THE INFLUENCE OF SEASON OF SELECTION OF EXPLANTS OF INTRODUCED SPECIES OF RHODODENDRONS (*RHODODENDRON* L.) ON THEIR VIABILITY *IN VITRO*

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ABSTRACT

There were presented results of investigations about viability of explants of introduced species of rhododendrons depending on season of the year, when explant is isolated. It was shown, that more favourable time for isolation of explants of investigated overgreen species of rhododendrons (*Rhododendron brachycarpum*, *Rh. ponticum*, *Rh. discolor*, *Rh. catawbiense*, *Rh. smirnowii*) should be consider spring-summer period (from March to June); for deciduous shrub (*R. japonicum*) more favourable time is winter-spring period (from January to April).

Key words: season, buds, rhododendrons, steriled culture.

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INTRODUCTION

Season, when explant is isolated, plays enormous importance in the process of micropropagation of plants: Robb (1957), Bondok et al. (1986), Sebanek and Sladky (1987), Leontiev-Orlov et al. (1988), Beaujard and Viemont (1989), Prasad and Chaturvedi (1989), Sehgal et al. (1989), Bondok (1990), Sidorovich and Kutas (1995), Carlo et al. (2003), Kuznetcova (2008). This factor takes on special significance according to introduced species of rhododendrons, because they belong to family Ericaceae Juss., plants of which are characterized by high content of phenol compounds.

It is known, that at the isolation of explant, mechanical damages of tissues with the sharp strengthening of biosynthesis of phenol compounds are to be observed.

When phenols are going out of vacuoles, where they are localized, into protoplasm , owing to damage of tissues during isolation of explant, they are exposed to fermentative oxidation, as the result of which toxic compounds (chinons), inducing necrosis and destruction of explants, are formed (Baraboi, 1976; Zaprometov, 1993).

In spite of what, that necrotic reaction is examined as a protective supersensitive reaction, realizing with the aid of polyphenol compounds, because products of oxidation of phenols create chinon barriers, owing to which protective necrosises appear. Such necrosises are undesirable at the introduction of steriled explant into culture in vitro.

In this connection it was necessary to find a favourable time of the year for selection of exlants, when necrosis of tissue will be minimum.

MATERIALS AND METHODS

The investigation were carried out with the plants of 6 species of rhododendrons: *Rhododendron brachycarpum* D.Don. (syn. *Azalea brachycarpa* D.Don), *Rhododendron ponticum* L., *Rhododendron discolor* Franch., *Rhododendron catawbiense* Michaux, *Rhododendron japonicum* (A.Gray) Suring, *Rhododendron smirnowii* Trautv. We used buds of 6 rhododendron species as explants, isolated from young shoots in different times of the year.

Buds were sterilized in 0, 1% solution of diacid during 8 minutes with preliminary immersion in 70% ethanol during 5 seconds (Kutas and Ogorodnik, 2011). This plant material was washed three times in distillationed water for 15 minutes. Steriled buds were planted on modifiable Andersen"s medium (Anderson, 1975), which contains 4 mg/l indolylacetic acid and 15 mg/l isopentenyladenine. Cultures were put under conditions of fluorescent illumination – 4000 lk with photoperiod - 16 hours, at the temperature 25^0C and relative humidity of air was 76%. Experimental data are presented in the Table 1.

RESULTS AND DISCUSSION

According to Table 1 percentage of viable and oxidized explants depends on season, when explants were isolated and depends on species belonging of plants. Highest percentage of viable explants of species of rhododendrons (*Rh. brachycarpum* D.Don., *Rh. ponticum* L., *Rh. discolor* Franch., *Rh. catawbiense* Michaux, *Rh. smirnowii* Trautv.) obtained at the isolation in March and April. Lowest percentage (%) of viable explants of the same species of plants was obtained at the isolation in November and December; for *Rh. japonicum* (A.Gray) Suring in May-June. Low percentage of viable explants was obtained in July-August (50%

and 40% accordingly) in comparison with another months (in January-February - 85%, in March-April - 95%, in September-October - 60%, in November - December - 70%). Explants of another fifth rhododendron species, isolated in November-December, were unviable owing to phenol oxidation.

Therefore, optimum time for selection of explants for fifth introduced rhododendron species (*Rh. brachycarpum, Rh. ponticum, Rh. discolor, Rh. catawbiense, Rh. smirnowii*) should be consider spring - summer period from March to August. Probably, in this time lowering of level of phenol growth inhibitors, which were accumulated in the buds in autumn and winter for provision with relative dormant state is to be abserved.

Maybe in autumn-summer period natural auksines and cytokinines pass to the active form, which promote conservation of viability of explants and regeneration of shoots from them.

To our opinion, absence of regeneration of explants, isolated in November-December is conditioned by accumulation of phenol inhibitors in buds, which can lid to sharp lowering of endrogenic auksines and cytokinines and their transition to unactive form.

The optimum time of selection of *Rh. japonicum* explants should be consider winter-spring months from January to April, because in this time oxidation of explants is minimum (5-15% relatively).

Therefore, it is necessary to select plant material for introduction into culture *in vitro* in period of minimum oxidation of buds in order to protect explants of introduced species of Rhododendron against phenol oxidation and to get healthy regenerants from explants.

It is possible to use antioxidants against the phenol oxidation. Confirmation of above-mentioned are our results, obtained under investigation of various antioxidants action on

survival of explants of introduced plants of Rhododendron at the introduction of them into culture in vitro.

Results of experiments are presented in Table 2 for one species, because analogical data were obtained and for another species of Rhododendron (*Rh. brachycarpum, Rh. ponticum, Rh. discolor, Rh. catawbiense, Rh. smirnowii, Rh. japonicum*). Analysis of material, obtained under investigation allowed to determine, that more effective among used sterilizing compounds was polyvinylpyrrolodone in quantity 10 g/l.

CONCLUTION

Optimum time for selection of explants for introduced overgreen rhododendrons species (*Rh. brachycarpum, Rh. ponticum, Rh. discolor, Rh. catawbiense, Rh. smirnowii*,) should be consider spring-summer period from March to August; for deciduous shrub (*Rh. japonicum)* – winter-spring months from January to April.

For prevention of phenol oxidation of investigated rhododendron species explants (*Rh. brachycarpum, Rh. ponticum, Rh. discolor, Rh. catawbiense, Rh. smirnowii, Rh. japonicum)* it is expediently to add 10 g/l of polyvinylpyrrolidone into nutrient medium.

Table 1. The influence of season of selection of explants of introduced species of rhododendrons on their viability in culture in vitro(data from 20 explants with repeatability)

Time of selection	Species of rhododendron											
	Rh. brachycarpum		*Rh. ponticum*		*Rh. discolor*		*Rh. catawbiense*		*Rh. japonicum*		*Rh. smirnovii*	
	1	2	1	2	1	2	1	2	1	2	1	2
January-February	25	75	30	70	10	90	35	65	85	15	40	60
March-April	45	55	55	45	35	65	60	40	95	5	65	35
May-June	35	65	40	60	20	80	50	50	50	50	50	50
July-August	30	70	35	65	15	85	40	60	40	60	30	70
September-October	15	85	10	90	5	95	25	75	60	40	20	80
November-December	0	100	0	100	0	100	0	100	70	30	0	100

Conventional signs: 1 - percentage of viable explants, 2 - percentage oxidized explants

Table 2. The influence of antioxidants on prevention of phenol oxidation of explants of rhododendron catawbiense (medium of Andersen)

Antioxidant	Quantity of compound in medium, mg/l	Quantity of explants			
		exemplars		percentage	
		in all	alive	in all	alive
Ascorbic acid	50	50	1	100	2
	75	50	2	100	4
	100	50	10	100	20
Diethylditiocarbamate	10	50	2	100	4
	30	50	3	100	6
	50	50	12	100	24
Polyvinylpyrrolidone	1000	50	4	100	8
	5000	50	16	100	32
	10 000	50	28	100	56

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