GENETIC DIVERSITY ANALYSIS OF EASTERN COTTONWOOD USING RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS

Xiaohong Ma and Michael Stine [1]

Abstract:--The genus *Populus* is one of the most economically important wood resources in the northern hemisphere. Eastern cottonwood *(Populus deltoides* Bartr. ex Marsh) is a native poplar in the southeastern United States. DNA-based molecular marker techniques are playing increasingly important roles in genetic diversity analysis. In this research, we studied the random amplified polymorphism DNA (RAPD) variation of 259 eastern cottonwood individuals, including 57 clones from a clonal bank and 202 individual trees from natural populations along the geographic regions. In total, there were 112 fragments with 110 polymorphic bands amplified by 14 primers, with the polymorphic rate of 98.21%. Genetic distance and identity were calculated using Nei's formula, and phylogenetic relationships among subregions, river systems, and population were developed.

Keywords: *Populus deltoides* Bartr. ex Marsh, genetic diversity analysis, molecular marker, RAPD

INTRODUCTION

Eastern cottonwood *(Populus deltoides* Bartr. ex Marsh) is a native poplar in the southeastern United States, is widely grown in natural populations and plantations, and has adapted to diverse environmental conditions. After the establishment of breeding programs in the early 1960s, the focus on genetic variation within populations and among populations of eastern cottonwood has been on characteristics such as physiological adaptations, disease resistance, and morphology. Because of its rapid growth rate and good fiber quality, there is increased interest in intensive culture plantations for pulp production (Lin et al. 1994).

Molecular marker techniques, especially polymerase chain reaction (PCR)-based DNA markers, are playing increasingly important roles in genetic diversity analysis. Random amplified polymorphic DNA (RAPD) markers, which are easy to process and require only trace amounts of DNA, have been used to study genetic diversity of forest trees (Lin et al. 1994; Yeh et al. 1995). The objectives of this research were to develop RAPD markers for fingerprinting individual eastern cottonwood clones and to estimate genetic variation in eastern cottonwood in the southeastern United States.

MATERIALS AND METHODS

Leaf tissue was collected from 259 eastern cottonwood individuals, including 57 clones from clonal banks, and 202 individuals from natural populations in the southeast United States (Figure 1). The leaf tissue was stored at –80°C before DNA extraction. DNA samples were extracted using standard CTAB procedures. A total of 111 RAPD primers were screened, using 40 primers from Operon Technologies (Alameda, CA) and 61 primers from J. E. Carlson (Univ. of British Columbia, Vancouver, B. C., Canada). The RAPD analysis followed Yeh et al. (1995). RAPD bands were scored as either present (1) or absent (0). Calculations of the distance matrix and phylogenetic analysis were carried out using Popgene v 1.21 (Yeh et al. 1997).

[1] Graduate Research Assistant and Associate Professor, School of Forestry, Wildlife, and Fisheries, Louisiana Agricultural Experiment Station, LSU Agricultural Center, Baton Rouge, LA 70803

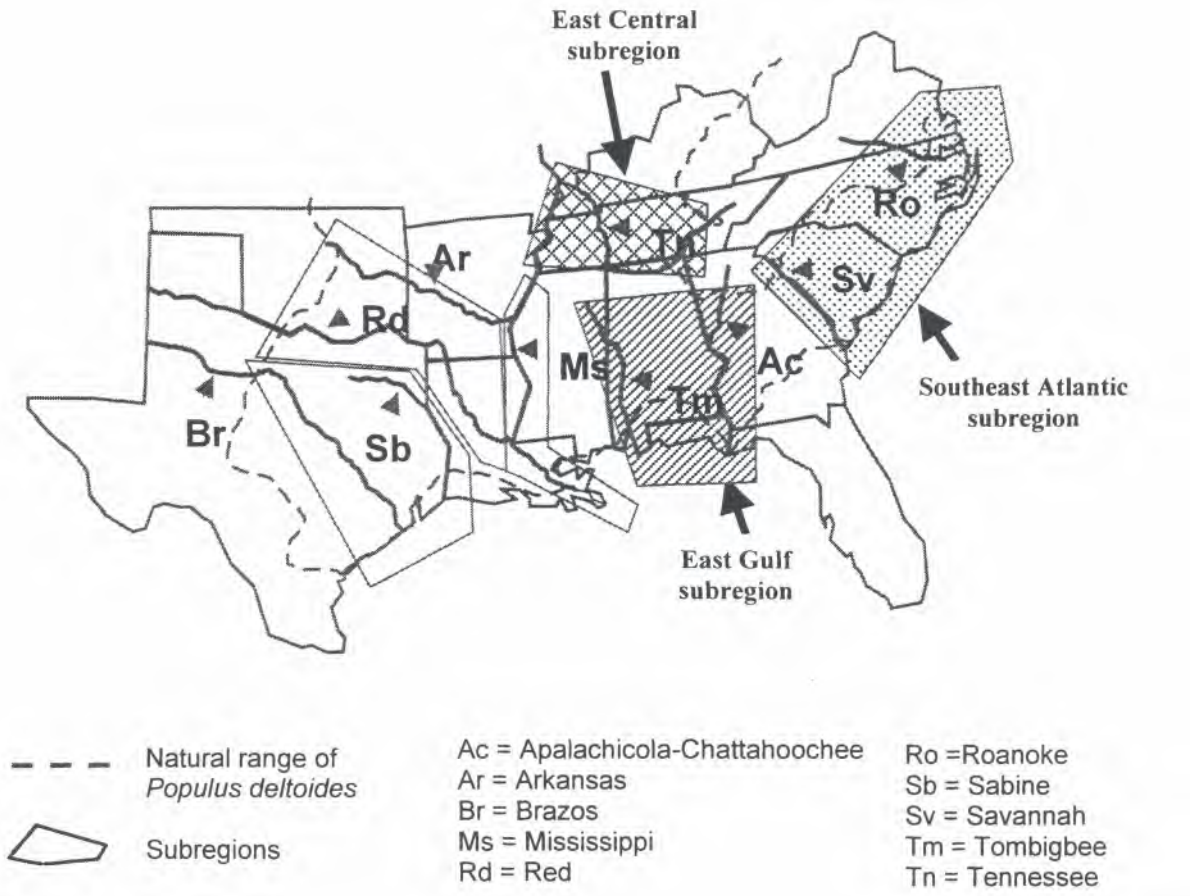

Figure 1. Subregions and river systems were represented in collections and breeding for *Populus* crop development in the southeast United States.

RESULTS

Among the 111 RAPD primers screened, 14 primers, which amplified the most scorable and polymorphic fragments, were selected for further analysis. The RAPD fragments amplified by each primer ranged from 6 to 11. A total of 110 polymorphic loci were identified, along with 2 loci that were monomorphic across the 259 eastern cottonwood individuals.

The RAPD genotypic data were used to calculate pairwise genetic distances based on Nei's formula (1978). Cluster analysis was performed using the UPGMA (unweighted pair group method average) to analyze genetic variation in eastern cottonwood. Dendrograms were produced by treating individuals from the same subregion, river system, population, and stand as groups, respectively. Figure 2 shows the dendrogram by subregion that is consistent with the geographic distributions.

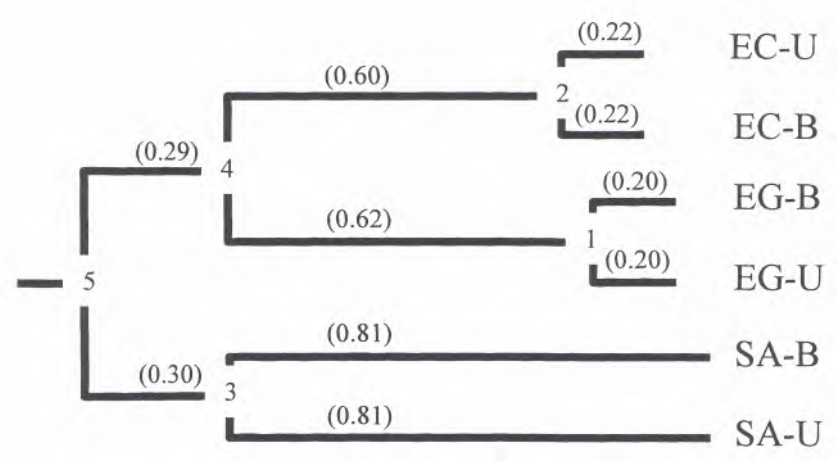

Figure 2. UPGMA dendrogram of eastern cottonwood by subregions. EC = east central; EG = east gulf; SA = south Atlanta; U = upland; B = bottomland. Values in parenthesis indicate the genetic distance.

DISCUSSION

Fusion of molecular evolution and population genetics began with the availability of protein sequence and electrophoretic data. Molecular markers developed in the last two decades allow genetic analysis directly at the DNA level. Intraspecific variation detected by genetic markers provides insights into genome evolution. The enormous amount of variation detected by RAPD markers in eastern cottonwood within and among populations is consistent with results based on isozymes (Rajaro 1990). An important factor affecting the genetic diversity assessed by molecular marker techniques is the number of markers used in the data analysis. Precision improves as more marker loci are detected in the analysis (Tivang et al. 1994). With greater capability to detect polymorphisms than traditional markers such as isozymes, and much easier to process than molecular markers such as RFLP and microsatellites, RAPD markers have great potential in genetic analysis.

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LITERATURE CITED

Lin, D, M. Hubbes, and L. Zsuffa. 1994. Differentiation of poplar and willow clones using RAPD fingerprintings. Norw J Agri Sci Sup: 67-81.

Nei, M. 1978. Genetic distance between populations. Am Nat 106: 283-292.

Rajora, 0. P. 1990. Genetics of allozymes in Populus deltoides Marsh., *P. nigra* L., and *P. maximowiczii* Henry. Journal of Heredity 81: 301-308.

Tivang, J. G., J. Nienhuis, and 0. S. Smith. 1994. Estimation of sampling variance of molecular-marker data using the bootstrap procedure. Theor Appl Genet 89: 259-264.

Yeh, F. C., K. X. Chong, and R. C. Yang. 1995. RAPD variation within and among natural populations of trembling aspen *(Populus tremuloides* Michx.) from Alberta. J of Heredity 86: 454-460.

Yeh, F. C., R. C. Yang, and T. Boyle. 1997. Popgene ver. 1.21: Microsoft Window-based freeware for population genetic analysis.