CONSIDERATIONS FOR MICROPROPAGATION OF PLANT CHIMERAS

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Abstract. A woody periclinal chimera, *Rhododendron* 'President Roosevelt', was micropropagated to study *in vitro* bud development Shoot tips were best for maintaining phenotypic stability, while leaf and floret explants gave segregated variant shoots A new chimera was captured with a reversed variegation pattern Periclinal chimeras have good potential to serve as micropropagation models, aiding in refining techniques applied to other cultivars. Phenotypic stability during micropropagation was possible in these and other chimera cultures

INTRODUCTION

This paper is partly based on a project that took place at Clemson University from 1987 to 1989, involving the micropropagation of the woody periclinal chimera, *Rhododendron* 'President Roosevelt'. Our goal was to better understand the *in vitro* bud development of a woody plant. Also included are observations of chimeras in culture at the tissue culture lab at Losely Nursery.

Plant chimeras are comprised of two or more genetically distinct layers of tissue growing adjacently in the same plant. Chimeras have been discussed in a previous IPPS article (6). Periclinal plant chimeras, with whole cell layers being genetically distinct from other whole cell layers in the apical meristem, are the most stable type of chimera. Periclinal chimeras, the topic of this paper, are maintained true-to-type by formation of axillary buds and shoots, which retain the special organization of the chimeral meristem. The formation of adventitious shoots off such plants usually results in segregation to a solid phenotype. There are quite a few horticulturally important plants which are periclinal chimeras (11). The most obvious chimeral trait is leaf color; other traits include flower color, epidermal structures, fruit color, and chromosome number. In theory, a plant could be chimeral for any trait; these traits need not be limited to only those we can visually detect.

Recent work on tissue culture of herbaceous periclinal plant chimeras has shown that these plants often do not behave stably

in vitro. Chimeral segregation has occurred in a number of systems, including pinwheel flowering African violets (*Saintpaulia ionantha*) (4), variegated strawberry plants (*Fragaria vesca*) (7), *Chrysanthemum* (2), and *Episcia* (3). Few reports exist on the culture of woody periclinal chimeras, however (1, 8, 10).

MATERIALS AND METHODS

Young budded (plants with flower buds) stock plants of *Rhododendron* 'President Roosevelt' were obtained from Bruce Briggs, Briggs Nursery, in 1987. Two studies were performed. One study involved culturing *in vivo*-derived florets, shoot tips, and vegetative buds, and *in vitro*-derived leaves, buds, and shoot tips on Woody Plant Medium (WPM) (5), 40 μM (8 mg/l) 2iP, 3% sucrose, and 0.15% Gelrite (Kelco, San Diego, CA) at pH 5.3. Explants were subcultured at 5 to 6-week intervals for about 7 months. The second study examined the effects of growth regulators on the resulting phenotypes of multiplying shoot cultures. Five 10-mm-long true-to-type *in vitro*-derived shoots per treatment were cultured on WPM, 3% sucrose, 0.15% Gelrite with 20, 40, 80, or 160 μM (4, 9, 16, or 32 mg/l) 2iP in combination with 0 or 0.5 μM (0.1 mg/l) IBA, or with a control treatment of no growth regulators. Three consecutive subcultures were done at 5-week intervals, after which shoots were transferred to growth-regulator free WPM, 3% sucrose, 1.2% Difco Bacto-agar, and 0.2% activated charcoal for one month prior to determination of phenotypes. The growth regulator experiment was done twice.

RESULTS AND DISCUSSION

Best Explant for Chimera Culture. The best explant for maintenance of a true-to-type culture was an explant containing a pre-existing meristem. *In vitro*-shoot tips were the best explants in this study (Table 1). No variegated shoots were observed in cultures of 'President Roosevelt' initiated from leaves or florets. All of the shoots from florets apparently were adventitiously regenerated. In contrast with this result is the case of chimeral pinwheel flowering African violets where true-to-type plantlets were produced from whole inflorescences (4). In *Hosta sieboldiana* 'Frances Williams', some chimeral plantlets were obtained from floret explants (9).

Effect of High Multiplication Rates. The highest multiplication rates for 'President Roosevelt' occurred on 40 μM 2iP (Table 2); this optimal level of cytokinin also resulted in the lowest percentage of true-to-type shoots. Indeed, the addition of any level of 2iP resulted in a reduction of the percentage of true-to-type shoots, relative to

Table 1. Percent true and percent variant microcuttings from six explant sources of *Rhododendron* 'President Roosevelt' cultured on WPM with 40 μM 2iP.

Explant source	Number of microcuttings	Percent true	Percent variant
In vivo source			
Floret	112	0	100
Vegetative bud	566	6.2	93 8
Shoot tip	70	21 4	78 6
In vitro source			
Shoot tip	263	50.2	49.8
Axillary bud	62	17.7	82.3
Leaf	142	0	100

Table 2. Percent true and percent variant microcuttings during shoot tip culture of *Rhododendron* 'President Roosevelt' on nine combinations of 2iP and IBA[1].

Treatment (μM) 2iP	IBA	Total shoot number	Percent true	Percent variant
0	0	10	100.0	0
20	0	199	40 7	59 3
20	0 5	86	61.6	38.4
40	0	385	36.4	63.6
40	0.5	189	53.4	46.6
80	0	184	57.1	42 9
80	0 5	106	50.0	50 0
160	0	172	55.8	44.2
160	0.5	42	59.5	40 5

[1] Totals of two experiments, 5 original shoots/treatment per experiment

the control. During these experiments, all shoots were subcultured each time; i.e., the green variants were not selectively removed from the cultures. Over time, the faster growth rate of these green shoots could result in a completely segregated culture. With observant selection against such variant shoots (in stock cultures) it was possible to keep the culture multiplying and true-to-type. Very high 2iP levels stunted shoot growth and, in the case of 160 μM 2iP with 0.5 μM IBA, were toxic.

Potential for Off-Types. As propagators, we want to use methods of increasing plants which give us clonal copies of the starting material. Of course, we also like to keep an eye out for something new, whether it be a promising seedling, an interesting sported branch, etc. During culture of periclinal chimeras, an infrequent reversal, or rearrangement, of the original variegation pattern may occur. One such reversed shoot appeared in a stock culture during this project, named *R.* 'Carolina Jewel'. This cultivar is evidently very stable *in vitro*, but occasionally produces entirely yellow variant shoots. The flower color of 'Carolina Jewel' is not yet known.

Effect of Vitrification on a Chimera Culture. A problem with culturing 'President Roosevelt' shoots on Gelrite was vitrification of some shoots, which obscured the variegated leaf pattern. This was reversible by putting Difco Bacto-agar into the medium. Even though the vitrification process itself apparently did not change the phenotype of variegated shoots, it did allow the potential for inadvertent subculture of similarly appearing green shoots, and thus could lead to subsequent loss of the chimeral condition. Experience with other variegated plants, such as *Rhododendron* 'Carolina Jewel', *Cornus kousa* 'Snowboy', and *C. kousa* 'Gold Star' has indicated this phenomenon occurs in a variety of plants.

Stabilization of Periclinal Chimera Cultures. Can periclinal chimeras be stabilized during micropropagation? Based on the cultures I have worked with, the answer is yes. Cultures of *R.* 'President Roosevelt' have been maintained for almost four years. Cultures of the new chimera, *R.* 'Carolina Jewel', have been stable for 2½ years. A variegated dogwood, *C. kousa* 'Snowboy', has been stable and multiplying in culture for seven months. A new variegated miniature rose sport has multiplied true-to-type for over one year. Based on our experience with periclinal chimeras, it is evident that they can be stabilized and multiplied with success. Attention to detail concerning the appropriate culture medium and subculture technique is essential, but this should be true for every plant in culture.

Use of Periclinal Chimeras to Test Techniques. The results of this study show that plant chimeras are useful for determining the best explant source. Also, even at low cytokinin levels in the medium, adventitious shoot formation does take place to some extent. The highest percentages of variant shoots were found on the optimal 2iP concentration. This should tell us that, certainly in the case of chimeral plants, we need to consider reducing multiplication rates, or else run the risk of ending up with aberrant cultures.

Interesting areas for further exploration using shoot cultures of periclinal chimeras include: effects of various light intensities, use of various vessel types, response to refrigeration, variations of solidifier kind and concentration, and different methods of culture subdivision.

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