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Morphogenesis of introduced varieties of Surfinia and Calibrachoa on various modifications of nutrient media

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Abstract

The paper presents the results of experimental studies obtained to study the morphogenesis of introduced varieties of surfinia and calibrachoa on six modifications of nutrient media, differing in the content of macro- and microsols, hormonal additives. The media of the 2nd and 4th modifications containing macro- and microsols according to MS, as well as hormonal additives: 0.4 mg/l of indolylbutyric acid and 1.0 mg/l of benzylaminopurine, turned out to be the best for the morphogenesis of introduced surfinia varieties. On these media (of the 2nd and 4th modifications), surfinia obtained the maximum number of shoots per explant from 2 to 4, depending on the variety. For the morphogenesis of introduced calibrachoa varieties, media of the 1st and 4th modifications containing macro- and microsols according to MS, as well as hormonal additives (0.1 mg/l indolylbutyric acid and 1 mg/l benzylaminopurine) turned out to be the best.

Keywords: morphogenesis, nutrient media, surfinia, calibrachoa

Introduction

An extensive literature is devoted to the issue of morphogenesis in cell and tissue culture. Its analysis allows us to conclude that morphogenesis is a complex and multifactorial process that depends 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 additives), as well as on the pH of the medium, cultivation conditions, and a number of others. factors. This can be confirmed by numerous experimental studies (Egorova et al., 2011; Noreldaim, 2012; Miyamoto et al. 2015; Kaveri and Srinath, 2017; Gilani et al., 2019; Tevfik and Egorova, 2019; Jaiswal al., 2022; Misal et al., 2023; Pallavi and Sangeeta, 2025).

According to the research results of Shor and Papazyan (1989), obtained when studying the processes of morphogenesis in the culture of isolated rose tissues on five media differing in the concentration of macro salts and a combination of hormonal additives, the implementation of morphogenesis consisted in the development of shoots from axillary buds and the formation of a callus on sections of the stem and petiole of the leaf. The most intensive development of shoots was noted on the Murashige-Skuga medium of full mineral composition with the addition of 1 mg/l of NAA.

It follows from the publication of Vilor and co-authors (1987) that the morphogenetic processes occurring in sunflower in vitro culture depend on the type of nutrient medium and explant. They found that the callus formed best on the Erickson and Murashige-Skoog media from the apical meristem of the stem, and on the White medium from the 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 conducted 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 calli form better on explants taken from adult plants grown in the field with a medium content of 1 mg/l benzyladenine and 1.2 NAA. Shoot formation was observed on Murashige-Skuga medium containing 2 mg/l of benzyladenine.

Gupta and Chandra (1985) studied the effect of growth regulators (BAP, IAA, 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 internode; strips of epidermal tissue with several adjacent layers of cells isolated from young internodes. Experimental data allowed the authors to conclude that GA at a concentration of 0.5 mg/l stimulated kidney formation only on explants of leaf fragments. Kinetin and IAA promoted the formation of vegetative buds on stem explants, and kinetin − on leaf explants.

As a result of studying the effect of growth regulators on the regeneration of chickpea shoots of *C. arietinum* L., Doskina and Suvorova (2013), it was found that the growth and development of isolated segments of chickpea seedlings in culture in vitro depend on the genotype, mineral composition of nutrient media and the content of growth regulators in the nutrient medium.

Regeneration of plants from callus tissue can be carried out as a result of such types of morphogenesis as organogenesis (hemogenesis, shoot formation, rhizogenesis) or somatic embryogenesis (embryoidogenesis). Based on the results of experimental studies of factors determining the ratio of types of morphogenesis in corn callus tissue, Derkach and co-authors (2013) concluded that the type of morphogenesis in in vitro culture in corn depends mainly on the genotype of the explant, as well as on the duration of cultivation of the callus tissue on a nutrient medium.

Kulkhanova et al. (2013) established in the course of their work that for the two studied species of fritillaries, their development along the path of somatic embryogenesis is possible. During long-term cultivation of *Fritillaria dagana* Turcz. on the Gamborg medium with 5.0 μm of NAA, a callus is formed, which exhibits morphogenic activity. In the future, for the development of somatic embryoids, the callus must be cultured on a medium with 5.0 BAP. *F. sonnikovae* Shaulo & Erst forms a dense callus culture on Dunstan and Short medium (BDS), characterized by high embryogenic activity, including without growth regulators.

It is well known that auxins act as an inducer of rhizogenesis in plants (Butenko, 1975), although there are cases when rooting of shoots occurs without growth regulators (Lakshmi et al., 1986; Bovo et al., 1986). According to theory Skoog and Miller (1957), with the predominance of auxin in the nutrient medium, it is possible to

induce root growth, cytokinin − shoots, with the same ratios of cytokinin and auxin – the growth of an undifferentiated callus. This theory underlies the regulation of morphogenesis in cell and tissue culture.

Thus, as a result of a study of the effect of phytohormones on the morphogenesis of apple cotyledons in tissue culture, Stanis et al. (1991) established that cytokinin caused direct bud formation and suppressed rhizogenesis, auxins inhibited stem organogenesis and induced rhizogenesis.

A detailed study of the effect of NAA, IAA, and kinetin on the morphogenesis of tomato leaf tissues in vitro was conducted by Santana and Ramirer (1989). The authors determined the effect of hormones (NAA, IAA, kinetin), as well as their combinations on root growth and root system formation. The best results were observed on a medium containing both NAA and kinetin in the concentration range of 0.1−1.0 mg/l.

The study of the morphogenesis of introduced surfinia and calibrachoa varieties on various modifications of nutrient media will make it possible to determine the composition of the nutrient medium for the course of this physiological process in vitro.

Materials and Methods

The objects of the study were beautifully flowering introduced varieties of surfinia (Surfinia x hybrida hort "Purple", Surfinia x hybrida hort "Double Red", Surfinia x hybrida hort "Star Yellow Violet ") and calibrachoa (Calibrachoa x hybrida hort "Kabloom denim", Calibrachoa x hybrida hort "Million Bells Red", Calibrachoa x hybrida hort "Bloomtastic Lavender Quartz", Calibrachoa x hybrida hort "Aloha Kona Dark Red", Calibrachoa x hybrida hort "Mini Famous Uno Double White"). The experiments were performed on nutrient media on six different modifications (Table 1).

Table 1 − Composition of nutrient media used to study the morphogenesis of introduced surfinia and calibrachoa varieties

Component, mg/l	Modification of the medium, no.					
	1	2	3	4	5	6
Microsols by MS	f.n.	f.n.	f.n.	f.n.	-	f.n.
Macrosols by MS	f.n.	f.n.	f.n.	f.n.	-	f.n.
Microsols by Andersen	-	-	-	-	п.н.	-
Macrosols by Andersen	-	-	-	-	п.н.	-
Mesoinosite	100,0	100,0	100,0	100,0	100,0	100,0
Adenine sulfate	-	-	-	-	80,0	-
Thiamine (B1)	0,1	0,1	0,1	0,1	0,4	0,1
Pyridoxine (B6)	0,5	0,5	0,5	0,5	-	0,5
Nicotinic Acid (PP)	0,5	0,5	0,5	0,5	-	0,5
Glycine	2,0	2,0	2,0	2,0	-	2,0
Indolylacetic acid	-	-	0,4	0,1	1,0	1,5
Indolyl Butyric acid	0,1	0,4	-	-	-	-
Benzylaminopurine	0,5	1,0	0,5	1,0	-	-
Sucrose, g/l	30,0	30,0	30,0	30,0	30,0	30,0
Agar, g/l	9,0	9,0	9,0	9,0	9,0	9,0

Note. f.n. – the full norm of the component in the medium, - the component is missing in the medium

Micro gears of three introduced surfinia varieties (Surfinia x hybrida hort "Purple", Surfinia x hybrida hort "Double Red", Surfinia x hybrida hort "Star Yellow Violet") and five calibrachoa varieties (Calibrachoa x hybrida hort "Kabloom denim", Calibrachoa x hybrida hort "Million Bells Red", Calibrachoa x hybrida hort "Bloomtastic Lavender Quartz", Calibrachoa x hybrida hort "Aloha Kona Dark Red", Calibrachoa x hybrida hort "Mini Famous Uno Double White") introduced into a sterile culture were used as explants.

Sterile explants were planted on Murashige-Skoog and Andersen culture 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 hours, and an illumination of 4.000 lux. The repetition of experiments is threefold. The number of shoots per explant (pcs.) and callus formation (mg) were taken into account 45 days after the explants were planted on the nutrient medium. Statistical data processing was carried out based on 10 explants per repeat. The experimental data are summarized in Tables 1-5. They contain arithmetic averages and their standard errors.

Results and Discussion

After 45 days of cultivation, an average of 1 to 4 microgrowths were formed from one microgrowth in surfinia (Table 2) and up to 5 microgrowths in calibrachoa (Table 3), depending on the composition of the nutrient medium and the variety of the plant.

Table 2 – Direct organogenesis in introduced surfinia varieties on various modifications of nutrient media

Number of the medium modification	Number of shoots per explant, pcs.		
	Surfinia x hybrida hort "Purple"	Surfinia x hybrida hort "Double Red"	Surfinia x hybrida hort "Star Yellow Violet"
1	2,0±1,0	3,0±1,0	1,0±1,0
2	3,0±1,0	2,0±1,0	3,0±1,0
3	2,0±1,0	3,0±1,0	2,0±1,0
4	4,0±2,0	3,0±1,0	3,0±1,0
5	1,0±1,0	1,0±1,0	1,0±1,0
6	1,0±1,0	1,0±1,0	1,0±1,0

Note. The calculation was made based on 10 explants for each variety.

As follows from Table 2, the surfinia variety (Surfinia x hybrida hort "Purple") had the highest organogenic potential, which amounted to 4 shoots per explant on MS medium of the 2nd modification containing the following hormonal additives: 1.0 mg/l BAP+0.4 mg/l IBA. The lowest organogenic potential of 1 shoot per explant is typical for all three varieties of surfinia (Surfinia x hybrida hort "Purple", Surfinia x hybrida hort "Double Red", Surfinia x hybrida hort "Star Yellow Violet") on media of the 5th and 6th modifications containing 1.0 and 1.5 mg/l of IAA, respectively.

Table 3 – Direct organogenesis in introduced calibrachoa varieties on various modifications of nutrient media

Number of the medium modification	Number of shoots per explant, pcs.				
	Calibrachoa x hybrida hort "Million Bells Red"	Calibrachoa x hybrida hort "Mini Famous Uno Double White"	Calibrachoa x hybrida hort "Aloha Kona Dark Red"	Calibrachoa x hybrida hort "Kabloom denim"	Calibrachoa x hybrida hort "Bloomtastic Lavender Quartz"
1	3,0±1,0	3,0±1,0	3,0±1,0	2,0±1,0	2,0±2,0
2	4,0±2,0	3,0±1,0	4,0±1,0	3,0±1,0	3,0±1,0
3	3,0±1,0	2,0±1,0	2,0±2,0	2,0±1,0	3,0±1,0
4	4,0±2,0	5,0±2,0	5,0±2,0	4,0±2,0	3,0±2,0
5	2,0±1,0	2,0±1,0	2,0±2,0	1,0±1,0	1,0±1,0
6	2,0±1,0	2,0±1,0	2,0±1,0	1,0±1,0	1,0±1,0

Note. The calculation was made based on 10 explants for each variety.

It follows from Table 3 that the studied calibrachoa varieties had a relatively high morphogenetic potential of up to 4-5 shoots per explant on media of the 2nd and 4th modifications supplemented with 1.0 mg/l BAP+0.4 mg/l IBA and 1.0 mg/l BAP+0.1 mg/l IAA, respectively. On the media of the 5th and 6th modifications, this indicator decreased to 1 escape per explant.

Table 4 − Morphogenesis in the introduced Surfinia variety (Surfinia x hybrida hort "Star Yellow Violet") depending on the composition of the nutrient medium

Number of the medium modification	Callus, mg	Shoots, pcs.	The source of explants (parts of the microprobe)		
			Upper	Middle	Lower
1	430,0±6,0	1,0±1,0	+ +	+	+
2	1208,0±5,3	3,0±1,0	+ ++	+ +	+ +
3	450,3±2,9	2,0±1,0	+ +	+	+ +
4	1256,0±4,0	3,0±1,0	+ + +	+ +	+ +
5	215,0±3,0	1,0±1,0	+	+	+
6	213,3±1,8	1,0±1,0	+	+	+

Note: + + + − morphogenesis is high, + + − middle, + − low

Table 5 − Morphogenesis in the introduced Calibrachoa variety (Calibrachoa x hybrida hort "Million Bells Red") depending on the composition of the nutrient medium

Number of the medium modification	Callus, mg	Shoots, pcs.	The source of explants (parts of the microprobe)		
			Upper	Middle	Lower
1	220,0±1,0	3,0±1,0	++	+	+
2	644,7±8,3	4,0±2,0	+ ++	+ +	+ +
3	225,5±2,5	3,0±1,0	+ +	+ +	+
4	576,6±9,5	4,0±2,0	+ + +	+ + +	+ + +
5	117,8±2,5	2,0±1,0	+	+	+
6	112,0±2,0	2,0±1,0	+	+	+

Note: + + + − morphogenesis is high, + + − middle, + − low

Tables 4-5 present the results of experimental studies of the morphogenesis of the introduced Surfinia x hybrida hort "Star Yellow Violet" and Calibrachoa x hybrida hort "Million Bells Red". In this case, the morphogenesis of surfinia and calibrachoa is based on the ability of explant cells 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 of which the cells lost during differentiation (Butenko, 1975).

According to the theory of Skoog and Miller (1957), the process of morphogenesis begins from the cell transition to the initiation of organized development and is the result of a change in the balance between phytohormones. They found that an excess of auxin over cytokinin in the medium causes root induction; the opposite ratio, i.e., an excess of cytokinin over auxin, leads to the formation of buds and shoots. It can be assumed that differences between cells and tissues in the content of endogenous phytohormones determine the different nature of their behavior in an isolated culture and the different requirements for environmental components.

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 processes, therefore they need exogenous growth regulators. Callus cells can only proceed to organized growth and shoot formation with a certain ratio of cytokinins and auxins in the medium. This ratio is established experimentally for each plant species. This can be confirmed by numerous studies concerning the regulation of morphogenesis in cell and tissue culture using a certain ratio of auxins and cytokinins in the nutrient medium (Stanis et al., 1991; Santana and Ramier, 1989; Hala, 2016; Martínez et al., 2016).

Our research has shown that cytokinins and auxins must be added to the nutrient medium in the following ratios for the formation of shoots in the introduced Surfinia x hybrida hort "Star Yellow Violet" and Calibrachoa x hybrida hort "Million Bells Red" from callus tissue: 2.5:1 (medium No. 2); 10:1 (medium No. 4); Tables 4-5.

As shown by the analysis of the results of experimental studies obtained to study the morphogenesis of introduced surfinia varieties on six modifications of nutrient media differing in the content of macro- and microsols, hormonal additives, the media of the 2nd and 4th modifications containing macro- and microsols according to MS, as well as Hormonal supplements: 0.4 mg/l of indolylbutyric acid and 1.0 mg/l of benzylaminopurine (Table 1). On media of the 2nd and 4th modifications, in comparison with those of the 1st, 2nd, 3rd, 4th, 5th, 6th, the maximum number of shoots per explant was obtained from 2 to 4, depending on the plant variety (Table 4); for introduced varieties calibrachoa are media of the 1st and 4th

modifications containing macro- and microsols according to MS, as well as hormonal additives (0.1 mg/l indolylbutyric acid and 1 mg/l benzylaminopurine).

Conclusion

Based on the study of morphogenetic processes occurring in explants of beautifully flowering introduced varieties of surfinia and calibrachoa on various modifications of nutrient media, the fundamental possibility of regeneration of introduced varieties of surfinia and calibrachoa by two methods is shown.: 1) by activating the axillary meristems, 2) through the proliferation of the callus and the subsequent formation of shoots from it. The method of activation of axillary meristems can be used for clonal micropropagation of the studied varieties, and the method of callus proliferation followed by the formation of shoots from it in the system of genetic transformation and plant breeding.

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