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Reproductive organography of *Bougainvillea spectabilis* Willd

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ABSTRACT

Bougainvillea spectabilis Willd. is of prime importance for horticulture, as well as potentially for pharmaceutical industries, agriculture and environmental industries. However, its floral development is not yet well understood. A detailed study on floral structure and floral organography in the species was first completed using microscopy of paraffin microtome sections of buds. The results were indicated as follows: first, the three trumped flowers in the cymose inflorescence develop asynchronously. Secondly, Varieties with multi-whorl bracts do not develop any sexual organs, i.e., perianth, pistil and stamens. Thirdly, the wall of the two-loculus anther consists of two kinds of cells: the inner wall, consisting of thick-cytoplasmed cells and the outer wall, consisting of fibrous cells. Fourthly, the pollen grains, with three germination colpi, vary substantially in the form and size in summer under the highest day temperature of 40 °C. Fifthly, the pistil is characterized with betalain-acumulating stylar brush. Followed the developmental course, only one basal ovule is developed in the superior ovary. Finally, organs of one flower develop consecutively from the outer to the inner, i.e., from bracts, to calyx, stamen, and carpel while the three flowers bloomed one by one in one cymose inflorescence. It almost takes 1 week from first bud to the third flower blooming. Our research showed a series of special characteristics of reproduction organography of *B. spectabilis* which can be useful for understanding its reproduction biology and its sterility.

1. Introduction

Bougainvillea, a member of the Nyctaginaceae family, comprises 18 species of spiny trees, shrubs, and vines (Mabberley, 1997). Bougainvilleas are among the most floriferous shrubs of the tropics, which can hardly be exceeded by any other plant. Of 18 species, only *Bougainvillea spectabilis* Willd. and *Bougainvillea glabra* Choisy. are most commonly used in horticulture. Recently, it indicated that *B. spectabilis* also showed great potential in virus-resistance (Balasaraswathi et al., 1998; Schlein et al., 2001; Joseph et al., 2005; Ali et al., 2005) and pollution-tolerance (Sharma et al., 2005). The insulin-like compound, pinitol, was first isolated from leaves of *B. spectabilis* and evaluated that it can be developed into dietary supplements as insulin substitutes in curing diabetes (Narayanan et al., 1987; Sarah et al., 2000; Campbell et al., 2004). The studies above indicated that *B. spectabilis* showed great importance in a few fields besides in horticulture. However, the interesting species is almost sterile which causes the difficulty in sexual reproduction. Fortunately, vegetative multiplication helps to circumvent the disadvantages of a high level of sterility. Thus the survival potential of the species is not affected by the sterility (Zadoo and Khoshoo, 1968). However, the high level of sterility makes the cross breeding very difficult, which greatly narrows the gene pool of the species (Zadoo and Khoshoo, 1968; Khoshoo and Zadoo, 1969).

The previous studies on *B. spectabilis* focused mainly on taxonomic aspects (Standley, 1931; Toursarkissian, 1975), or on inflorescence enhancement under phytohormone treatments at the physiological level (Hackett and Sachs, 1968; Steffen et al., 1988; Baraskar et al., 1990; Norcini et al., 1994), or on development and propagation of new cultivars (Anonymous, 1961). So far researches on reproductive biology of *B. spectabilis* were limited. Sattler and Perlin (1982) investigated three morphological problems, epiphylly, phyllotaxis and placentation, among *B. spectabilis*, *Boerhaavia diffusa*, and *Mirabilis jalapa*. Although their research gave a clue to inflorescence development of *B. spectabilis* based on the comparison among the three species, knowledge of reproductive biology of *B. spectabilis* is still little.

The aim of this study was to characterize the developmental patterns of the floral organs of *B. spectbilis* at the anatomical and

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morphological level and better understand its unusual reproductive biology, as well as to reveal the factors underlying its poor sexual reproduction.

2. Materials and methods

Plants of *B. spectabilis* were grown in the Bougainvillea garden of the Fujian Institute of Subtropical Botany (altitude 118°04′04″, latitude 24°26′46″, annual average temperature 22 °C). In this study 10 branches from 10 individual plants were tagged to study the floral structures in the early summer of 2007. In order to investigate fruit set and seed set, we examined the numerous withering flowers in branches or dropped ones in the garden of the Fujian Institute of Subtropical Botany. We chose eight typical stages during floral bud development to make microtome sections for observation of floral organ development in 2007. The fresh anthers were collected for paraffin microtome sections in different developmental stages (during anthesis and after anthesis). Fresh buds and anthers were collected and fixed in a mixture of FAA (formalin, acetic acid, 50% ethanol, 5:6:89 (v/v/v)). After subsequent dehydration in a graded ethanol series, floral buds and anthers were rinsed several times in distilled water and embedded in paraffin (melting point, 56–57 °C, Sigma, USA). Thin sections (8 μm) were cut with a diamond knife on a Leica RM2235 microtome (Nussloch, Germany) and usually double-stained with 0.5% Delafield's Hematoxylin (60 min) with aqueous safranin as a counterstain (Sattler and Perlin, 1982). Observations were made using a Zeiss photomicroscope (Carl Zeiss Microimaging, Göttingen, Germany).

We observed fresh pollen grains from 10 anthers of five plants in autumn and in summer of 2007, respectively. The average day temperature of our city is often 40 °C in the middle of summer. The average day temperature is 24 °C in autumn. We carried out the morphology investigation of pollen in summer and autumn considering the affects of high temperature. Pollen grains were collected through squeezing anthers in water and were observed under a Zeiss light microscope (Carl Zeiss Microimaging, Göttingen, Germany).

3. Results

3.1. Floral morphology

The inflorescences of *B. spectabilis* arise as terminal or axillary cymose represented by a three-flowered umbel. Within the inflorescence, three flowers are circled by three large paper-like bracts. The three bracts are closely appressed at their bases and attached at the top of the ultimate branches (Fig. 1a). The pedicel of each flower is confluent with the main vein of the supporting bract, respectively. In contrast with three conspicuous bracts, the three trumpet-shaped flowers are inconspicuous, which often mislead people to regard bracts as petals of a flower. Each flower is hermaphroditic and apetalous with a 5-lobed tubular perianth with short, ascending curved hairs (Fig. 1b). The tubular perianth is very constricted, which makes stamens very difficult to be observed by flower visitors. When the perianth is cut eight stamens and one carpel can be seen clearly (Fig. 1c). The eight stamens have unequal filaments which are fused only at their extreme base (Fig. 2). Each stamen raises a yellow two-egged anther at the top. The carpel includes a style, stigma and an ovary

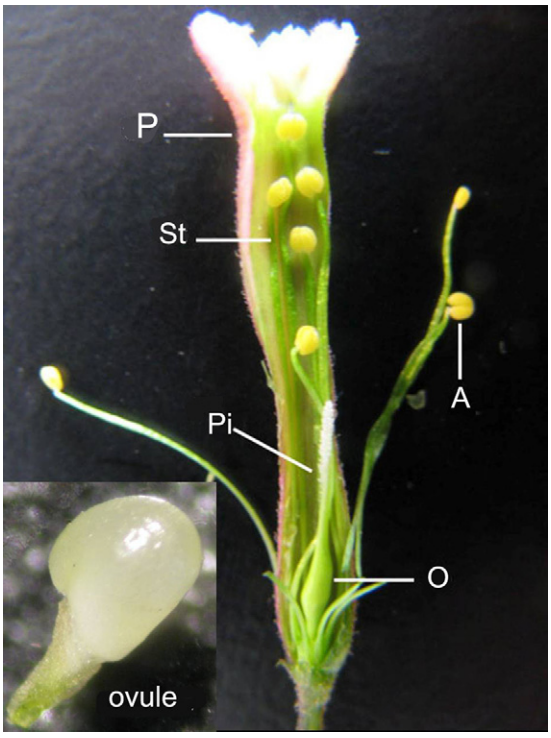

Fig. 2. Longitudinal section of a trumped-shaped flower. P = perianth, St = stamen, A = anther, Pi = pistil, O = ovary, the left below is the details of the ovule which takes a form, somewhat intermediate between anatropous and campylotropous types.

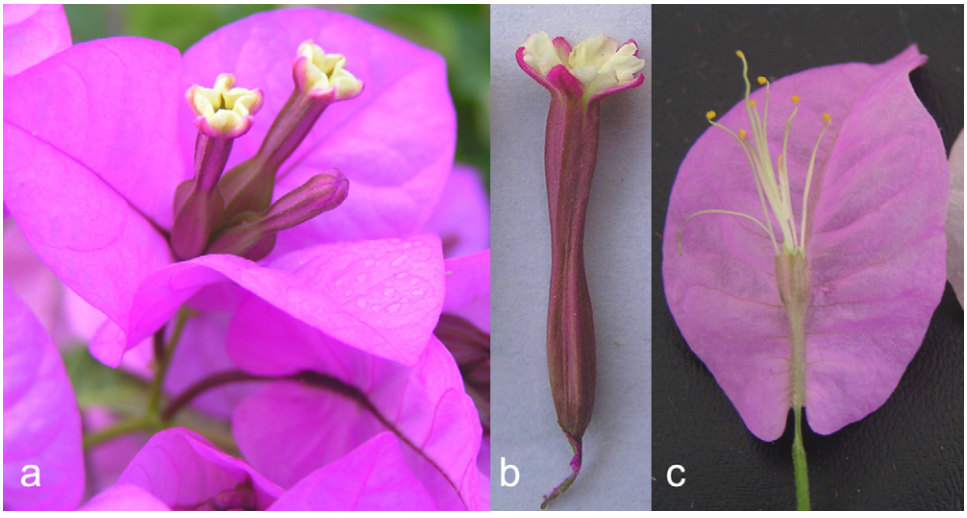

Fig. 1. Morphological view of a inflorescence (a), a trumped-shaped flower (b) and organs in the flower (c).