A Fertile Tetraploid *Anigozanthos* Hybrid Produced by in Vitro Colchicine Treatment

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Additional index words. kangaroo paws, polyploidy, allopolyploid

Abstract. Kangaroo paw is a new cut flower crop native to Australia. There are several interspecific hybrids with improved flower colors, heat tolerance, and growth habit. These hybrids are sterile due to divergent evolution of the parent species. Colchicine was used to double the chromosome number of one important sterile hybrid. This hybrid is everblooming, dwarf, and heat tolerant. The resulting allodiploid was fertile, and progeny are now being evaluated.

Anigozanthos, or kangaroo paw, is an herbaceous, Australian perennial with a growth habit similar to a rhizomatous iris. The natural habitat is in a Mediterranean climate with summer drought and winter rain. *Anigozanthos* species are dormant in the summer and flower during the winter growing season (Roh & Lawson, 1987; Roh et al., 1986).

Eleven species have been described, including *A. bicolor*, *A. flavidus*, *A. fuliginosa*, *A. gabrelae*, *A. humilis*, *A. kalbarriensis*, *A. manglesii*, *A. onycis*, *A. pulcherrimus*, *A. rufus*, and *A. viridis* (Roh et al., 1986). Primary hybrids between all the *Anigozanthos* species have been produced, as well as a few secondary and tertiary hybrids. A single clone ('Bush Ranger') from Mervyn Turner's cross of *A. humilis* x *A. flavidus* is being distributed by Biotech Plants, Australia. Unlike the other *Anigozanthos* species and hybrids, this clone does not become dormant during the summer, and it flowers continuously all year. Since 'Bush Ranger' is a primary hybrid, it is genetically sterile. This project was undertaken to produce a fertile amphidiploid of 'Bush Ranger' through colchicine treatment.

Axillary vegetative buds of 'Bush Ranger' were aseptically removed from actively growing plants and placed on an in vitro proliferation medium (Ellyard, 1978) that contained Murashige and Skoog (1962) salts and vitamins, 30 g sucrose/liter, 8 g phytoagar

(Gibco)/liter and 0.5 mg NAA/liter. After 1 month, the newly formed shoots were placed on proliferation medium supplemented with 0.1% colchicine, as previously described by Griesbach (1981) for orchids. The single shoots were incubated on this medium for 1 week and then placed back on standard proliferation medium. After 6 months of continued growth and proliferation, individual shoots were collected and rooted on a medium (Ellyard, 1978) containing Murashige and Skoog salts and vitamins, 0.2 mg NAA/liter, 20 g sucrose/liter, and 8 g phytoagar/liter. All cultures were maintained at 25C with 16 hr daylight, 260 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ cool-white fluorescent illumination. Rooted plants were established in a greenhouse under natural conditions. Chromosomes were counted using the standard acetocarmine technique (McClintock, 1929).

Five shoots were treated with colchicine. About five lateral offshoots developed from each shoot tip after 6 months of culture on proliferation medium. Two of the shoots did not produce tetraploid offshoots. Of the other three shoots, two produced one tetraploid offshoot each and the other produced two.

Table 1. Floral characteristics of the diploid and tetraploid 'Bush Ranger'. Values are reported as the mean of five flowers plus or minus the standard deviation times the 1 value at 95% probability.

Flower dimension (cm)	Diploid Bush Ranger	Tetraploid Bush Ranger
Flower width	3.33 $\pm$ 0.12	3.77 $\pm$ 0.12
Ovary diameter	0.48 $\pm$ 0.06	0.68 $\pm$ 0.06
Petal width	0.25 $\pm$ 0.10	0.33 $\pm$ 0.06
Petal length	2.12 $\pm$ 0.06	2.47 $\pm$ 0.12

Received for publication 3 Aug. 1989. I thank Ron Beck for his technical assistance and the Fred C. Gloeckner Foundation for support. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked **advertisement** solely to indicate this fact.

Fig. 1. Comparison of diploid and tetraploid plants (**top**) and flowers (**bottom**) of 'Bush Ranger' *Anigozanthos*. Diploid form is on the left.

The tetraploid offshoots were further proliferated in culture to assure their nonchimeral nature. No diploid offshoots developed from any of the tetraploid shoots. The tetraploid plants were rooted and evaluated in the

greenhouse.

The tetraploid form of 'Bush Ranger' had larger, fuller, flatter florets than the diploid (Table 1), taller inflorescences and a stouter plant habit (Figs. 1 and 2). Besides its im-

Fig. 2. Comparison of the chromosome number of 'Bush Ranger' *Anigozanthos*. (A) diploid $2n = 2x = 12$; (B) tetraploid $2n = 4x = 24$.

proved horticultural merit, the tetraploid 'Bush Ranger' is fertile. It is now being evaluated for pot plant production and will be released through the USDA-SAF New Crops Program.

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