Sandersonia: Towards the New Generation

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Abstract
Sandersonia is a mono-specific genus in the family Colchicaceae. In recent years it has become an important flower crop in New Zealand with both cut stems and tubers being exported. Considerable investment has been made in research on agronomy, tuber storage and post-harvest handling of this crop and much of this information has been published. A number of techniques have been employed to produce new forms of Sandersonia. They include inducing and identifying mutants, producing polyploid plants and performing wide crosses using in-ovulo embryo culture. Current work is aimed at characterising various mutants that have appeared. To date two new cultivars have been assigned plant variety rights in New Zealand. These are Santonia cv ‘Golden Lights’ and Sandersonia aurantiaca cv. ‘Phoenix’.

INTRODUCTION
Sandersonia is a mono-specific genus in the family Colchicaceae. The recent development of Sandersonia as a cut flower crop in New Zealand has seen the popularity of this crop increase markedly. If New Zealand is to retain its dominance in the market for this crop, new proprietary varieties are needed. Opportunities exist to develop new cultivars of ornamental plants by applying a range of in vitro techniques, e.g. the generation and identification of mutant plants, polyploidy and interspecific hybridisation.

The generation and identification of mutant plants provides a valuable tool for obtaining new varieties. Sandersonia is seed propagated and occasionally variants arise. A number of mutations have appeared, including at least two cream-yellow forms. Such plants offer useful opportunities to further develop this crop.

Polyploidy can be used to generate variation from which new varieties can be selected. In addition triploid plants are likely to be sterile limiting opportunities for further breeding or propagation from newly released cultivars of Sandersonia. The use of colchicine and oryzalin to generate tetraploid plants is well documented, and tetraploid plants can be crossed with diploid plants to generate triploid plants using embryo rescue techniques. In New Zealand winter-grown Sandersonia tends to have thin weak stems. The development of polyploid plants may offer an opportunity to produce quality stems during the New Zealand winter.

Embryo rescue or ovule culture techniques have been used to produce new cultivars of flower crops, e.g. Lilium (Van Tuyl et al., 1990, 1991), Gypsophila (Kishi et al., 1994), Limonium (Morgan et al., 1995, 1998) and Sandersonia (Morgan et al., 1999). Littonia modesta is related to Sandersonia, and hybrids between the two plants may be valuable as cut flower crops.

In this review we describe 2 colour mutants and polyploid forms of Sandersonia and review the production of hybrids between Sandersonia and Littonia.
MATERIALS AND METHODS

*Sandersonia* tubers were obtained from commercial sources and the cream-yellow flowered lines were provided by SANZA. These tubers were grown in a greenhouse (heated from 15°C and vented at 25°C) under ambient light conditions.

**Colour Mutants**

Flowers were collected at various stages of development. Epidermal peels were made from these flowers for examination of chromoplast development. Pigment profiles of the orange and two cream-yellow lines were made with tissues from young leaves and two different stages of flower development. Carotenoid pigment analysis was performed as described by Lewis et al. (1998) with total carotenoid content being determined by spectrophotometric absorbance measurements. Individual pigments were identified using HPLC with lutein, zeaxanthin, \( \beta \)-cryptoxanthin and \( \beta \)-carotene being identified by comparing retention lines and on-line spectral data with standard samples.

**Polyploidy**

A tetraploid *Sandersonia* (Morgan et al., 1999) plant provided the pollen used to pollinate diploid *Sandersonia* plants. Enlarged ovules were rescued from swollen ovaries 14-30 days after pollination and transferred to in vitro culture using procedures and media similar to those described by Morgan et al., (1995, 1998). The triploid nature of the plants derived from ovule culture was confirmed using flow cytometry and later by chromosome counts and morphological comparison of diploid, triploid and tetraploid plants. The internal standard used for the flow cytometry was *Hordeum vulgare* cv. Sultan with a mean 2C nuclear DNA content of 11.12 pg DNA (Bennet and Smith, 1991).

**Wide Crosses**

Pollination barriers between diploid *Sandersonia* and *Littonia* were investigated using each species as male and female parents and studied following the procedures of Kho and Baer (1968).

Pollinated *Sandersonia* and *Littonia* flowers were collected 14-30 days after pollination, the ovary was surface sterilised and enlarged ovules dissected out and transferred onto modified KM media as described by (Morgan et al., 1995, 1998). The ovules continued to grow and produced a callus from which a shoot eventually appeared. Flow cytometric analysis of a small piece of tissue (approx. 20 mg) removed from the callus provided the first evidence of the hybrid nature of the tissues derived from ovule-culture. Upon transfer to a growth-regulator free, MS-based medium a shoot grew from the callus, produced 3-5 leaves and then died back leaving a small dormant or quiescent tuber. For unspouted tubers to survive acclimatisation they needed a dormancy breaking treatment of 5°C for 8-12 weeks, as required for *S. aurantiaca* (Clark, 1995) or to have shoot growth initiated before transfer to the greenhouse. The hybrid nature of the acclimatised plants was confirmed using flow cytometry, comparison of morphological features and chromosome counts. Pollen was stained with Alexander stain (Alexander, 1969).

RESULTS AND DISCUSSION

**Colour Mutants**

The wild type plant has a golden orange flower whereas the mutant plants were pale yellow. Examination of chromoplasts at different stages of flower development revealed no difference between the wild type and the mutants in the early stages of flower development (while tissues were still green). The development of colour in the flowers corresponded with significant differences becoming apparent in chromoplast structure. Chromoplasts in the wild type flowers were elongated orange-yellow organelles, but in the cream-yellow mutants they were small, colourless globular structures.
There was little difference in total pigment profile in young leaf tissue although a higher overall carotenoid level was observed in young leaf tissue of one of the cream-yellow types. Both of the cream-yellow types had lower levels of carotenoids in the floral tissues than the golden orange wild type. One type contained the same pigments as the wild type and the other accumulated different types of pigments. Flowers from wild type flowers and one of the cream-yellow types accumulated mainly $\beta$-cryptoxanthin and xeaxanthin with trace levels of $\beta$-carotene. The other cream-yellow type accumulated mainly $\beta$-carotene and lutein with low levels of $\beta$-cryptoxanthin.

**Polyplloid Sandersonia**

The ploidy status of the diploid, triploid and tetraploid plants was indicated by flow cytometry and confirmed by chromosome counts. The mean nuclear DNA contents of 2C nuclei from glasshouse-grown diploid, triploid and tetraploid forms of *S. aurantiaca* were 6.86 pg, 10.04 pg and 13.55 pg respectively. Chromosome counts of 24, 36 and 48 were recorded for the diploid, triploid and tetraploid plants respectively.

The triploid plants are to be slightly bigger and have stronger stems than the diploid plants.

**Wide Crosses**

There was abundant pollen germination on the stigma of both female parents with pollen tubes growing to the ends of the styles. This observation was made with crosses in both directions.

With *Sandersonia* as the female parent a 75 putative hybrids were produced and most of the ovaries that contained enlarged ovules had 2 or 3 and at most 6 enlarged ovules. For the *Littonia x Sandersonia* crosses only 2 of the enlarged ovules produced hybrids. Although many enlarged ovules were brought into culture from the *Littonia x Sandersonia* crosses and almost all of them grew, almost all of them eventually discoloured and were discarded.

Flow cytometry proved to be an efficient method for confirming the hybrid nature of the plants. Measurements of channel number for the 2 parents and 2 of the hybrids are given in Table 1. The channel number of the 2 hybrids are midway between those of the parents, confirming their hybrid nature. Channel numbers or nuclear DNA contents of hybrids midway between those of the parents have been reported in other hybrids, e.g. *Limonium* (Morgan et al., 1995, 1998).

Mitotic chromosome counts from root tips gave 2C chromosome numbers of 24 for *S. aurantiaca*, 22 for *L. modesta* and 23 for the hybrids. Pollen staining of the hybrid plants was 0% (Alexander stain) and all pollen appeared shrunken. In addition, anther dehiscence was poor and anthers in senescent flowers were only partly dehiscent. The poor pollen viability can be attributed to the formation of micronuclei after the second meiotic division. Although attempts have been made to restore pollen fertility through chromosome doubling this has not yet been achieved.

The hybrid plants had leaf and flower morphologies intermediate between the two parents. The leaf tips of *L. modesta* and the two hybrids form tendrils whereas those of *S. aurantiaca* do not, although the tendrils of the hybrids are not as well formed as those of *L. modesta*. A clear distinguishing characteristic that separated the hybrids from the parents was flower morphology. The tepals of *S. aurantiaca* flowers are fused, except at the outwardly curving tips whereas those of *L. modesta* are entirely separate. By contrast, the tepals of the flowers of the hybrids are fused for half of their length. The flower colours of the hybrids and parents were the same. The stem lengths of the putative hybrids grown in the greenhouse (1.8 m) were closer to those of *L. modesta* than *S. aurantiaca* (0.8 m), although stem length is dependent on both environment and tuber size. No morphological differences were observed between *Sandersonia x Littonia* and *Littonia x Sandersonia* hybrids.
ACKNOWLEDGEMENTS

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Literature Cited

ABBREVIATIONS
KM - Kao and Michayluk (1975) KM8p medium; MS - Murashige and Skoog (1962) medium.
### Tables

Table 1. Channel number values of 2C nuclei from *S. aurantiaca, L. modesta* and their putative hybrids and from the internal standard, *Gloriosa superba*. The standard errors of the means are given (n=3). (*No standard error is given as only one observation is available.*)

<table>
<thead>
<tr>
<th>Plant</th>
<th>Channel number of 2C nuclei</th>
<th>Channel number of <em>Gloriosa</em> 2C nuclei</th>
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<tbody>
<tr>
<td><em>S. aurantiaca</em></td>
<td>109.2 ± 0.4</td>
<td>222.6 ± 2.2</td>
</tr>
<tr>
<td>S x L hybrid</td>
<td>128.2 ± 0.7</td>
<td>215.0 ± 1.5</td>
</tr>
<tr>
<td>L x S hybrid*</td>
<td>127.1</td>
<td>215.0</td>
</tr>
<tr>
<td><em>L. modesta</em></td>
<td>151.7 ± 1.4</td>
<td>214.9 ± 1.6</td>
</tr>
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