Available online at www.sciencedirect.com
ScienceDirect
Flora 204 (2009) 463–470
FLORA
www.elsevier.de/flora

Abnormalities in sexual development and pollinator limitation in *Michelia coriacea* (Magnoliaceae), a critically endangered endemic to Southeast Yunnan, China

Xingfeng Zhao[a,b], Weibang Sun[a,*]

[a]*Kunming Botanic Garden, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China*
[b]*Graduate School of the Chinese Academy of Sciences, Beijing 100049, China*

Received 16 April 2008; accepted 5 July 2008

Abstract

Michelia coriacea (Magnoliacae) is a critically endangered tree, endemic to Southeast Yunnan province, China. Most of the individuals in the extant populations normally bear flowers, but fruit set and fertile seed production rates were only 6.7% and 0.2%, respectively. To identify possible causes of reproductive barriers, the following studies were carried out: examination of stamen and pistil development; experiments using controlled pollination; observations of behavior and frequency of floral visitors in both cultivated and natural populations. The results revealed that about 60% of pollen was abnormal and approx. 70% of ovules had delayed development. Hand pollination could effectively enhance the fruit set ($F_{4,25} = 35.139$, $P < 0.0001$) and seed set per fruit ($F_{4,25} = 85.022$, $P < 0.0001$). Both cultivated and wild *M. coriacea* had an extremely low frequency of floral visitors. Some beetles, a few species of Andrenidae and some *Bombus sp*. are likely to be the effective pollinators. The fruit set and seed set per fruit from controlled self-pollination and cross-pollination were significantly different ($P < 0.05$), and thus it is inferred, that inbreeding depression may be a contributing factor in the very low seed production. It appears that low seed set in *M. coriacea* is due to a combination of factors: abnormalities in pollen and ovules, low number of effective pollinators, and inbreeding depression.

Keywords: *Michelia coriacea*; Critically endangered tree species; Yunnan of China; Rarity of species; Pollen limitation

Introduction

Michelia coriacea Chang and B. L. Chen (Magnoliaceae) is a newly described species distributed in southeast Yunnan Province of China (Chen, 1988; Liu and Wu, 1988). This species is a diploid ($2n = 38$; Zhang and Xia, 2005) usually small tree up to 10 m high and 20–45 cm diameter (Chen, 1988; Chen and Nooteboom, 1993). Recent detailed surveys confirmed that the species can grow to an excellent timber tree, up to 30 m high and 60 cm in diameter. It bears scented whitish or creamy yellow flowers with 6–7 tepals (originally described nine tepals; Chen, 1988). Anthesis lasts from February to April (Cicuzza et al., 2007; Sun and Yan, 2006).

Michelia coriacea is considered to be a critically endangered endemic tree (Cicuzza et al., 2007). Since 2003, we have carried out a comprehensive investigation of this species. The existing population of the species consists of about 300–500 individuals scattered on limestone mountain slopes or secondary shrubby woods

*Corresponding author. Fax: +86 871 5223622.
E-mail address: wbsun@mail.kib.ac.cn (W. Sun).

0367-2530/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.flora.2008.07.001

and on roadsides at altitudes of 1400–1700 m (Cicuzza et al., 2007). The habitats have been badly degraded and fragmented due to heavy logging and vegetation destruction in the past decade, resulting in sub-populations that are isolated from each other. Although this species flowered well, its fruit and seed set were very low and seedlings/saplings were rarely found in the past three years that we have studied it. Thus, conservation measures based on scientific studies are urgently needed for this taxon.

Knowledge of reproductive biology is necessary for effective protection of endangered plants, especially for species with low seed set and small populations (Evans et al., 2004; Kaye, 1999; Kruckeberg and Rabinowitz, 1985; Spira, 2001; Xiao and Xu, 2006), and this is especially true when regeneration occurs exclusively via seeds (Bond, 1994; Schemske et al., 1994). In such species, population viability might be closely linked to seed dynamics, and conservation might depend on understanding the factors that limit seed production (Pavlik et al., 1993). When seed production is mediated by pollinators, seeds can be limited by pollen availability, scarcity and/or inefficiency of pollination vectors, and poor pollen quality (Bierzychudek, 1981; Burd, 1994; Larson and Barrett, 2000; Wilcock and Neiland, 2002). Pollen quantity and quality as well as abnormal gametic development can often result in limited seed production and subsequent population decline and eventual extinction. The study of plant species with low reproductive capabilities has been the subject of much recent research and has become an important aspect in plant conservation (Byers, 2004; Colling et al., 2004; Evans et al., 2004; Hedrick and Kalinowski, 2000; Pan et al., 2003; Rocha and Aguilar, 2001). In addition, self pollen can limit seed production through inbreeding depression (Bosch and Waser, 1999; Brown and Kephart, 1999). Fragmentation of habitat, human activities, and especially low sexual reproductive capability will limit regeneration and population maintenance (Evans et al., 2004; Pan et al., 2003).

It is well known that the production of well-developed seeds of a species plays an important role in population dynamics and persistence. Undoubtedly, the effective and long-term conservation of *M. coriacea* will depend on an understanding of causes of its poor seed production. Thus, this paper focuses on observations/examinations of sexual development and mating system of *M. coriacea*, while trying to identify the factors that lead to its low fruit/seed set. The following questions are particularly emphasized: (1) Do pollen and ovule of the sampled individuals have normal development? (2) If not, which process and what percent of abnormalities occurred? (3) Which pollinators were efficient in fruit and seed production? (4) Were pollinators sufficient or did they limit seed production?

Materials and methods

Study sites

This study was carried out at the Seedling Station of Wenshan Forest Bureau (SSWFB) (lat. 23°24′23.4″N, long. 104°15′5.9″E) and in a wild population in Xichou County (lat. 23°23′–23°25′N, long. 104°25′–105°57′E) in southeast Yunnan Province, China. The sampled *M. coriacea* cultivated at SSWFB were 15 years old.

Methods

Stamen and pistil development

Flower buds at successive developmental stages were collected every two weeks during the period from November 2006 to the following April at SSWFB. Each time, 15 flowers buds were collected and fixed in FAA (formalin:acetic acid:50% ethyl alcohol = 5:5:90 v/v). The customary methods of dehydration, infiltration, and embedding in paraffin were used. Samples were sectioned at a thickness of 5–7 μm for microspores and male gametophytes and 9–12 μm for macrospores and female gametophytes. The sections were stained with Heidenhain's iron-alum hematoxylin, and then observed with Olympus BX-51.

Breeding system

Pollination treatments were performed on six randomly chosen individual trees at SSWFB in March of 2007. Five pollination treatments were used on each tree to determine the mating system. Each pollination treatment was conducted on 10–15 flowers per tree. These five treatments were: (1) Floral buds were labeled and left untouched under open pollination conditions (''Open pollination''); (2) Floral buds were labeled and bagged to test for autogamy (''Autogamic pollination''); (3) The flowers were emasculated, labeled and bagged to test for agamospermy (asexual reproduction in flower, e.g. apomixis) (''Agamospermic test''); (4) Hand self-pollinations (pollen from the same tree) was performed on emasculated flowers; (5) Hand cross-pollinations (pollen come from different trees) were done on emasculated flowers. The treated flowering organs were bagged with water-proof paper bags. These bags were removed after anthesis of the target flowers.

Labeled fruits were harvested in late September of 2007. Seeds from the fruits were collected and viable seeds were distinguished by the color of the aril. Arils of fertile seeds were red, whereas those of aborted seeds were dark brown. The effect of pollination treatments was analyzed for two properties: (1) fruit set percentage [(number of fruits/number of flowers) × 100]; (2) percentage of seed set per fruit [(number of viable seeds/number of initial ovules) × 100].