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In vitro induction of polyploidy in *Ribes*

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Abstract

To restore the fertility of interspecific currant hybrids and to obtain tetraploidic varieties, we investigated currant polyploidization *in vitro* using microshoots and isolated embryos. Explants of *Ribes nigrum*, *R. hudzonianum*, *R. aureum*, *R. americanum*, *R. uva-crispa* and their hybrids were used for the studies. Explants were treated with colchicine and orysalin solutions in various concentrations. Colchicine (0.25 %), to a lesser extent than orysalin (from 20 to 40 μM), decreased the vitality of isolated microshoots of currants and the output of rooted plantlets. Systems of isolated embryos and microshoots are equally effective for creating polyploids currant. Chimeric plants were obtained during polyploidization *in vitro*. The total number of regenerants decreased with polyploidogen treatment, but the total number of regenerants with a higher number of chromosomes did not depend on the kind of polyploidogen and its concentration. The biggest output of regenerants was obtained by treating isolated explants with 20 μM orysalin. The frequency of regeneration after polyploidogen treatment reached 19.3 % from embryos and 32.3 % from microshoots.

Key words: colchicine, *in vitro*, oryzalin, polyploidisation, *Ribes* sp.

Introduction

The induction of chromosome doubling allows to restore the fertility of the distant hybrids, to strengthen the heterosis effect, and to create polyploidic plant cultivars (Keep 1981). Polyploidic rows have not been determined in *Ribes* sp. All species have a diploidic (2n = 16) chromosome number (Brezhnev, Korovina 1981). Polyploidy has been used episodically in the breeding of currants (Kursakov 1986). The first alotetraploidic cultivars were created by the method of seed and bud colchicination (Bauer 1978; Nilsson 1978). The investigations of currant polyploidization *in vitro* have not been carried out.

The aim of the present investigations was to test methods of currant polyploidization *in vitro* using microshoots and isolated embryos, and to evaluate the efficiency of polyploidization.

Materials and methods

Isolated embryos of currant species *R. nigrum* (cvs. 'Titania', 'Vakariai', 'Ben Alder', 'Ojebyn', 'Saniuta'), interspecific hybrids *R. nigrum* × *R. uvae-crispa* (cvs. 'Kaptivator', 'Beloruskij', 'Kuršu dzintars'); *R. nigrum* × *R. aureum* (cv. 'Corona'); *R. nigrum* × *R. petraeum* (cvs. 'Jonkheer van Tets', 'Random'); *R. nigrum* × *R. americanum* and microshoots of *R. hudzonianum* grown *in vitro* were investigated. In the different variants, from 22 to

693 embryos and from 20 to 73 microshoots in three replications were used.

The explants were treated for four days with 0.25 % colchicine or for one day by 20 μM or 30 μM orysalin. Microshoots were grown in Murashige and Skoog (1962) nutrient medium supplemented with 1 mg l^{-1} benzylaminopurine. Isolated embryos were grown in White (1943) nutrient medium adapted for currant embryos (Stanys 1997). The ploidy level of the rooted regenerants was evaluated by counting the chromosome numbers in the cells of root meristems. Significant differences between the treatment means were determined by the Duncan's multiple range test.

Results

The polyploidogens colchicine and orysalin inhibited the regeneration of currant embryos. In the control variant, 50 % of explants regenerated (Table 1). With colchicine treatment 0.8 to 8.1 % embryos regenerated, depending on the variant of the experiment. Orysalin inhibited the regeneration to the lower extent. Under orysalin treatment 15.6 to 19.3 % of the embryos regenerated.

The frequency of regeneration reached 32.3 % when microshoots of *R. hudzonianum* were treated with orysalin. The highest number (from 12 to 16 of polyploidic plants) was obtained when embryos were treated with orysalin solution and from 0 to 3 of polyploidic shoots when colchicine was used.

Table 1. The effect of colchicine and orysalin on plant regeneration *in vitro* of different *Ribes* sp. and hybrids and on the output of polyploids (explants were treated for four days with colchicine or for one day by orysalin when embryos were 45 days and microshoots 28 days old). Means followed by the same letter are not significantly different (p = 0.05)

Species or hybrid	Polyploidogen, concentration	No. of affected explants	Regenerants (%)	No. of polyploids
		Embryos		
R. nigrum	Colchicine 0 %	30	50.0[a]	0
R. nigrum	Colchicine 0.25 %	295	6.1[e]	3
R. nigrum × *R. uvae–crispa*	Colchicine 0.25 %	693	4.5[e]	1
R. nigrum × *R. aureum*	Colchicine 0.25 %	172	8.1[d]	0
R. nigrum × *R. petraeum*	Colchicine 0.25 %	358	2.5[f]	0
R. nigrum × *R. americanum*	Colchicine 0.25 %	263	0.8[f]	0
R. nigrum	Orysalin 0 μM	22	50.0[a]	0
R. nigrum	Orysalin 20 μM	202	19.3[b]	16
R. nigrum	Orysalin 30 μM	147	15.6[c]	12
LSD (1 %)			1.9	
		Microshoots		
R. hudzonianum	Orysalin 0 μM	20	40.0[a]	0
R. hudzonianum	Orysalin 20 μM	73	32.3[a]	4
LSD (1 %)			14.1	

Discussion

The polyploidogens colchicine and orysalin can be used to induce polyploids *in vitro*. In our treatments as in experiments with rhododendrons (Väinölä 2000) oryzalin was more efficient for the induction of chromosome doubling. The highest number of polyploidic plants was obtained by orysalin treatment. The number of regenerants with an increased chromosome number was directly correlated with their output. Systems of isolated embryos and microshoots *in vitro* can be used to create currant polyploids.

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