Chromosomal Mapping of 18S-25S and 5S Ribosomal Genes on 15 Species of Fagaceae From Northern Thailand

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Abstract

Fifteen species of Fagaceae from Chiang Mai province, northern Thailand, were investigated: eight Castanopsis, four Lithocarpus and three Quercus species. The species were generally diploid with the chromosome number 2n = 24, and the basic number x = 12 was confirmed in some species with meiosis. One tree belonging to Q. lentiscellatus had 2n = 14. Chromosomal mapping of the highly repetitive 18S-25S and 5S ribosomal genes by fluorescence in situ hybridisation (FISH) was performed. Most species (from all three genera) showed four 18S-25S rDNA sites (two pairs: one subterminal major and one paracentromeric/intercalary minor loci) and two 5S rDNA sites (one pair: paracentromeric loci). Quercus kerrii also had two pairs of 18S-25S rDNA sites, but both were subterminal major loci. Two species, C. argentea and Q. brandisianus, only had one pair of 18S-25S rDNA sites. Two species, C. calathiformis and L. vestitus, showed an odd number of (unpaired) sites, and this indicated hybrid origin and/or polyploidy. Polyploid cells were detected in these species. The ribosomal gene maps based on both sequences together were genus-specific. In Castanopsis, the 18S-25S and the 5S genes were localized on three different chromosome pairs, and comprised species-specific maps. On the other hand, the ribosomal genes in Lithocarpus and Quercus were found only on two chromosome pairs, because one of the two 18S-25S rDNA loci was localized on the same chromosome as the 5S rDNA locus. The FISH markers may be used to clarify discrepancies arising from morphological assessments.

Key words: Fagaceae, Castanopsis, Lithocarpus, Quercus, fluorescence in situ hybridisation (FISH), ribosomal gene mapping, 18S-25S and 5S rRNA genes.

Introduction

Fagaceae (beech family) includes 7–12 genera and 600–1000 species distributed worldwide, apart from tropical and southern Africa (SOEPADMO, 1972; SCOGGAN, 1978; CHENGJIU et al., 1999). Fagaceae dominates forests in the temperate, seasonally dry regions of the Northern Hemisphere, with a centre of diversity found in tropical South-east Asia (SOEPADMO, 1972; MANOS et al., 2001). In Thailand (FORMAN, 1964; GARDNER et al., 2000; PHENGKLAI et al., 2005), this family comprises four genera: Castanopsis (D. Don) Spach. (chestnut, mostly evergreen, 33 species); Lithocarpus Blume (stone oak, mostly evergreen, 56 species); Quercus L. (oak, mostly deciduous, 29 species); and Trigonobalanus Forman (evergreen, one species). SOEPADMO (1972) emphasized that South-east Asia, Indo-China in particular, maintained the greatest assemblage and most primitive forms of Castanopsis and Lithocarpus as well as Quercus (subgenus Cyclobalanopsis), compared to other regions of the world. Many of these species, especially in the genus Castanopsis, form part of the montane forest distribution east of Himalaya, including eastern Nepal,
north-eastern India, Myanmar, Thailand, southern China, western Indo-China and southward to the Malay Peninsula. The species diversity of this family in South-east Asia is high and the taxonomy is known to be difficult.

Based on studies of Fagaceae from the temperate regions, the genetic diversity in this family is substantial, due to morphological variation, ecological adaptation, clinal differentiation, hybridisation, gene flow and introgression. These phenomena have been well documented, especially in economically important tree genera like oaks (*Quercus*), beeches (*Fagus*) and chestnuts (*Castanea*) from Europe, North America and East Asia (e.g. MÜLLER-STARCK et al., 1992; DOW and ASHLEY, 1996; DUMOLIN-LAPEGUE et al., 1997; STREEPF et al., 1998; WANG, 2003; KANNO et al., 2004; BELLAROSA et al., 2005; CHUNG et al., 2005; LANG et al., 2006; MAGRI et al., 2006). According to floral (e.g. SCHWARZ, 1964; SCOGGAN, 1978; NIXON, 1997), species within each genus of Fagaceae, wind-pollinated species in particular, are often interfertile and hybrids are therefore common in regions where related species grow together. Such hybridisation, together with gene flow and introgression, makes taxonomic delimitation difficult.

Molecular cytogenetics has proven to be a useful tool in plant taxonomy: for identification of variation due to hybridisation and polyploidy; for studying genome and species relationships; and for identifying ancestral origin of natural hybrids and allopolyploids (ANAMTHAWAT-JÖNSSON et al., 1990; ANAMTHAWAT-JÖNSSON et al., 2001; BENNETT, 2004; KATO et al., 2005). However, advances in plant cytogenetics have been made essentially from studying large monocot chromosomes. For tree species, especially those with small chromosomes, cytogenetic data is not as extensive. The oak species examined so far have diploid chromosome number 2n = 24, and very similar karyotypes, regardless of the substantial genetic diversity within the genus *Quercus* (e.g. MEHRA et al., 1972; OHRI and AHUJA, 1990; D’EMERICO et al., 1995). In general, the family Fagaceae shows a very stable chromosome number with all genera and species having 2n = 24 except *Nothofagus* with 2n = 26 (CARR and McPHERSON, 1986; OHRI and AHUJA, 1990 and 1991). Striking similarities in terms of genome size among different *Quercus* species, and genome organization based on mapping of the ribosomal repeats (18S-25S and 5S rRNA genes), have also been reported (ZOLDOS et al., 1998; ZOLDOS et al., 1999). Differentiation among some temperate oak species at the chromosome and whole genome level appears to be small, particularly between the two European white oak species, *Q. robur* and *Q. petraea*. The species status has been questioned by some oak specialists, who proposed that the two oaks should be ecophysiological types rather than good species. They are interfertile, as many pairs of oak species are, and the evidence based on biochemical and molecular markers supports the hypothesis that any differences, allelic or genomic, are too small for differentiation at species level (GOMORY et al., 2001; MUIR et al., 2001; ZOLDOS et al., 2001).

The knowledge about genetic differentiation among Fagaceae species in tropical South-east Asia is limited. The species have been identified taxonomically based on morphological characteristics (e.g. PHENGKLAI, 2004), but genetic or evolutionary relationships among taxonomically related species have not been systematically investigated. Most studies on tropical Asiatic species of Fagaceae involve analysis of genetic diversity and phylogeography based on molecular markers (CANNON and MANOS, 2003; BLAKESLEY et al., 2004; CHENG et al., 2005). The objective of the study reported here was therefore to characterize genetic relationships among selected Fagaceae species from northern Thailand, using molecular cytogenetic markers from 18S-25S and 5S ribosomal gene families. The ribosomal gene maps were expected to reveal species differentiation within this region, which could be used to clarify discrepancies arising from morphological assessments.

**Materials and Methods**

**Plant materials**

Plant samples were from *Khun Mae Kuang* Forest in Doi Saket district, Chiang Mai province, at approximately 18.87N/99.14E, northern Thailand. All samples were collected in the field during the period 2002 to 2005 by authors of this paper. Six locations, representing three types of habitats, were selected: hill-evergreen forest (code names PA & NK) and hill-evergreen forest with pine (BA & RD), both at relatively high elevation (1000–1800 m), and dry-deciduous forest at altitudes below 800 m (PS & HH). At each location, twenty trees or more, 5–30 m tall and 30–100 m apart, were randomly selected, marked with identification number, and local names recorded together with their position/map within the forest. Leaf buds, and flower buds if available, from individual trees were collected for chromosome isolation. In a few cases it was also possible to obtain root tips from just below the soil surface.

Twigs with fully grown leaves, fruits (acorns) and flowers were collected for taxonomic identification and kept as voucher specimens. Taxonomic identification followed Flora Malesiana (SOEPAITO, 1972) and the report on Fagaceae that was prepared for the Flora of Thailand (PHENGKLAI et al., 2005). The plants were identified to genus based on flowers, acorns, leaves and other vegetative characteristics, but species identification was based primarily on acorns. Acorns of *Castanopsis* are usually covered by spiny cupules, whereas acorns of *Lithocarpus* and *Quercus* are mostly or partly covered by non-spiny cupules (Fig. 1). Thirty different Fagaceae species were identified from this collection (unpublished results), but only fifteen species were included in this chromosome study: eight *Castanopsis*, four *Lithocarpus* and three *Quercus* species (Table 1).

**Chromosome preparation**

In the field, samples of leaf buds, flower buds or root tips were placed in iced water (4°C) for 23–27 h, to arrest metaphases. After this, the samples were fixed in a 3:1 mixture of absolute ethanol and glacial acetic acid, and the samples kept at −20°C in this fixative until use. Chromosomes from leaf buds were isolated according to ANAMTHAWAT-JÖNSSON (2003), with slight modification in the enzyme digestion step and the hypotonic treatment. Each sample was digested for at least 3–4 h at room
temperature in 100 µl of the enzyme mixture. Ten ml of this enzyme mixture contained 500 units of Cellulase Onozuka R10 (no. 102321, Merck, Germany), 280 units of Pectinase (P4716, Sigma, USA) in a buffer containing 75 mM KCl and 7.5 mM EDTA, pH 4. After digestion, the filtered protoplast suspension was treated with hypotonic solution (1.5 ml of cold 75 mM KCl) for 15 min at room temperature. The protoplasts were cleaned with fresh fixative 3–4 times, before being dropped onto microscopic slides. After staining with the fluorochrome 4, 6-diamidino-2-phenylindole (DAPI), chromosome number was determined under 1000x magnification in an epifluorescence microscope Nikon Eclipse 800. The images were captured with a Nikon DXM 1200F digital camera. The DAPI stained preparations were then used for fluorescence in situ hybridisation (FISH) experiments. Chromosomes from flower buds and root tips were isolated using the squash method as in ANAMTHAWAT-JÖNSSON (2001).

**Fluorescence in situ hybridisation (FISH)**

FISH was performed using a method modified from SCHWARZACHER and HESLOP-HARRISON (2000) and ANAMTHAWAT-JÖNSSON (2001). Two ribosomal DNA probes were used for double-target FISH: (1) Clone pTa71, a 9-kb fragment from wheat, which contained a part of 18S and the entire 5.8S and 25S coding region, together with non-transcribed spacers (GERLACH and BEDBROOK, 1979), was used as an 18S-25S rDNA probe. (2) Clone pTa794, which contained a complete 410-bp BamHI fragment of the 5S rRNA gene and spacer regions from wheat (GERLACH and DYER, 1980), was used as a 5S rDNA probe. The rDNA probes were labelled by standard nick translation, using linearized cloned fragments as templates. Fluorescent labels used in this study were the followings: (1) Red labels from Rhodamine-4-dUTP (Amersham, no longer available), SpectrumRed-dUTP (Vysis, USA), ChromaTide Alexa Fluor 568-5-dUTP (Molecular Probes, USA), and Cy3-dUTP (Amersham, GE Healthcare, Sweden). (2) Green labels from Fluorescein-11-dUTP (Roche Applied Science, Germany), and ChromaTide Alexa Fluor 488-5-dUTP (Molecular Probes). The labelled probes were purified through ProbeQuant G-50 Micro Column (Amersham – GE Healthcare) following the manufacturer’s protocol.

Before performing FISH experiments, the chromosome preparations were first treated with fresh fixative for 10 min at room temperature, washed twice with 96% ethanol, and air dried. The preparations were then treated with RNase-A (5 µg/ml) for 1 h at 37°C, proteinase-K (4–10 µg/ml) for 20 min at 37°C, and paraformaldehyde (4%, w/v) for 20 min at room temperature. In FISH experiments, 50 ng each of the 5S and the 18S-25S rDNA probes, which were labelled with different fluorescent colours, was applied to a chromosome preparation, together with 50% formamide, 20% dextran sulphate, 2xSSC and 0.5% SDS. The probe and the slide were denatured together at 89°C for 20 min, in a PTC-100 thermocycler with slide chambers (MJ Research, MA, USA), after which hybridisation was allowed to take place overnight at 37°C. The post-hybridisation washing steps included a stringent wash in 0.1xSSC at 60°C for 15 min. The chromosomes were stained again with DAPI and examined in an epifluorescence microscope using appropriate filters.

**Results**

The Fagaceae species under study had the expected 2n chromosome number of 24 (Table 1, an example shown in Fig. 2a). Each species was represented by at

<table>
<thead>
<tr>
<th>Species</th>
<th>Tree number</th>
<th>2n chromosome number</th>
<th>Number of 18S-25S rDNA sites</th>
<th>Number of 5S rDNA sites</th>
<th>Number of cells analysed for FISH</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castanopsis acuminatissima (Blume) A.D.C.</td>
<td>BA22, NK15</td>
<td>24, 24 ^w(NK15)</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>a</td>
</tr>
<tr>
<td>Castanopsis argentea (Blume) A.D.C.</td>
<td>PA14, RD24</td>
<td>24</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>a</td>
</tr>
<tr>
<td>Castanopsis armata (Roxb.) Schap.</td>
<td>NK16, PA17</td>
<td>24</td>
<td>4</td>
<td>2</td>
<td>11</td>
<td>a</td>
</tr>
<tr>
<td>Castanopsis calathiformis (Skan.) Rehder &amp; Wilson</td>
<td>NK5, PA25</td>
<td>36 ^w(NK5)</td>
<td>3</td>
<td>2</td>
<td>13</td>
<td>a</td>
</tr>
<tr>
<td>Castanopsis cerebrina (Hickel &amp; A. Camus) Barnett</td>
<td>NK23, PA26</td>
<td>24</td>
<td>4</td>
<td>2</td>
<td>12</td>
<td>a</td>
</tr>
<tr>
<td>Castanopsis diversifolia (Kurz) King &amp; Hook.f.</td>
<td>NK1, PA12</td>
<td>24</td>
<td>4</td>
<td>2</td>
<td>8</td>
<td>a</td>
</tr>
<tr>
<td>Castanopsis indica (Roxb.) A.D.C.</td>
<td>PA4, PA18</td>
<td>24</td>
<td>4</td>
<td>2</td>
<td>6 (±2 meiotic)</td>
<td>a</td>
</tr>
<tr>
<td>Castanopsis tribuloides (Sm.) A.D.C.</td>
<td>RD4, RD14</td>
<td>24</td>
<td>4</td>
<td>NA</td>
<td>6</td>
<td>a</td>
</tr>
<tr>
<td>Lithocarpus ceriferus (Hickel &amp; A. Camus) A. Camus</td>
<td>BA19, RD1</td>
<td>24</td>
<td>4</td>
<td>NA</td>
<td>3</td>
<td>a</td>
</tr>
<tr>
<td>Lithocarpus elegans (Blume) Harus ex Soepadmo</td>
<td>BA20, NK24</td>
<td>24</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>a</td>
</tr>
<tr>
<td>Lithocarpus polyacanthus (A.D.C.) Rehder</td>
<td>BA9, RD7</td>
<td>24</td>
<td>4</td>
<td>2</td>
<td>7</td>
<td>a</td>
</tr>
<tr>
<td>Lithocarpus vestitus (Hickel &amp; A. Camus) A. Camus</td>
<td>NK10, PA11</td>
<td>24, 48 ^w(NK10)</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>a</td>
</tr>
<tr>
<td>Quercus bradidentus Kurz</td>
<td>RD3, PS9</td>
<td>24</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>Quercus kerrii Craib</td>
<td>HH9, PS10</td>
<td>24</td>
<td>4</td>
<td>2</td>
<td>12</td>
<td>a</td>
</tr>
<tr>
<td>Quercus lentiscusus Barnett</td>
<td>HH18, RD17</td>
<td>24, 14 ^w(RD17)</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>a</td>
</tr>
</tbody>
</table>

Table 1. – Fagaceae species and individual trees examined. They were from different forest types: hill-evergreen forest (NK & PA), hill-evergreen forest with pine (BA & RD) and dry-deciduous forest (HH & PS). Chromosome number was determined from metaphases from leaf buds, and in some samples also from root-tips (*). FISH mapping of the 18S-25S and 5S ribosomal genes revealed the number of hybridisation sites per cell. Note: a) One pair major and one pair minor 18S-25S rDNA sites.
Figure 1. – Typical acorns of Fagaceae species in this study: (a) Castanopsis argentea, tree RD24; (b) Lithocarpus polystachyus, tree BA9; and (c) Quercus kerrii, tree HH9.
least two trees from different locations. The 2n chromosome number for each tree was determined from 10–30 well-spread metaphases isolated from several leaf buds, which were in some cases collected in the field at different times of the year. No variation in chromosome number within sample (tree) was detected using this type of tissue, i.e. leaf buds. Samples of root tips were collected from a few trees in the NK forest and two of these trees had polyplody roots. The tree NK5 (Castanopsis calathiformis) had triploid root cells with 36 chromosomes (Fig. 2p), while the tree NK10 (Lithocarpus vestitus) had tetraploid root cells with 48 chromosomes (Fig. 2t). Metaphases from root tips of other trees (for example Fig. 2d) showed the normal 2n = 24 as in leaf cells.

One sample belonging to Quercus lenticellatus (tree RD17) had the 2n chromosome number of 14 (Fig. 2w), whereas another tree from this species (HH18) had the normal 2n = 24. All other Fagaceae species and samples had 24 chromosomes in their diploid cells and therefore the basic number was x = 12. This was supported by a meiotic analysis of two species of Castanopsis, i.e. C. indica and C. tribuloides, whereby 12 bivalents were observed (Fig. 2g).

Fluorescence in situ hybridisation (FISH) mapping of the 18S-25S and 5S ribosomal genes on chromosomes of five Fagaceae species was performed and the results were brought together in Table 1. Examples of FISH results are shown in Fig. 2 and ideograms showing ribosomal gene maps on selected species are presented in Fig. 3. Most species had four sites (two pairs) of 18S-25S rRNA genes and two sites (one pair) of 5S rRNA genes (Fig. 2). Based on FISH signal intensity, the four 18S-25S rDNA sites in Castanopsis and Lithocarpus consisted of one pair major and one pair minor sites. The major locus was subterminal (subtelomeric) and the minor locus paracentromeric or intercalary (Fig. 3). The major locus coincided with nucleolar organizing region (NOR) at the secondary constriction of satellite (SAT)-chromosomes (results not shown). Quercus kerrii also had two pairs of the 18S-25S rDNA sites, but both pairs were subterminal (Fig. 2v and Fig. 3g). Both were probably major loci, but this required confirmation with silver staining. Two species, C. argentea and Q. brandisianus, only had one pair of 18S-25S rDNA sites (Fig. 2i). The sample with 2n = 14 (Q. lentiscus) showed two pairs of 18S-25S rDNA sites (Fig. 2x) and one pair of 5S rDNA sites, as did most of the Fagaceae species under study.

Odd numbers of ribosomal sites, or unpaired sites, were detected in two species, C. calathiformis and L. vestitus (Table 1). This observation was consistent in all cells examined, and in both trees of each species. Castanopsis calathiformis had three (unpaired) 18S-25S rDNA sites (Fig. 2n and Fig. 2o), whereas the 5S rRNA genes seemed to be in one pair of hybridisation sites, like in other species. Root tip chromosome preparations of one tree in this species (tree NK5) revealed a triploid chromosome complement (Fig. 2p); this triploid number (36) was seen in all metaphases examined and from several roots. However, leaf chromosome preparations produced variable results. The tree NK5 had a diploid chromosome number of 24 in the leaf cells (Fig. 2m), but the other tree belonging to this species (PA25) appeared to have two chromosome numbers, 24 and 28. The aneuploidy could cause reduced fertility of that particular tree. Neither of these trees produced flowers or acorns during the four-year period of field work. These two trees were from different locations, several kilometres apart. Lithocarpus vestitus was another species that showed multiple and unpaired ribosomal sites: six 18S-25S rDNA sites with variable FISH signal intensity and three sites of the 5S rRNA genes (Table 1, Fig. 2e). Chromosome preparations from root tips of the tree NK10 showed a tetraploid number of 2n=48 (Fig. 2t), although the leaf chromosome number was 2n = 24. FISH results of C. calathiformis and L. vestitus indicated polyplody in Fagaceae, but more trees need to be examined before a definite conclusion can be made.

Among the Fagaceae species under study, the 18S-25S ribosomal gene maps were variable both in terms of chromosomal location and the chromosomes bearing these genes, while the 5S rDNA maps were more conserved (Fig. 3). The 5S rDNA locus was always paracentromeric (see for example in Fig. 2h and Fig. 2v), and was localized on one pair of large submetacentric chromosomes. In Castanopsis (represented by five species in

**Figure 2.** – Fluorescence in situ hybridisation (FISH) of the 18S-25S and 5S rRNA genes to chromosomes of Castanopsis (a–p), Lithocarpus (q–t) and Quercus (u–x). The scale bar represents 5–8 μm. *Castanopsis acuminatissima* (a–d), the most common Castanopsis species in this region, has two pairs of (green) 18S-25S rDNA sites and one pair of (red) 5S rDNA sites. The 18S-25S rDNA loci consist of one pair of major and one pair of minor sites (b & c). The major locus is subterminal, as seen in DAPI-stained metaphase (a), and in this cell the major sites lie very close to each other (b). This species, like most species in this study, is diploid with 2n = 24 in both leaf buds (a) and root tips (d). *Castanopsis indica* (e–h) also has two (major and minor) pairs of 18S-25S rDNA sites and one pair of (red) 5S rDNA sites. The major 18S-25S locus is also subterminal (e & f), which is the case in all Fagaceae species. The 18S-25S and 5S rDNA genes in Castanopsis are on different chromosome pairs, and this is confirmed in meiosis (g & h), whereby all three ribosomal loci are on different bivalents. *Castanopsis argentea* (i) only has one pair of (green) 18S-25S rDNA sites, the major locus, and one pair of (red) 5S rDNA sites. *Castanopsis cerebrina* (j & k) and *C. diversifolia* (l) have two (major and minor) pairs of 18S-25S rDNA sites (red in j & k, green in k) and one pair of 5S rDNA sites (red in k, green in l). *Castanopsis calathiformis* (m–p) is mainly diploid in its leaf buds (m) but triploid in root tip cells (p). In the leaf metaphases, three sites of (red) 18S-25S rRNA genes can be seen (n & o), whereas the 5S rDNA locus (green) is on one chromosome pair (o). *Lithocarpus polyplastyxus* (q), like *Castanopsis*, has two (major and minor) pairs of 18S-25S rDNA sites. *Lithocarpus vestitus* (r–t), unlike all other species, has multiple ribosomal sites, i.e. six (green) 18S-25S rDNA sites with varying FISH signal intensity and three (red) 5S rDNA sites in its diploid leaf bud cells (r & s). Root tip cells are tetraploid (t). *Quercus kerrii* (u & v), the most common *Quercus* species in this region, is diploid with 2n = 24 (u) and has two sites of the 18S-25S rDNA sites (red, both are subterminal major loci) together with one pair of (green) paracentromeric 5S rDNA sites (v). The 5S rDNA locus is on the same chromosome arm as one of the major 18S-25S loci. *Quercus lentiscus* (w & x), tree RD17, has 14 chromosomes in all leaf metaphases examined (w). This tree has two pairs of 18S-25S rDNA sites (red) together with one pair of (green) 5S rDNA sites (w). The 5S locus is adjacent to one of the 18S-25S loci (indicated with an arrow).
Subterminal (another major locus in homologous pairs. The basic number (x) for this tree status of these samples, as the chromosomal loci were in mapping of the ribosomal genes supported the diploid Q. lenticellatus basic number x = 12. The exception is that one species, diploid with the chromosome number 2n = 24 and the basic number x = 12. Other studies have shown that species of Quercus in Europe and Asia are also diploid with chromosome number 2n = 24 and the basic chromosome number x = 12 (e.g. Wang, 1986; Ohri and Aihua, 1990; D’Ememico et al., 1995; Zoldos et al., 1999). The same chromosome number was found in two Castanopsis species (Huang et al., 1989), but there are no records on chromosome number of Lithocarpus. Cytogenetic data on tropical and subtropical species of Fagaceae has so far been limited, probably due to difficulty in obtaining plant materials for the conventional root-tip chromosome preparation together with difficulty in counting small chromosomes accurately. In the present study, a newly developed protocol for chromosome preparation from leaf buds (Azamthawat-Jonsson, 2003) was used. This type of plant material is always available in the field. In addition, the protoplast dropping technique can produce well-spread metaphases suitable for counting and karyotyping, as well as for molecular cytogenetic experiments.

The basic chromosome number x = 12 found in this study falls within the general range of the basic number mode of most woody plant genera. In the floras of the temperate zone, trees and shrubs have, on average, lower frequencies of polyploidy within a genus than perennial herbs, but they have higher basic numbers (Stebbins, 1971). Woody plants of tropical regions resemble those of the temperate zone in the rarity of polyploid series within a genus, and their basic numbers are similar, with a mode at x = 11, 12, 13 and 14, which is significantly higher than the mode for temperate herbs, which has basic numbers x = 7, 8 and 9 (Darlington and Wylie, 1955). Stebbins (1971) explained that basic numbers of modern woody genera were derived by ancient polyploidy, and that the original basic numbers of angiosperms, both woody and herbaceous, were x = 6 and x = 7. Although tropical floras are still much more poorly known cytogenetically than temperate ones, it is becoming evident that most woody families of angiosperms include basic numbers of x = 7–9, and that the great majority of these genera are tropical, in agreement with the hypothesis that temperate woody groups.
have, in general, been derived from tropical ancestors (BRIEGS and WALTERS, 1997). Molecular data has begun to reveal the polyploid nature of many present-day diploid plant species (reviewed in SOLTIS and SOLTIS, 1999; SOLTIS et al., 2003; BENNETT, 2004). Our discovery that one Quercus tree has the somatic (leaf) chromosome number of 14 may indicate the existence of the basic chromosome number x = 7 among tropical Fagaceae trees. More trees from this region will be examined.

Two species, C. calathiformis and L. vestitus, showed unpaired rDNA sites in diploid cells. Some cells in these samples were polyploid or aneuploid. Unexpectedly, the polyploid cells were obtained from root-tip meristems, not from leaf tissues. On the other hand, the aneuploidy occurred in leaf buds. Such variation within a plant is difficult to explain. A genetic distance analysis using molecular markers (unpublished results) has indicated that C. calathiformis could be an interspecific hybrid involving Lithocarpus. This species has non-spiny acorns, which is an unusual character for Castanopsis (see typical acorns in Fig. 1). Castanopsis and Lithocarpus co-exist in hill-evergreen forests of this region and both are pollinated by generalist insects, whereas the wind-pollinated Quercus is dominant in deciduous forests. Further study will be carried out, both to verify the hybridisation or polyploidy in these samples and to explore the extent of such variation among Fagaceae trees in these forests.

Ribosomal gene maps of selected Fagaceae species from northern Thailand were found to be highly variable, both in terms of location of the 5S and 18S-25S rRNA genes and the chromosomes bearing these genes. A typical Castanopsis map consisted of three pairs of chromosomes bearing, separately, one paracentromeric 5S rDNA locus and two 18S-25S rDNA loci, one of which was a subterminal major locus and the other a paracentromeric or intercalary minor locus. In the few species of Lithocarpus and Quercus studied, the location of these genes varied considerably, and was completely different from the Castanopsis maps in that all of the loci were localized on only two pairs of chromosomes. The 5S rDNA locus was on the same chromosome arm as the minor or the second major 18S-25S rDNA locus. The ribosomal gene map of several European Quercus species was shown to be highly conserved (ZOLDOS et al., 1999), but the only similarity to our Fagaceae maps seems to be in the 5S rDNA gene. Genetic diversity within the family Fagaceae is undoubtedly large, especially within Quercus, and this is often reflected in the complex taxonomic classification. For example, Quercus spp from Europe belong to the subgenus Quercus while the subgenus Cyclobalanopsis comprises species from South-east Asia (MANOS et al., 2001). Present distribution of this genus is also extensive, covering both temperate and tropical regions, whereas Castanopsis and Lithocarpus are mainly found in the tropical and subtropical areas (SOEPADMO, 1972).

Ribosomal gene maps, constructed using both 18S-25S and 5S rRNA genes, could be used for tracing species origin, analysing species relationships and resolving taxonomic discrepancies. Ribosomal gene mapping has been shown to be useful, for example in differentiating between closely related tree species such as those found within Picea and Pinus genera (BROWN and CARLSON, 1997; SILIJA-YAKOVLEV et al., 2002; LIU et al., 2003). These species have large chromosomes, which are convenient for FISH karyotyping. Although broadleaf tree species tend to have small and compact chromosomes, the FISH application is becoming more visible, as shown in the present study and in others (e.g. ANAMTHAWAT-JÓNSSON and HESLOP-HARRISON, 1995; ZOLDOS et al., 1999; CORREDOR et al., 2004). A number of taxonomic questions arising from the present study will be examined further. For example, Castanopsis fissa appears to have a ribosomal gene map similar to that of Lithocarpus. Other evidence, molecular and morphological, also indicates that C. fissa is more closely related to Lithocarpus than to other species of Castanopsis (MANOS et al., 2001). Molecular cytogenetic markers offer distinct advantages as they can screen cryptic, cytotypic and/or ploidy variation that influence genetic architecture and reproductive potential of a population (LAVANIA, 2002). Some cytotypic variation has been detected in Fagaceae for the first time in the present study, and it is interesting to carry out further research on the extent and impact of this variation. It is important to know the structure and behaviour of chromosomes and genomes in order to elucidate the evolutionary potential of a population, which can help complement conservation plans.

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References


Evaluation of Early Rooting Traits of Eastern Cottonwood That Are Important For Selection Tests

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Abstract

Vegetative propagation of superior genotypes via stem cuttings depends on their development of strong vigorous root system. Fourteen characters of cutting rooting were examined in multiannual tests with 12 genotypes of eastern cottonwood (Populus deltoids BARTR. EX MARSH) in course of evaluation of their utilization in selection tests. Variability and relationship among examined characters, and cutting survival rate were analyzed according to contribution of expected variances to the total variance and results of principal component analysis, stepwise regression analysis and path analysis. Along with total number and length of first-order roots, the characters that are regularly used in the assessment of rooting potential, our results signify dynamic shoot growth and uniform arrangement of roots on cutting at the beginning of growing period. The best results were obtained for shoot length at the second half of May. A rapid and non-destructive way of shoot characters' measurement allows testing of larger material and prevents losses in propagation material of interesting genotypes. Alone or together with total root number and length these alternative characters could be used for the improvement of selection tests and procedures for cultivar technology design.

Key words: Populus deltoids, cutting rooting characters, cutting survival rate, multivariate analysis.

Introduction

The hardwood cuttings of black poplars (section Algeiros DUBY) are characterized by good rooting