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Rhizogenesis of introduced Surfinia and Calibrachoa varieties in sterile culture

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Abstract

The effect of hormonal supplements on rhizogenesis of introduced surfinia and calibrachoa varieties in sterile culture has been studied. It was shown that an increase in the concentration of naphthylacetic acid, indolylbutyric acid, and indolylacetic acid from 0.25 to 1.00 mg/l contributed to an increase in the number of rooted micro shoots of the studied varieties from 10 to 90%, respectively. The addition of 1,00 mg/l hormonal additives to the nutrient medium ensured a high percentage of rooted micro shoots of introduced surfinia and calibrachoa varieties in a sterile culture.

Keywords: rhizogenesis, hormonal supplements, surfinia, calibrachoa, varieties.

Introduction

There is no doubt that auxins act as an inducer of rhizogenesis in plants (Xu et al., 2022; Barros et al., 2005; Karpov, 2004; Butenko, 1975; Abidin and Metali, 2015; Jasfeeda et al., 2017; Erst, 2017; Yemelyanova, 2010; Dmenko et al., 2010; Bryshten and Kasipskau, 2018; Kastritskaya et al., 2018; Venkatachalam et al., 2015; Arab et al., 2018; Mohamed et al., 2018; Hongthongkham et al., 2025; Jabbour and Alzahrani, 2025), although there are cases where rooting of shoots occurs without growth regulators (Sengupta and Sumitra, 1987; Lakshmi et al., 1986; Bovo et al., 1986; Yasodha et al., 2008).

According to the theory of Skoog and Miller (1957), with the predominance of auxin in the nutrient medium, root growth can be induced, 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) found 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. Similar studies were conducted by Sadeghi et al. (2015) for pear, Wang et al. (2025) for oak.

In vitro rooting is a key stage of the micropropagation process. The study of rhizogenesis of introduced varieties of surfinia and calibrachoa, which have high decorative properties, on nutrient media containing various hormonal additives, will allow us to determine their concentration for the course of this physiological process in vitro.

Materials and Methods

The research was aimed at studying rhizogenesis in three varieties of surfinia (Surfinia x hybrida hort "Purple", Surfinia x hybrida hort "Double Red", Surfinia x hybrida hort "Star Yellow Violet") and five varieties of 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 Doube White") on a modified 1/2 MS nutrient medium with a different content of hormonal additives.

Micro shoots of the listed surfinia and calibrachoa varieties with a height of 1 cm were planted in flasks of the same volume of 10 pieces each on 1/2 MS medium containing half the dose of macro- and microsols, as well as indolylacetic acid (IAA), naphthylacetic acid (NAA), indolylbutyric acid (IBA) at a concentration of 0.25; 0.50; 1.0 mg/l for each hormone. The material was placed in a culture room at a temperature of 24 ° C, an illumination of 4000 lux, and a photoperiod of 16 hours. The experimental readings were taken 8 weeks after they were performed. The processed data is summarized in Tables 1-6.

Results and Discussion

The figures presented in Table 1 indicate a high percentage of rooted shoots of the studied surfinia varieties with a nutrient content of 1.00 mg/l of naphthylacetic acid. Thus, the rooting rate was 70% for the Surfinia x hybrida hort "Purple" variety; 80% − for the Surfinia x hybrida hort "Double Red" and Surfinia x hybrida hort "Star Yellow Violet" varieties. A relatively low rooting rate of 10 to 30% was observed in micro shoots of the same varieties with a NAA content of 0.25 mg/l in the nutrient medium; an intermediate position in this indicator of 50-60% of rooted micro shoots was noted at a concentration of 0.50 mg/l of naphthylacetic acid in the nutrient medium.

Therefore, with an increase in the concentration of NAA in the nutrient medium from 0.25 to 1.00 mg/l, the percentage of rooted microgrowths increased from 10 to 80%, respectively. The minimum rooting percentage is typical for the Surfinia x hybrida hort "Purple" variety (10%) at 0.25 mg/l of NAA; the maximum is 80% for the remaining Surfinia x hybrida hort "Double red" and Surfinia x hybrida hort "Star Yellow Violet" varieties at 1.00 mg/l of NAA. Therefore, it is advisable to use NAA at a concentration of 1.00 mg/l to root the three introduced surfinia varieties in a sterile culture.

Table 1− The effect of naphthylacetic acid on the rooting of micro shoots of introduced surfinia varieties on a nutrient medium of ½MS

Variety	NAA, mg/l	Number of shoots		
		total, pcs	rooted	
			pcs	%
Surfinia x hybrida hort "Purple"	0,25	10	1	10
	0,50	10	5	50
	1,00	10	7	70
Surfinia x hybrida hort "Double Red"	0,25	10	3	30
	0,50	10	6	60
	1,00	10	8	80
Surfinia x hybrida hort"Star Yellow Violet"	0,25	10	2	20
	0,50	10	6	60
	1,00	10	8	80

The experimental material presented in Table 2 indicates a similar pattern characteristic of the rooting of the studied micro shoots of the five introduced calibrachoa varieties.

That is, with an increase in the concentration of NAA in the nutrient medium to 1.00 mg/l, an increase in the percentage of rooted micro shoots was observed in all the studied calibrachoa varieties from 50 to 70%, depending on the variety.

The maximum number (70%) of rooted micro shoots was observed on a medium containing 1 mg/l of NAA in Calibrachoa x hybrida hort "Million Bells Red" variety, the minimum was 50% in other varieties with the same concentration of NAA.

At a concentration of NAA 0.25 mg/l in the nutrient medium, micro shoots of three calibrachoa varieties lacked rooting: Calibrachoa x hybrida hort "Kabloom denim", Calibrachoa x hybrida hort "Bloomtastic Lavender Quartz", Calibrachoa x hybrida hort "Mini Famous Uno Doube White"; micro shoots the other two varieties: Calibrachoa x hybrida hort "Million Bells Red", Calibrachoa x hybrida hort "Aloha Kona Dark Red" rooting was 20% and 10%, respectively.

Table 2– The effect of naphthylacetic acid on the rooting of micro shoots of introduced calibrachoa varieties on a nutrient medium of ½MS

Variety	NAA, mg/l	Number of shoots		
		total,pcs	rooted	
			pcs	%
Calibrachoa x hybrida hort "Kabloom denim"	0,25	10	0	0
	0,50	10	1	10
	1,00	10	5	50
Calibrachoa x hybrida hort "Million Bells Red"	0,25	10	2	20
	0,50	10	4	40
	1,00	10	7	70
Calibrachoa x hybrida hort "Bloomtastic Lavender Quartz"	0,25	10	0	0
	0,50	10	3	30
	1,00	10	5	50
Calibrachoa x hybrida hort "Aloha Kona Dark Red"	0,25	10	1	10
	0,50	10	2	20
	1,00	10	6	60
Calibrachoa x hybrida hort "Mini Famous Uno Doube White"	0,25	10	0	0
	0,50	10	2	20
	1,00	10	5	50

At a concentration of NAA 0.50 mg/l in the nutrient medium, micro shoots of the studied 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 Doube White") occupied an intermediate position in this indicator: 10, 40, 30, 20 and 20%, respectively (Table 2). Therefore, the process of rhizogenesis proceeded better in the studied calibrachoa varieties at a NAA concentration of 1 mg/l in the nutrient medium.

Table 3 presents experimental data obtained during the study of the effect of indolylbutyric acid (IBA) on the rooting of micro shoots of introduced surfinia varieties on a nutrient medium of ½ MS.

Table 3− The effect of indolylbutyric acid on the rooting of micro shoots of introduced surfinia varieties on a nutrient medium of ½MS

Variety	IBA, mg/l	Number of shoots		
		total,pcs	rooted	
			pcs	%
Surfinia x hybrida hort "Purple"	0,25	10	5	50
	0,50	10	7	70
	1,00	10	9	90
Surfinia x hybrida hort "Double Red"	0,25	10	4	40
	0,50	10	9	70
	1,00	10	8	80
Surfinia x hybrida hort "Star Yellow Violet"	0,25	10	4	40
	0,50	10	7	70
	1,00	10	9	90

As shown by the analysis of the digital material in Table 3, the micro shoots of the studied surfinia varieties (Surfinia x hybrida hort "Purple", Surfinia x hybrida hort "Double Red", Surfinia x hybrid hort "Star Yellow Violet") showed maximum rhizogenesis at a concentration of 1 mg/l of IBA in the nutrient medium, which amounted to 90, 80, 90%, respectively. Rhizogenesis decreased by an insignificant amount, up to 70%, in micro shoots of the studied surfinia varieties at an IBA concentration of 0.50 mg/l in the nutrient medium; at a concentration of 0.25 mg/l of IBA, this indicator decreased and amounted to 40-50%, depending on the variety.

Table 4 shows experimental material indicating the effect of IBA on the rhizogenesis of micro shoots of five introduced 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 Doube White"). The analysis of the digital material allows us to conclude that the maximum figure (60-90%) of rooted micro shoots is typical for all the studied calibrachoa varieties at a concentration of 1.00 mg/l of IBA contained in the nutrient medium; the minimum (10-20%) – at 0.25 mg/l of IBA; the intermediate (40-60%) at 0.50 mg/l of IBA.

Table 4– The effect of indolylbutyric acid on the rooting of micro shoots of introduced calibrachoa varieties on a nutrient medium of ½MS

Variety	IBA, mg/l	Number of shoots		
		total,pcs	rooted	
			pcs	%
Calibrachoa x hybrida hort "Kabloom denim"	0,25	10	1	10
	0,50	10	4	40
	1,00	10	8	80
Calibrachoa x hybrida hort "Million Bells Red"	0,25	10	2	20
	0,50	10	6	60
	1,00	10	9	90
Calibrachoa x hybrida hort "Bloomtastic Lavender Quartz"	0,25	10	3	30
	0,50	10	6	60
	1,00	10	6	60
Calibrachoa x hybrida hort "Aloha Kona Dark Red"	0,25	10	2	20
	0,50	10	5	50
	1,00	10	7	70
Calibrachoa x hybrida hort "Mini Famous Uno Doube White"	0,25	10	2	20
	0,50	10	6	60
	1,00	10	8	80

As shown by the analysis of the experimental material presented in Table 5, in the studied micro shoots of introduced surfinia varieties, maximum rhizogenesis (30-50%) was observed at a concentration of 1.00 mg/l IAA in the nutrient medium; 20-30% – at 0.50 mg/l IAA; 0-20% – at 0.25 mg/l IAA.

A similar pattern was observed in root formation at different concentrations of IAA in the nutrient medium in calibrachoa micro shoots (Table 6). Thus, with an increase in the concentration of IAA from 0.25mg/l to 1.00 mg/l, the number of rooted shoots increased from 0% to 70%-80%, depending on the variety.

Concluding the analysis of the results of experimental studies obtained during the study of the effects of various hormonal supplements (NAA, IBA, IAA at a concentration of 0.25; 0.50; 1,00 mg/l) for the rhizogenesis of micro shoots of three

Table 5− The effect of indolylacetic acid on the rooting of micro shoots of introduced surfinia varieties on a nutrient medium of ½MS

Variety	IAA,mg/l	Number of shoots		
		total,pcs	rooted	
			pcs	%
Surfinia x hybrida hort "Purple"	0,25	10	1	10
	0,50	10	2	20
	1,00	10	5	50
Surfinia x hybrida hort "Double Red"	0,25	10	0	0
	0,50	10	3	30
	1,00	10	3	30
Surfinia x hybrida hort "Star Yellow Violet"	0,25	10	2	20
	0,50	10	3	30
	1,00	10	5	50

Table 6−Theeffectofindolylaceticacidontherootingofmicroshootsof introduced calibrachoa varieties on a nutrient medium of ½MS

Variety	IAA,mg/l	Number of shoots		
		total,pcs	rooted	
			pcs	%
Calibrachoa x hybrida hort "Kabloom denim"	0,25	10	0	0
	0,50	10	3	30
	1,00	10	2	20
Calibrachoa x hybrida hort "Million Bells Red"	0,25	10	2	20
	0,50	10	3	30
	1,00	10	8	80
Calibrachoa x hybrida hort "Bloomtastic Lavender Quartz"	0,25	10	0	0
	0,50	10	4	40
	1,00	10	6	60
Calibrachoa x hybrida hort "Aloha Kona Dark Red"	0,25	10	1	10
	0,50	10	4	40
	1,00	10	7	70
Calibrachoa x hybrida hort "Mini Famous Uno Doube White"	0,25	10	0	0
	0,50	10	3	30
	1,00	10	7	70

Introduced varieties of surfinia (Surfinia x hybrida hort "Purple", Surfinia x hybrida hort "Double Red", Surfinia x hybrida hort "Star Yellow Violet") and five varieties of 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 dependence of rhizogenesis on the content of hormonal additives in the nutrient medium can be stated. With an increase in the concentration of NAA, IBA, and IAA in the nutrient medium from 0.25 to 1.00 mg/l, the number of rooted micro shoots of the studied varieties increased from 10 to 90%.

Conclusion

1. The addition of 1,00 mg/l hormonal additives to the nutrient medium ensured a high percentage of rooted micro shoots of introduced surfinia and calibrachoa varieties in a sterile culture.
2. A direct dependence of rhizogenesis in introduced varieties of surfinia and calibrachoa on the content of hormonal additives in the nutrient medium was found. An increase in the concentration of NAA, IBA, and IAA from 0.25 to 1.00 mg/l contributed to an increase in the number of rooted micro shoots of the studied varieties from 10 to 90%, respectively.
3. For the rooting of the studied introduced varieties of surfinia and calibrachoa in a sterile culture, it is advisable to use NAA, IBA, IAA at a concentration of 1.00 mg/l.

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