Conservation and Recovery of *Cheesemania* 'Chalk Range' an Endangered New Zealand Brassicaceous Plant

C.L. Hargreaves
New Zealand Forest Research Institute Limited, Private Bag 3020, Rotorua

D.R. Smith
MetaGenetics, State Highway 30, RD4, Rotorua, <dalesmith@xtra.co.nz>

M.N. Foggo
Department of Science, Central Institute of Technology, P O Box 40 740, Upper Hutt

M.E. Gordon
School of Biological Sciences, Victoria University, P O Box 600, Wellington

INTRODUCTION

The Marlborough chalk cress *Cheesemania* 'Chalk Range' is a critically endangered New Zealand plant (Cameron et al., 1995). It is a small herb (10 to 15 cm tall, with inflorescences up to 25 cm) and the surviving plants were found on mostly south-facing montane bluffs. The leaves form a single rosette on a large root which extends above the ground giving the plant the appearance of a small palm tree (Anon, 1992). The plant is a monocarpic perennial. It is highly palatable and introduced browsing animals such as goats, possums, sheep, and hares were thought to have eaten the plant to extinction by the 1970s (Anon, 1992).

However, in 1992, 45 chalk cress plants were found clinging to steep bluffs on private land in the Chalk Range in Eastern Marlborough by Department of Conservation staff (Anon, 1992). In March 1992, at the Forest Research Institute (FRI) Rotorua, New Zealand, a propagation programme was initiated, with the aim of testing the viability of field-collected seed and of establishing protocols for in vitro multiplication of plants of diverse genotypes. Subsequently the propagation programme included an investigation into cryopreservation of in-vitro-grown plants and long-term storage of seed.

The importance of cryopreservation of endangered species is that it facilitates the long-term preservation of plants which may produce seed erratically, have threatened natural habitats, and which might have genetic value for crop improvement. Long-term cryopreservation has the potential to preserve a wide range of genotypes for genetic evaluation and intercrossing while using a minimum of space and cost.

MATERIALS AND METHODS

Propagation. Seed capsules were collected from three plants and some of them were sterilised in 270 mg litre^{-1} mercuric chloride with surfactant followed by two rinses in sterile water. Seeds (38) were placed on plant nutrient medium in petri dishes and incubated with a 16-h light (24C), 8-h dark (18C) photoperiod. After 20 weeks seeds were assessed for germination. Plantlets from germinated seeds were transferred to a Murashige and Skoog medium (MS) (Murashige and Skoog, 1962),

containing 2 mg litre^{-1} Merck activated charcoal, in 600-ml jars. At 4-weekly intervals, plants were cut into 10-mm nodal segments and placed in fresh medium. Nodal segments with roots were transferred to potting mix in the glasshouse.

Cryopreservation. A cryopreservation experiment was carried out using a vitrification solution designated PVS2 (Sakai et al., 1990; 1991a; 1991b; Yamada et al.,1991). A small number of in-vitro-grown shoots were cut into 3- to 5-mm nodal segments and pretreated for 2 days at 4C on preconditioning media (a modified Quoirin and Lepoivre medium, Horgan, 1987) containing 50 g litre^{-1} glucose or 73 g litre^{-1} sorbitol (0.4 M), with or without 5% dimethylsulphoxide (DMSO). There were 2 to 7 nodal segments per treatment. Following preconditioning, PVS2 was added to nodal segments contained in 2-ml cryopreservation vials. These were cooled in a Nalgene^{TM} Cryo 1C Freezing Container held in a -80C freezer for 1.5 h prior to immersion in liquid nitrogen (-196C), control segments were removed after 1.5 h cooling. After 1 week of storage in liquid nitrogen (LN) tissue was thawed rapidly (2 min in a 40C water bath) and PVS2 rinsed from segments with a high osmoticum sucrose solution (340 g litre^{-1}). Nodal segments were placed on MS medium and returned to standard growing conditions. Segments were assessed for growth 4 weeks after thawing.

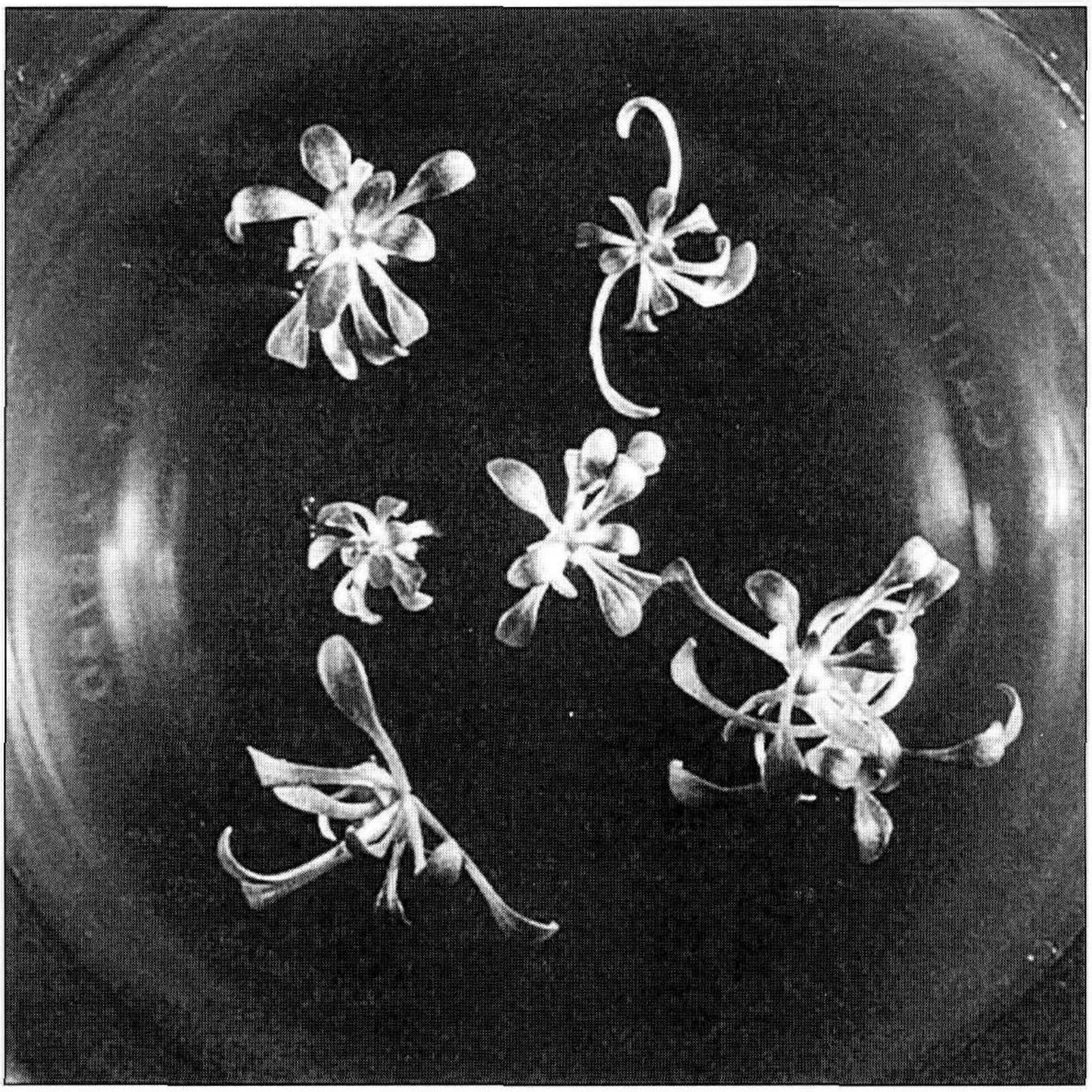

Figure 1. Vigorous shoot cultures of *Cheesemania* 'Chalk Range' on Murashige and Skoog medium.

Seeds which were not sterilised in the initial 1992 germination experiment were stored at 4C. In 1996, these seeds were surface sterilised, placed on medium and assessed for germination at 20 weeks (total of 326 seeds).

RESULTS AND DISCUSSION

Propagation. Chalk cress plants were successfully propagated using the techniques described. The multiplication factor was high with a four-fold amplification per nodal segment possible at each 4-weekly transfer. No contamination was observed at any stage during the in-vitro culture of *Cheesemania* 'Chalk Range'. After 20 weeks 81% of the fresh seed had germinated and shoot cultures established from these were vigorous and subcultured well (Fig. 1). Nodal segments spontaneously produced roots and these plants were transferred to the glasshouse. However, some problems were experienced with both damping-off fungi and white cabbage butterfly and none of the plants survived to produce seed. These problems could be overcome by spraying with fungicide and establishing plants in the glasshouse in winter so that they would be hardened and less palatable to the white cabbage butterfly.

CRYOPRESERVATION

Table 1. Percentage of *Cheesemania* 'Chalk Range' nodal segments showing axillary meristem development 3 to 4 weeks after storage in liquid nitrogen.

Preconditioning Media	(Control) Preculture + PVS2	Preculture + PVS2 + LN
50 g litre^{-1} glucose	100 (2)*	0(7)
50 g litre^{-1} glucose + 5% DMSO	50 (2)	33(6)
73 g litre^{-1} sorbitol	50 (2)	0(6)
73 g litre^{-1} sorbitol + 5% DMSO	50 (2)	17(6)

* Number of nodal segments/treatment

Plants of the chalk cress were successfully regenerated from cryopreserved tissue. Cryopreservation preculture treatments that included 5% DMSO gave up to 33% survival following immersion and storage in liquid nitrogen (Table 1). No survival was observed in nodal segments precultured without 5% DMSO (treatments 1 and 3, Table 1). Dead tissue remained bleached in appearance and green shoots elongated from meristems at the base of the leaf axils (Fig. 2). Other researchers have also observed the beneficial effects of 5% DMSO in preculture media with angiosperm in-vitro-grown axillary or shoot-tip meristems (Kartha et al., 1979 and 1980; Fukai et al., 1991; Touchell et al., 1992).

It was noticeable that seed viability was reduced on storage at 4C and after 4 years the germination had reduced from 81% to 43% after 20 weeks in germination conditions. The storage of seeds thus seems an unlikely long-term conservation practice.

Cryopreservation has been used with other endangered species with variable success. The Australian plant *Grevillea scapigera* was thought extinct until 1989

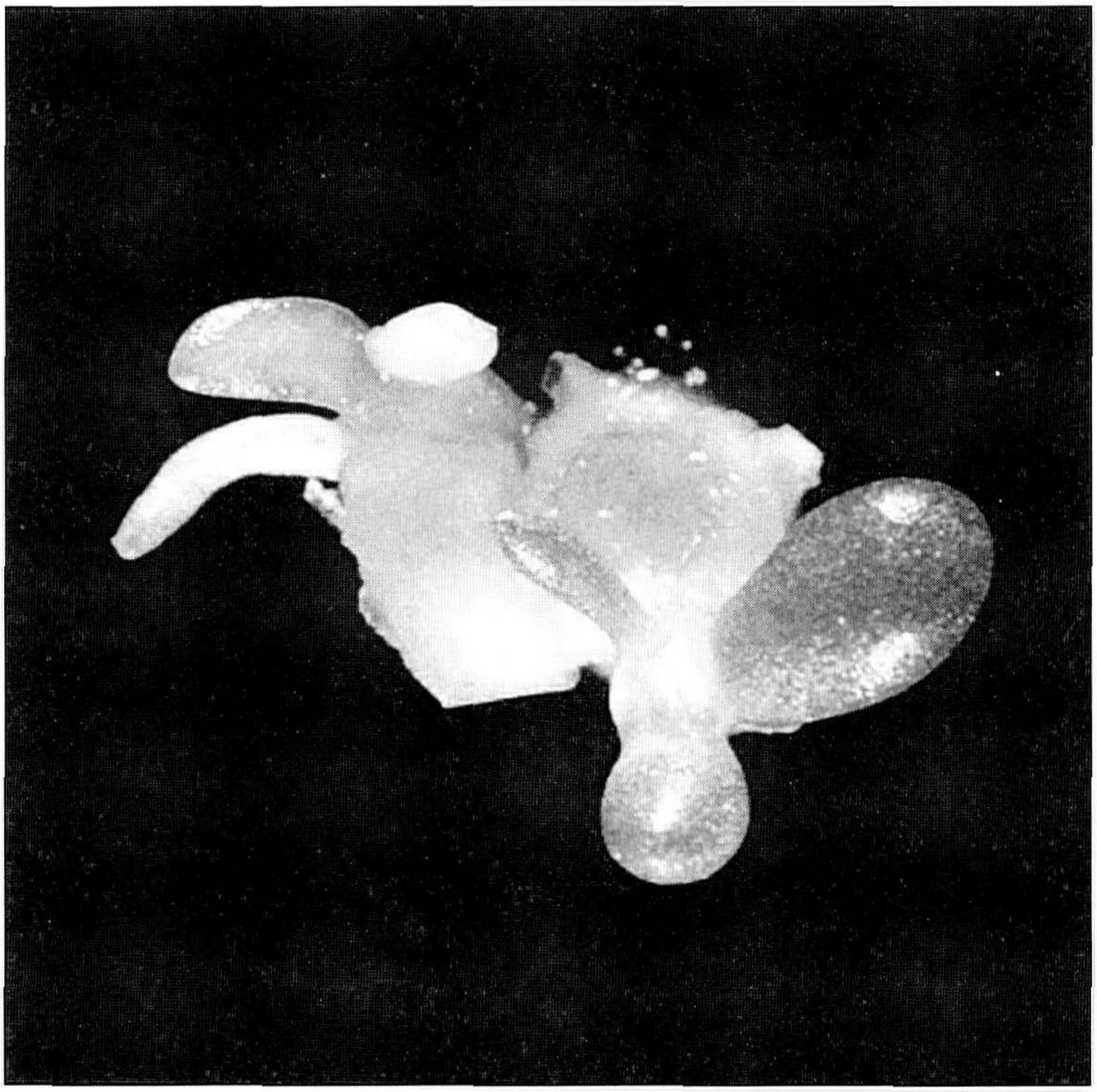

Figure 2. *Cheesemania* 'Chalk Range' development following cryopreservation (4 weeks following thawing).

when six plants were found and an in vitro propagation programme was established. Chrysanthemum [*Dendranthema* ×*grandiflorum* (syn. *Chrysanthemum* ×*morifolium*)] related species are also seriously endangered in Japan. Shoot tips of both these species were precultured with media containing 5% DMSO (Fukai et al., 1991; Touchell et al., 1992). Up to a 100% of the *D.* ×*grandiflorum* and related species produced plants and 20% of the *G. scapigera*. Long-term preservation of in-vitro-grown *Cheesemania* can thus be justified for all the reasons of erratic seed production, threatened habitat, possible importance to crop improvement programmes, and economy in terms of space and duration of storage required.

CONCLUSIONS

These results are encouraging for the long-term survival prospects of the critically endangered New Zealand *Cheesemania* 'Chalk Range'. Over 125 genotypes and more than 400 plants are now growing in vitro. Plants have been grown in the glasshouse for up to 2 months. Improved glasshouse techniques should allow such explants to grow to maturity. Successful plant regeneration from cryopreserved meristems has been demonstrated. Such storage will provide an economic method of holding in-vitro-grown *Cheesemania*.

Acknowledgments. Phil Clerke, Shannel Courtney and Cathy Jones of the New Zealand Department of Conservation are thanked for their enthusiastic support of the project.

LITERATURE CITED

Anon. 1992. Rarer than the kakapo. Forest and Bird Magazine N.Z. 256:5.

Cameron, E.K., P.J. De Lange., D.R. Given., P.N. Johnson, and **C.C. Ogle.** 1995. New Zealand Botanical Society, Threatened and local plant lists (1995 Revision). N.Z. Bot. Soc. Newsletter, March 1995. 39:15-28.

Fukai, S., M. Goi and **M. Tanaka.** 1991a. Cryopreservation of shoot tips of *Chrysanthemum morifolium* and related species native to Japan - chrysanthemum shoot tip culture; propagation germplasm preservation. Euphytica 54(12):201-204.

Horgan, K. 1997. *Pinus radiata.* pp.128-145. In: Cell and tissue culture in forestry. Vol. 3. J.M. Bonga and D.J. Durzan (Eds.). Martinus Nijhoff Publishers, Dordrecht, Netherlands.

Kartha, K.K., N.L. Leung and **O.L. Gamborg.** 1979. Freeze-preservation of pea meristems in liquid nitrogen and subsequent plant regeneration. Plant Sci. Letters 15:7-15.

Kartha, K.K., N.L. Leung, and **K. Pahl.** 1980. Cryopreservation of strawberry meristems and mass propagation of plantlets. J. Amer. Soc. Hort. Sci. 105:481-484.

Murashige, T. and **F. Skoog.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Sakai, A., S. Kobayashi, and **I. Oiyama.** 1990. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. var. *Brasiliensis* Tanaka) by vitrification. Plant Cell Rept. 9:30-33.

Sakai, A., S. Kobayashi, and **I. Oiyama.** 1991a. Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* var. *Brasiliensis* Obs.) by a simple freezing method. Plant Sci. 74:243-248.

Sakai, A., S. Kobayashi, and **I. Oiyama.** 1991b: Survival by vitrification of nucellar cells of navel orange (*Citrus sinensis* var. *Brasiliensis* Tanaka) cooled to -196C. J. Plant Physiol. 137:465-470.

Touchell, D.H., K.W. Dixon, and **B. Tan.** 1992. Cryopreservation of shoot tips of *Grevillia scapigera* (Proteaceae): A rare and endangered plant from Western Australia. Aust. J. Bot. 40:305-310.

Yamada, T., A. Sakai., T. Matsumura, and **S. Higuchi.** 1991. Cryopreservation of apical meristems of white clover (*Trifolium repens* L.) by vitrification. Plant Sci. 78:81-87.