Micropropagation of Eastern Redbud (*Cercis canadensis* L.)

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INTRODUCTION

Eastern redbud (*Cercis canadensis*) is an important nursery crop native to eastern North America. It is a deciduous small tree in the legume family. Eastern redbud is a variable species with cultivars selected for lavender, pink, or white flowers (Raulston, 1990). In addition, cultivars have been selected for purple ('Forest Pansy') and variegated foliage ('Silver Cloud'). The inherent variability in this species (Robertson, 1976) indicates a potential to select additional traits such as disease resistance and drought tolerance to improve marketability. However, production of cultivars of eastern redbud have been limited because they are difficult to propagate from cuttings or grafts (Dirr and Heuser, 1987). Progress in the propagation of eastern redbud has recently suggested that cutting propagation can be successful for cuttings taken from mature trees during a narrow developmental window during early shoot development (Tipton, 1990) or with cuttings treated with relatively high concentrations of auxin (Dillion and Klingaman, 1992). Tissue culture offers a commercial alternative for the propagation for cultivars of eastern redbud (Bennett, 1987; Burkhart and Meyer, 1990; Yusnita et al., 1990). Unfortunately, commercial tissue culture production of eastern redbud has been limited by the difficulty in rooting microcuttings of this species. The objective of this communication is to detail procedures for the micropropagation of eastern redbud and the successful rooting of five mature clones.

ESTABLISHMENT OF CULTURES

Establishment into culture of actively expanding spring growth has been difficult because of contamination inherent with tissue growing in the outdoors environment. Successful cultures have been established at a high rate by selecting budwood and forcing shoots to expand in the greenhouse or growth chamber. This technique has worked very well and we have been able to utilize budwood sent through the mail for forcing. Budwood was forced in February by placing 10 to 12 stems per 250 ml of a solution containing 1.0% florist's preservative. This solution was changed as necessary. Vigorous shoots were selected when they reached 3 to 4 cm long. Leafless shoots were disinfected by washing in running tap water for 1 h. This was followed by sequentially treating the shoots with 70% ethanol (10 sec), 1,500 ppm benomyl (10 min), 10% Clorox containing 0.1% detergent (15 min), and rinsing explants with three changes of autoclaved, deionized water. Cultures were established on either WPM (Lloyd and McCown, 1980) or DKW (Driver and Kuniyuki, 1984) medium containing 0.7% agar and 10 µM benzylaminopurine (BAP) in Magenta containers. All cultures have been grown at 24°C (75°F) and a 16 h photoperiod at 30 µmol sec^{-1} m^{-2} provided by fluorescent lamps.

MULTIPLICATION OF MICROSHOOTS

Initially shoots were multiplied on WPM medium containing BAP (Fig. 1). BAP at 10 or 15 µM provided optimum microshoot multiplication from primarily axillary shoots. Thidiazuron was not effective for redbud cultures because of the induction of multiple shoots which were fasciated and failed to elongate. However, Burkhart and Meyer (1990) found suitable shoot multiplication with a combination of Thidiazuron and BAP. Redbud cultures grown on WPM medium soon developed a problem with shoot-tip necrosis. This was adequately alleviated by switching cultures to DKW medium, although additional salt substitutions may be required to completely alleviate this problem. The original work in our lab with redbud micropropagation was performed on a white flowering form (Yusnita et al., 1991). Subsequently, this protocol has also been used successfully to culture both lavender and pink flowering forms, and the cultivars 'Forest Pansy' and 'Silver Cloud'.

ROOT FORMATION IN MICROCUTTINGS

Root formation has been reported to be difficult in redbud microcuttings. Microcuttings did not root without an auxin treatment and failed to respond to quick dip treatments (Yusnita et al., 1990). We were able to achieve high rooting percentages by pulse treating microcuttings with auxin in vitro (Table 1). Previous work indicated that IBA was a more effective auxin than NAA for root induction (Yusnita et al., 1990). Microcuttings rooted at a higher percentage with IBA and the

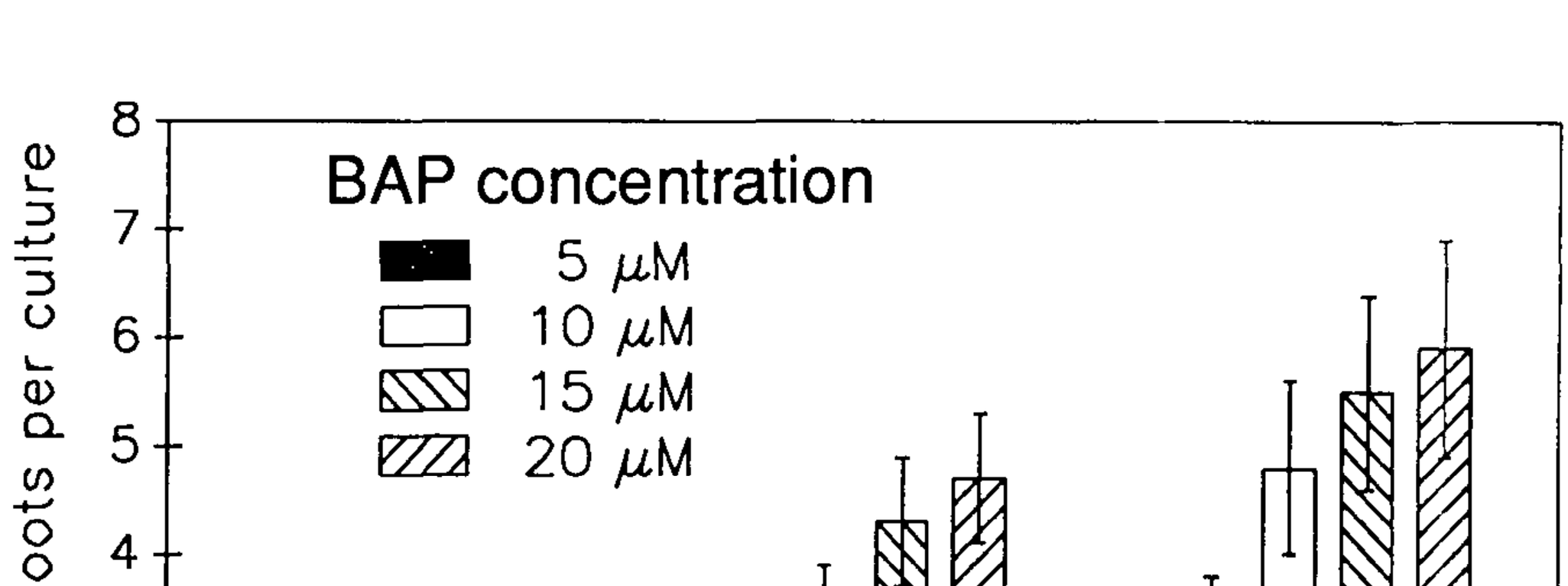

Figure 1. The effect of BAP on shoot formation in explants from white flowering eastern redbud.

subsequent roots formed were more normal with a tendency to branch. The procedure for pulse treating microcuttings consists of sticking 3- to 6- cm microcuttings on half-strength WPM medium salts containing 150 to 300 µM IBA. After 15 days, root primordia have been initiated and microcuttings can be moved to an ex vitro environment. Root development proceeds in a peat and perlite medium in cell packs under high (approx. 100%) relative humidity. Acclimatization can begin after three weeks by gradually reducing the humidity. This procedure has been very successful for rooting several mature clones of eastern redbud (Table 1). Experience with these redbud clones indicates that microcutting size influences the success of this procedure. Larger microcuttings (3 to 6 cm) root and acclimatize at the highest percentages.

Table 1. Root formation in microcuttings from four mature clones of eastern redbud treated in vitro with IBA for 15 days and subsequently rooted ex vitro in a peat and perlite medium.

Treatment IBA [µM]	Rooting (%)	No. roots per cutting
White		
Control	70	1.4
150	93	4.9
300	70	3.8
Lavender		
Control	20	0.3
150	67	3.8
300	53	1.7
Pink		
Control	40	1.0
150	83	6.7
300	83	4.3
'Forest Pansy'		
Control	0	0
300	77	2.2

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