

Article

Male Parent Identification of Triploid Rubber Trees (*Hevea brasiliensis*) and the Mechanism of $2n$ Gametes Formation

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Abstract: Eight triploids were screened among offspring of the rubber tree clone GT1 $\times$ different clones by flow cytometry and chromosome counting. Twenty-five simple sequence repeat (SSR) markers were screened to identify the origin of $2n$ gametes, to determine the male parents of these triploids, and to evaluate the mechanism of $2n$ gamete formation using band configurations and microsatellite DNA allele counting peak ratios (MAC-PR). The results showed that $2n$ gametes originated from the maternal rubber tree clone GT1, contributing the extra genome copy present in the triploids. It was confirmed that GT1 is able to produce a $2n$ megagametophyte spontaneously. Many male parents were shown to provide pollen for formation of triploid rubber trees, including clones RRIC 103, Yunyan 277-5, and three other clones. The second division restitution (SDR) was likely the main mechanism involved in formation of megagametophytes in GT1, as the rate of maternal heterozygosity restitution (HR) of all eight triploids varied from 27.78% to 75.00%, with a mean of 51.46%, and all 25 markers varied from 0% to 100%, with a mean of 51.69%. Elucidation of the origin and formation of $2n$ gametes will help optimize further sexual hybridization of polyploid rubber trees.

Keywords: rubber trees; triploids; SSR; $2n$ gamete; male parents

1. Introduction

The rubber tree, *Hevea brasiliensis*, is the only cultivated species for latex [1,2], and has often been described as an out-breeding species that is pollinated by insects such as thrips and midges [3,4]. Natural rubber is one of the most important raw materials in industry, agriculture, defense, transportation, and daily life [5,6]. Demand for rubber is increasing with economic development; however, the regions where rubber trees are planted are limited due to the stringent environmental requirements for their growth [7]. In addition, alternatives to natural rubber are still limited because synthetic rubber produced from petroleum does not match its resilience, elasticity, and abrasion resistance [8,9].

Substantial efforts have been expended to solve the problem of the imbalance between rubber supply and demand. Efforts by breeders led to many rubber tree cultivar clones being selected and planted in non-traditional planting areas, such as Chinese rubber plantations. These were established in Hainan and Yunnan Provinces, in areas as far north as 22° N, while rubber plantations are typically located in latitudes that range from 10° N to 10° S [6,10]. Two new rubber tree cultivars, Yunyan 77-2 and Yunyan 77-4, were selected and confirmed as triploids which were largely planted in Yunnan,