MORPHOGENESIS OF SELECTIVE HYBRIDS (FAM. VACCINIACEAE S.F.GRAY) IN CULTURE *IN VITRO*

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ABSTRACT

It was studied morphogenesis of selective hybrids in different modifications of nutrient media, and determined the optimal composition of nutrient medium for the occurrence of this process. Best for morphogenesis of studied hybrids were media of 8th and 9th modifications, containing in its composition macro and microelements on WPM and Andersen, as well as hormonal supplements: 4 mg / l indolyl acetic acid and 15 mg / l isopentenyladenine.
It was shown a principal possibility of regeneration of selective hybrids in two ways: 1) through the activation of axillary meristems, 2) through the proliferation of callus and subsequent formation of shoots from it.

Key words: morphogenesis, selective hybrids, aseptic culture.

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INTRODUCTION

An extensive literature was devoted to the question of morphogenesis in culture of cells and tissues. Its analysis allows us to conclude that the morphogenesis is a complex and multifactorial process, depending on the type and physiological state of explants composition of nutrient medium, that is, components, which are contained in it (macro- and microsalts,

vitamins, carbohydrates, hormonal supplements), and from pH of the medium, cultivation conditions and many other factors. Proof of this can serve numerous experimental studies.

According to the results of research of Shore and Papazian (1989), obtained in the study of morphogenesis in culture of isolated roses tissues in five media with different concentrations of macrosalts and combinations of hormonal supplements, realization of morphogenesis was in the development of shoots from axillary buds and the formation of callus on sections of the stem and 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.

According to 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. They found that the best callus was formed on media Erickson and Murashige - Skoog medium from apical meristem of the stem, and on the medium White – from leaf. The authors observed a formation of shoots with roots only from the apical meristem.

The experimental studies of Budagovskaya et al. (1990) testify about the role of auxin and cytokinin in the regulation of morphogenesis. As used explants of the young leaves and tops of young shoots of cereals, grown in aseptic conditions and also leaves of mature plants, cultivated under field conditions. The authors conclude that the calluses are formed on explants, taken from adult plants, grown in the field, when the content of 1 mg / l benzyladenine and 1.2 mg / L naphthyl acetic acid (NAA) in the medium. Shoot formation was observed on Murashige-Skoog medium, containing 2 mg / l benzyladenine.

Guta and Chandra (1985) studied the effect of different growth regulators benzylaminopurine (BAP), indolyl acetic acid (IAA), gibberellic acid (GA) on the morphogenesis of different types of tobacco explants: leaf pieces without central vein, isolated from 2-4 upper leaves; internode segments, isolated from the second upper internodes; strips of epidermal tissue with several adjacent layers of cells, isolated from young internodes. Experimental data of the authors have allowed to conclude that gibberellic acid (GA) in a concentration of 0.5 mg / l stimulated formation of buds only on the explants of leaf pieces; kinetin and NAA promoted the formation of vegetative buds on the stem explants, and kinetin - on the explants of leaf.

The purpose of the study - the selection of the optimal composition of nutrient media for induction of morphogenesis in selective hybrids of family Vacciniaceae S.F. Gray.

MATERIALS AND METHODS

As objects of study there were used four hybrids of four combinations: 1) Vaccinium vitis-idaea x Oxycoccus palustris, 2) V. vitis-idaea x O.macrocarpus (var. Stivens), 3) V. vitis-idaea x V.palustris, 4) V. vitis-idaea x V. uliginosum. Explants were micrografts of regenerants of these hybrids and epicotyl, hypocotyl, cotyledons, root, leaves of juvenile seedlings, obtained previously under aseptic conditions on a modified nutrient medium Andersen.

Sterile explants were plated on three nutrient media: Murashige-Skoog (1962), WPM (1981) and Andersen (1975) in nine modifications (Table. 1) in the flasks of the same volume by 15 ml of medium in each. Planted material was cultured at 26°C, 56% humidity, 16 h photoperiod, illuminance 4000 lux. Repeating of the experiments is thrice-repeated. There was considered the number of shoots per explant (pieces), callus formation (mg) after 45 days after landing of the explant on nutrient medium. Statistical analysis of data was carried out on the basis of 20 explants on repeating. Experimental data are summarized in Table. 2-3. There were given the arithmetic means and their standard errors.

RESULTS AND DISCUSSION

After four weeks of cultivation from one micrograft was formed in average from 1 to 8 microshoots depending on the composition of the nutrient medium (Table. 2). The rest of the explants (epicotyl, hypocotyl, cotyledon, root, leaves) in 5-6 weeks of cultivation formed organogenic callus with subsequent regeneration of the vegetative shoots from him. It should be noted that the formation of organogenic callus and subsequent regeneration of shoots are characteristic for the explants (root, epicotyl, hypocotyl, cotyledon, leaves), obtained from freshly collected from the seeds and for explants, obtained from seeds that have passed stratification, shoot formation occured directly from the explant tissue, passing the stage of

callus formation. It is logical to assume that it may be associated with varying the flow of physiological, biochemical, cytological, and other processes in the explant from freshly collected and stratified seeds, as well as with different content of endogenous phytohormone in it. Probably, all together formed the basis for the regeneration of shoots from callus without his preliminary passage on the nutrient medium of other composition. In other words, the induction of callusogenesis formation and then shoot formation occurred on the medium of the same composition.

Data of Table. 3 show, that all explants in two environments: WPM and Andersen of two modifications (№ 8, 9 see Table 1) possess by the highest morphogenetic potential. In this case, the ability of cells of explants to be dedifferentiated, lies in the base of morphogenesis, that is to lose their previous specialization and convert into callus cells. Conversion of specialized cells into callus cells is associated with the induction of cell division, the ability to which cells have lost during differentiation (Butenko, 1985).

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

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

Callus cells (except auksin- and cytokininundepended tumor cells) can not themselves synthesize phytohormones in sufficient quantities, necessary for the induction of morphogenesis, so in need of exogenous growth regulators. Callus cells only at a certain ratio of cytokinins and auxins in the medium can go to the organized growth and development of shoots. This ratio for each plant species is setting by experimentation. Proof of this can serve numerous studies of various plant species, concerning regulation of morphogenesis in culture tissue of cells and tissues with a help of certain ratio of auxins and cytokinins in the nutrient medium (Christopher et al., 1987, Makoveychuk, 1990, Mohamed and Alsadon, 2011, Sharaf et al., 2011, Abbas and Qaiser, 2012, Mir et al., 2014).

Our studies have shown that, for the formation of regenerants of selective hybrids of four combinations from callus tissue into nutrient medium is necessary to add cytokinins and auxins in the following ratio: 2.5: 1 (medium number 4), 2: 1 (medium number 5), 3.75 1 (medium number 8 and number 9).

As the analysis of the experimental results obtained by the study of morphogenesis of selective hybrids of four combinations in nine variants of nutrient media, differing in the content of macro- and microsalts, hormonal supplements best for morphogenesis of studied hybrids were media of 8th and 9th modifications, containing in their content macro and microelements on WPM and Anderson, hormonal supplements: 4 mg / l IAA and 15 mg / l isopentenyladenine (Table. 1). On media of 8th and 9th modifications in comparison with those of the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th was obtained maximum number of shoots per explant from 13 to 17, depending on the combination of the hybrid (Table. 2). On the base of the study of morphogenetic processes, occurring in explants on different modifications of nutrient media, showed a possibility of regeneration of selective hybrids in two ways: 1) through the activation of axillary meristems, 2) through the proliferation of callus and subsequent formation of regenerants from it.

CONCLUSION

As a result of research, carried out to study the processes of morphogenesis of selective hybrids (fam. Vacciniaceae) on different modifications of nutrient media, was determined the optimal composition of the nutrient medium for the occurrence of this process. It was shown the fundamental possibility of regeneration of selective hybrids in two ways: 1) through the activation of axillary meristems, 2) through the proliferation of callus and subsequent formation of his shoots from it. Best for morphogenesis of studied hybrids were media of 8th and 9th modifications, containing in its composition of macro and microelements on WPM and Andersen, as well as hormonal supplements: 4 mg / l of indolyl acetic acid and 15 mg / l of isopentenyladenine.

Table 1. The composition of the nutrient media for study of morphogenesis of selective hybrids (fam.Vacciniaceae)

Component, mg/l	Modification of media								
	1	2	3	4	5	6	7	8	9
Salts and vitamins by MS	+	-	1/2	+	-	-	-	-	-
Salts and vitamins by WPM	-	+	-	-	-	-	-	+	-
Salts and vitamins by Anderson	-	-	-	-	+	+	+	-	+
Mesoinosite	100	100	100	100	100	100	100	100	100
Adeninsulphate	-	80	80	80	80	40	60	80	80
Tiamin	0,4	-	-	0,4	-	0,1	0,1	0,4	0,1
Pyridoxin	-	-	-	0,4	-	-	-	-	-
Indolyl acetic acid	1,0	5,0	-	2,0	2,0	1,5	2,5	4,0	4,0
Hibberellic acid	-	4,0	-	-	-	-	-	-	-
Naphtyl acetic acid	-	-	-	-	-	-	-	-	-
Benzamilaminopurin	-	-	-	-	-	2,0	-	-	-
Isopentenyladenine	10	10	2,0	5,0	4,0	-	10	15	15
Sacharoza, г/л	20	20	20	30	30	20	20	30	30
Agar, г/л	9	9	9	9	9	9	9	9	9
pH	4,8	4,8	4,8	4,8	4,0	4,0	4,0	4,8	4,8

Notation: the sign (+) component is present in the medium; (-) sign component is absent in the medium; ½ is half the dose of the component in the medium.

Table 2. Shoot formation of selective hybrids depending on the composition of the nutrient medium

Modification number of media	Quantity of regenerants on explant, pieces			
	Vaccinium vitis-idaea x *Oxycoccus palustris*	*V. vitis-idaea* x *O.macrocarpus* (var.*Stivens*)	*V. vitis-idaea* x *V.palustris*	*V. vitis-idaea* x *V. uliginosum*
1	8,6±1,3	7,8±1,9	8,2±1,1	7,5±1,4
2	7,6±1,4	7,3±2,1	7,7±1,3	7,2±1,2
3	2,1±1,2	2,4±1,0	2,8±0,2	2,3±1,1
4	3,2±1,4	4,9±1,1	4,2±1,3	5,1±1,8
5	5,6±1,3	5,1±1,4	5,0±2,1	4,3±1,2
6	1,2±1,1	0,8±0,1	1,2±0,4	0,7±0,1
7	1,4±1,7	1,8±1,1	1,3±0,1	1,7±1,0
8	14±2,0	13±1,0	14,5±2,1	13,6±1,8
9	17±1,0	15±2,0	16,0±2,0	15,1±2,3

Table 3. Morphogenesis of selective hybrids depending on the composition of the nutrient medium as an example *Vaccinium vitis-idaea* x *Oxycoccus palustris*

Modification number of medium	Quantity of regenerants on one explant, (pieces)						
	callus, mg	shoots, pieces.	Source of explants				
			root	hypocotyl	epicotyl	cotyledon	leaves
1	42,7±4,1	1,0±0,0	+	+	+	+	+
2	176,6±3,9	13,0±2,0	+ +	+ +	+ +	+ +	+ +
3	140,0±4,2	10,0±1,0	+ +	+ +	+ +	+ +	+ +
4	220,0±3,5	24,0±3,0	+ + +	+ + +	+ + +	+ + +	+ + +
5	116,7±17,1	17,0±2,0	+ + +	+ + +	+ + +	+ + +	+ + ++
6	45,9±1,5	2,0±1,0	+	+	+	+	+
7	90,0±2,0	6,0±1,0	+	+	+	+	+
8	129,0±1,5	9,0±1,0	+ +	+ +	+ +	+ +	+ +
9	310,0±7,0	22,0±2,0	+ + +	+ + +	+ + +	+ + +	+ + +

Notation: the sign (+) – morphogenesis is low; sign (+) - medium; sign (+ + +) - high.

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