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Impact of the Leaf Cut Callus Development Stages of *Populus* on the Tetraploid Production Rate by Colchicine Treatment

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Abstract

This paper examined the impact of the leaf incision callus developmental status of *Populus* on the tetraploid production efficiency. Using diploid full-sib progeny [(*Populus pseudo-simonii* × *P. nigra* 'zheyin3#') × (*P.* × *beijingensis*)] as experimental material, leaf explants were gathered from seedlings of five genotypes selected randomly from full-sib hybrid progeny cultured in Murashige and Skoog basal medium with 0.4 mg/L 6-benzyladenine and 0.05 mg/L 1-naphthaleneacetic acid. The morphological and cytological characteristics of the incision callus (from the callus origin and adventitious bud development) were observed and divided into five stages based on the characteristics of the callus. The incision callus from each of the five stages was treated with 30 mg/L colchicine for 3 days. Then, the polyploidy level of the regenerated plants was confirmed by flow cytometry analysis and chromosome number counting. The results indicate that the rate of tetraploid production was significantly correlated with the callus development stage of *Populus* leaves; the most likely stage for chromosome doubling was Stage II, in which the calli initially formed around the cut end. To validate that callus developmental Stage II was the optimal callus developmental stage for chromosome doubling of diploid full-sib progeny, ten full-sib progeny genotypes were treated with 30 mg/L colchicine for 3 days at Stage II. All ten genotypes of the diploid progeny obtained tetraploid with no mixoploid production; the percentage of tetraploid induction was 7.9–13.2%. There were significant differences in the morphological characteristics of the leaves and roots of diploid and tetraploid plantlets.

Keywords *Populus* · Tetraploid induction · Callus development · Adventitious bud regeneration

Introduction

The genus *Populus* is an economically important model system for tree research and is widely distributing in the Northern Hemisphere (Bradshaw and others 2000). Due to the high growth performance, high-yield fiber production and resistance traits of triploid *Populus* (Kang and Zhu 2002; Yang and others 2006; Zhang and others 2012), triploid breeding programs have played an increasingly important role in *Populus* improvement. Therefore, as a parent for producing triploid trees, tetraploids have become an important breeding goal.

The major cytological mechanisms of polyploidy formation are the union of unreduced gametes, 2n pollen and somatic doubling. Of these, somatic chromosome doubling is the most common approach for obtaining tetraploids. The in vitro somatic chromosome doubling technique has been used to produce tetraploid plants artificially in forestry trees, including *Paulownia* (*Paulownia fortune*, Fan and others 2007), Betulaceae (*Betula*, Sarkilahti and Valanne 1990),

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