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Morphogenesis Introduced Varietes of Vaccinium Vitis-Idaea L. in Culture in Vitro

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Abstract
The results of experimental studies of morphogenesis of four introduced varietes cowberry ('Koralle', 'Masovia', 'Erntedank', 'Erntekröne') in culture *in vitro* on three types of nutrient media of nine modifications. It was found, that of all the investigated types of nutrient media among the most active shoot formation and rhizogenesis was observed on the medium WPM (№ 8) and Anderson (№ 9). This demonstrates a proof of universality of these types of media for both morphogenetic processes: shoot formation and rhizogenesis. On the basis of study of morphogenesis of explants proceeding from the introduced varietes Vaccinium vitis-idaea in aseptic culture on various types of nutrient media was shown a principal possibility to regenerate it in two ways: 1) by activation of axillary meristems , 2) through the callus proliferation and subsequent formation of a shoot. Research results obtained in the study of morphogenesis in four different types of explants of introduced varietes of cowberry on modified nutrient media, used by us in the elaboration of technology of micropropagation of these varietes.

Keywords: morphogenesis, introduced varietes of cowberry, aseptic culture

INTRODUCTION

An extensive literature was devoted to question of morphogenesis in cell and tissue culture. Its analysis allows to conclude that the morphogenesis is a complex and multifactorial process, depending on the type and physiological state of the explant, the composition of nutrient medium, components, contained in it (macro-and microelements, vitamins, carbohydrates, hormonal edditions) and a number of other factors. It can be confirmed by numerous experimental studies.

According to research of Shor and Papazyan (1989), obtained in the study of morphogenesis in isolated tissue culture of rose tissues on five nutrient media, which differ by concentration of macrosalts and combination of hormonal additions, implementation of morphogenesis was in development of shoots from axillary buds and callus formation on slices of the stem and leave petiole . The most intensive development of shoots was observed on Murashige-Skoog medium of full mineral composition with the addition of 1 mg/l NAA.

From the publication of Vilor et al. (1987) follows, that morphogenetic processes occurring in sunflower in culture in vitro, are dependent on the type of nutrient medium and the explant. Authors found that the best of all callus was formed on Erickson medium and Murashige - Skoog medium from apical meristem of the stem, and on the White medium from the leaves. Formation of shoots with roots authors observed only from the apical meristem .

Experimental studies, conducted by Budagovskava et al. (1990), indicate about the role of auxin and cytokinin in the regulation of morphogenesis.

In the capacity of explants we used leaves and tops of the young shoots of cereals, grown under aseptic conditions, as well as the leaves of adult plants cultivated in the field. The authors conclude that better callus formed on explants, taken from adult plants grown in the field, when the content in the medium 1 mg/l benzyladenine and 1.2 mg/l naphthalene acetic acid (NAA) . Shoot formation is marked on Murashige-Skoog medium, containing 2 mg/l benzyladenine.

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Guta and Chandra (1985) studied the effect of different growth regulators, benzylaminopurine (BAP), indole acetic acid (IAA), gibberellic acid (GA) on morphogenesis of various types of tobacco explants: leaf pieces without midrib, isolated from 2-4 of upper leaves; internodes segments, isolated from the second upper internodes; epidermal strips of tissue with several adjacent layers of cells isolated from the young internodes. The experimental data allowed the authors to conclude that GA concentration of 0,5 mg/l stimulated the formation of buds only on explants of pieces of the leave; IAA and kinetin promoted the formation of vegetative buds on the explants of stem, and kinetin − on explants of leave.

Thorough study of morphogenesis with the aim of elaboration of technology of introduced varietes cowberry was conducted by us for its four sorts ('Koralle', 'Masovia', 'Erntedank', 'Erntekröne') on three types of nutrient media of various modifications.

MATERIALS AND METHODS

As objects of study we used different types of explants of numerated varietes. Explants were epicotyl, hypocotyl, cotyledon, root, leaves of juvenile seedlings obtained previously under aseptic conditions on a modified medium Anderson and buds of young shoots of adult parent plant.

Buds with the stem pieces of 3-4 mm length were sterilized in a 0,1 % solution of the diacid over 10 min, previously dipped in a 70^0-degree ethanol, followed by a wash in three changes of sterile double distilled water (15 min in each).

Sterile material (buds, epicotyl, hypocotyl, cotyledon, leaves, roots) seeded into flasks of equal volume (15 ml medium in each) on three nutrient media: Murashige - Skoog medium (1962), WPM (Lloyd G, McCown) (1981), and Anderson (1975). Each medium has several variants, differing concentrations of macro-and microsalt, combination of hormonal supplements and other components (Table 1).

The level of pH of the medium was to 4,8 before autoclaving at 1,06 kg/cm^2 pressure for 20 min at 121^0 C.

Plant explants were cultivated at a temperature 26^0 C, relative humidity 56 %, 16 h photoperiod, illumination 4000 lux.
Experimental data are statistically processed and presented in the Table 2. The figures in the Table 2 are arithmetic means with their standard errors.

RESULTS, DISCUSSION AND CONCLUSION

After 5 weeks from buds developed vegetative shoots of all varietes of cowberry. After their transplantation to fresh medium we observed proliferation of new shoots of the third to fourth orders. During four weeks of cultivation one micrograft formed an average of 5 to 10 microshoots depending on the composition of the nutrient medium (Table 2). Of all the investigated types of media the most active shoot formation was observed on the medium WPM (№ 8) and Anderson (№ 9) (Table 2) containing the full composition of the macro-and microsalt with the following additions (mg /l): mezoinozitol − 100, adenine sulfate − 80, thiamine − 0.4, indoleacetic acid, −4, izopenteniladenin−15, sucrose −30g /l agar − 6g /l, pH of medium 4.0 (Table 1).
This fact indicates that by varying the amount and ratio of the components in the nutrient medium, one can achieve a high level of morphogenesis. In this instance, was able to activate the development of axillary meristems by removing apical dominance and to get regenerants.

After 4-5 passages almost all micrografts, planted for shoot formation, we observed rhizogenesis on medium (№ 8) and N 9, that was not mentioned on media of other modifications. This gives a proof about universality of these types of media for both morphogenetic processes of shoot formation and rhizogenesis.

Formation roots of regenerants of introduced varietes cowberry on medium for shoot formation does not exclude the supposition that they contain enough endogenous auxin, capable of causing of rhizogenesis.

The remaining explants (epicotyl, hypocotyl, cotyledon, root, leaves) after 5-6 weeks of cultivation formed organogenic callus with subsequent regeneration of vegetative shoots from him.

Table 1. Composition of modified culture media, used for the study of morphogenesis of introduced varietes of *Vaccinium vitis-idaea*

Components of nutrient media, mg / l	Medium modification number								
	1	2	3	4	5	6	7	8	9
Macrosalts on MS	+	–	1/2	–	–	1/2	1/2	–	–
Microsalts on MS	+	–	1/2	–	–	1/2	1/2	–	–
Macrosalts on WPM	–	+	–	+	–	–	–	+	–
Microsalts on WPM	–	+	–	+	–	–	–	+	–
Macrosalts by Anderson	–	–	–	–	+	–	–	–	+
Microsalts by Anderson	–	–	–	–	+	–	–	–	+
Mezoinositol	100	100	100	100	100	100	100	100	100
Adenine sulfate	–	80	80	80	80	40	60	80	80
Thiamine (B_1)	0,4	–	–	0,4	–	0,1	0,1	0,4	0,4
Pyridoxine (B_6)	–	–	–	–	0,4	–	–	–	–
Indoleacetic acid (IAA)	1,0	5,0	–	2,0	1,0	1,5	2,5	4,0	4,0
Benzylaminopurine (BAP)	–	–	–	–	–	2,0	–	–	–
Gibberellic acid (GA)	–	4,0	–	–	–	–	–	–	–
Izopenteniladenin (2-ip)	10	10	2,0	5,0	4,0	–	10	15	15
Saccharose, g / l	20	20	20	30	30	20	20	30	30
Agar, g / l	6	6	6	6	6	6	6	6	6
pH	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0

Note: Sign (+) component presents in the medium; sign (–) component is absent in the medium; ½ half of component dose in the medium

Table 2. Morphogenesis of *Vaccinium vitis-idaea*, depending on the composition of the nutrient medium.

Number of modifications of medium	Quantity of shoots per explant of varietes				Note
	'Koralle'	'Masovia'	'Erntedank'	'Erntekröne'	
1	8,5 ± 1,2	7,9 ± 2,0	8,0 ± 1,0	7,6 ± 1,5	
2	7,5 ± 1,5	7,0 ± 2,0	7,8 ± 1,4	7,4 ± 1,3	
3	2,0 ± 1,0	2,5 ± 1,5	2,9 ± 0,0	2,4 ± 0,0	
4	3,3 ± 1,5	5,0 ± 1,0	4,5 ± 1,2	5,0 ± 2,0	Shoots with elongated internodes
5	5,5 ± 1,0	5,0 ± 1,2	5,4 ± 2,0	4,1 ± 1,1	Shoots with large leaves
6	1,0 ± 1,0	0,9 ± 0,2	1,1 ± 0,5	1,7 ± 1,2	
7	1,5 ± 1,9	1,8 ± 1,3	1,0 ± 0,0	1,9 ± 1,0	
8	15 ± 2,0	14 ± 1,3	15,2 ± 2,7	41,7 ± 1,9	
9	16 ± 2,5	15 ± 3,2	16,3 ± 2,3	15,5 ± 2,7	

It should be noted that the formation of organogenic callus and further regeneration of shoots are characteristic for explants (root, epicotyl, hypocotyls, cotyledons, leaves) obtained from freshly collected seeds, and in explants from stratified seeds of shoot formation occurred by passing the stage of forming a callus that is directly from tissue explant.

We can assume that this is connected with the different physiological, biochemical, cytological , and other processes in explants from freshly collected and stratified seeds, as well as with different content of endogenous phytohormones. Probably all together formed the basis for shoot regeneration from callus without preliminary his passage to nutrient medium

of other composition. In other words, the induction of callus formation and then shoot formation occurred on the medium of the same composition.

All explants had a high morphogenetic potential on two media: WPM and Anderson of three modifications (№ 4 , № 5 , № 9). In this case in the base of morphogenesis lies the ability of cell explants to be differentiate, in other words, lose their previous specialization and transformate into callus cells. Transformation of specialized cells into callus cells is associated with the induction of cell division, the ability to which cells lost during differentiation (Butenko, 1975).

According to the theory Skoog and Miller, morphogenesis process starts from the transition of cell to initiation of organized development and is the result of changes in the balance between plant hormones. They found that an excess of content auxin over cytokinin in the medium causes induction of roots; inverse relationship that is excess cytokinin over auxin leads to the formation of buds and stem shoots (Skoog and Miller, 1957).

It can be assumed that the differences of the content of endogenous phytohormones in cells and tissues determine different character of their behavior in an isolated culture and different needs in the components of the medium.

Callus cells (except auxin- and cytokinindepended tumor cells) can not themselves synthesize plant hormones in sufficient quantities, required for the induction of morphogenesis, therefore require exogenous growth regulators.
Callus cells only at a certain ratio of cytokinins and auxins in the medium can go to the organized growth and the formation of shoots. This ratio for each plant variety was set experimentally.

It can be confirmed by numerous studies concerning the regulation of morphogenesis in cell and tissue culture with a help of certain ratio of auxin and cytokinin in the nutrient medium.

Our studies have shown that for the formation of regenerants Vaccinium vitis-idaea of callus tissue in nutrient medium it is necessary to add auxin and cytokinin in the following ratios : 2.5:1 (Medium number 4) , 4:1 (Medium number 5), 3.75:1 (Medium number 9).

Thus, by studying the morphogenesis, proceeding from Vaccinium vitis-idaea explants in cell and tissue culture on various types of nutrient media (9 series), was shown principal possibility to regenerate it in two ways: 1) by activating axille meristems 2) via callus proliferation and the subsequent formation of his shoots.

In conclusion, it should be noted that the research results obtained in the study of morphogenesis in different types of explants of introduced varieties of cowberry on modified nutrient media used by us in the development of technology micropropagation of these sorts.

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