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Research Article

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Rhizogenesis of introduced varieties of edible honeysuckle (*Lonicera edulis* Turcz. ex Freyn), Korean chrysanthemum (*Chrysanthemum coreanum* (H. Levl. et Vaniot) Nakai ex T. Mori *in vitro*

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Abstract

The paper presents the results of experimental studies obtained to study the rhizogenesis of introduced varieties of edible honeysuckle and Korean chrysanthemum on various modifications of nutrient media. It is shown that the media of ½ MS and ½ WPM containing a half dose of macro- and microsols were the best for the rhizogenesis of the studied plants. The rooting of introduced varieties of edible honeysuckle and Korean chrysanthemum depends on the concentration of auxins in the nutrient medium, the content of macro- and microsols in it, as well as on the varietal belonging of the plant.

Keywords: rhizogenesis, honeysuckle, chrysanthemum, varieties, nutrient media

Introduction

It is well known that auxins act as an inducer of rhizogenesis in plants (Lakshmi et al., 1986), although there are cases when the rooting of shoots occurs without growth regulators (Bovoet al., 1986; Sengupta and Sumitra, 1987). According to the theory of Skoog and Miller (1957), with the predominance of auxin in the nutrient medium, it is possible to induce the growth of roots, cytokinin − shoots, with the same ratios of cytokinin and auxin – the growth of undifferentiated callus. This theory underlies the regulation of morphogenesis in cell and tissue culture.

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

The results of the studies of Shor and Papazyan (1989), obtained by 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, allowed the authors to induce rhizogenesis on the MS medium of full mineral composition with the addition of 1 mg/l of NAA.

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

Soroka(2004) studied the regeneration processes of two hybrid genotypes of oilseed flax on nutrient media N6 and LMA-1 at different concentrations of 6-benzylaminopurine (BAP). It shows that the growth and development of callus occur better at a concentration of 2 mg/l in the medium of BAP compared to 4 and 6 mg/l. Regeneration of shoots and roots was observed only in the F1 genotype 6-8-nest × M 22 and did not depend on the concentration of BAP in the medium and on the type of medium.

The study of morphogenesis in flax hypocotyl tissues is devoted to the work of Kaul and Williams (1987). The authors conclude that yeast extract delayed root growth. According to Browning et al. (1987) the dominance of cytokinin over auxin led to the formation of shoots from explants of embryonic cotyledons of pear, the predominance of auxin over cytokinin caused the formation of roots, and the intermediate ratio of hormones – the development of callus.

Extensive literature is devoted to the issue of root formation in regenerants on a nutrient medium (Demenko et al., 2010; McClellandet al., 1990; Berardiet al., 1992;Al-Maarri et al.,1994; Cheng, 1979;Yeo and Reed, 1995; Reed, 1995; De Klerk et al., 1997;Barros et al., 2005; D'Onofrio and Morini, 2006; Abdullah, 2010; Drew et al., 1993; Zhuang et al.,2006; Babaei et al., 2014; Yasodha et al., 2008; Fogaça and Fett-Neto, 2005; Aygun and Dumanoglu, 2015; García-Anguloet al., 2018). Unfortunately, we have not found any information about the formation of roots in regenerants of introduced varieties of edible honeysuckle and Korean chrysanthemum in vitro culture in the available literature.

Based on this, studies were conducted to study the effect of various concentrations of auxins (indolylbutyric, indolylacetic, naphthylacetic acid) on the rooting of regenerants of introduced varieties of edible honeysuckle: 'Leningrad Giant', 'Azure'; two varieties of Korean chrysanthemum: 'Garnet bracelet', 'Natalie' in culture in vitro on nutrient media of various modifications.

Materials and Methods

Two varieties of edible honeysuckle ('Leningrad Giant', 'Azure') and two varieties of Korean chrysanthemum ('Garnet bracelet', 'Natalie') were used as objects of research.

Micro-shoots of the listed varieties with a height of 1 cm were planted in flasks of the same volume of 15 pieces each on a medium of ½ WPM containing a half dose of macro- and microsols, as well as indolylacetic acid (IAA) and naphthylacetic acid (NAA) at a concentration of 0.25; 0.50; 1.0 mg/l. The control version did not contain IAA. The material was placed in a culture room at a temperature of 24 ° C, illumination of 4000 lux, photoperiod of 16 hours. The readings of the experiments were taken after 8 weeks from the moment of their staging. The data are presented in Tables 1, 2. Similar studies were conducted to study the effect of various concentrations of 0.25; 0.50; 1.0 mg/l indolylbutyric acid (IBA) on the rooting of regenerants of the studied varieties on the Murashige and Skoog (MS) nutrient medium containing the full norm of macro- and microsols and ½ MS with a half norm of macro- and microsoleums (table 3). There is no control in tables 1-4, since in the control variant, which did

not contain auxins, rhizogenesis was not observed in regenerants.

Results and Discussion

The digital material presented in Table 1 indicates a relatively high percentage of rooted shoots in the studied varieties of edible honeysuckle 'Leningrad Giant', 'Azure' and two varieties of Korean chrysanthemum ('Garnet bracelet', 'Natalie') at a concentration of NAA of 1.00 mg/l contained in the nutrient medium of ½ WPM. The highest percentage of rooted shoots (100%) is characteristic of the Leningrad Giant variety and the Azure variety. The varieties of Korean chrysanthemum "Garnet bracelet" and "Natalie" occupied an intermediate position in this indicator (96% and 94%, respectively).

Table 1 − The effect of naphthylacetic acid on the rooting of micro-shoots of introduced varieties of edible honeysuckle, Korean chrysanthemum on the nutrient medium ½ WPM

Variety	NAA, mg/l	Number of shoots		
		total, pcs	rooted	
			pcs	%
'Leningrad giant'	0,25	50	30	60
	0,50	50	40	80
	1,00	50	50	100
'Azure'	0,25	50	32	64
	0,50	50	44	88
	1,00	50	50	100
'Garnet bracelet'	0,25	50	35	70
	0,50	50	42	84
	1,00	50	48	96
'Natalie'	0,25	50	36	72
	0,50	50	40	80
	1,00	50	47	94

An analysis of the results of experimental studies presented in Table 2 showed that a relatively high percentage of rooted shoots from the total number of planted ones is characteristic of all the studied plants at a concentration of 0.50 mg/l of IAA in a nutrient medium of ½ WPM. So the rooted shoots in percentage terms made up the following row in descending order: 100% − 'Garnet bracelet', 98% − 'Natalie', 92% − 'Leningrad giant', 90% − 'Azure'.

The number of rooted shoots decreased in varieties of Korean chrysanthemum 'Garnet bracelet', 'Natalie' and varieties of edible honeysuckle 'Leningrad Giant', 'Azure' at concentrations of 0.25 and 1.00 mg/l of IAA. In the variety of edible honeysuckle 'Leningrad Giant' at 0.25 and 1.00 mg / l IAA, a decrease in the percentage of rooted shoots was noted, which amounted to 54 and 62%, respectively; in the variety 'Azure' – 58 and 66%. In Korean chrysanthemum varieties, a similar pattern was observed in a decrease in the percentage of rooted shoots with a decrease in the concentration of IAA in the nutrient medium. So in the variety 'Garnet bracelet' the percentage of rooted shoots was 35 and 39%, respectively, in the variety 'Natalie' – 40 and 44%.

Table 2 − The effect of indolylacetic acid on the rooting of micro-shoots of introduced varieties of edible honeysuckle and Korean chrysanthemum on the nutrient medium ½ WPM (Woody Plant Medium)

Variety	IAA, mg/l	Number of shoots		
		total, pcs	rooted	
			pcs	%
'Leningrad giant'	0,25	50	27	54
	0,50	50	46	92
	1,00	50	31	62
'Azure'	0,25	50	29	58
	0,50	50	45	90
	1,00	50	33	66
'Garnet bracelet'	0,25	50	35	70
	0,50	50	50	100
	1,00	50	39	78
'Natalie'	0,25	50	40	80
	0,50	50	49	98
	1,00	50	44	88

The data presented in Table 3 indicate a different reaction of regenerants of edible honeysuckle varieties 'Leningrad Giant' and 'Azure', as well as chrysanthemums of the Korean variety 'Natalie' and 'Garnet bracelet' to the presence of different concentrations of IBA in the medium. The highest percentage of rooted shoots is characteristic of edible honeysuckle variety 'Azure' (98%), 'Leningrad Giant' (96%) and varieties of Korean chrysanthemum 'Natalie' (98%), 'Garnet bracelet' (100%) on 1/2 MS medium containing half the norm of macro- and microsols, with concentrations of 0.50 mg/l IBA. With an increase in the dose of macro- and microsols in the nutrient medium to the full norm, the rooting of shoots in the studied varieties decreased by an insignificant amount, depending on the variety and the concentration of IBA in the medium.

These facts indicate that the rooting of introduced varieties of edible honeysuckle, Korean chrysanthemum depends on the concentration of IBA in the nutrient medium, the content of macro- and microsols in it, as well as on the species of the plant.

Table 3 − The influence of indolylbutyric acid and the composition of nutrient media on the rooting of micro-shoots of introduced varieties of edible honeysuckle, Korean chrysanthemum

Variety	Medium	IBA, mg/l	Number of shoots		
			total, pcs	rooted	
				pcs	%
'Leningrad giant'	½ MS	0,25	50	35	70
		0,50	50	48	96
		1,00	50	36	72
	MS	0,25	50	30	60
		0,50	50	42	84
		1,00	50	34	68
'Azure'	½ MS	0,25	50	45	90
		0,50	50	49	98
		1,00	50	39	78
	MS	0,25	50	35	70
		0,50	50	34	68
		1,00	50	40	80
'Garnet bracelet'	½ MS	0,25	50	45	90
		0,50	50	50	100
		1,00	50	43	86
	MS	0,25	50	42	84
		0,50	50	44	88
		1,00	50	43	86
'Natalie'	½ MS	0,25	50	48	96
		0,50	50	49	98
		1,00	50	46	92
	MS	0,25	50	40	80
		0,50	50	43	86
		1,00	50	42	84

A comparative analysis of the effect of various auxins (IBA, IAA, NAA) and their concentrations on root formation in regenerants of introduced varieties of edible honeysuckle, Korean chrysanthemum showed that the best result in rooting of the studied plants was obtained in two varieties of edible honeysuckle 'Leningrad Giant' and 'Azure' when using NAA (naphthylacetic acid) at a concentration of 1.00 mg/l on a medium of ½ WPM containing a half dose of macro- and microsols. The rooting of the listed varieties was 100% (Table 4).

As follows from the data presented in Table 4, for the Korean chrysanthemum varieties 'Garnet Bracelet' and 'Natalie', the best rooting result (rooting was 100%) was obtained using IAA and IBA at a concentration of 0.50 mg/l on media ½ WPM and ½ MS containing a half dose of macro- and microsols.

Table 4 − Influence of auxins and nutrient media on the rooting of micro-shoots of introduced varieties of edible honeysuckle, Korean chrysanthemum

Variety	Medium	IBA, mg/l	Number of shoots			Medium	IAA, mg/l	Number of shoots			Medium	NAA, mg/l	Number of shoots		
			total, pcs	rooted				total, pcs	rooted				total, pcs	rooted	
				pcs	%				pcs	%				pcs	%
'Leningrad giant'	½ MS	0,25	50	35	70	½ WPM	0,25	50	27	54	½ WPM	0,25	50	30	60
		0,50	50	48	90										
		1,00	50	36	72		0,50	50	46	92		0,50	50	40	80
	MS	0,25	50	30	60										
		0,50	50	42	89		1,00	50	31	62		1,00	50	50	100
		1,00	50	34	68										
'Azure'	½ MS	0,25	50	45	90	½ WPM	0,25	50	29	58	½ WPM	0,25	50	32	64
		0,50	50	49	98										
		1,00	50	39	78		0,50	50	45	90		0,50	50	44	88
	MS	0,25	50	35	70										
		0,50	50	34	68		1,00	50	33	66		1,00	50	50	100
		1,00	50	40	80										
'Garnet bracelet'	½ MS	0,25	50	45	90	½ WPM	0,25	50	35	70	½ WPM	0,25	50	35	70
		0,50	50	46	92										
		1,00	50	43	86		0,50	50	50	100		0,50	50	42	84
	MS	0,25	50	42	84										
		0,50	50	44	88		1,00	50	39	78		1,00	50	48	96
		1,00	50	43	86										
'Natalie'	½ MS	0,25	50	48	96	½ WPM	0,25	50	40	80	½ WPM	0,25	50	36	72
		0,50	50	49	98										
		1,00	50	46	92		0,50	50	49	98		0,50	50	40	80
	MS	0,25	50	40	80										
		0,50	50	43	86		1,00	50	44	88		1,00	50	47	94
		1,00	50	42	84										

A relatively low rooting result for the introduced varieties of edible honeysuckle 'Leningrad Giant' and 'Azure' was obtained on a medium of ½ WPM supplemented with 0.25 mg/l of IAA and amounted to 54 and 58%, respectively; for the varieties of Korean chrysanthemum 'Garnet Bracelet' and 'Natalie' − 70 and 72%, respectively (Table 4).

Thus, the analysis of the results of experimental studies showed that for rooting regenerants of introduced varieties of edible honeysuckle 'Leningrad Giant' and 'Azure', a medium of ½ WPM containing a half dose of macro- and microsols supplemented with NAA at a concentration of 1.00 mg/l should be used; for introduced varieties of Korean chrysanthemum 'Garnet Bracelet', 'Natalie' – media ½ WPM and ½ MS containing a half dose of macro- and microsols supplemented with IAA and IBA at a concentration of 0.50 mg/l (Table 4).

Conclusion

Analysis of the results of experimental studies obtained to study the effect of various concentrations of auxins (indolylbutyric, indolylacetic, naphthylacetic acid) on the rooting of regenerants of introduced varieties of edible honeysuckle and Korean chrysanthemum on nutrient media differing in the content of macro- and microsols, hormonal additives, showed that the media of ½ MS and ½ WPM turned out to be the best for the rhizogenesis of the studied plants containing half a dose of macro- and microsols. The rooting of introduced varieties of edible honeysuckle and Korean chrysanthemum depends on the concentration of auxins in the nutrient medium, the content of macro- and microsols in it, as well as on the taxonomic affiliation of the plant. Regenerants of introduced varieties of edible honeysuckle 'Leningrad Giant' and 'Azure' should be rooted on a medium of ½ WPM containing a half dose of macro- and microsols supplemented with NAA at a concentration of 1.00 mg/ l; regenerants of introduced varieties of Korean chrysanthemum 'Garnet bracelet', 'Natalie' – on media of ½ WPM and ½ MS containing a half dose of macro- and microsols supplemented with IAA and IBA at a concentration of 0.50 mg/l.

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