SHOOT CULTURE OF *IN VITRO* MICROTUBERISATION OF POTATO (*Solanum tuberosum* L.)

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Abstract: This paper presents results of shoot culture of *in vitro* microtuberisation of potato. Were researched induction of callus and roots of shoot explants on seed varieties of potatoes like: *Dido and Marabel* and mercantile varieties of potatoes like: *Agria BE and Agria SR.*

During this research the initial explants – sprouts, further are transfer (passeges) to new nutrirent medium, fresh, with new hormonal composition. These explants transferred to the new fresh medium are called shoots.

Key words: explants, shoots, potato, in vitro, medium

Introduction

Potato is the fourth important crop in the world after wheat, rice and maize. Potatoes are thought to have originated from high - mountain ranges of the Andes in South America. This crop is grown in 180 countries worldwide. According to the FAO statistic (https://faostat.fao.org), the largest producer of potatoes is Asia, then Europe, South America and North and Central America.

The very early beginning of potato cultivation in Macedonia is dating back 150-170 years ago. Today in the country, potatoes are grown on more than 13,000 hectares with an average yield of 20-40 t/ha, and every year the area of potato cultivation is extended (Statistical Yearbook of Republic of Macedonia, 2014).

According to the research Nistor et al. (2010), the genotype of potato under *in vitro* conditions have a positive impact of obtaining microtubers. In their research they perceived that, first needs to have a healthy material for obtaining microtuberisation in the laboratory, then with healthy material will planted on medium and will get also healthy reclaimed microtubers.

For obtaining microtubers influenced by many factors: external factors (temperature, light, heat), type of medium, the contents of the medium, genotype, explants used for tuberization, their physiological maturity and the effect of regulators of growth (Dobranszki, 2008).

Microtuberisation of potato (*Solanum tuberosum* L.) is a complex evolutionary process that is influenced by photoperiod (Seabrook et al., 1993), temperature (Leclerc et al., 1994), sources of carbohydrates (Simko, 1994), inorganic nutrition (Sarkar and Naik, 1998) and even the physiological age of the parent tuber (Villafranca et al., 1998).

These factors directly or indirectly influence the formation of *in vitro* microtubers by regulating the effects of the application of exogenous growth substances or by endogenous changes in the hormonal balance (Ewing and Struik, 1992).

In vitro cultures are produced that can be used for rapid multiplication (*in vitro*), microtuberisation *(in vitro*), as well as producing minitubers (in glasshouses, greenhouses) (Struik and Lommen, 1990).

Material and methods

The experiment was conducted in the Laboratory of Plant Biotechnology, Faculty of Agriculture, Goce Delcev University – Stip, Macedonia. The following potato varieties were used as starting material for the experiment:

- seed potatoes: Dido, Marabel, Agria, Ambition and Agriko;
- mercantile potatoes: Agria SR, Agria BE and Andrea.

The variety Agria SR is cultivated in Strumica region, while the variety Agria BE is cultivated in Berovo region. The two regions differ in altitude, soil types and climate, thus the commercial potatoes of the same variety were treated as different starting material.

Shoot culture

During the research initial explants – sprouts further transfered (passaged) to new nutritional medium, i.e. the fresh new medium with new hormonal composition. These explants passaged on fresh medium are called shoot culture. First shoots passaged was made on a new medium enriched with cytokinins and auxin.

The combination of cytokinin and auxin was very effective for organogenesis under *in vitro* conditions in different potato genotypes (Gudeva Koleva et al., 2012).

Data analysis

All data were subjected to statistical analysis with IBM SPSS Statistical 21, one-way ANOVA and Duncan *posthoc* test, with the level of significance 0.05%.

Results and discussion

In recent time are used many synthetic auxin and cytokinins for obtaining undifferentiated callus tissue, which is widely used in cultures *in vitro* and in cultures that breed generative (Grbic et al., 2007; Grbic, 2007).

From Table 1 can be seen that the genotype dido (30 mm) and agria SR (27,65 mm) have a length of sprout that is significantlly greater than the length of the sprout genotype marabel (18,68 mm).

A while the thickness of the sprouts, genotypes that were tested showed no significant difference in the values obtained.

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In Table 1 shows the results of the formation of callus in mercantile potato genotypes: agria, agria BE and agria SR and seed potato: dido and marabel.

Shoot explants					Formation of callus			
Genotype	MS medium (mg/L)	Number of shoots	Lentgh of shoot (mm)	Thickness of shoots (mm)	Height of callus (mm)	Thickness of callus (mm)	Number of callus	% of callusing
Seed potato								
Dido	2 BAP + 1 IAA	17	30,00a	1,00a	1,45a	1,38a	18	72,61ab
Marabel	2 BAP + 1 IAA	16	18,68b	1,18a	0,94b	1,43a	16	93,75a
Mercantile potato								
agria BE	2 BAP + 1 IAA	44	22,31ab	1,0a	1,18ab	1,25a	22	50,44b
agria SR	2 BAP + 1 IAA	20	27,65a	1,01a	0,71b	0,59b	11	80,00ab

Table 1. Formation of callus –shoot explants

Genotype agria BE (22) formed the most calluses, apart other genotypes: agria SR (11), marabel (16) and dido (18).

The highest callus from 1,45 mm had genotype dido, which values significantly different from the amount of callus at genotype marabel (0,94 mm) and agria SR (0,71 mm).

Genotype agria SR (0,59 mm) in thickness of the callus showed statistical minimum value to the values of other genotypes: agria BE (1,25 mm), marabel (1,43 mm) and dido (1,38 mm).

During induction of the callus medium MS + 2 mg / L BAP + 1 mg / L IAA at all potato genotypes showed callusogenesis.The biggest values showed genotype marabel (93,75%) value which is significantly better than genotype agria SR (80,00%), and genotype dido (72,61%) and genotype agria BE (50,44%).

In vitro regenerants obtained by micropropagation of potato are less transmissible of bacteria, fungi and viruses. *In vitro* regeneration of potatoes proved one of the most widely used technique in several countries worldwide. This method has great advantages in the creation of new genotypes unlike conventional breeding. According to this survey import of potatoes with much positive results decreased by 50% (Karim, 2009).

To get well entrenched, callusogenesis and the formation of shoots should investigate which hormone, growth regulator would be best for these parameters (Karim, 2009).

The results of the percentage of rooting in shoot culture are shown in Table 2.

At genotypes marabel and agria SR were formed lowest number of roots (2 per shoot) apart genotype dido which have 8 roots and genotype agria BE had 14 roots per shoot.

With the longest roots featured shoots of genotypes marabel and dido (15,00 mm) that was significantly with highest value than 3,50 mm at genotype agria SR.

In percentage of rooting no significant differences in the values of all tested genotypes: marabel (31,25%), agria BE (30,03%), agria SR (29,58%) and dido (26,90%).

Shoot explants					Formation of roots			
Variety	MS medium (mg/L)	Number of shoots	Lentgh of shoot (mm)	Thickness of shoots (mm)	Number of shoots which formated roots	Number of roots	Lentgh of root (mm)	% of rooting
Seed potato								
Dido	2 BAP + 1 IAA	17	30,00a	1,00a	4,50	8	15,00a	26,90a
Marabel	2 BAP + 1 IAA	16	18,68b	1,18a	5,00	2	15,00a	31,25a
Mercantile potato								
agria BE	2 BAP + 1 IAA	44	22,31ab	1,10a	13,21	14	8,00ab	30,03a
agria SR	2 BAP + 1 IAA	20	27,65a	1,01a	5,91	2	3,50b	29,58a

Table 2.Formation of roots – shoot explants

Callusogenesis and rhyzogenesis in shoot culture

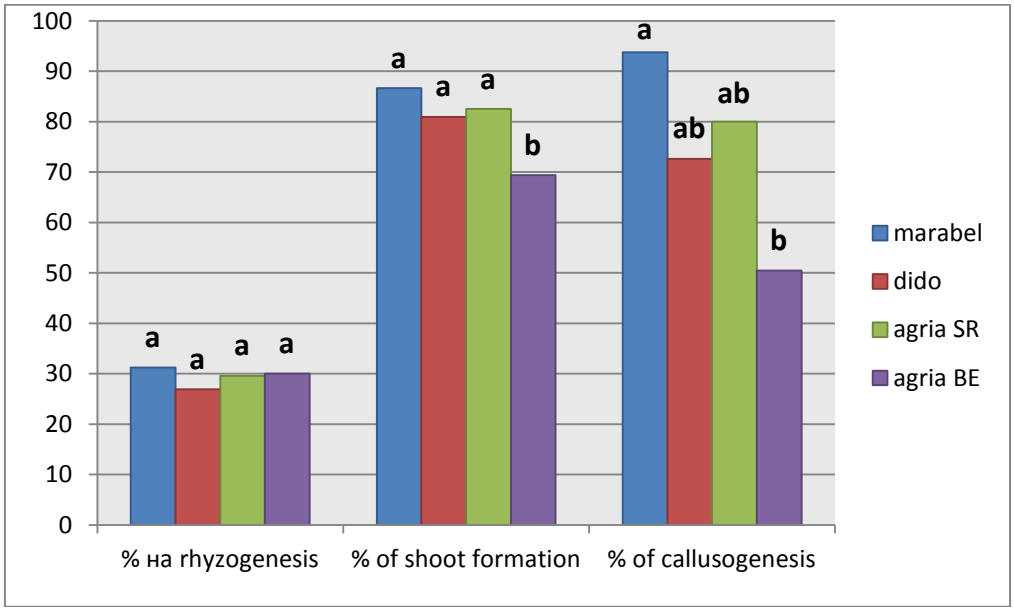
During the research performed to examine the impact of hormonal medium MS + 2 mg / L BAP + 1 mg / L IAA of shoots culture of potato till the process of callusogenesis. The impact is determined by the percentage of callused explants.

The impact of the above medium and hormonal composition of the process of rhyzogenesis is determined with the percentage of rooted explants.

Also was examined the percentage of shoot formation of genotypes: marabel, dido, agria SR and agria BE, which were transfered on the same medium MS + 2 mg / L BAP + 1 mg / L IAA.

From the results (Graph 1) are obvious:

- No significant differences in values at the percentage of rooting. The percentage of rooting varies from 26.90 to 31.25%;
- At the precentage of shoots formation have significant differences between genotypes. Genotypes marabel (86,66%), dido (80,95%) and agria SR (82,50%) showed values significantly different from genotype agria BE (69.41%) of formed shoots;
- In proportion to the formation of calluses we have significant differences in values among genotypes. The least percentage of callusogenesis (50.44%) features agria BE genotype is significantly lower value of 93.75% of formed calluses at genotype marabel (Figure 1).



Graph 1. Callusogenesis, rhyzogenesis and shoots formation of shoots culture on MS + 2 mg/L BAP + 1 mg/L IAA

Figure 1. a) Formation of shoots, roots and callus from mercantile genotype *agria BE* on medium MS + 2 mg/L BAP + 1 mg/L IAA, b) Formation of shoots, roots and callus from seed genotype *marabel* on medium MS + 2 mg/L BAP + 1 mg/L IAA

Conclusion

The best shown medium MS + 2 mg / L BAP + 1 mg / L NAA, which genotype dido, marabel, agria SR and agria BE showed good induction of callus and roots. On this medium those 4 genotypes are shown the best results of making callus, roots and also shoot culture. Were presented % of rhysogenesis, % of shoot culture and % of callusogenesis.

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