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Tissue Culture Propagation of Some Temperate Woody Ornamentals

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Summary

Plant tissue culture is a technique of growing isolated plant parts and tissues in aseptic condition on a chemically defined medium under controlled conditions of light, temperature, and humidity. Mass propagation of many woody plants and trees that are difficult to propagate through usual cutting production methods in nursery can be better multiplied in tissue culture. Tissue culture not only offers rapid multiplication but also generates disease free clones. In vitro propagation also allows uninterrupted propagation of plants even in peak winter when the temperate plants generally embrace dormancy. Common tissue culture methods applied for mass propagation include shoot tip / nodal cultures; direct or indirect organogenesis and meristem culture. In this paper, tissue culture propagation of woody ornamentals such as redbud (*Cercis*) and birch (*Betula*) that are generally grown in temperate regions of Australia are described.

INTRODUCTION

Plant tissue culture is a method of rapidly cloning plants from isolated cells (e. g. microspores/ mesophyll cells), tissue (pith / cambium) or parts of organs (leaf segments/ nodal segments, root segments) under aseptic conditions in controlled condition of culture media, light, temperature and humidity. Tissue culture becomes an efficient and cost-effective method for rapid cloning of

many woody ornamentals when cutting production is less efficient due to poor strike rates as observed with many tree species. Tissue culture is also a preferred method when the precious mother stock is very limited in supply, for example plants coming out of quarantine during the import process. Tissue culture can provide disease free stock of plants if the cultures are initiated from disease indexed mother plants free of contagious viral/ bacterial diseases. For example, the Quality Approved Banana Nursery (QBAN) program employing this method successfully supplies millions of Bunchy Top Virus (BBTV) free banana saplings across Australia. Disease free nature of carefully cultured plants also assists with overcoming quarantine barriers for international exchange of germplasm.

Tree species are less amenable to tissue culture cloning compared to herbaceous species. Temperate woody ornamentals like redbud, birch, maple and sycamore are species that are high in commercial demand but difficult to clone in large numbers. Although there are a few published papers on tissue culture of these species (Bowen-O'Connor et al. 2007; Cheong and Pooler, 2003; Girgžde, 2017; Huang et al. 2009; Wayne et al. 1995), the published protocols are not efficient for commercial cloning of specific commercial cultivars and varieties of these species. Therefore, I undertook this research to develop protocols suitable for commercial cloning of these plants.

MATERIALS AND METHODS

Published tissue culture formulations, MS Medium (Murashige and Skoog, 1962) and Woody Plant Medium (WPM) of Lloyd and McCown (1980) were modified with phytohormones and addenda to achieve efficient cloning of these species. Commercial supply of MS medium with vitamins and WPM medium with vitamins, plant hormones, laboratory grade sucrose and other addenda were sourced from Phytotech lab (https://phytotechlab.com), USA. Pure water prepared with RO system was used throughout the experiments. A factorial system trial with hormones and addenda was followed to determine suitable media combinations to achieve best results at stages 1-4 of tissue culture.

Culture media were sterilized at 121C at 1.2 kg/cm^2 pressure for 15 minutes before use. Sterilised media were stored at 22°C in the dark around 70% humidity for at least a week before use.

Disposable, commercial food grade containers (www.genfac.com.au) were used as culture vessels throughout. Cultures were incubated in an airconditioned clean room at 25±2°C, ≤70% relative humidity illuminated to 4000 lux with cool daylight fluorescent tubes.

Plants evaluated included three cultivars of redbud (*Cercis canadensis* 'Forest Pansy', 'Merlot' and Lavender Twist ['Covey']) and purpleleaf birch (Betula 'Royal Frost'). Young, 3-4 cm nodal explants from spring sprouts were used to initiate micropropagation of all the species studied. Detergent washed explants were disinfected for 5 min with 5% (v/v) commercial bleach (Clorox ®) followed by 5 rinses with sterile water before inoculating to initiation media.

RESULTS AND DISCUSSION

Culture initiation was better under dark incubation as browning of the explants followed by tissue death was significantly higher when incubated under light. Shoot growth and multiplication was invariably better under light incubation. To achieve faster multiplication the shoots were allowed to grow for up to 6 weeks, then used

nodal segments from the axenic cultures for further multiplication. This method supplied 200-250 nodal explants per 500 ml culture vessel containing 10-12 multiplying cultures of redbud. Purple leaf birch was most proliferative and could generate 2,000 or more nodal segments per shoot cultures in a 500 ml jar in 6 weeks period. Purple leaf birch rooted on WPM medium supplemented with 0.1 mg/l IBA while redud cultivars rooted better ex vitro following incubation in MS medium supplemented with 2-5 mg /l IBA.

All the species were acclimatised in green house fitted with fine sprinklers to supply necessary levels of humidity. All the species acclimatised in a porous potting medium, when 90% or more relative humidity was maintained in the first week following the transfer. Gradual reduction in the humidity to 80% and 70% in the following weeks reduced fungal and bacterial infections on the plantlets and improved recovery of acclimatised plants.

CONCLUSION

Protocols for commercial cloning of a few redbud cultivars, purple leaf birch, maple and sycamore were developed. Micropropagation ability was higher in birch compared to redbud cultivars. Millions of in vitro rooted plantlets of these woody ornamentals per year can be generated at 10-20 US cents / plantlet using this protocol.

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