Change in shoot proliferation with repeated in vitro subculture of shoots of woody species of Rosaceae

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Abstract. Shoots of 6 ornamental species and cultivars of Rosaceae were repeatedly subcultured in vitro for 9 generations on Linsmaier and Skoog (1965) medium with the addition of BA. Shoot proliferation increased over the first few generations and then gradually declined in all species and at all BA concentrations tested with the exception of Chaenomeles japonica in which a decline in shoot formation occurred only at 5.0 mg L⁻¹ BA. A decrease in shoot length and leaf size and an increase in the incidence of callus formation was observed after several subcultures. This apparently irreversible decline could be due to either genetic or epigenetic change resulting from repeated fluxes in cytokinin, nutrient status or sucrose, or to elimination of seasonal environmental fluctuations.

Introduction

Shoot cultures have not received much study during long-term culture or repeated subculture, although it has been reported that (genetically) deviant plants result from repeated subculture [4] and it is generally accepted that it is unwise in commercial micropropagation to repeatedly subculture shoots for more than three or four generations [9, 10]. Genetic aberrants probably appear spontaneously, but the possibility of more subtle change with propagative generation has not previously been examined. Benzyladenine (BA) has previously been shown to stimulate both axillary and adventitious shoot formation in species of Rosaceae [12]. This paper examines the effect of repeatedly culturing shoots of ornamental Rosaceae on BA containing media.

Materials and methods

The following species were used: Chaenomeles japonica (Thunb.) Spach, Crataegus brachycantha Sarg. and Engelm., Potentilla fruticosa L. cv. Coronation Triumph, Potentilla fruticosa L. cv. Sutter's Gold, Prunus cerasifera Ehrh. cv. Thundercloud and Prunus tomentosa Thunb.

Initial cultures were established using actively growing shoot tips (15 mm
in length) selected from field-grown plants. Shoot tips were surface sterilized after leaf removal by immersion in 0.5% sodium hypochlorite (10% Clorox bleach) for 15 minutes, and then rinsed twice in sterile distilled water. Explants were placed horizontally on the nutrient medium and were embedded to a depth of approximately one-third of their thickness. Culture tubes were slanted at an angle of 20° from horizontal.

Shoots were subcultured every four weeks (one shoot per culture tube and four replicates per treatment). Shoots were selected from the treatment which had formed the greatest number of shoots during the previous culture period.

Linsmaier and Skoog [6] nutrient medium with sucrose (30 g l⁻¹) as the carbon source and Difco Bacto agar (7 g l⁻¹) was used both for initial culture and subcultures. BA was added to the nutrient medium at each of the following concentrations: 0, 0.1, 0.5, 1.0, 2.5, 5.0 or 10.0 mg l⁻¹. The pH of the medium was adjusted to 5.8 prior to autoclaving for 20 minutes at 121 °C.

Temperature was maintained at 25 °C ± 2 °C and cool-white fluorescent light was provided at 73 μmol m⁻² s⁻¹ for 16 hours per day. Shoot number was recorded at the end of each four week culture period for a total of 9 generations.

Results

Shoot number increased over the first few generations and then gradually declined in all species and at all BA concentrations except in *Chaenomeles* in which a decline in shoot formation only occurred at 5.0 mg l⁻¹ BA. Figures 1 to 6 show fitted polynomial curves of shoot number plotted against propagative cycle. The significance of fit is given beside each figure (p < 0.05 to p < 0.001).

Other changes (to be reported in a separate paper) were also observed, particularly a decrease in shoot length and leaf size and an increase in the incidence of callus formation in later generations.

Discussion

An increase in shoot formation was observed in the first one to three generations depending on species. A similar increase has been reported for other species [15].

A decline in shoot number formed was observed beyond a certain number of subcultures for each species. However, no change in resultant plants from the seventh proliferation culture was found in blackberry [16], *Prunus* shoots (reduced in size) could be cultured for two years on medium containing BA and NAA [14], and shoot proliferation increased over 29 subcultures in *Actinidia* [15]. Although others involved in commercial