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Morphogenesis of introduced varieties of *Lonicera edulis* Turcz. ex Freyn depending on composition of nutrient media

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Abstract

The paper presents the results of experimental studies on the morphogenesis of introduced varieties of honeysuckle edible on nine modifications of nutrient media that differ in the content of macro- and microsalts, hormonal supplements. It was shown that the media of the 8th and 9th modifications, which contain macro- and microelements according to WPM and Anderson, and also hormonal supplements: 4 mg / l of indolylacetic acid and 15 mg / l of isopentenyladenine, were better for the morphogenesis of the studied honeysuckle edible varieties.

Keywords: morphogenesis, honeysuckle edible, varieties, nutrient media

Introduction

The issue of morphogenesis in culture of cell and tissue was devoted the extensive literature. Her 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. components, contained in it (macro- and microelements, vitamins, carbohydrates, hormonal supplements), as well as the pH of the medium, the conditions of cultivation and a number of other factors. This can be confirmed by numerous experimental studies.

According to the results of studies by Shor and Papazyan (1989), obtained during the study of the processes of morphogenesis in the culture of isolated tissues of rose in five media, differing in the concentration of macrosalts and the combination of hormonal supplements, the realization of morphogenesis consisted in the development of shoots from the axillary buds and the formation of callus on sections of the stem and leaf petiole. The most intensive development of shoots was observed on the Murashige-Skooga medium of complete mineral composition with addition of 1 mg / l NAA.

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

The role of auxins and cytokinins in the regulation of morphogenesis is evidenced by experimental studies, conducted by Budagovskava et al. (1990). As explants were used leaves and tops of young shoots of cereals grown under aseptic conditions, as well as leaves of adult plants, cultivated under the field conditions. The authors came to the conclusion, that calluses are better formed on explants, taken from adult plants, grown in the field with a content of 1 mg / l of benzyladenine and 1.2 mg / l 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 various growth regulators (BAP, IAA, GA) on the morphogenesis of different types of tobacco explants: pieces of leaf without a central vein, isolated from 2-4 upper leaves; internode segments isolated from the second upper internode; strips of epidermal tissue with several adjacent cell layers, isolated from young internodes. Experimental data allowed the authors to come to the conclusion that GA (gibberellic acid) in a concentration of 0.5 mg / l stimulated the formation of the buds only on explants of pieces of leaf; kinetin and IAA promoted the formation of vegetative buds on the explants of the stem, and kinetin - on the explants of leaf.

The study of the morphogenesis of introduced varieties of honeysuckle edible on various modifications of nutrient media will allow to determine the optimal composition of the nutrient medium for the course of this physiological process under conditions *in vitro*.

Materials and Methods

The objects of the study were introduced varieties of honeysuckle edible: 'Leningrad Giant', 'Early', 'Lazurnaya', 'Kamchadalka'. The experiments were performed on three types of nutrient media: MS (Murashigeand Skoog, 1962), WPM (Lloyd and McCown, 1981), Anderson (Anderson, 1975), represented by 9 different modifications (Table 1). As explants were used microrootlets of four introduced varieties of honeysuckle edible ('Leningrad Giant', 'Early', 'Lazurnaya', 'Kamchadalka') introduced into a sterile culture, as well as epicotyl, hypocotyl, cotyledons, rootlet, leaves of juvenile seedlings of the variety "Early", thatwe received previously under aseptic conditions on the modified Andersen nutrient medium. Sterile explants were planted on Murashige-Skooga, WPM and Andersen nutrient media in flasks of the same volume with 15 ml of medium in each. The planted material was cultivated at a temperature of 26 ° C, an air humidity of 56%, a photoperiod of 16 h, an illumination of 4,000 lux. The repetition of the experiments is threefold. The number of shoots per explant (pcs), callus formation (mg) after 45 days from the moment of explant planting on the nutrient medium was taken into account. Statistical processing of data was carried out, based on 20 explants for replication. Experimental data are summarized in tables 2-3. They show the arithmetic mean and their standard errors.

Results and Discussion

At the end of four weeks of cultivation, from 1 rootlet was formed 11 microshoots an average, depending on the composition of the nutrient medium (Table 2). In explants of honeysuckle edible (variety 'Leningrad Giant') (epicotyl, hypocotyl, cotyledon, rootlet, leaves), after 5-6 weeks of cultivation an organogenic callus was formed, followed by regeneration of vegetative shoots from it. It should be noted that the formation of organogenic callus and further regeneration of shoots are typical for explants (rootlet, epicotyl, hypocotyl, cotyledons, leaves) obtained from freshly harvested seeds, and for explants obtained from seeds that have undergone stratification, shoot formation occurred directly from the tissue of explant, bypassing the stage of callus formation. It is logical to assume that this may be due to the unequal flow of physiological, biochemical, cytological and other processes in explants from freshly harvested and stratified seeds, as well as with different content of endogenous phytohormones in them. Probably all taken together served as a basis for the regeneration of sprouts from callus without preliminary passing it onto the nutrient medium of another composition. In other words, the induction of callusogenesis and then shoot formation took place on a medium of the same composition.

From Table 3 it follows that all honeysuckle edible explants ('Leningrad Giant variety') on the WPM and Anderson media of two modifications (No. 8, 9, see Table 1) have the highest morphogenetic potential. In this case, in the morphogenesis of honeysuckle edible "Leningrad Giant" lies the ability of the cells of explants to dedifferentiate, in other words, to lose their former specialization and turn into callus cells. The transformation of specialized cells into callus cells is associated with the induction of cell division, the ability for which the cells lost in the process of differentiation (Butenko, 1975).

Table 1 – Composition of nutrient media for investigation of morphogenesis of introduced varieties of honeysuckle edible

Component, mg/l	Modification of medium								
	1	2	3	4	5	6	7	8	9
Macro- and microelements on MS	+	-	1/2	+	-	-	-	-	-
Macro- and microelements on WPM	-	+	-	-	-	-	-	+	-
Macro- and microelements on Anderson	-	-	-	-	+	+	+	-	+
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
Gibberellic 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	6,0	6,0	6,0	6,0	5,6	5,6	5,6	5,8	5,8

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 of introduced varieties of honeysuckle edible depending on composition of nutrient medium

Number of modification of medium	Quantity of shoots on one explant, piece			
	'Leningrad Giant'	'Lazurnaya'	'Kamchadalka'	'Early'
1	3,4±1,1	3,6±1,7	3,6±1,2	3,1±0,9
2	2,7±1,3	2,1±0,9	3,1±1,0	2,6±1,1
3	1,5±1,0	1,2±1,0	1,4±0,7	1,7±0,1
4	1,6±1,2	1,9±0,8	2,0±1,0	2,1±0,1
5	2,4±0,2	2,1±0,3	1,1±0,6	2,0±1,1
6	1,2±0,1	1,3±0,1	1,6±0,1	1,0±0,3
7	1,1±0,3	1,5±1,0	1,4±0,5	1,1±0,2
8	6,0±0,9	8,0±1,0	5,0±1,6	9,5±1,1
9	8,0±1,4	10,0±1,3	6,0±1,2	11,0±1,0

Table 3 – Morphogenesis of introduced variety 'Leningrad Giant' of honeysuckle edible depending on composition of nutrient medium

Number of modification of medium	callus, mg	shoots, piece	Source of explants				
			rootlet	hypocotyl	epicotyl	cotyledons	leaves
1	29,6±2,2	2,0±0,0	+	+	+	+	+
2	148,5±1,4	9,0±2,0	+ +	+ +	+ +	+ +	+ +
3	125,0±2,0	7,0±1,0	+	+	+	+	+
4	180,0±2,9	10,0±1,0	+ + +	+ + +	+ + +	+ + +	+ + +
5	107,1±1,5	8,0±3,0	+ + +	+ + +	+ + +	+ + +	+ + +
6	34,6±1,7	4,0±1,0	+	+	+	+	+
7	62,1±2,3	6,0±2,0	+	+	+	+	+
8	109,0±2,1	11,0±2,0	+ +	+ +	+ +	+ +	+ +
9	202,7±5,0	14,0±1,0	+ + +	+ + +	+ + +	+ + +	+ + +

Notation: + −morphogenesis is low, + + −average morphogenesis, + + + −high morphogenesis

According to the theory of Skoog and Miller, the process of morphogenesis starts 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 the auxin content over the cytokinin in the medium causes induction of the roots; the inverse relationship; that is excess of cytokinin over auxin leads to the formation of buds and shoots (Skoog and Miller, 1957).

It can be assumed that the 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 the components of the medium.

Callus cells (with the exception of auxin and cytokinin-independent tumor cells) cannot themselves synthesize phytohormones in sufficient quantities necessary for inducing of morphogenesis processes, therefore they need exogenous growth regulators. Callus cells only with a certain ratio of cytokinins and auxins in the medium can get across to organized growth and shoot formation. This ratio is established experimentally for each plant species. This is 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 (Christopher et al., 1987, Makoveychuk, 1990; Drefahl et al., 2007; Papafotiou and Antoniou, 2008; Zulfiqar et al., 2009; Soundararajan and Karrunakaran, 2010; Sun Y. et al., 2010; Sun X. et al., 2010; Sujata et al., 2010; Egorova et al., 2011; Mao et al., 2011; Abbas and Qaiser, 2012 , Sellathurai and Rathinavel, 2012, Noreldaim, 2012, Shirdel et al., 2013; Timofeeva et al., 2014; Miyamoto et al., 2015; Matushkina et al., 2015; Martínez et al., 2016; El-Sayed et al., 2016; Kaveri and Srinath, 2017).

Our studies show, that for formation of shoots of honeysuckle edible (variety'Leningrad Giant') from callus tissue in the nutrient medium, it is necessary to add cytokinins and auxins in the following proportions: 2.5: 1 (medium No. 4), 2: 1 (medium No. 5), 3.75: 1 (medium No. 8 and No. 9).

An analysis of the results of experimental studies on the morphogenesis of introduced varieties of honeysuckle edible on nine modifications of nutrient media differing in the content of macro- and microsalts, hormonal supplements has shown that the best for the morphogenesis of the studied plants were the media of the 8th and 9th modifications, containing its composition of macro- and microelements on WPM and Anderson, as well as hormonal supplements: 4 mg / l indolylacetic acid and 15 mg / l isopenteniladenine (Table 1). On the media of the 8th and 9th modifications, in comparison with those of the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th, was received the maximum number of shoots per explant from 5 to 11 depending on the plant variety (Table 2).

Thus, based on the study of the morphogenetic processes taking place in explants on various modifications of nutrient media, it is shown that it is possible to regenerate introduced honeysuckle edible varieties by two methods: 1) by activation of axillary meristems; 2) through the proliferation of callus and the subsequent formation of shoots from it.

Conclusion

Analysis of the results of experimental studies on the morphogenesis of introduced varieties of honeysuckle edible on nine modifications of nutrient media that differ in the content of macro- and microsalts, hormonal supplements showed that the best for the morphogenesis of the studied varieties were the media of the 8th and 9th modifications, containing in their composition macro- and micro elements for WPM and Anderson, as well as hormonal supplements: 4 mg / l indolylacetic acid and 15 mg / l isopentenyladenine.

Based on the study of morphogenetic processes taking place in explants on various modifications of nutrient media, it is shown that it is possible to regenerate introduced varieties of honeysuckle edible by two methods: 1) by activation of axillary meristems; 2) through proliferation of callus and the subsequent formation of shoots from it.

It has been established that cytokinins and auxins must be added in order to form honeysuckle edible shoots from callus tissue in the nutrient medium in the following proportions: 2.5: 1 (medium No. 4), 2: 1 (medium No. 5), 3.75: 1 (medium No. 8 and No. 9). It has been established , that for formation of shoots of honeysuckle edible from callus tissue into the nutrient medium it is necessary to add cytokinins and auxins in the following proportions: 2,5:1 (medium No.4) 2:1 (medium No.5) 3,75:1 (medium No.8 and No.9).

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