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The influence of the composition of the nutrient medium on the morphogenesis of *Rhododendron luteum* Sweet, introduced varieties of *Vaccinium corymbosum* L. and *Vaccinium vitis-idaea* L.

Elena Kutas[1]*, Vladimir Titok[1], Lyubov Ogorodnyk[2]

[1]Central Botanical Garden of the NAS of Belarus, 220072 Minsk, Surganova, 2v, Republic of Belarus.
E-mail: *vinogradova-kira@tut.by*. Tel: (+375 17) 284-15-89.Fax: (+375 17) 284-14-84.
[2]Kyiv Taras Shevchenko University, 01601 MSP Kyiv, Volodymyrska street, 64, Ukraine.

Abstract

The morphogenesis of the introduced varieties of *Vaccinium corymbosum*, *Vaccinium vitis-idaea*, *Rhododendron luteum* on various modifications of the nutrient media was studied, the optimal composition of the nutrient medium for this process was determined. The principal possibility of regeneration of introduced varieties of *Vaccinium corymbosum*, *Vaccinium vitis-idaea* by the method of activation of axillary meristems was shown; for *Rhododendron luteum*− by two methods: 1) by activation of the axillary meristems, 2) through the proliferation of callus and the subsequent formation of shoots from it.

Keywords: morphogenesis, nutrient media, introduced varieties

Introduction

Extensive literature is devoted to the question of morphogenesis in the culture of cells and tissues. Its analysis allows us to conclude that morphogenesis is a complex and multifactorial process, depending on the type and physiological state of the explant, the composition of the nutrient medium, i.e. the components, contained in it (macro- and microelements, vitamins, carbohydrates, hormonal supplements), as well as on the pH of the medium, cultivation conditions and a number of other factors. This is confirmed by numerous experimental studies.

Gupta and Chandra (1985) studied the effect of various growth regulators (BAP, NAA, GA) on the morphogenesis of various types of tobacco explants: leaf pieces without a central vein, isolated from 2–4 upper leaves; segments of internodes isolated from the second upper internodes; stripes of epidermal tissue with several adjacent layers of cells isolated from young internodes. Experimental data allowed the authors to conclude that GA in a concentration of 0.5 mg / L stimulated the formation of buds only on explants of leaf pieces; kinetin and NAA promoted to the formation of vegetative buds on stem explants, and kinetin on leaf explants.

According to the results of studies by Shor and Papazyan(1989), obtained by studying the processes of morphogenesis in a culture of isolated rose tissues on five media, differing in the concentration of macrosalts and a combination of hormonal supplements, the realization of morphogenesis consisted in the development of shoots from axillary buds and the formation of callus on sections of the stem and petiole of the leaf . The most intensive shoot development was noted on the Murashige-Skoog medium of full mineral composition with the addition of BAP and NAA under a 16-hour photoperiod.

From a publication by Vilor et al. (1987), it follows, that the morphogenetic processes, occurring in sunflower in an in vitro culture depend on the type of nutrient medium and explant. They found, that callus was formed best of all on Erickson and Murashige-Skoog media from the apical meristem of stem, and on the medium of White - from a leaf. The authors observed the formation of shoots with roots only from the apical meristem.

The role of auxins and cytokinins in the regulation of morphogenesis is evidenced by experimental studies by Budagovskava et al. (1990). The leaves and tops of young shoots of cereals grown under aseptic conditions, as well as the leaves of adult plants cultivated in the field, were used as explants. The authors conclude that calliare formed better on explants, taken from adult plants, grown in the field, with 1 mg / L benzyladenine and 1.2 NAA in the medium. Shoot formation was noted on Murashige-Skoog medium, containing 2 mg / l benzyladenine.

A study of the morphogenesis of introduced varieties of *Vaccinium corymbosum*, *Vaccinium vitis-idaea*, *Rhododendron luteum*, on various modifications of the nutrient media, will determine the optimal composition of the nutrient medium for passing of this physiological process in vitro.

Materials and Methods

The objects of study were introduced varieties of *Vaccinium corymbosum* 'Elizabeth', *Vaccinium vitis-idaea*' Ammerland', 'Red Pearl', *Rhododendron luteum*. The experiments were performed on three types of nutrient media:MS (Murashige and Skoog, 1962), WPM (Lloyd and McCown, 1981), Anders (Anderson, 1975), represented by 9 different modifications (Table 1).

As explants there were used micrografts of introduced varieties of *Vaccinium corymbosum* 'Elizabeth', *Vaccinium vitis-idaea*' Ammerland', 'Red Pearl', *Rhododendron luteum*, introduced into a sterile culture, and also epicotyl, hypocotyl, cotyledon, root, leaves of juvenile seedlings of *Rhododendron luteum*, obtained by us earlier under aseptic conditions on modified Anderson’s nutrient medium. Sterile explants were planted on nutrient media: Murashige-Skoog, WPM and Anderson in flasks of the same volume of 15 ml of medium in each. The planted material was cultivated at a temperature of 26 ° C, humidity of 56%, a photoperiod of 16 hours, and illumination of 4,000 lux. The repetition of experiments was three times. The number of shoots per explant (pcs.), callus formation (mg) after 45 days from the moment of planting of explants on a nutrient medium was taken into account. Statistical data processing was carried out on the basis of 20 explants for repetition. The experimental data are summarized in the table. 2–3. They show arithmetical means and their standard errors.

Results and Discussion

After four weeks of cultivation from one micrograft was formed on the average of 1 to 13 microshoots, depending on the composition of the nutrient medium (table 2). In explants of *Rhododendron luteum* (epicotyl, hypocotyl, cotyledons, root, leaves), after 5-6 weeks of cultivation, organogenic callus was formed with subsequent regeneration of vegetative shoots from it. It should be noted that the formation of organogenic callus and the subsequent regeneration of shoots are typical for explants (root, epicotyl, hypocotyl, cotyledons, leaves,) obtained from freshly harvested seeds, and for explants from germinated seeds that have passed stratification, shoot formation occurred directly from explant tissue, by passing the stage of callus formation. It is logical to assume, that this may be due to the unequal course of physiological, biochemical, cytological and other processes in explants from freshly harvested and stratificated seeds, as well as with a different content of endogenous phytohormones in them. Probably, all taken together served as the basis for the regeneration of shoots from callus without preliminary passaging it on a nutrient medium of other composition. In other words, the induction of callusogenesis, and then the formation of shoots, took place on a medium of the same composition.

From table 3 it follows that the highest morphogenetic potential is possessed by all explants of *Rhododendron luteum* on media: WPM and Anderson of two modifications (No. 8, 9, see table 1). In this case, the morphogenesis of *Rhododendron luteum* is based on the ability of explant cells to be dedifferentiated, in other words, to lose their previous specialization and turn into callus cells. The transformation of

Table 1− Composition of nutrient media for studying the morphogenesis of introduced varieties of *Vaccinium corymbosum*, *Vaccinium vitis-idaea*, *Rhododendron luteum*

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 on Andersen	-	-	-	-	+	+	+	-	+
Mesoinositol	100	100	100	100	100	100	100	100	100
Adenine sulphate	-	80	80	80	80	40	60	80	80
Thiamine	0,4	-	-	0,4	-	0,1	0,1	0,4	0,1
Pyridoxine	-	-	-	0,4	-	-	-	-	-
Indolylacetic acid	1,0	5,0	-	2,0	2,0	1,5	2,5	4,0	4,0
Gibberelic acid	-	4,0	-	-	-	-	-	-	-
Naphtylacetic acid	-	-	-	-	-	-	-	-	-
Benzylaminopurine	-	-	-	-	-	2,0	-	-	-
Isopenteniladenine	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

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

Table 2− Shoot formation in introduced varieties of *Vaccinium corymbosum*, *Vaccinium vitis-idaea, Rhododendron luteum*, depending on the composition of the 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 − Morphogenesis in *Rhododendron luteum*, depending on the composition of the nutrient medium.

Number of modification of medium	Quantity of regenerants on one explant, piece						
	callus, mg	shoots, piece.	Source of explants				
			rootlet	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	+ + +	+ + +	+ + +	+ + +	+ + +

Notation: (+) – morphogenesis is low, (+ +)–average morphogenesis, (+ + +)–high morphogenesis specialized cells into callus cells is associated with the induction of cell division, the ability of which cells lost in the process of differentiation (Butenko,1975).

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

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

Callus cells (with the exception of auxin- and cytokinin-independent tumor cells) cannot synthesize phytohormones themselves in sufficient quantities necessary for the induction of morphogenesis, therefore, they need exogenous growth regulators. Callus cells only with a certain ratio of cytokinins and auxins in the medium can go on to organized growth and shoot formation. This ratio for every species of plant is established experimentally. This can be confirmed by numerous studies on the regulation of morphogenesis in cell and tissue culture using a certain ratio of auxins and cytokinins in a nutrient medium (Grozeva and Nankar, 2020; Sharaf et al., 2011; Mohamed and Alsadon, 2011; Abbas and Qaiser, 2012; Dadvar et al., 2013; Kakarla et al., 2014; Nqobile et al., 2015; Christopher et al.,1987; Makoveychuk, 1990; Cheruvathur et al., 2015; Hala and Almobasher, 2016; Sweety and Rahman, 2016; Ali et al.,2017; Shete et al., 2017; Kaveri and Srinath, 2017; Yandia et al.,2018; Fikadu and Tileye, 2019; Khan et al., 2019; Choudhari et al., 2020; Dereje et al., 2020).

Our studies have shown, that for the formation regenerants of *Rhododendron luteum* from callus tissue into nutrient medium, it is necessary to add cytokinins and auxins in the following ratios: 2.5: 1 (medium No. 4), 2: 1 (medium No. 5), 3.75: 1 (medium No. 8 and No. 9).

As shown by the analysis of the results of experimental studies on the morphogenesis of introduced varieties of *Vaccinium corymbosum*, *Vaccinium vitis-idaea*, *Rhododendron luteum* on nine modifications of nutrient media, that differ in the content of macro- and microsalts, hormonal supplements, best for morphogenesis of studied plants were the 8th and 9th modifications, containing macro- and microelements of WPM and Anderson media, as well as hormonal supplements: 4 mg / l indolylacetic acid and 15 mg / l isopentenyladenine (Table 1). On media of the 8th and 9th modifications, in comparison with those of the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th, the maximum number of shoots per explant was obtained from 6 to 13 depending on the variety and type of plant (Table 2).

Conclusion

The best for morphogenesis of introduced varieties of *Vaccinium corymbosum*, *Vaccinium vitis-idaea* and *Rhododendron luteum* were media of the 8^{th} and 9^{th} modifications, containing macro- and microelements of WPM and Andersen media, as well as hormonal supplements: 4 mg / l indolylacetic acid and 15 mg / l isopentenyladenine. The principle possibility of regeneration of *Rhododendron luteum* by two methods has been shown: 1) by activating the axillary meristems, 2) through the proliferation of callus and the subsequent formation of regenerants from it.

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