P 19: Characterization of the flower morphology of three *Duboisia* species

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Abstract

The tropane alkaloid scopolamine, an important precursor of active pharmaceutical ingredients due to its anticholinergic properties, can be extracted from leaf material of *Duboisia* R. Br., a member of the *Solanaceae* family. Robust and high-yielding *Duboisia* plants are a major requirement in cultivation because of challenges such as climatic changes and the rising demand of the active pharmaceutical ingredient. Therefore, breeding activities are carried out to improve *Duboisia* plants with regard to leaf and scopolamine yield maximization as well as a higher tolerance to environmental conditions.

Molecular genetic analysis of highly conserved plastid sequences was used to prove the identity of the species *Duboisia hopwoodii*, *D. myoporoides* and *D. leichhardtii*. To evaluate different breeding options various flower characteristics have been analyzed by means of stereo microscopy for the three species. For *D. hopwoodii* and *D. leichhardtii*, all characteristics occur stably with nearly no variation. In contrast, the number of flower organs such as petals and anthers varied for *D. myoporoides*.

Keywords: flower morphology, *Duboisia* sp., microscopy, molecular genetic analysis, *Solanaceae*

Introduction

The genus *Duboisia* R. Br. belongs to the tribe *Anthocercidiae* within the family *Solanaceae*. Four different species are comprised within this genus: *D. arenitensis* Craven et al., *D. hopwoodii* F. Muell., *D. myoporoides* R. Br. and *D. leichhardtii* F. Muell. (Craven et al., 1995). *Duboisia* species are woody plants that are distributed in Australia while *D. myoporoides* also occurs in New Caledonia (Craven et al., 1995). *D. arenitensis* is native to Arnhem Land in the Northern Territory (Craven et al., 1995) and *D. hopwoodii* to the arid interior region of Australia.

The distribution areas of *D. myoporoides* and *D. leichhardtii* in Australia are overlapping. In these areas naturally occurring interspecific hybrids exist (Barnard, 1952). Hybrids between these two species are used in large-scale production since the leaves of *D. leichhardtii* and *D. myoporoides* contain pharmaceutically important tropane alkaloids such as scopolamine (Foley, 2006) which is used as an active pharmaceutical ingredient to treat motion sickness and as a precursor for partially synthetic anticholinergics.

Conventional breeding approaches via crosses are used to optimize plants with regard to leaf and scopolamine yield maximization, resistance and tolerance against environmental influences. A detailed knowledge of flower biology is an important prerequisite for crosses and/or possible hybridization.

The aim of this study was the characterization of the flower morphology of the three different species *D. hopwoodii*, *D. myoporoides* and *D. leichhardtii*.

Materials and Methods

Plants were propagated via cuttings from material originally derived from Australia. Cultivation of *D. hopwoodii*, *D. myoporoides* and *D. leichhardtii* took place in the greenhouse at 20/24 °C heating / ventilation set points. Flowers for morphological studies were taken from about ten month old plants.

Selected sequences of three plastid genes, psbA (photosystem II protein D1), ndhF (NADH dehydrogenase F) and matK (maturase K) (SHAPCOTT ET AL., 2015), were amplified using Polymerase Chain Reaction (PCR) (primer sequences: psbA 5´ CTTCCCTCTAGACCTAGCTGCT, psbA 3´ CTCGCCTACTTACATTCCAT, ndhF 5´ TCTATTCAATATCTCTATGGGG, ndhF 3´ AATGAGTAAAATCAGCTAATCCTC, matK 5´ ACATTTATTACGATTCTTTCTCCAC, matK 3´ ACTCCCCACAAACTAGAAGAAGCT; PCR: 1 min/94 °C, [30 s/94 °C, 30 s/55 °C, 1 min/72 °C] x 40, 7 min/72 °C). After restriction enzyme digestion (*Dde*I, *Cac*8I and *Bam*HI; 90 min/37 °C) the fragments were separated on 2 % agarose gels.

For each species 30 flowers were analyzed regarding various flower characteristics. The flowers of *D. myoporoides* and *D. leichhardtii* were collected from three different inflorescences, one from the upper, middle and lower section of the plants. Due to a more growth habit of *D. hopwoodii*, 15 flowers each were taken from the upper and lower section.

After harvesting the plant material in the greenhouse, measurement and description of *Duboisia* flower characteristics was realized by means of stereo microscopy. The magnification was adjusted to the flower organs sizes and ranged between 6.5 and 50 fold.

Results

Sequences of three different plastid genes were used to verify species identity. The banding pattern that was received after restriction digestion correlated exactly with the banding pattern that was expected. Therefore, the three species could be distinguished and the identity of *D. hopwoodii*, *D. myoporoides* and *D. leichhardtii* was proven.

The analysis of flower morphology illustrated the variation in several flower characteristics of the investigated *Duboisia* species *D. hopwoodii*, *D. myoporoides* and *D. leichhardtii* (Fig. 1).

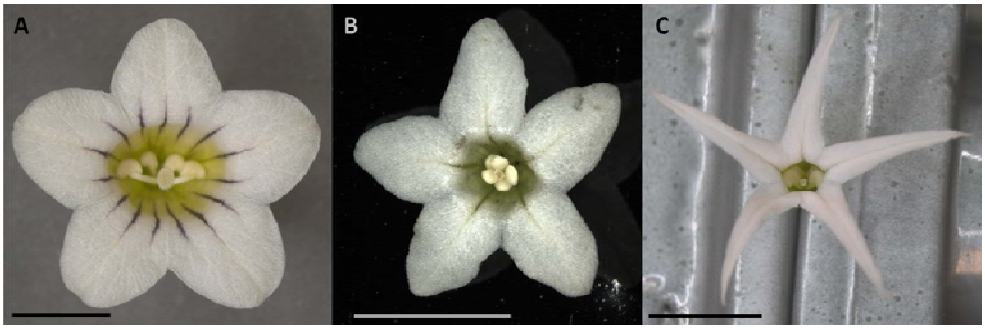

Fig. 1 Flower morphology of D. hopwoodii (A), D. myoporoides (B) and D. leichhardtii (C). Bar: 5 mm.

The flowers of *D. hopwoodii* appeared to be nearly consequently symmetric and widely open. Petals of the *D. myoporoides* and *D. leichhardtii* flowers more often differed in their orientation so that almost every flower had unique appearance. The pronounced star-like structure was typical for *D. leichhardtii*.

The data collected from flowers of the three species is shown in Tab 1.

Tab. 1 Flower characteristics of Duboisia spec. Means of observations of 30 flowers are presented.

Characteristic	*D. hopwoodii*	*D. myoporoides*	*D. leichhardtii*
Diameter of corolla [mm]	11.73	9.73	14.43
Number of petals	5 (100 %)	5 (87 %)	5 (100 %)
		6 (13 %)	
Number of anthers	4 (100 %)	4 (10 %)	4 (100 %)
		5 (77 %)	
		6 (13 %)	
Length of flower tubes [mm]	9.27	4.01	5.89
Diameter of flower tubes [mm]	4.62	2.43	2.54
Length of style [mm]	5.45	1.25	2.09

Regarding the diameter of corollas the data presented in this study is confirming the proportions published by CRAVEN ET AL. (1995). *D. myoporoides* showed the smallest corolla diameter. In contrast, *D. leichhardtii* offered the largest diameter due to its peaked corolla lobes. For *D. hopwoodii* and *D. leichhardtii* the number of petals and anthers was stable with five and four, respectively. Interestingly, *D. myoporoides* deviated from the two other species, because a small percentage (13 %) of flowers with six petals was recorded. Also, there was variation in the number of anthers in this species: while most flowers contained five anthers (77 %), there were some with four (10 %) or six (13 %) anthers. These unstable flower organ numbers were unique for *D. myoporoides*.

Measurements of the flower tubes emphasized the flowers of *D. hopwoodii*: in comparison to *D. myoporoides* and *D. leichhardtii*, *D. hopwoodii* had the longest and widest flower tubes. Also the longest styles were recorded for flowers of *D. hopwoodii*.

There was nearly no variation in the number of ovules (17 ± 1 ovule/ovary) for *D. myoporoides* and *D. leichhardtii* (data not shown).

In summary, for each *Duboisia* species typical flower characteristics were found. By their flower morphology, the three species *D. hopwoodii*, *D. myoporoides* and *D. leichhardtii* which had been confirmed by molecular markers can be easily distinguished.

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