IN VITRO CULTIVATION OF *TODEA BARBARA*—FROM SPORE TO SPOROPHYTE

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Abstract. The in vitro cultivation of *Todea barbara*, a fern endangered in New Zealand, is described. Spores were collected from the wild, disinfested, and grown in sterile water on half strength modified Murashige and Skoog medium with Linsmaier and Skoog vitamins without growth hormones. Culture conditions were: light intensity, 2000 lux; photoperiod, 16 hours; and temperature, 23°C. After six months sporophytes began to appear and were pricked out and replated on the above medium. Alternatively, this tissue was macerated, the dissected pieces of gametophyte tissue regenerating to increase the number of sporophytes available. When frond and root growth was sufficient the ferns were deflasked and hardened off under glasshouse conditions. After one year spore formation had occurred on the fronds of the ferns still held in culture. The cultivation of *T. barbara* from spore to sporophyte was repeated entirely under in vitro conditions. This process has been repeated annually over the last four years.

INTRODUCTION

T. barbara is found in South Africa, Australia, and New Zealand. A fern from the old world tropics, it is restricted to the milder climates of the North Island in New Zealand. It is a large attractive fern with fronds up to one metre long, dark green to yellowish green in colour, leathery, and smooth. Sporangia form at the base of the frond on the under surface, the remainder of the frond is barren. Versatile in habitat, *T. barbara* can grow in full sunlight or in shade, in rich humus, or in clay soil. Thus it is very suitable for the home garden. *T. barbara* is classified as an endangered species in New Zealand (1); that is, it may become extinct, as survival in the wild is unlikely.

The Black Hill Native Flora Centre in South Australia has named *T. barbara* amongst its rare and difficult to propagate plants. *T. barbara* can be propagated from spore, by normal methods, or by in vitro techniques, and consequently it will survive in the nursery trade.

MATERIALS AND METHODS

In autumn, spores of *T. barbara* were collected from the wild. Disinfestation was achieved by an agitated wash in 0.6% sodium hypochlorite followed by a rinse in sterile distilled water. Spores were grown in sterile distilled water on a half strength modified Murashige and Skoog medium supplemented with Linsmaier and Skoog vitamins, 20 g/l of sucrose, 7 g/l Davis agar, with the pH adjusted to 5.7. No growth hormones were used. Plant material was subcultured every two months, and held in culture conditions of

temperature: 23°C; photoperiod, 16 hours; and light intensity, 2000 lux.

The gametophyte tissue was macerated using a French "Mouli" parsley cutter that had been steam sterilised at 121°C for 20 min. The macerated tissue was resown on half strength Murashige and Skoog medium, as above.

An attempt was made to find a multiplication medium suitable for the proliferation of the sporophytes. Half strength Murashige and Skoog medium as above, was used with an addition of growth hormones, 0 to 1.5 mg/l benzylaminopurine (BAP), and 0 to 0.2 mg/l kinetin, with 0 to 2.0 mg/l NAA.

The sporophyte tissue formed spores while still in culture and these spores were sown on half strength Murashige and Skoog medium as above.

RESULTS

The disinfested spores of *T. barbara* gathered from the wild, germinated after ten days and grew slowly. After six months sporophyte fronds were showing amongst the gametophytes.

The sporophytes were pricked out, replated at two-month intervals, until after six months the root growth and fern size were adequate for deflasking. Ferns were planted in seed trays containing 50/50 peat/pumice-sand and covered with a plastic bag. After four weeks the plastic was slowly removed and the ferns gradually hardened off under glasshouse conditions.

The use of growth hormones to stimulate the proliferation of sporophytes of *T. barbara* was unsuccessful. The plants grew slowly and multiplication was limited.

The macerated gametophytes regenerated with a 4 to 6 fold increase in tissue substance, and within six months sporophytes were beginning to appear.

After one year in culture the spores that formed on the fronds of the ferns still held in culture were germinated and grown in a similar manner to the spores collected from the wild.

DISCUSSION

T. barbara may be continuously cultivated from spore to sporophyte under in vitro conditions. This method obviates the necessity to collect and resterilise fern spore from the wild.

The maceration of gametophytes was a method of increasing by 4 to 6 fold the available fern supply but the attempt at micropropagation of sporophytes using growth hormones was unsuccessful.

With the continuous supply of in vitro fern spore assured, and aided by the maceration technique, a feasible regime for the cultivation of *T. barbara* can be achieved.

LITERATURE CITED

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2. Murashige, T. and Skoog, P. 1962. Revised media for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plant.* 15:473–97.

THE NECESSITY FOR NEW ZEALANDERS TO KEEP UP WITH THE LATEST PLANT SCIENCE TECHNOLOGY

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As a plant breeder I find the late 1980s an exciting time. Right now we are on the threshold of a major technological breakthrough which will be comparable in impact to the development of air transport, television, and computers.

As this breakthrough is very much in our area of activity it is essential that everyone involved in horticulture appreciates what is happening and understands the implications it has for New Zealand. I am referring to what is commonly termed "Biotechnology" or "Genetic Engineering".

The development of "improved" plants and animals has traditionally been severely restricted by a whole range of biological barriers. Even in cases where it has been possible to bypass a barrier the methods have usually taken a long time. Dr Legro's development of the red delphinium is a good example. This has taken the whole of his working lifetime.

In essence, biotechnology embraces a number of related disciplines that have reached a stage of development, and have come together, so that things which plant breeders have long wanted to do are now starting to become possible. A whole range of techniques are covered by the term "biotechnology" and central to all of these is tissue culture, a process now familiar to most of you. Some techniques are now considered "Low Tech" like embryo rescue, endosperm culture, and anther culture. Others are considered to be "High Tech" and involve unravelling and understanding the genetic code itself.

One of the big attractions of the "High Tech" end is the process of "transformation". Here it is possible to identify individual genes and to move them from one organism to another. As the genetic code is essentially common to plants, animals, and micro-organisms it is possible to put animal genes into plants and *vice versa*. It will also be possible to manufacture genes.