

Final Report to the Sir Victor Davies Foundation

Saving our native plants: Development of cryopreservation protocols for *Dysoxylum spectabile*.

Project Title: Saving our native plants: Development of cryopreservation protocols for *Dysoxylum spectabile*

Project Description: Seed banking is an important component of *ex-situ* conservation of endangered plants species. Seeds of a number of species in the New Zealand flora are classified as recalcitrant where they do not tolerate drying to the moisture contents (approximately 5%) required for conventional long term seed bank storage. For these species cryopreservation where material is desiccated using cryoprotectants and stored at ultra low temperatures (e.g. liquid nitrogen, -196°C) offers potential as an alternative conservation strategy. However, before this potential can be realised, research needs to be conducted to develop protocols suited to these species as this information does not currently exist. Several options are available for cryopreservation techniques in terms of both type of explants (e.g. seed, somatic embryos, shoot tips) and technique.

One desiccation-intolerant species in the New Zealand flora is kohekohe (*Dysoxylum spectabile*). The objectives of this project were to:

1. Determine the optimum seed developmental stage for cryopreservation.
2. Determine the desiccation sensitivity of the seeds and excised embryos.
3. Develop cryopreservation protocols necessary for successful long term storage of *D. spectabile*.

The facilities needed to carry out these studies are not available in New Zealand. This work was undertaken by Myoung Joo Park at the Millennium Seed Bank (Royal Botanic Gardens, Kew) in West Sussex, United Kingdom from June to August 2009 under the supervision of Dr Jayanthi Nadarajan, a leading expert on seed cryopreservation. The results of this work will form part of Myoung Joo Park's PhD. thesis.

Project Results: Prior to departing for the Millennium Seed Bank, development of *D. spectabile* seed was monitored from March to June 2009 to determine seed development completion and identify a suitable seed collection period for cryopreservation. Maximum seed dry weight, indicating seed was mature, was not reached until 26 June. In contrast, the seed embryo reached maximum dry weight on 23 May. Germination percentage remained above 90% for the entire March to June period that development was monitored, but, the rate of germination increased from March until May after which it remained constant. Seed was therefore considered fully mature in May and June 2009 and collected for cryopreservation.

The following research was undertaken during Myoung Joo Park's time at the Millennium Seed Bank:

Determination of the desiccation sensitivity of the seed and embryo axes of *D. spectabile* seed: Neither seed nor embryo axes of *D. spectabile* tolerated desiccation to low moisture contents. *D. spectabile* seed and embryo axes lost viability at 21% and 34% moisture contents respectively. This confirms *D. spectabile* seed's recalcitrant storage behaviour and hence their inability to be stored at the low moisture contents (approximately 5%) used in conventional seed bank conditions. The rate of drying influenced loss of desiccation tolerance. The faster the rate of drying the lower the moisture content at which the seed or embryo axis lost viability. A small proportion (10%) of embryo axes survived desiccation to 8% seed moisture content when dried rapidly. These results suggest that embryo axes rather than the whole seed should be cryopreserved, particularly as moisture can be removed more rapidly from excised embryo axes than from whole seeds.

Cryopreservation of rapidly dried embryo axes: Embryo axes were rapidly desiccated prior to cryopreservation. As in the preliminary study a small percentage (10%) of embryo axes survived desiccation to 15% moisture content. After desiccation and cryopreservation 10% of the embryo axes remained viable. However the embryo axes could not be recovered post-cryopreservation, i.e., when axes were cultured on a growing medium a normal seedling did not develop because no shoot development occurred.

Encapsulated dehydration of excised embryo axes: Surface sterilised *D. spectabile* embryo axes were encapsulated in sodium-alginate beads before dehydration. The advantages of encapsulated dehydration are that it avoids the use of toxic cryoprotectants and gives extra mechanical protection to the embryos during dehydration. Embryo axis viability declined from 59% immediately after encapsulation to 17% after 6 hours dehydration. At 6 hours the embryo axis moisture content was 18%. The survival of some embryo axes at 18% moisture suggests that encapsulation of embryo axes may also be an option for cryopreservation of *D. spectabile* seed tissue. A significant percentage (23%) of encapsulated dehydrated embryo axes survived subsequent dehydration. Survival at the end of cryopreservation was 20%, but again none of the surviving embryo axes could be recovered because of the failure of shoots to develop.

Vitrification of embryo axes: One method of avoiding ice crystal formation during cryopreservation is to induce the formation of a non-crystalline glassy state (vitrification). Vitrification is achieved by pre-treating embryo axes in a loading solution which includes a high concentration of sucrose (a cryoprotectant) before exposing the axes to a 'vitrification cocktail' containing dimethyl sulphoxide, glycerol, ethylene glycol and sucrose followed by cryopreservation. Survival of *D. spectabile* embryo axes was 55% after sucrose pre-treatment. Survival of vitrified embryo axes after cryopreservation was 20%.

Conclusions: This study was designed to develop cryopreservation protocols for embryo axes of *D. spectabile* using commonly used protocols; rapid desiccation, encapsulation dehydration and vitrification. Although this work demonstrated that cryopreservation of *D. spectabile* embryo axes is possible, a number of technical problems remain to be solved. These include the low (20%) post-cryopreservation survival of embryo axes and the failure of surviving axes to develop shoots. This failure to develop shoots is the main limitation to cryopreservation of embryo axes of *D. spectabile*. Further research will be required to identify the reasons for the failure of post-cryopreservation shoot development and to identify appropriate tissue culture protocols for recovering embryo axes *in vitro* and encourage normal seedling development.