THAI FOR. BULL. (BOT.) 35: 38–44. 2007.

Karyotypes of some species of *Castanopsis, Lithocarpus* and *Quercus* (Fagaceae) from Khun Mae Kuong Forest in Chiang Mai province, northern Thailand

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ABSTRACT. Karyotypes of 18 species belonging to three genera, *Castanopsis* (9), *Lithocarpus* (5) and *Quercus* (4) of Fagaceae from northern Thailand were constructed. All species showed diploid (2n = 2x = 24) chromosome numbers, with the basic number being 12. Meiosis from anthers of two *Castanopsis* species confirmed the diploid status. Metaphase chromosomes are relatively small, about 2–5 ìm in size, and all are metacentric and submetacentric chromosomes. The arm-ratio measurement revealed karyotypic variation among the species investigated – the chromosome complement consists of 8–12 pairs of metacentric and 0–4 pairs of submetacentric chromosomes. This is the first report of karyotypes of *Castanopsis* and *Lithocarpus* from Thailand. The karyotypic description of the *Quercus* species examined here is substantially different from that of European species.

KEYWORDS: *Castanopsis*, Fagaceae, karyotype, *Lithocarpus*, *Quercus*.

INTRODUCTION

Fagaceae includes 7–12 genera and 600–1000 species distributed worldwide except in tropical and southern Africa (Soepadmo, 1972; Nixon, 1997; Chengjiu et al., 1999). The family dominates forests in the temperate, seasonally dry regions of the Northern Hemisphere, with a centre of diversity in tropical South-east Asia. In Thailand the family comprises four genera: *Castanopsis* (D. Don) Spach., *Lithocarpus* Blume, *Quercus* L. and *Trigonobalanus* Forman. Altogether there are 119 species, one subspecies and one variety (Phengklai et al., 2005). Based on studies of Fagaceae from the temperate regions, genetic diversity in this family is expected to be substantial, due to morphological variation, ecological adaptation, clinal differentiation, hybridisation, gene flow and introgression. Such complex diversity patterns have made taxonomic delimitation of species a difficult task.

Cytogenetics has been used primarily as a tool in cytotaxonomy of plants, as the plant genomes are extremely variable. A very large part of this variation is due to hybridisation and polyploidy (Bennett, 2004; Soltis et al., 2003). The difference in chromosome number alone is in certain circumstances sufficient to differentiate species, because such difference will create meiotic pairing abnormality and infertility in the hybrids,

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and as a consequence restricting gene flow between species. As for the family Fagaceae, most chromosome data are available from some investigated species of *Quercus* in Europe (Ohri & Ahuja, 1990; D'Emerico et al., 1995; Zoldos et al., 1999). Cytogenetics of tropical species of Fagaceae has never been reported. This study therefore presents the first report on karyotypes of 18 species from three genera of Fagaceae from northern Thailand.

MATERIALS AND METHODS

Plant samples were collected from *Khun Mae Kuong* Forest in Doi Saket district, Chiang Mai province, at approximately 18.87 N/99.14 E, northern Thailand. Taxonomic identification followed the family treatment in Flora Malesiana (Soepadmo, 1972) and the report on Fagaceae prepared for the Flora of Thailand (Phengklai et al., 2005). A total of 18 species were examined cytogenetically in this study: nine *Castanopsis*, five *Lithocarpus* and four *Quercus* species (Fig. 1, Table 1). Leaf buds from all samples were collected for chromosome isolation, chromosome number determination and karyotyping. Male flower buds were also collected when available for meiotic analysis. In the field, samples (leaf buds) were placed in iced water (4°C) for 23–27 h, to arrest metaphase. The samples were then fixed in a 3:1 mixture of absolute ethanol and glacial acetic acid, and were stored at -20°C in this fixative until investigation.

The chromosome isolation procedure followed Anamthawat-Jónsson (2003)'s a protoplast dropping method developed for leaf buds. Important modifications included the enzyme digestion step and the hypotonic treatment. Each sample was digested for at least 3–4 h at room temperature in 100 ml of the protoplast enzyme mixture. Ten ml of this enzyme mixture contained 500 units of Cellulase Onozuga R10 (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. The protoplasts were cleaned with fresh fixative 3–4 times, before dropping onto microscopic slides. After staining with the fluorochrome DAPI (4, 6-diamidino-2-phenylindole), chromosome number was determined under 1000x magnification in epifluorescence microscope Nikon Eclipse 800 using the UV filter sets. The images for karyotype analysis were captured with Nikon DXM 1200F digital camera using the maximum resolution of 12.5 megapixels. Chromosome pairs were identified and arranged on the basis of chromosome length and arm-ratio (Levan et al., 1964). Karyotypes were constructed from at least five metaphases in each sample.

RESULTS AND DISCUSSION

Karyotypes were constructed from *Castanopsis* (9 species), *Lithocarpus* (5 species) and *Quercus* (4 species) (Fig. 2). All species had 24 chromosomes and the diploid genome consisted of 12 pairs of homologous chromosomes (2n = 2x = 24). Twelve bivalents were observed in meiosis of male flowers from two species of *Castanopsis*, *C. indica* and *C. tribuloides*, and this confirmed the diploid status 2n = 2x = 24 (Fig. 3). Previous studies on some *Quercus* species in Europe showed the basic chromosome number x = 12 (D'Emerico

Table. 1 Chromosome number, arm ratios and karyotypic description of 18 Fagaceae species.

Species Name	2n	Mitotic arm ratio												Karyotypic
	number	1	2	3	4	5	6	7	8	9	10	11	12	description
Castanopsis acuminatissima (Blume) A.DC	24	1.27	1.13	1.82	1.70	1.17	1.36	1.27	1.20	1.38	1.50	1.25	2.00	18m + 6sm
Castanopsis argentea (Blume) A.DC.	24	1.00	1.30	1.75	1.50	1.71	1.25	1.83	1.00	1.00	1.14	1.33	1.00	18m + 6sm
Castanopsis armata (Roxb.) Spach	24	1.09	1.28	1.50	1.00	1.83	2.20	1.67	1.67	1.60	1.00	1.20	1.20	20m + 4sm
Castanopsis calathiformis (Skan.) Rehder & Wilson	24	1.08	1.18	1.69	1.75	1.67	1.89	1.60	1.40	1.20	1.75	1.50	1.25	18m + 6sm
Castanopsis cerabrina (Hickel & A. Camus) Barnett	24	1.00	1.14	1.40	1.75	1.50	1.25	1.67	1.00	1.00	1.33	1.67	1.33	22m + 2sm
Castanopsis diversifolia (Kurz) King & Hook.f.	24	1.40	1.10	1.33	1.25	1.29	1.21	1.80	1.08	1.40	1.40	1.00	1.00	22m + 2sm
Castanopsis fissa (Champ) Rehder & Wilson	24	1.05	1.00	1.33	1.19	1.83	1.67	1.80	1.33	1.60	1.17	1.00	1.00	20m + 4sm
Castanopsis indica (Roxb.) A.DC.	24	1.12	1.22	1.63	1.56	1.00	1.33	1.33	1.33	1.60	1.60	1.27	1.25	24m
Castanopsis tribuloides (Sm.) A.DC.	24	1.27	1.44	1.50	1.71	1.25	1.57	2.00	1.67	1.50	1.07	1.14	1.17	20m + 4sm
Lithocarpus ceriferus (Hickel & A. Camus) A. Camus	24	1.20	1.00	1.33	2.25	1.00	1.00	1.50	1.13	1.13	1.00	1.29	1.33	22m + 2sm
Lithocarpus elegans (Blume) Harus ex Soepadmo	24	1.33	1.40	1.00	1.17	1.00	1.20	1.20	1.50	1.00	1.00	1.00	1.14	24m
Lithocarpus harmandianus (Hickel & A. Camus) A. Camus	24	1.23	1.33	2.00	2.00	1.30	1.50	1.11	1.25	1.25	1.13	1.00	1.17	20m + 4sm
Lithocarpus recurvatus Barnett	24	1.17	1.67	2.00	1.07	1.80	1.08	2.00	2.00	1.40	1.63	1.25	1.29	16m + 8sm
Lithocarpus vestitus (Hickel & A. Camus) A. Camus)	24	1.00	1.38	1.13	1.00	1.07	2.00	1.33	1.00	1.20	1.00	1.20	1.00	22m + 2sm
Quercus brandisianus Kurz	24	1.35	1.75	1.26	1.50	1.50	1.11	2.20	1.21	1.50	1.60	1.17	1.22	20m + 4sm
Quercus kerrii Craib	24	1.30	1.20	1.25	1.46	1.33	1.29	1.17	1.17	1.60	1.17	2.00	1.20	22m + 2sm
Quercus mespilifolius Wall. ex DC.	24	1.11	1.19	1.67	1.67	1.80	2.00	1.18	1.40	1.10	2.50	1.00	1.50	18m + 6sm
Quercus rex Hemsl.	24	1.22	1.24	1.13	1.33	1.00	1.40	2.00	1.20	1.00	1.20	1.11	1.13	22m + 2sm

et al., 1995; Zoldos et al., 1999). The chromosome number and karyotypic description together with the arm-ratio data of each species is shown in Table 1. Metaphase chromosomes of these species are small (2–5 ìm), as expected based on the genome size of some species of *Quercus* (Zoldos et al., 1998; Loureiro et al., 2005). There is no record of genome or chromosome size of *Castanopsis* or *Lithocarpus* species available in the published databases. In the present study, chromosomes of *Lithocarpus* appear to be smaller than those of *Castanopsis* and *Quercus* (Fig. 2). However, genome size measurement such as by flow cytometry will be required in order to confirm this observation.

Figure 1. Acorns and flowers of some Fagaceae species: *Castanopsis acuminatissima* (Blume) A.DC. (a), *C. argentea* (Blume) A.DC. (b), *C. diversifolia* (Kurz) King ex Hook.f. (c), *C. indica* (Roxb. ex Lindl.) A.DC. (d), *C. tribuloides* (Sm.) A.DC. (e), *Lithocarpus ceriferus* (Hickel & A.Camus) A.Camus (f), *L. elegans* (Blume) Hatus. ex Soepadmo (g), *L. harmandii* (Hickel & A.Camus) A.Camus(h), *L. vestitus* (Hickel & A.Camus) A.Camus (i), *Quercus brandisiana* Kurz (j), *Q. mespilifolia Wall. ex A.DC.* (k), *Q. kerrii* Craib (l), *Q. rex* Hemsl. (m). The scale bar represents one centimetre.

Figure 2. Karyotypes of *Castanopsis acuminatissima* (Blume) A.DC. (a), *C. argentea* (Blume) A.DC. (b), *C. armata* (Roxb.) Spach (c), *C. calathiformis* (Skan) Rehder & Wilson (d), *C. cerebrina* (Hickel & A.Camus) Barnett (e), *C. diversifolia* (Kurz) King ex Hook.f. (f), *C. fissa* (Champ. ex Benth.) Rehder & E.H.Wilson (g), *C. indica* (Roxb. ex Lindl.) A.DC (h), *C. tribuloides* (Sm.) A.DC. (i), *Lithocarpus ceriferus* (Hickel & A.Camus) A.Camus (j), *L. elegans* (Blume) Hatus. ex Soepadmo (k), *L. harmandii* (Hickel & A.Camus) A.Camus (l), *L. recurvatus* Barnett (m), *L. vestitus* (Hickel & A.Camus) A.Camus (n), *Quercus brandisiana Kurz* (o), *Q. kerrii* Craib (p), *Q. myrsinaefoius* Blume (q), and *Q. rex* Hemsl. (r). The scale bar represents five micrometers.

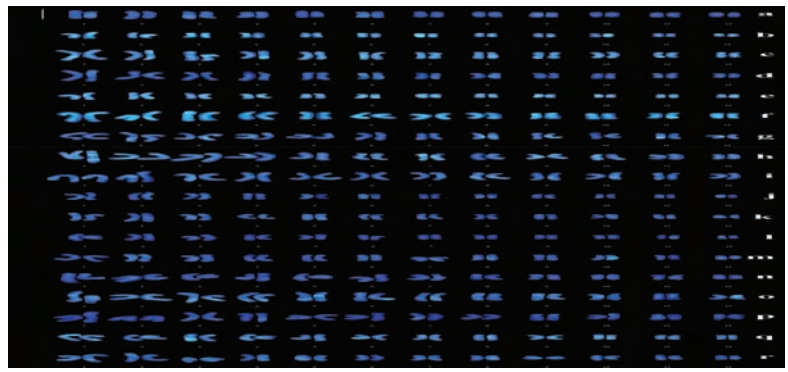

Figure 3. Meiotic chromosomes at metaphase-I from anthers of *Castanopsis indica* (a) and *C. tribuloides* (b).

Karyotypes of the species examined are very similar in that they consist mainly of metacentric chromosomes (8–12 pairs) whereas submetacentric chromosomes are not as common (0–4 pairs). There is usually one pair of chromosomes with secondary constriction, so called satellite (SAT-chromosomes). The comparison of karyotypes of *Quercus* species in the present study and those from Europe reveals different number ratios of metacentrics and submetacentrics in the chromosome complement. The European oak species have variable ratios, which are thought to be due to unequal chromatin condensation, different chromosome preparation and small chromosome size (Ohri & Ahuja, 1990; Zoldos et al., 1999). Nevertheless, the European oak species clearly have higher number of submetacentric chromosomes (3–6 pairs), while there are only 1–3 pairs of submetacentrics in this study. The species of *Castanopsis* and *Lithocarpus* in our study also show low number of submetacentrics, i.e. 0–3 and 0–4 pairs respectively. It is therefore likely that the differences between karyotypes of temperate and tropical oaks are significant and have evolutionary origin, rather than being artifacts in the preparation.

This is the first report on karyotypes of Fagaceae species from Thailand. The study has confirmed the diploid 2n number of 24 and the basic number of 12 for *Quercus* and shows the same 2n = 2x = 24 for *Castanopsis* and *Lithocarpus* for the first time. The karyotypes of these tropical Fagaceae species are similar in the composition of metacentric and submetacentric chromosome pairs, but there may be significant variation among the genera in the chromosome size and morphology.

ACKNOWLEDGMENTS

The work was supported by the Thai Royal Golden Jubilee PhD Program (RGJ Grant No. PHD/0073/2546). We are most grateful for the assistance from the staff of the Icelandic Laboratory for Plant Genetics of the University of Iceland; the research students and fellows at the Botany Department of Chulalongkorn University; the staff of *Huai Hong Khrai* Royal Development Study Centre in Chiang Mai; and all the friends who helped with the field work.

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