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Morphogenesis of rhododendron yellow, introduced varieties of high bush blueberry, red bilberry ordinary, depending on the composition of the nutrient media

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Abstract

It was studied the morphogenesis of introduced varieties of high bush blueberry (*Vaccinium corymbosum* L.), red bilberry ordinary (*Vaccinium vitis-idaea* L.), rhododendron yellow (*Rhododendron luteum* Sweet) on various modifications of nutrient media, determined the optimal composition of nutrient medium for the development of this process. It was shown the fundamental possibility of regeneration of introduced varieties of high bush blueberry, red bilberry ordinary with the help of activation method of axillary meristems; rhododendron yellow - in two ways: 1) through the activation of axillary meristems, 2) through the proliferation of callus and subsequent formation of shoots from this callus.

Keywords: Morphogenesis, nutrient media, rhododendron yellow, varieties of red bilberry ordinary, high bush blueberry.

Introduction

An extensive literature is devoted to the question of morphogenesis in cultured 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, i.e., components, contained in it (macro- and microelements, vitamins, carbohydrates, hormonal supplements), and on the pH of the medium, conditions of cultivation and many other factors. Proof of this are the numerous experimental studies.

According to the research of Shor and Papazian (1989), obtained in the study of morphogenesis in culture of isolated tissues of roses on five media with different concentrations of macrosalts and combinations of hormonal supplements, realization of morphogenesis was lied in the development of shoots from axillary buds and formation of callus on the sections of the stem and leaf petiole. The most intensive development of shoots was observed on Murashige-Skoog medium of full mineral composition with the addition of benzylaminopurine and naphthyl acetic acid under conditions of 16-hour photoperiod.

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

About the role of auxins and cytokinins in the regulation of morphogenesis indicate the experimental studies of Budagovskaya et al. (1990). As the explants were used the young leaves and tops of cereal shoots, grown under aseptic conditions and leaves of mature plants, cultivated under field conditions. The authors conclude that the best of all calluses are formed on explants, taken from adult plants grown in the field, when the content in the medium 1 mg / l benzyladenine and 1.2 - the NAA. Shoot formation was observed on Murashige-Skoog medium, containing 2 mg /l benzyladenine.

Gupta and Chandra (1985) studied the effect of different growth regulators 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 tissues with several adjacent layers of cells, isolated from young internodes. Experimental data have allowed the authors to conclude, that gibberellic acid at a concentration of 0.5 mg /l stimulated a formation of buds only on explants of the leaf pieces; kinetin and naphthalene acetic acid promoted the formation of vegetative buds on the explants of stem, and kinetin - on explants of leaves.

The study of the morphogenesis of introduced varieties of high bush blueberry, red bilberry ordinary, rhododendron yellow on various modifications of nutrient media will allow to determine the optimal composition of the nutrient medium for the development of this physiological process under conditions *in vitro*.

Materials and Methods

The objects of study were introduced varieties of high bush blueberry “Elizabeth”, red bilberry ordinary “Ammerland”, “Red Pearl”, rhododendron yellow (*Rhododendron luteum* Sweet). Experiments were carried out on three types of nutrient media: MS (Murashige and Skoog, 1962), WPM (Lloyd and McCown, 1981), Anders (Anderson, 1975), submitted by the 9 different modifications (Table 1).

Table 1. The composition of nutrient media for investigation of morphogenesis of introduced varieties of high bush blueberry, red bilberry ordinary, rhododendron yellow

Component, mg/l	Modification of medium								
	1	2	3	4	5	6	7	8	9
Salts and vitamins on MS	+	-	1/2	+	-	-	-	-	-
Salts and vitamins on WPM	-	+	-	-	-	-	-	+	-
Salts and vitamins according to Anderson	-	-	-	-	+	+	+	-	+
Mesoinositol	100	100	100	100	100	100	100	100	100
Adeninesulfate	-	80	80	80	80	40	60	80	80
Thiamine	0.4	-	-	0.4	-	0.1	0.1	0.4	0.1
Pyridoxine	-	-	-	0.4	-	-	-	-	-
Indoleacetic acid	1.0	5.0	-	2.0	2.0	1.5	2.5	4.0	4.0
Gibberellic acid	-	4.0	-	-	-	-	-	-	-
Naphthylacetic acid	-	-	-	-	-	-	-	-	-
Benzylaminopurine	-	-	-	-	-	2.0	-	-	-
Izopentiladenine	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	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

Annotation. Sign (+) − component is present in the medium; sign (-) − component is absent in the medium; ½ − half of dose of component in the medium

As explants were used micrografts of introduced varieties high bush blueberry “Elizabeth”, red bilberry ordinary “Ammerland”, “Red Pearl”, rhododendron yellow, entered into a sterile culture, and epicotyl, hypocotyl, cotyledons, root, leaves of juvenile seedlings of rhododendron yellow, obtained previously under aseptic conditions on a modified nutrient medium of Andersen. Sterile explants were planted on nutrient media: Murashige-Skoog, WPM and Andersen into flasks of equal volume of 15 ml medium in each. Planted material was cultured at 26°C, 56% of humidity, 16 h photoperiod, illuminance 4000 lux. Repetition of the experiments is three times. There were considered the number of shoots per explant (pcs.), callus formation (mg) after 45 days of planting of the explants on nutrient medium. Statistical analysis was carried out on the basis of 20 explants on repetition. The experimental data are summarized in Table 2-3. They are given the arithmetic means and standard errors.

Results and Discussion

After four weeks of cultivation from one micrograft was formed in average from 1 to 13 microshoots depending on the composition of the nutrient medium (Table 2). In explants of rhododendron yellow (epicotyl, hypocotyl, cotyledon, root, leaves) 5-6 weeks of cultivation was formed organogenic callus with followed 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 explants (root, epicotyl, hypocotyl, cotyledons, leaves), derived from freshly collected seeds. In explants, isolated from germinated seeds, that have passed stratification, shoot formation occurred directly from tissue of explant, by passing the stage of callus formation. It is logical to assume, that it may be associated with varying development of physiological, biochemical, cytological, and other processes in the explants from freshly collected stratified seeds, as well as with different concentrations of endogenous phytohormones in it. Probably, all together taken served by the basis for the regeneration of shoots from callus without his preliminary passage in the nutrient medium of other composition. Thus, induction of callus formation and then shoot formation occurred on the medium of the same composition.

Table 2. Shoot formation of introduced varieties of high bush blueberry, red bilberry ordinary, rhododendron yellow, depending on composition of nutrient medium

Number of modification of medium	Quantity of regenerants on one explant, piece			
	‘Elizabeth‘	‘Ammerland‘	‘Red Pearl‘	*Rhododendron luteum*
1	4.5±1.2	4.7±1.8	4.2±1.0	4.5±1.3
2	3.5±1.4	3.2±1.0	3.7±1.2	4.2±1.0
3	1.1±1.0	1.3±1.0	1.8±0.2	1.3±1.0
4	1.2±1.3	2.9±1.1	3.2±1.1	3.1±1.7
5	3.6±1.2	3.1±1.3	3.0±2.1	2.1±1.2
6	1.3±1.1	0.7±0.1	1.0±0.3	0.6±0.1
7	1.4±1.0	1.6±1.1	1.2±0.1	1.5±1.0
8	7.0±1.0	10.0±1.0	11.5±2.1	6.0±1.7
9	9.0±1.0	12.0±2.0	13.0±2.0	7.0±2.2

Table 3 shows, that all rhododendron yellow explants possess by highest morphogenetic potential on media: WPM and Andersen of two modifications (№ 8, 9, see. Table1). In this case, in the basis of morphogenesis of rhododendron yellow is the ability of cells of explants to be dedifferentiate, i.e. to loose their previous specialization and turn 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, 1975).

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 in the balance between phytohormones. They found, that an excess of content of auxin above cytokinin in the medium, causes the induction of roots; inverse relationship, i.e. excess of cytokinin above auxin leads to the formation of buds and stem shoots.

Table 3. Morphogenesis of rhododendron yellow depending on composition of nutrient medium

Number of modification of medium	Quantity of regenerants on one explant, piece						
	callus, mg	shoots, piece	Source of explants				
			root	hypocotyl	epicotyl	cotyledons	leaves
1	30.7±3.1	1.0±0.0	+	+	+	+	+
2	165.6±3.8	10.0±3.0	+ +	+ +	+ +	+ +	+ +
3	130.0±3.2	9.0±1.0	+ +	+ +	+ +	+ +	+ +
4	210.0±3.0	16.0±1.0	+ + +	+ + +	+ + +	+ + +	+ + +
5	110.5±16.1	13.0±2.0	+ + +	+ + +	+ + +	+ + +	+ + ++
6	40.8±1.4	2.0±1.0	+	+	+	+	+
7	85.0±2.5	7.0±2.0	+	+	+	+	+
8	119.0±1.7	8.0±2.0	+ +	+ +	+ +	+ +	+ +
9	305.0±6.1	19.0±3.0	+ + +	+ + +	+ + +	+ + +	+ + +

Annotation.Sign (+) − morphogenesis is low, sign (+ +) − medium, sign (+ + +) – high.

It can be assumed, that the differences between the cells and tissues on 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 auxin- and cytokinine depended and tumor cells) cannot themselves synthesize phytohormones in sufficient quantities, required for the inducing of morphogenesis, so they need exogenous growth regulators. Callus cells only at a certain ratio of cytokinin and auxin in the medium can go to the organized growth and development of shoots. This ratio for each plant species is set by experimentation. Proof of this are the numerous studies on the regulation of morphogenesis in culture of cells and tissues with the help of specific ratio of auxin and cytokinin in the medium (Christopher et al. 1987, Makoveychuk 1990, Mohamed and Alsadon 2011, Sharaf et al. 2011, Abbas and Qaiser 2012 , Dadvar et al. 2013, Kakarla et al. 2014, Nqobile et al. 2015).

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

The analysis of the experimental results, obtained by the study of the morphogenesis of introduced varieties of high bush blueberry, red bilberry ordinary, rhododendron yellow, on nine modifications of nutrient media, differing in the content of macro- and microsalts, hormonal supplements showed that the best for morphogenesis of studied plants were medium of 8th and 9th modifications, which contain in the composition macro- and microelements for WPM and Andersen, and also hormonal supplements: 4 mg/L indoleacetic acid, and 15 mg/l isopenteniladenine (Table 1). On media of 8th and 9th modifications in comparison to those 1, 2, 3, 4, 5, 6 and 7th was obtained by the maximum number of shoots per explant from 6 to 13 depending on the plant species and varieties (Table 2).

Conclusion

Best for morphogenesis of introduced varieties of high bush blueberry, red bilberry ordinary and rhododendron yellow 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 indoleacetic acid and 15 mg/l isopenteniladenine. It was shown the fundamental possibility of regeneration of rhododendron yellow in two ways: 1) through the activation of axillary meristems, 2) through the proliferation of callus and subsequent formation of regenerants from it.

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