sup>-1</sup> BAP	3.62 <sup>b</sup>	16.81 <sup>b</sup>	2.86 <sup>b</sup>	15.71 <sup>a</sup>
WPM + 0,5 mg/l-1 <sup>-1</sup> BAP	1.42 <sup>a</sup>	5.21 <sup>a</sup>	-	-

<0.05.

Values with different letters in the same column are significantly different at p < 0.05.

### Rooting

Micro plants of both species rooted easily on various nutrient media with or without the presence of auxins (Table 6, Fig. 1 (6; 6; 8)).

( 6, . 1 (6;7;8)).

The highest percentage of rooting of the royal pelargonium plants was recorded for plants cultivated on WPM basal salt medium solidified with perlite.

WPM,

## 6.

## NAA.

**Table 6. Rooting of micro plants of both studied species pelargonium on various nutrient media, containing activated carbon and NAA.**

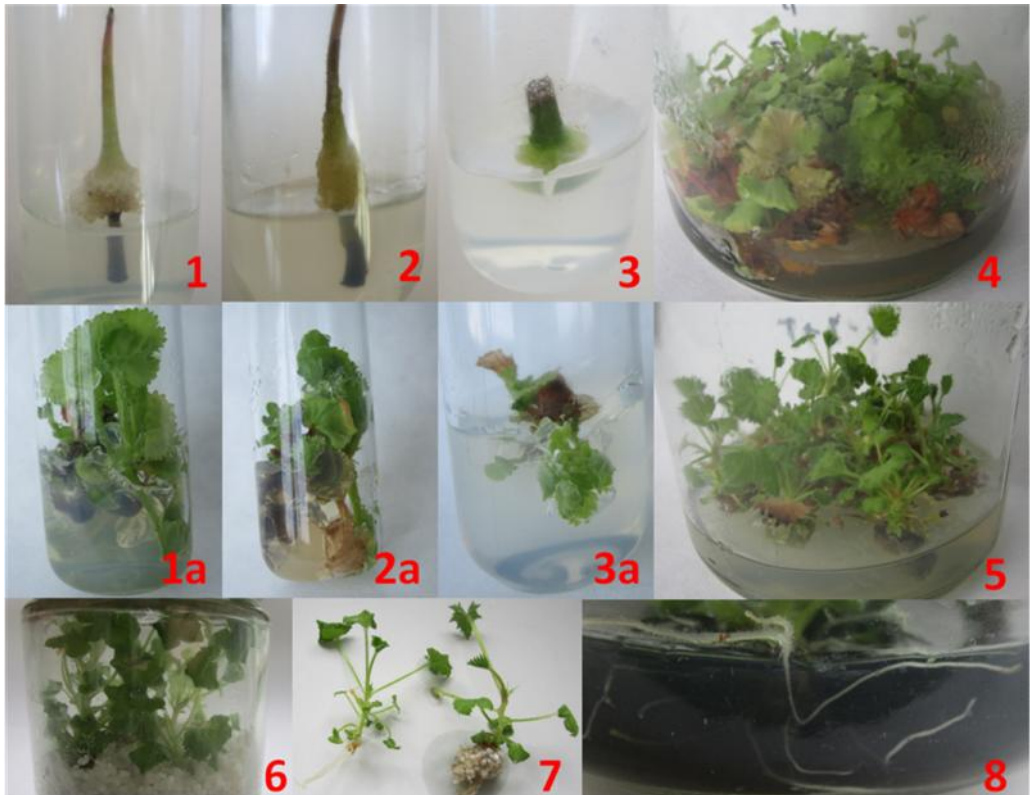
Basal salt medium / Growth regulators	<i>P. grandiflorum</i>	<i>P. zonale</i>
	% % rooting	% % rooting
MS . . / MS without growth regulators	88 <sup>c</sup>	80 <sup>a</sup>
MS . . / MS without g. r. + 2 g/l <sup>-1</sup> / activated carbon	94 <sup>d</sup>	86 <sup>ab</sup>
/Perlite + MS / liquid MS	96 <sup>d</sup>	94 <sup>bc</sup>
MS + 0,2 mg/l-1 <sup>-1</sup> NAA	88 <sup>c</sup>	96 <sup>c</sup>
MS + 0,5 mg/l-1 <sup>-1</sup> NAA	62 <sup>a</sup>	78 <sup>a</sup>
WPM . .	92 <sup>cd</sup>	86 <sup>ab</sup>
WPM . . / WPM without g. r. + 2 g/l <sup>-1</sup> / activated carbon	96 <sup>d</sup>	90 <sup>b</sup>
/ Perlite + WPM/liquid WPM	98 <sup>d</sup>	96 <sup>c</sup>
WPM + 0,2 mg/l-1 <sup>-1</sup> NAA	92 <sup>cd</sup>	100 <sup>c</sup>
WPM + 0,5 mg/l-1 <sup>-1</sup> NAA	76 <sup>b</sup>	84 <sup>a</sup>

<0.05.

Values with different letters in the same column are significantly different at  $p < 0.05$ .

MS	Similar results were obtained on MS medium without growth regulators supplemented with 2 g/l <sup>-1</sup> activated carbon, on MS medium solidified with perlite, as well as on WPM medium without growth regulators supplemented with 2 g/l <sup>-1</sup> activated carbon.
2 g/l <sup>-1</sup>	
MS	
WPM	
2 g/l <sup>-1</sup>	
(100%)	The highest percentage of rooting of the zonal pelargonium plants (100%) was obtained on WPM basal salt medium supplemented with 0.25 g/l <sup>-1</sup> NAA.
WPM( 0,2	Statistically similar results, however, were accounted for on WPM and MS solidified with perlite, as well as on MS supplemented with 0.25 mg/ l <sup>-1</sup> NAA.
g/l <sup>-1</sup> NAA.	
WPM MS,	
0,2 mg/l <sup>-1</sup> NAA.	For both species it was recorded that the plants rooted on

liquid nutrient media, solidified with perlite, are healthier with smaller leaves, thicker stems and more extensive root system. All this gives us reason to assume that these plants will adapt successfully to conditions in vivo.



1. E  
WPM, 0,5 mg/l-1<sup>-1</sup>BAP; 1. ;  
1. ; 2  
2  
; 3.  
; 3a.  
; 4.  
WPM,  
0,25 mg/l-1<sup>-1</sup>BAP; 5.  
MS, 0,25 mg/l<sup>-1</sup>  
1<sup>-1</sup>BAP; 6.  
;



WPM, . 7. WPM,  
; 8. , 2 g/l<sup>-1</sup>  
WPM

**Fig. 1. Explant of pedicel callus on WPM medium supplemented with 0.5 mg/l<sup>-1</sup> BAP. 1. Explant of callus from royal pelargonium; 1a. Regeneration on callus of pedicel of royal pelargonium; 2. Explant of zonal pelargonium; 2a. Regeneration on explant of petiole on the same medium; 3. Petiole from royal pelargonium not forming callus; 3a. Direct regeneration without formation of callus on petiole of royal pelargonium; 4. Micro plants of royal pelargonium in the stage of multiplication on WPM medium, supplemented with 0.25 mg/l<sup>-1</sup> BAP; 5. Micro plants of zonal pelargonium in multiplication stage on a MS medium supplemented with 0.25 mg/l<sup>-1</sup> BAP; 6. Micro plants royal pelargonium set for rooting on WPM liquid medium solidified with perlite; 7. Micro plants of zonal and royal pelargonium rooted on liquid medium WPM solidified with perlite;. 8. roots of zonal pelargonium on WPM nutrient media without plant growth regulators supplemented with 2 g/l<sup>-1</sup> activated carbon.**

### CONCLUSIONS

- |   |   |
|---|---|
| <p>1. % ,</p> <p>2. - ,</p> <p>3. ,</p> <p>4. -</p> | <p>1. In both studied pelargonium species a significant difference between the different genotypes in the % sterility was established, which is probably due to the presence of endogenous bacterial contamination.</p> <p>2. The highest rate of sterility was found for the pedicels and plants were successfully regenerated from pedicels for both species.</p> <p>3. Satisfactory sterility was established for apical buds which grow easily in in-vitro conditions.</p> <p>4. For callus tissue from pedicels, for both species, the most suitable nutrient medium is MS with vitamins supplemented with 30 g/l sucrose, 0.1 mg/l-1 2,4-D and 0.25 mg/l-1 BAP, solidified with 7 g/l agar. The highest</p> |
|---|---|

5. WPM, 30 g/l<sup>-1</sup> sucrose, 0,25 mg/l<sup>-1</sup> BAP, 7 g/l<sup>-1</sup> agar, MS, 30 g/l sucrose, 0,5 mg/l<sup>-1</sup> BAP, 7 g/l<sup>-1</sup> agar.

6. WPM, 0,25 mg/l<sup>-1</sup> BAP, MS, 0,25 mg/l<sup>-1</sup> BAP.

7. WPM.

8.

percentage of explants forming callus and largest average volume of callus of explant were reached on this medium.

5. Regeneration of callus from pedicels from royal pelargonium was most effective on WPM nutrient medium with vitamins, enriched with 30 g/l sucrose and 0.25 mg/l-1 BAP, solidified with 7 g/l agar. For zonal pelargonium the best variant was MS medium with vitamins, enriched with 30 g/l sucrose and 0.5 mg/l-1 BAP, solidified with 7 g/l agar. Zonal pelargonium showed lower capacity for regeneration compared to the royal.

6. The most suitable medium for multiplication of explants of royal pelargonium is WPM supplemented with 0.25 mg/l-1 BAP, while best results for zonal pelargonium were obtained on MS supplemented with 0.25 mg/l-1 BAP.

7. Very high percentages of rooting were observed for both species, and as the two best results are recorded on the WPM basal medium.

Both showed very good results on liquid nutrient media solidified with perlite. Very good rooting was reported as well on media containing activated charcoal.

8. The results show that the cost of a plant propagated in

vitro from each of the two species has a value comparable to that of other ornamental species that are mainly propagated through this method.

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## YIELDS OF GREEN BEANS IN ORGANIC FARMING

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### SUMMARY

2011-2013 .  
 “ ”-  
 2  
 - 20 l/da.  
 2  
 60 80 cm  
 10 cm.  
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 - ,  
 - ,  
 - .  
 -  
 kg/da 1705,3 2057,4  
 .  
 ,

During the period 2011-2013, at the Vegetable Crops Research Institute “Maritza”-Plovdiv, field trials with 2 Bulgarian varieties Tangra and Nikos and the hybrid Dutch variety Lodi, fertilized with the liquid organic fertilizer Emosan – 20l/da were carried out. The seeds were sowed after a predecessor - spinach. They were tried two schemes of sowing with a width between the rows 60 and 80 cm and distance between plants in rows 10 cm. The plants were grown in conformity with the principles of the organic production.

It was found that the level of yields of green beans in organic production largely depends on the varietal characteristics and the technological element - width between the rows has less impact. The received data are statistically proven.

Variety Lodi has the highest yields in both schemes of sowing from 1705.3 till 2057.4kg/da. It is appropriate for receiving of organic production and recommended for growing in the organic farms. Variety Tangra has comparatively lower productivity but it can be used for organic production too while Nikos variety is not recommended for organic farming.

**Key words:** organic farming, green beans, fertilizing, schemes of growing, yields

## INTRODUCTION

Beans are preferred predecessor for most agricultural crops, because in developing plant roots tuberos (Nitrogen-Fixing) bacteria of the genus *Rhizobium* enrich the soil with nitrogen.

The production of organic N from the bean crops is the greatest benefit from their usage as cover crops and the decay of the ploughed for green fertilization vegetative mass (leaves and stems) they release nutrients in quantities that are equal to fertilizing with 3 t/da natural fertilizer (Mnasri et al., 2007; Nakhone and Tabatabai, 2008.). That makes the green beans particularly valuable as an element of science-based crop rotations in integrated and organic production and technologies for conservation of soil fertility.

Worldwide they are work out many experiments connected with the organic agriculture. The green beans participate in the most experimental schemes included in crop rotation or independently (Venturini et al., 2005).

The yields in organic farming of green beans are lower but the

*Rhizobium*

N

3 t/da

(Mnasri et al., 2007; Nakhone and Tabatabai, 2008).

(Venturini et al.,

2005).

,  
 (Abubaker et al., 2007).  
 (Karungi et al. 2006).  
 .  
 ,  
 ( , 2003)  
 ,  
 .  
 .

- quality of the output is higher (Abubaker et al., 2007).

-

This crop is very appropriate for growing in organic vegetable farms (Karungi et al. 2006). The interest towards the organic production in our country continuously increases.

Increased demand for organic production in the EU, where the market is unlimited (Yordanova, 2003) imposes the extension of the production and expansion of the range of vegetable crops grown in accordance with the standards of the biological method in Bulgaria rapidly.

The aim of the research is determination of the productiveness of the green beans varieties in growing of the conditions of organic agriculture in the region of Trakiya Lowlands.

### **MATERIAL AND METHODS**

2011-2013 .  
 ” “-  
 ,  
 -  
 10.05.-17.06.,  
 ( ) .

-

The field trials were conducted in the period 2011-2013 in the field of Vegetable Crops Research Institute “Maritza”- Plovdiv, on highly leached meadow cinnamon soil after its conversion period. The sowing was made in the period 10.05 – 17.06., after predecessor spinach (autumn sowing). The spinach was grown in accordance with the principals of the organic production, too. Two Bulgarian varieties beans were





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 ;  
 ;  
 .  
 (N) – 5%;  
 (N) – 5 %;  
 (C)  
 – 14 %; – 34  
 / ; – 65 / ; – 0,4  
 / ; – 0,06 / ; –  
 7,0-10,0(Farpromodena, ).

forms, it does not soil the  
 groundwater, it neutralizes the  
 alkaline ground, it creates  
 conditions for the growth of the  
 useful microflora. Ingredients: Total  
 Nitrogen (N) – 5%, Organic  
 Nitrogen (N) – 5%, Organic Carbon  
 (C) with biological origin – 14%,  
 Protein – 34 p/p, Humidity – 65  
 p/p; K – 0,4 p/p; P – 0,06 p/p and  
 others; pH – 7,0-10,0  
 (Farpromodena, Italy).

## RESULTS AND DISCUSSION

1).  
 , 2011 .  
 2-3 -  
 2-3 -  
 -  
 .  
 2012 .  
 ,  
 .  
 ,  
 ( 19.05.)  
 .

As to the terms of the  
 occurrence of the single  
 phenophases during the three  
 years of experience were not  
 registered significant differences  
 between studied varieties (Table  
 1). From phenological information  
 it reveals that in 2011, the  
 beginning of germination is  
 registered with 2-3 days earlier in  
 Lodi. Phenophase mass fruit set  
 was observed by 2-3 days earlier  
 also in Lodi. Also the technological  
 maturity phase is registered earlier  
 in Lodi compared with variety  
 Tangra. The speed of Nikos variety  
 for phenological development is  
 almost at the same level compared  
 with the variety Tangra.

Several days after the sowing  
 in 2012 fell torrential rain and it  
 formed a thick soil cover, which  
 hampered the germination of the  
 plants. The density of the plants in  
 rows was not good enough and  
 became necessary (on 19.05.), a  
 part of the attempt with Lodi to be  
 resowed again. Therefore,

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		-	
	2013	.	
	3	-	
			.

regarding the terms of starting of individual phenophases from Table 1 it reveals that the earliest mass blossoming is registered with Nikos variety. With three days later this phase occurred in Tangra variety. The relevant phase was registered at the latest due it Lodi variety because of the additional drilling.

During the third year, due to late sowing period the phenological phases were recorded later in comparison with previous years. It is noteworthy that the technological maturity occurs earlier in Nikos variety and two days later in Lodi variety. In the Tangra variety the growth of pods proceeds more slowly and this phase in 2013 was reported 3 days later compared with the other varieties.

1.

**Table 1. Phenological observations in green beans**

Sowing:	Date of advent of phenophases								
	Lodi			Tangra			Nikos		
	2011	2012	2013	2011	2012	2013	2011	2012	2013
10.05.2011									
06.05. 2012									
17.06. 2013									
Starting of germination	18.05	14.05	21.06	20.05	13.05	20.06	22.05.	14.05	20.06
Mass germination	24.05	26.05	23.06	24.05	16.05	22.06	30.05	16.05	22.06
Mass blossoming	06.06	17.06	30.07	04.06	12.06	28.07	08.06	09.06	28.07
Mass knitting	27.06	24.06	07.08	29.06	19.06.	06.08	30.06	16.06	04.08
Technical ripeness	10.07	04.07	19.08	13.07	18.07	21.08	12.07	02.07	18.08

( 2) ,

.

253,3 kg/da.

166,7%.

- Following the information for the received yields (Table 2) it is noticed that the lowest average yield is for Nikos variety. In control trial which is organic production too, the average value of the yield is only 253.3kg/da. As a result of the fertilization with Emosan the yield of Nikos variety rose by 166.7%.

2. (2011-2013)

Table 2. Average Yield of Green Beans (2011-2013)

Variant	Standard yield kg/da	Proven Reliability	Relative yield % towards the control trial
%			
/ Lodi Variety			
1. ( ) Not Fertilized (control trial)	469,1		100
2. - 80 cm Emosan – 80 cm	1705,3	+++	363,5
3. - 60 cm Emosan – 60 cm	2057,4	+++	438,6
GD 5%	371,54		
GD 1%	616,21		
GD 0.1%	1152,37		
/ Tangra Variety			
1. ( ) Not Fertilized (control trial)	336,8		100
2. - 80 cm Emosan – 80 cm	1197,6	+	327,1
3. - 60 cm Emosan – 60 cm	1548,5	++	459,8
GD 5%	556,45		
GD 1%	922,87		
GD 0.1%	1725,88		
/ Nikos Variety			
1. ( ) Not Fertilized (control trial)	253,3		100
2. - 80 cm Emosan – 80 cm	675,5	++	266,7
3. - 60 cm Emosan – 60 cm	893,5	+++	352,7
GD 5%	153,88		
GD 1%	255,21		
GD 0.1%	477,26		

-

83,5 kg/da.

-

- 215,8

kg/da.

,

166,7

263,5%.

,

-

,

,

-

(60 cm).

,

1705,3      2057,4 kg/da

,

1197,6

1548,5 kg/da.

,

,

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-

Significantly higher average yield without fertilization was received from the control trial in Tangra variety. The difference is 83.5kg/da. However the biggest difference is in Lodi variety - 215.8 kg/da.

The results show that the average yields towards the control trials are increased from 166.7 till 263.5% after fertilization with Emosan for the three of the varieties.

Comparing the efficient abilities of the studied varieties it is seen that Lodi variety has the highest average yields, irrespective of the pattern of planting. It makes an impression that better results are received in growing of plants with smaller width between the rows (60cm). This trend is typical for all three varieties. The received average organic yields after the usage of Emosan according to the width between the rows is in the range from 1705.3 to 2057.4 kg/da for Lodi variety. For Tangra variety the values are significantly lower and vary from 1197.6 to 1548.5 kg/da. Nikos variety has the lowest productivity in comparison with the other two varieties which shows that it is not appropriate for organic production.

The green beans from the studied varieties are at high yields for the variants with smaller width

(80 cm)

- between the rows. The larger width (80 cm) between the rows, however, is suitable for organic farms due to the fact that it is possible to use small size processing technique of crops against weeds.

The obtained differences in the yields of biological production between the variants due to different food area defined by the width between the rows in the varieties Lodi and Tangra are statistically unproved. In Nikos variety however the resulting difference is reliable at the level of 5%.

5%.

In conclusion we will specify that in our studies for biological production of green been with greater force of impact on the rate of yield is the variety (Table 3).

( 3).

### 3.

**Table 3. Variation of the green beans yields under the influence of the scheme of growing and fertilization**

	SS	df	MS	F	P-value	F crit
Variety	3615460	2	1807730	38,413	*** 78,22	3,885
Scheme	424151,2	1	424151,2	9,013	* 9,18	4,747
Casual	17827,72 564719,4	2 12	8913,861 47059,95	0,189	0,39 12,22	3,885
Total	4622159	17				

## CONCLUSIONS

It was found that on average yields of green beans in organic farming, a decisive influence has the choice of an appropriate variety, and the effect of the tested schemes of sowing is definitely less pronounced. The results are statistically proven.

With the highest productivity in both schemes of growing is variety Lodi. In organic cultivation, the average yields of green beans in Lodi are from 1705,3 to 2057,4 kg/da. It is suitable for organic production and it is also recommended for growing in organic farms.

Variety Tangra has relatively a lower productivity, but it also can be used for the preparation of organic products, however, the variety Nikos is not suitable for such production.

1705,3      2057,4 kg/da.

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