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Original article

Leaf cuticle micromorphology of *Fagus* L. (Fagaceae) species

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ABSTRACT

Cuticle micromorphology of all eight species of *Fagus* and an outgroup were examined in the present study. The genus *Trigonobalanus* was selected as the outgroup. Thirteen characteristics of the inner surface and five of the outer surface of the cuticle were described. Some characteristics, such as the subsidiary cell shape, size of stomata, arrangement of subsidiary cell, shape of anticlinal and periclinal cell walls, texture of periclinal cell wall, development epicuticular wax, and presence of papillae, were considered important for infrageneric classification. The topology was obtained from the analysis using two major lineages: (1) *Fagus engleriana* + *Fagus japonica* + *Fagus longipetiolata* and (2) *Fagus sylvatica* + *Fagus crenata* + *Fagus lucida* + *Fagus hayatae* + *Fagus grandifolia*. The first clade supported a bootstrap value of 98% and the second clade a bootstrap value of 97%. Based on the cuticle morphology, our results support the previous study, by revealing *F. engleriana*, *F. japonica*, and *F. longipetiolata* with long peduncles in one group and the remaining extant species of short- to medium-length peduncles in another group. In addition, molecular phylogenetic study of *Fagus* based on ribosomal DNA ITS and chloroplast DNA sequences data supports this assemblage. This study shows that cuticle micromorphological characteristics provide useful and important information for analyzing the evolutionary aspects of *Fagus*.

Introduction

The genus *Fagus* L. is distributed over China, Japan, Korea, Taiwan, the USA, and Ukraine in the Northern Hemisphere (Chang and Huang, 1988; Kvaćek and Walther, 1991) (Figure 1). This genus grows under conditions of average temperature, rainfall, altitude, and soil, and also in environments consisting of a mixture of a pine grove, a low-temperature evergreen grove, and tropical deciduous forest (Jones, 1984, 1986; Shen, 1992). The tree has a beautiful shape and is considered a living cultural asset (Shen, 1992).

In taxonomic studies of this genus, since Linnaeus (1753) had reported three species, additional 10–20 species were added, and then many kinds of taxonomic systems have been proposed (Kolakowski, 1960; Tralau, 1962; Tanai, 1974; Zetter, 1984; Kvaćek and Walther, 1991; Shen, 1992; Denk, 2003). Recently, molecular phylogenetic relationships based on chloroplast (cp) DNA and nuclear ribosomal DNA sequences of *Fagus* were examined (Stanford, 1998; Manos and Stanford, 2001; Li et al., 2003). However, the relationships between species are vague and taxonomic schemes suggested by researches are different to each other. Therefore, through the various taxonomic accesses, a more improved taxonomic system should be established along with the development of new beneficial taxonomic features.

Researchers are generally reluctant to adopt taxonomic features because of the variation in leaf characteristics in different environments (Barthlott et al., 1998). However, diverse analysis techniques have accumulated continuously, and now more profound research on leaf variation is being conducted.

Especially, cuticle layer the boundary of environment and plant, can exactly duplicate the characteristic of micromorphology of epidermal cell, so it can be helpful in taxonomic research (Stace, 1965; Hill and Jennifer, 1991; Stocky and Ko, 1988; Whang et al., 2001). Research on the cuticle layer of the genus *Fagus* has mostly been carried out using light microscopy; some of the recent research works by scanning electron microscopy (SEM) have also identified extremely limited information and did not perform the taxonomic treatment (Bandullska, 1924; Smiley and Huggins, 1981; Jones, 1984, 1986; Shen, 1992).

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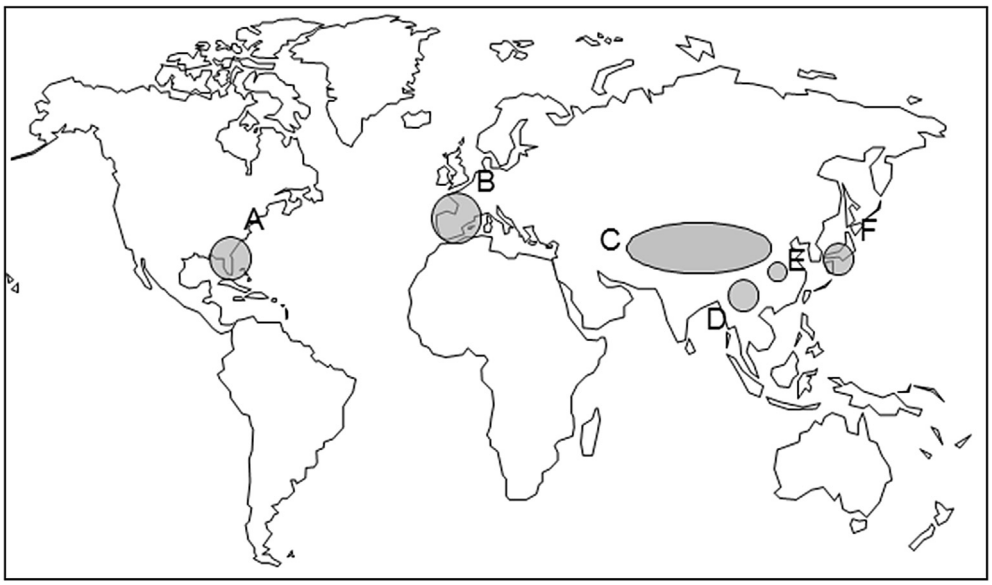

Figure 1. World map of *Fagus* regions. A region represents the location where *Fagus grandifolia* is available; B region, *Fagus sylvatica*; C region, *Fagus lucida*; D region, *Fagus longipetiolata*; E region, *Fagus engleriana*; F region, *Fagus crenata* and *Fagus japonica*; and G region, *Fagus hayatae*.

It has been known that the inner surface of the cuticle has more useful micromorphological features than its outer surface (Wells and Hill, 1989, Stocky and Ko, 1988, Whang et al., 2001).

In this study, we reported the leaf cuticle micromorphology of *Fagus* (Fagaceae) using SEM, carried out cladistic analysis on the basis of researched features, and proposed a new phylogenetic relationship based on the results.

Materials and methods

The genus *Fagus* consists of two subgenera: subgen. *Fagus* and *Engleriana*, according to Shen (1992). He divided subgenus *Fagus* into four sections: sect. *Fagus,* sect. *Grandifolia*, sect. *Longipetiolata*, and sect. *Lucida*. He also suggested that sect. *Lucida* comprises three series: ser. *Lucida*, ser. *Crenata*, and ser. *Hayatae*. Materials for this study included all eight species of the genus *Fagus* and one species of the genus *Trigonobalanus*, which is a sister group of *Fagus* (Forman, 1966). Leaves of most of the species used for this study were obtained from the herbarium specimens deposited at Royal Botanical Gardens Kew (K; Richmond, England), New York Botanical Garden (NY; New York. American), Kyungpook National University Herbarium (KNU; Daegu, Korea), Herbarium of Kunming Institute of Botany (KUN; Yunnan, China), Kyoto University Herbarium (KYO; Kyoto, Japan), Southwest Forest College Herbarium (SWFC; Yunnan, China), and Chollipo Arboretum(Taean, Korea) (Table 1). Leaf fragments (about 5 mm long) were excised from the middle portion of approximately two to six mature leaves of each specimen. Cuticles were prepared by soaking leaf fragments in 5% aqueous chromium trioxide until all organic matters, except the cuticle, were dissolved (Alvin and Boulter, 1974). Isolated cuticles were mounted on aluminum stubs with double-sided adhesive tape and were air dried. The stubs were then sputter coated with gold to a maximum thickness of 15 nm, and examined with an S-4300 (Hitachi; Tokyo, Japan) SEM operated at 15 kV. Leaf architectural analyses were performed following the procedures of Hickey (1973), Dilcher (1974), and Hardin (1979). The architectural terminology used was that proposed by Dilcher (1974) and Hardin (1979).

Cuticle characteristics listed in Table 2 were coded for cladistic analysis (Table 3). The data matrix was analyzed using the parsimony program PAUP 4.0b10 (Swofford, 2002).

Results

Cuticle micromorphology and anatomical structure of *Fagus* and an outgroup are presented in Tables 2 and 4, respectively.

Features of cuticle's inner surface in Fagus

The shape of the anticlinal wall on the upper epidermis can be of three different types: straight, sinuous, and undulate. For *Fagus japonica*, *Fagus sylvatica*, and *Fagus longipetiolata* the anticlinal wall is sinuous and straight (Figures 2B and 2D), for *Fagus grandifolia* it is undulated (Figure 2F); and for *Fagus engleriana*, *Fagus hayatae*, *Fagus lucida*, and *Fagus crenata* it is straight (Figures 2C and 2E). *F. engleriana* and *F. japonica* have ball-shaped ornaments at the upper epidermis (Figures 2A and 2B).

The anticlinal wall and periclinal wall texture of the lower epidermis can, respectively, be thick or thin, and smooth or rough. In *F. engleriana*, *F. japonica*, and *F. longipetiolata*, the anticlinal wall is relatively thick and the periclinal wall texture is smooth (Figures 3A–C). In the rest of the species, these are relatively thin and rough, respectively (Figures 3D–F).

The subsidiary cell number is common (4–7) in all species, except for *F. longipetiolata* for which it is irregular. The shape of subsidiary cells' anticlinal wall can be of three different types: irregular circular, lunate, and rectangular. For *F. engleriana*, *F. japonica*, and *F. longipetiolata*, the anticlinal wall is irregular circular; for *F. sylvatica*, *F. lucida*, *F. grandifolia*, and *F. crenata*, it is lunate; and for *F. hayatae* it is rectangular.

Subsidiary cells show two types of arrangements: anomocytic and cyclocytic types. *F. engleriana*, *F. japonica*, and *F. longipetiolata* have anomocytic-type arrangement (Figures 3A–C), and *F. sylvatica*, *F. hayatae*, *F. lucida*, *F. crenata*, and *F. grandifolia* have almost cyclocytic and randomly anomocytic-type arrangement (Figures 3D–F). All species have stomatal apparatus only on the abaxial surface. The stomatal apparatus is almost elliptical and randomly circular. The