Breeding Approaches to the Development of Selected Australian Native Daisies for Pot Culture

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INTRODUCTION

In the period 1990-94 Plant Growers Australia P/L conducted an extensive breeding program aimed at improving members of the Australian Asteraceae (daisies) for commercial pot culture. The specific aim was to develop novel and proprietary cultivars for markets in the northern hemisphere where a number of Australian daisy species had already achieved considerable success. Since commencement, over a dozen new cultivars have been commercialised and many are protected in this country by plant breeders rights. Cultivars derived from the project are now cultivated in Europe, North America, New Zealand, and Japan. This paper summarises the approaches adopted, both successful and otherwise, to improve this diverse flora. The aim is to provide some guidelines for horticulturalists wishing to pursue a program of plant improvement by breeding, and has particular reference to other Australian taxa which are yet to realise their full commercial potential.

PARENTAL BREEDING AND BREEDING OBJECTIVES

There are in excess of 100 Australian daisy species. Many Australian taxa are currently the subject of botanical revision, the results of which will be incorporated into the forthcoming volume of "Flora of Australia" dealing with this group. Recent publications (Wilson, 1992; Short, 1994; Salkin et al., 1995) provide useful insight into the current status of the genera targeted by this study. The broad aims of the breeding program were to develop compact, brightly coloured, free-flowering, vegetatively propagated, perennial types which would perform well in containers and garden situations. Specifically targeted was the widow box or "basket stuffer" market in the northern hemisphere, where there is growing demand for rapidly flowering vegetative material.

Over 150 taxa from all over Australia were assembled in a collection and comprehensively assessed according to the criteria above. Although perennial species were favoured, annuals with useful characters (e.g. flower colour) were also included. At the completion of this phase the genera *Brachycome*, *Rhodanthe* (syn. *Helipterum*), and *Bracteantha* (syn. *Helichrysum*) were selected for further breeding. Preliminary cytological analysis in *Rhodanthe* was useful in identifying likely "crossable" gene pools and eliminating others. Chromosome counts were obtained by observing mitosis in root tips (Salmon, 1995).

ASSESSMENT OF BREEDING SYSTEMS

Members of the Asteraceae are distinguished by their distinct combination of floral features. The inflorescence, often mistaken as a single flower, is a capitulum composed of numerous small flowers (florets) arranged on a compressed head. These florets can have a diverse morphology and sexuality, even across the same

capitulum, and the implications of this diversity for breeders is discussed in detail by Burtt (1977). In *Brachycome*, the ornamental feature of the inflorescence is the conspicuous outer whorl of ray florets. In *Rhodanthe* and *Bracteantha* the inflorescence is subtended by a series of brightly coloured involucral bracts which are papery, non-fertile, modified leaves (Sharman and Sedgley, 1988).

The complex nature of the capitulum provides many potential outcomes for pollination. It is generally accepted that the Asteraceae is well adapted for insect pollination and evidence from this study would support this view. Seed set in the absence of insect pollination was very low for most species of all genera studied and four species of *Rhodanthe* were found to be strongly self-incompatible (a failure to produce viable seed after self pollination). Detailed assessment of pollen/pistil relationships in *R. anthemoides* using florescence microscopy showed this species to possess a sporophytic type self-incompatibility system, where pollen tubes fail to penetrate the stigma (Salmon, 1995). Self-incompatibility would prove to be beneficial in hybridisation programs as it eliminates the need for emasculation (removal of anthers to prevent self-pollination) which is difficult with daisies where individual florets are often only millimetres long.

INTRA-SPECIFIC BREEDING

Several new cultivars were derived from intra-specific crosses (within a species) where naturally defined barriers to genetic exchange do not exist. Bulk pollination can be achieved by rubbing inflorescences of each plant together. More regularly however pollen was collected from florets where it had recently been presented at the tip of the anther tube, this can easily be achieved with a fine pair of forceps. Floral development is a fairly uniform process in this family. In *Rhodanthe*, pollen is shed from the anther tube well before the stigma is exposed. It is the extension of the style through the anther tube which presents the pollen as a globular mass at the tip of the anther tube, providing an ideal time to collect fresh uniform samples of pollen. Shortly after this time (within 24 h in *R. anthemoides*) the stigmatic lobes reflex and the pollen is dispersed. In *R. anthemoides* the stigma is receptive for at least 8 days after anthesis, with highest receptivity during the first 4 days (Salmon, 1995).

The mapping of floral development and identification of receptivity patterns on the stigma is an important preliminary objective with all new species. The aim of this study was to develop a uniform pollination protocol for all future cross pollination utilising fresh, viable pollen and receptive stigmas.

INTER-SPECIFIC HYBRIDISATION

Where there is insufficient natural variation within a species to meet certain breeding objectives, plant breeders often turn to wider crosses between species, and even genera, to bring new genes and character expressions to the fore. These methods were attempted with *Rhodanthe* and *Brachycome*, and whilst they were unsuccessful with the former some considerable success was achieved with the latter. Some members of this genus are known to hybridise freely (Salkin et al., 1995) and several cultivars were developed by simply crossing selected individuals, raising progeny, and selecting desirable forms. Novel flower colours, especially yellow in *B. multifida*, were introduced by inter-specific hybridisation.

The problem arises for plant breeders when inter-specific crosses are unsuccess-

ful, as one must decide how far to investigate the possible cause of the failure. Such investigations can be time consuming and expensive. Barriers to hybridisation prior to fertilisation often result from incompatibilities between pollen and pistil, often on the stigma or in the stylar tissue. Methods to observe pollen/pistil relationships in *Rhodanthe*, using fluorescence microscopy and the callose specific stain decolourised Aniline Blue, were developed and would most certainly have wider application among other Asteraceae (Salmon, 1995).

When fertilisation occurs but a viable embryo fails to develop it is often the result of; an arrest of embryo development, disintegration of the endosperm, or abnormal development of the ovular tissues (Raghaven, 1986). In such circumstances it is often possible to excise immature embryos (usually about 14 days old) and transfer these on to a nutrient medium where they can proceed to develop normally. These embryo rescue methods were adopted to derive various inter-specific hybrids of *B. formosa*. In this instance, mature seed from the cross *B. formosa* × *B. segmentosa* failed to germinate in the nursery. Upon dissection it was revealed that the embryo was withered and dead. Subsequent transfer under aseptic conditions of 14-day-old embryos onto half-strength M&S medium modified with 30 g litre^{-1} sucrose, 0.1 µm IBA, and 0.1 µm BAP, allowed for normal embryo development, germination and ultimately the selection of several new cultivars.

MUTATION BREEDING

The use of various physical and chemical agents to induce desirable mutations (genetic changes) in plant tissue has been practiced for some time but with limited success. Experiments with a cultivar of *R. anthemoides* aimed to use gamma irradiation to induce desirable colour change mutations in the involucre. Rooted cuttings were subjected to 6 krad (the dose was established in earlier trials) and 970 plants were subsequently propagated (by cutting) from regenerative shoots arising from these treated plants. Mutations were observed in 4.8% of the M1 progeny, however none were commercially important (Salmon, 1995).

Mutation is a single-celled event and treating the whole plant with a mutagen inevitably leads to problems with locating the mutated tissue, if it even exists. This problem can be compounded with recessive mutants which are difficult to isolate in the first generation and are usually revealed only after a generation of selfing. Methods such as those described above for *Rhodanthe* must be approached with a knowledge of the character(s) being targeted, the genetic inheritance of this character (which can be complex for flower colour) and an appreciation of the difficulty in locating and isolating the mutant. More recently these methods have moved into tissue culture laboratories where isolation problems can be overcome by regenerating plants from callus cultures, a method which in itself has been shown to promote mutagenesis (Larken and Scowcroft, 1981). Attempts to culture callus of *Rhodanthe* species on diverse media were unsuccessful.

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