RIBOSOMAL DNA ORGANIZATION AND COMPOSITION IN AMERICAN AND CHINESE CHESTNUTS

Nurul Islam-Faridi[1*], Tetyana Zhebentyayeva[2], George L Hodnett[3], Laura L Georgi[4], Paul H Sisco[5], Frederick V Hebard[4], and C Dana Nelson[6,7]

[1] USDA Forest Service, Southern Institute of Forest Genetics, SRS, College Station, TX USA
[2] Department of Ecosystem Science and Management, The Pennsylvania State University, University Park, PA USA
[3] Dept. of Soil & Crop Sciences, Texas A&M University, College Station, TX USA
[4] The American Chestnut Foundation, Meadowview, VA USA
[5]The American Chestnut Foundation, Asheville, NC USA
[6] Forest Health Research and Education Center, USDA Forest Service, SRS, Lexington, KY USA
[7] USDA Forest Service, Southern Institute of Forest Genetics, SRS, Saucier, MS USA:
*m.n.faridi@usda.gov

Introduction

The American chestnut (*Castanea dentata*, $2n = 2x = 24$), once a foundation forest species over 800,000 km^2 in eastern North America, was decimated by chestnut blight caused by an introduced fungal pathogen, *Cryphonectria parasitica*. The devastating disease was first reported in 1904 by Hermann Merkel, a forester at the New York Zoological Park (Murril 1906). The disease spread rapidly, covering the entire species range by the early 1950s and killing nearly 4 billion trees. (Hepting 1974). Chinese chestnut (*Castanea mollissima*), a species closely related to American chestnut, is relatively resistant to the blight pathogen. Efforts are underway to transfer resistance from Chinese chestnut to American chestnut, including a backcross breeding program operated by the American Chestnut Foundation (Hebard 2006; *www.acf.org*) and a biotechnology-based program sponsored by the Forest Health Initiative (Nelson et al. 2014; www.foresthealthinitiative.org). Recently an integrated genetic/physical map of Chinese chestnut was published (Kubisiak et al. 2013) and the species genome has been sequenced (Staton et al. 2020); however, little cytogenetic data are available to confirm and complement these genomic resources. Fluorescence *in situ* hybridization (FISH) is an important cytogenetic technique for assigning and orienting genetic markers to specific chromosomes. In this study we assign the major 35S rDNA to LG_H and compare this linkage group chromosome between American and Chinese chestnuts.

Materials and Methods

Root tip collection and pre-treatment to accumulate chestnut metaphase chromosome spreads were carried out as described by Staton et al. 2020. Whole plasmid DNA including the 18S-26S insert of maize (Zimmer 1988) and four BAC clones [BB134N22, 1.3 cM (C5); BB171MO4, 6.3 cM (G6); BD176N08, 50.2 cM (F12); and BB055C18, 57.9 cM (F2)] were selected from LG_H BAC contigs that genetically mapped (9), two from each extreme end of the genetic map, were labeled with biotin-16-dUTP (Biotin Nick Translation Mix, Roche, USA) and/or digoxigenin-11-dUTP (Dig Nick Translation Mix, Roche, USA) following the manufacturer's instructions.

Fluorescent *in situ* hybridization coupled with epi-fluorescence microscopy to capture digital images and subsequent processing were performed as described previously (Islam-Faridi et al.

2009a, 2020). Three FISH experiments were conducted. In the first experiment, Chinese chestnut chromosome spreads were probed with four BAC clones (C5, G6, F12 and F2). In the second, BAC probes were washed off and the second FISH with 35S rDNA probe were carried out as described elsewhere (Islam-Faridi et al. 2020). In the third, American chestnut chromosome spreads were probed simultaneously with two BAC clones (G6 and F2) and 35S rDNA.

Results and Discussion

Earlier we reported the major 35S rDNA site is located terminally and sub-terminally in American and Chinese chestnut trees, respectively (Islam-Faridi et al. 2009b; Staton et al. 2020). For the LG_H chromosome identification, we used four BAC clones (see material and Methods). While analyzing BAC-FISH images for LG_H, we observed that all four BAC clones hybridized to the NOR-associated satellite pair of chromosomes, and these were concordant to the LG map but leaving about 25% of the physical (structural) body of the chromosome (Fig. 1), which has not been assigned to this genetic map. After visual microscopic identification of the NOR-associated satellite for this LG_H chromosome, we recorded the co-ordinates of a few good chromosome spread cells from this FISH slide and conducted a second FISH with the 35S rDNA probe (see materials and methods). The 35S rDNA probe hybridized at the NOR region, which is considered to be the major site for the 35S rRNA gene, and a proximal portion the satellite (Fig. 1b). In a third FISH experiment, we used two BAC clones (one from each end of the LG_H) including the 35S rDNA region on American chestnut chromosome spreads to check the physical positions of these DNA probes on the NOR-bearing chromosome of this species. As expected, all three probes hybridized to the NOR-bearing major 35S rDNA chromosome of American chestnut (Fig. 1d). The 35S rDNA hybridized terminally, distal to BAC G6 (6.33 cM), covering the entire NOR and the satellite. This indicates that the AC satellite is relatively much shorter than that of its counterpart in Chinese chestnut. Further, this comparative FISH result supports the gene and/or molecular marker(s) synteny of these two chestnut species on LG_H (Kim et al. 2005). Additional research is needed to identify the chromatin composition of the respective satellite since the 35S rDNA covered the entire satellite of the American chestnut and the proximal portion of the Chinese chestnut.

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Disclaimer: The findings and conclusions in this chapter are those of the author(s) and should not be construed to represent any official USDA or US Government determination or policy.

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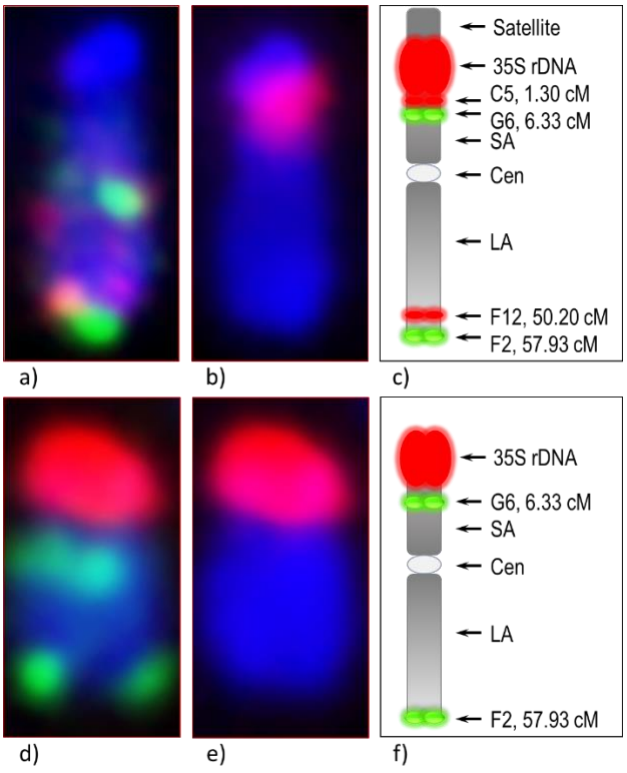

Fig. 1. The major 35S rDNA-bearing individual chromosome of American and Chinese chestnut show the physical location of the 35S rDNA locus and LG_H BAC clones. Four BAC clones on Chinese chestnut LG_H chromosome (a), the same chromosome with 35S rDNA (b, 2nd FISH); d) two BAC clones and 35S rDNA on American chestnut LG_H chromosome, e) AC LG_H chromosome with 35S signal; 'c' and 'f' are the diagrammatic illustrations of CC and AC LG_H chromosome, respectively. SA = short arm, Cen = centromere, LA = long arm.