



Article

# Megaspore Chromosome Doubling in *Eucalyptus urophylla* S.T. Blake Induced by Colchicine Treatment to Produce Triploids

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Received: 1 November 2018; Accepted: 20 November 2018; Published: 21 November 2018



**Abstract:** Triploids generally provide an advantage in vegetative growth in forest trees. However, the technique of triploid breeding is still an open field in the *Eucalyptus* tree species. This study aims to explore the colchicine treatment technique for megaspore chromosome doubling to establish triploids in this tree species. Cytological observation on microsporogenesis and megasporogenesis was carried out to guide megaspore chromosome doubling induced by colchicine treatment. Ploidy level in progenies was detected by flow cytometry and somatic chromosome counting. A relationship between microsporogenesis and megasporogenesis was established to guide the colchicine treatment. Seven triploids were obtained in the progenies, and the highest efficiency of triploid production was 6.25% when the flower buds underwent a 0.25% colchicine solution treatment for 6 h using an aspiration method seven days after the first observation of leptotene during microsporogenesis on the floral branch. Cytological analysis showed that the megasporocyte from leptotene to diakinesis may be the optimal period for megaspore chromosome doubling by colchicine treatment. Plant height, ground diameter, leaf area, and the photosynthetic parameter of triploid eucalypt were significantly higher than those of the diploid plant at 6 months old. Hybridization with 2n megaspores induced by colchicine treatment is an effective way for *Eucalyptus* triploid breeding. These results should accelerate the development of advanced germplasms in this tree species.

**Keywords:** polyploid breeding; 2n female gamete; microsporogenesis; megasporogenesis; stomata characteristics

## 1. Introduction

Eucalypts are one of the most important planted hardwoods worldwide [1]. The remarkable characteristics of this species, including their rapid growth, straight form, valuable wood properties, and wide adaptability, have driven them to be planted over 20 million hectares [2,3], having provided key renewable resources for the production of pulp, paper, biomaterials, and bioenergy in more than 100 countries across six continents [4–7]. In addition, they also have high concentrations of diverse essential oils for medicinal and industrial uses [8,9]. As a result of many years of study,