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RESEARCH NOTE

Floral Bud Length as Morphological Predictor for Microspore Developmental Stage in Sturt's Desert Pea (*Swainsona formosa*)

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ABSTRACT

This work was conducted to establish the relationship between microspore developmental stage and length of the floral bud in glasshouse-grown Sturt's desert pea, a native Australian legume. The stages of microspore development were segregated into tetrad, early-uninucleate, mid-uninucleate and late-uninucleate. The results showed that the stage of microspore development was highly dependent on the length of floral bud. The tetrad stage lasted longer than early-, mid- or late-uninucleate stages. The attempted induction of androgenesis in Sturt's desert pea using anthers from floral buds with similar size, as in the present work, was unsuccessful. However, our work showed that the floral bud length can be used as a reliable predictor of microspore developmental stage in Sturt's desert pea.

Key words: Sturt's desert pea, Swainsona formosa, androgenesis, legume.

Sturt's desert pea, *Swainsona formosa* (G.Don) J.Thompson, is one of Australia's most spectacular wild flowers, and is the floral emblem of South Australia. It is a papilionoid legume with chromosome number of 2*n* = 16 (Zulkarnain *et al.*, 2002), and self-compatible but self-pollination is often prevented by the presence of stigmatic cuticle that precluded pollen germination until ruptured (Jusaitis, 1994).

One of the economic importance of this plant is in its use as cut flower plant (Williams and Taji, 1991). However, its commercialisation is impeded by the production of a large amount of pollen grains that reduces flower quality (Barth, 1990) due to petal staining by pollen grains released by the anther during transportation. In addition, self pollination of the flowers during transportation would easily occur, especially by rough handling, resulting in rapid degeneration of flowers. Developing strategies to produce male-sterile plants is then becoming the most appropriate method to solve this problem. One approach to create such sterility is via androgenesis using anther culture method.

Androgenesis is determined by a number of factors, including the microspore developmental stage at the time of the introduction to the *in vitro* environment. Unfortunately, the exact stage for successful plant regeneration is species dependent. Romeijn and Lammeren (1999) found that first pollen mitosis was a suitable stage for the induction of androgenesis in *Scabiosa columbaria.* Tetrad to mid-uninucleate stages were found to be useful in androgenesis of *Helianthus annuus* (Coumans and Zhong, 1995; Zhong *et al.*, 1995). Meanwhile, the late-uninucleate to early-binucleate stages were believed to be more responsive in *Brassica napus* (Fan *et al.*, 1988). As the consequence, determining the correct stage of microspore development in Sturt's desert pea is a crucial step before anther culture initiation.

Conventionally, the determination of microspore developmental stage of a given floral bud has been using aceto-orcein or aceto-carmine staining technique prior to observation under a light microscope (Prakash, 2000). This method, however, is impractical and time-consuming, particularly for a large sample size such as in a routine anther or microspore culture programme.

To our knowledge, no practical and quick microspore staging protocol has been developed for Sturt's desert pea. The present study aimed at correlating the floral bud length as a morphometric attribute with microspore developmental stage for a better time prediction for Sturt's desert pea anther culture.

The experiment was conducted from July through to December 2001 at the Plant Biotechnology Laboratory, School of Rural Science and Agriculture, University of New England, Armidale, Australia. Plant materials were obtained from a field collection in South Australia, and grown in a temperate glasshouse. The

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voucher specimen was deposited at the NCW Beadle Herbarium, University of New England (accession number NE79130).

Floral buds (florets) 13 – 16 mm long were isolated from 60 glasshouse-grown plants during. The total number of sampled florets was 131. These were obtained from 131 umbels (from one umbel consisting of 5 – 7 florets, one floret was taken as sample). The length of the buds was measured, using graph paper, from the base to the uppermost tip.

Ten anthers from each individual bud were bulk-squashed in a few drops of 1% aceto-orcein (Prakash, 2000). The microspore stage was determined based on the presence of a nuclear stage, i.e. tetrad, early-, mid- and late-uninucleate (Figure 1), from at least 100 observations under a light microscope (Zeiss Standard 20).

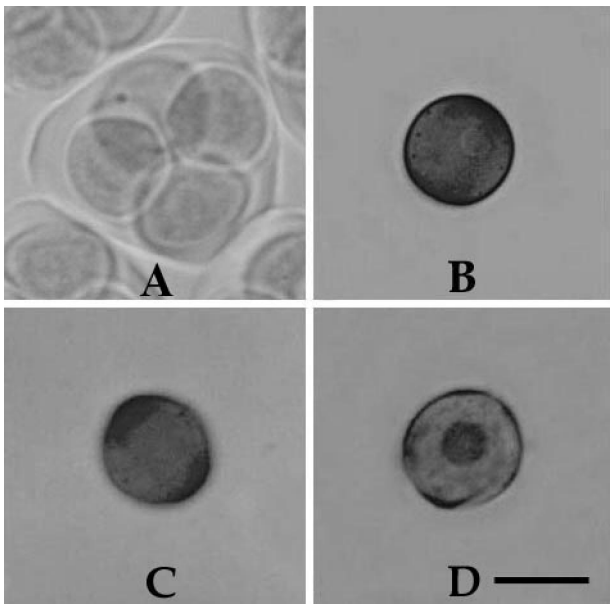

Figure 1. Cytological stages of *Swainsona formosa* microspore: A, tetrad; B, early-uninucleate; C, mid-uninucleate; D, late-uninucleate. Bar = 10 µm.

Since there could be microspores with various developmental stages within the anthers from the same bud, the stage of development was then determined based on at least by 50% observation. Data were analysed using analysis of variance with the aid of Microsoft Excel spreadsheet program (Microsoft-Corporation, 2000), followed by Fisher's protected least significant difference (FPLSD) (Petersen, 1985) to separate the means, and the standard deviation (SD) was calculated.

Results obtained from this study indicated that the stage of microspore development in Sturt's desert pea highly dependent on the size of floral buds ($P = 0.00$). The FPLSD test showed that the length of buds containing microspores at early-, mid- and late-uninucleate stages significantly differed from those containing tetrad microspores. In addition, significant difference in size was also recorded between buds containing early- and late-uninucleate microspores. However, the difference in size between buds containing early- and mid-, and between mid- and late-uninucleate microspores were not significant (Table 1). The reason for this was the fact that these three stages lasted only for a short period, while the tetrad stage lasted longer. Tomasi *et al.* (1999) used a similar morphological predictor for determining the microspore developmental stage in *Lasquerella* sp. Floral bud length was then used as a morphological predictor in investigating the effect of the microspore developmental stage in subsequent anther culture, though no microspore-derived embryo was initiated. In *Nicotiana tabacum* (Sunderland, 1974), *Linum usitatissimum* (Nichterlein and Friedt, 1993) and *Phleum pratense* (Guo *et al.*, 1999), however, microspores at late-uninucleate were proven to be useful in inducing androgenesis. If *S. formosa* is considered to be similar to these 3 species, the anthers should be obtained from floral buds 15.23 – 15.43 mm long. Concurrent anther culture of *S. formosa* used materials obtained from buds 13 – 16 mm long, which included all the microspore developmental stages, but unfortunately, resulted in no haploid plant regeneration except callus formation.

Table 1. The classification of microspore developmental stage based upon morphological measurements of floral bud length of glasshouse-grown *Swainsona formosa*

Stage of microspore development	Mean of floral bud (mm)[*)]	
Tetrad	14.73 ± 0.41	a[**)]
Early-uninucleate	14.98 ± 0.38	b
Mid-uninucleate	15.20 ± 0.34	b c
Late-uninucleate	15.33 ± 0.37	c

[*)] Means ± Standard Deviation.
[**)] Mean separation by FPLSD test at 0.05 protection level = 0.17.

Based on the work reported here, the floral bud length in *S. formosa* can be used as a reliable indicator of microspore developmental stage, eliminating the need for assessing every bud to ascertain its correct stage of microspore development at the time of culture initiation. This finding will also allow a quick staging for future *Swainsona formosa* anther or microspore culture.

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