CRYOPRESERVATION OF GREVILLEA SCAPIGERA


Grevillea scapigera, an endangered species from Western Australia, has been successfully stored in liquid nitrogen and revived. This is the first time in Australia that an endangered plant species has been cryopreserved. Cryopreservation may now offer an excellent means for long-term storage of rare or threatened Australian plants.

The need for genetic conservation of important crop species, and more recently, rare or threatened species has seen the establishment of gene banks around the world. However, conventional means of storing germplasm (seed, slow-growth storage, container and living collections) has many disadvantages including:

- loss of viability over time for seed collections
- exposure to natural calamities for vegetative propagules
- risk of infection by pathogens
- exposure to predators
- genetic drift

Cryopreservation (the storage of biological material at ultra low temperatures such as that of liquid nitrogen at -196°C) offers a means of long-term preservation when genetic populations are not subjected to the disadvantages inherent to conventional preservation techniques. However, in order to successfully cryopreserve plant species complex procedures involving the application of cryoprotectants to specimens and the subsequent freezing, thawing and regeneration of plants are required.

Cryopreservation is used as a means of long-term conservation of important crop species but has received scant attention for the conservation of rare or threatened species. The first successful cryopreservation of a rare or threatened species was of cell suspensions of Brunfelsia densifolia (Pence, 1990). As a part of the endangered species program at Kings Park and Botanic Garden, exploration of cryopreservation as a means of long-term maintenance of germplasm began in 1991.

The Corrigin Grevillea (Grevillea scapigera) is an endangered species, with only five remaining plants, from the south-west of Western Australia and is one of the 238 WA plants that are in a critical state of conservation and likely to become extinct in the next 10 years. The critical status of this species makes it an ideal candidate for cryopreservation. Shoot-tips taken from invitro maintained plantlets were treated with dimethylsulphoxide (DMSO), a chemical that acts to prevent freeze injury to material during a freeze-thaw cycle, before being frozen slowly to -40°C followed by plunging and storage in liquid nitrogen. After thawing two weeks later shoot-tips were placed on a solid medium. Twenty percent survival was obtained from the frozen shoot-tips, however survival wasn’t observed until at least 19 days after thawing. Callus proliferated from the thawed shoot-tips and shoots did not appear until 22 weeks after thawing. Shoots have continued to proliferate from this callus mass and are likely to be potted out soon. Although this is low compared to results obtained in other studies (Kartha et al 1979; Towill 1983), it is encouraging in that it shows that cryopreservation techniques are applicable to an endangered species.

Further work will centre on increasing the post-thaw survival and decreasing the length of time of the intermediary callus phase. It is hoped that the protocol for cryopreservation of Grevillea scapigera will be able to be adapted to other rare or threatened Australian species.

References

EX SITU CONSERVATION OF PULTENAEA PARVIFLORA
AT MOUNT ANNAN

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The Horticultural Research Section at Mount Annan Botanic Garden is researching techniques for the conservation of a threatened population of Pultenaea parviflora (2V). This species is endemic to Western Sydney and is currently considered to be inadequately conserved (Benson and McDougall, 1991).

The population of P. parviflora we have chosen to work on is at Badgery's Creek, near to the site of Sydney's proposed second airport and quite close to Mount Annan. Our project has been funded by the Federal Department of Transport and Communications. The population at Badgery's Creek is very degraded by semi-rural activity with plants now numbering 23 adults confined to a short narrow strip adjacent to both sides of a road. At this site there is regular dumping of household rubbish and stripping of stolen cars. A significant proportion of the area was burnt when a car was set alight there in early 1991 and no regrowth has been observed since. Grazing and market gardening in adjacent paddocks have further exacerbated decline. It is possible that reintroduction into well secured nearby bushland (ie not threatened) may be carried out in the future but this will only occur if we are assured that the plants are able to survive there and do not threaten other native species.

During 1990-1991 we collected material from this population to form an ex situ population at Mount Annan. The primary material taken was cuttings, from some of the 70 adult plants occurring there when we first surveyed the area. Some seeds were collected but in this situation we considered seeds to be a secondary source of material due to possible problems with longevity (they may have to be stored for decades) and the impact of removal of significant amounts of seeds on the viability of the existing population. Only a small amount of cutting material was removed and in some cases appears to have regenerated the plants to some extent.

We now have in our collection one or more plants of each of 46 clonal types, all of which are represented in a stock plant pot collection, field plantings and a seed collection. Because of the problems associated with

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