

## THE USE OF TISSUE CULTURE TECHNIQUES IN KIWIFRUIT BREEDING

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### ABSTRACT

Methods are being investigated in the use of tissue culture techniques as a tool in plant improvement of *Actinidia* species. Protocols have been defined for tissue culture and regeneration of plants using different plant parts as source material. Plantlets showing morphological variation have been obtained, and further assessment of these variants is proceeding.

### KEY WORDS

*Actinidia*, somatic variation, embryogenesis.

### INTRODUCTION

Tissue culture techniques are providing valuable additions and alternatives to conventional plant breeding practices. Somatic variation originating in tissue culture has been the source of novel genotypes which have proved to be of economic value in, for example, potato (Shepard *et al.*, 1980) and sugarcane (Liu and Chen, 1976; Liu, 1981). In plants with a complex and largely uncharacterised genome, and where generation time is long, the method offers a relatively simple and quick way of obtaining new cultivars. Furthermore, tissue culture methods can allow the rescue of aborting embryos from interspecific hybrids, as well as the production of haploid plants via anther or pollen culture, and triploid plants via endosperm culture.

An important prerequisite for the utilisation of tissue culture in plant improvement, is the development of reliable methods of callus formation and subsequent regeneration of plants. This paper describes protocols for the tissue culture of *Actinidia* species.

### MATERIALS AND METHODS

Tissue cultures of *Actinidia chinensis* Planch. var *chinensis* C.F. Liang, and *Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson, cultivars Hayward and Matua, were established from the following plant parts:

petioles, nodal and internodal segments, leaves, fruits, stamen filaments, anthers, petals, ovaries, and seeds. They were surface sterilised in hypochlorite 1.5%. The basic culture medium was LS salts (Linsmaier and Skoog, 1965) and vitamins, plus 3% sucrose and various growth regulator supplements depending on the response desired. The pH of the medium was 5.8 before autoclaving, and the gelling agent was 0.8% Difco Agar. All media ingredients were sterilised by autoclaving at 103.5 KPa for 20 minutes. Cultures were incubated at 26°C under cool white fluorescent illumination (20 microeinsteins m<sup>-2</sup>. s<sup>-1</sup>) with a 12-hour photoperiod.

### RESULTS AND DISCUSSION

The following protocols have been established for petiole segments of *A. deliciosa* cv. Hayward.

- To establish callus cultures capable of regeneration (Fig. 1). Culture petiole segments approximately 1 cm in length on basic medium plus 2 isopentenyl adenine (2iP) 5 mgm. 1<sup>-1</sup> plus indole acetic acid (IAA) 0.3 mgm. 1<sup>-1</sup> for two weeks. Subculture excised callus every two to three weeks. Shoots will appear after two to three weeks, and should be removed when approximately 1 cm long and subcultured on the same medium.
- To proliferate callus (Fig.2), culture segments on basic medium plus 2,4-dichlorophenoxyacetic acid (2,4-D) 1 mgm. 1<sup>-1</sup>. After removal of 2,4-D and sub-culturing on basic medium plus 2iP 10 mgm. 1<sup>-1</sup>, it is five to six months before this callus is capable of regenerating shoots.

Nodal and internodal stem segments show similar regenerative ability, while leaves, fruits and reproductive structures are significantly slower in their response. Different parts of the plant respond to changes in the protocols depending on the length of time in culture, and previous hormonal treatments.

To induce rooting of excised plantlets it is necessary to remove cytokinin from the medium, while further manipulation of the hormone additives is necessary to induce somatic embryogenesis (Fraser and Harvey 1986).

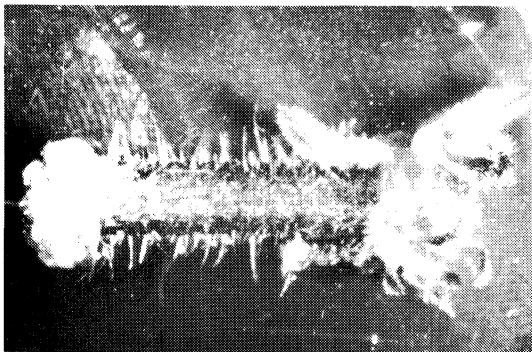


Figure 1. Petiole segment cultured on basic medium plus  $2iP$   $5 \text{ mgm. l}^{-1}$  plus  $1AA$   $0.3 \text{ mgm. l}^{-1}$ , showing shoot formation from callus after three weeks (x 6).

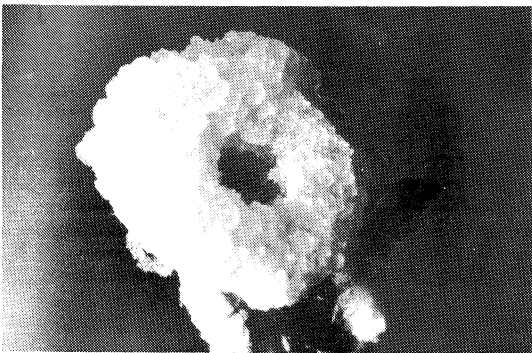


Figure 2. Proliferation of callus on basic medium plus  $2,4-D$   $1 \text{ mgm. l}^{-1}$  (x 5.6).

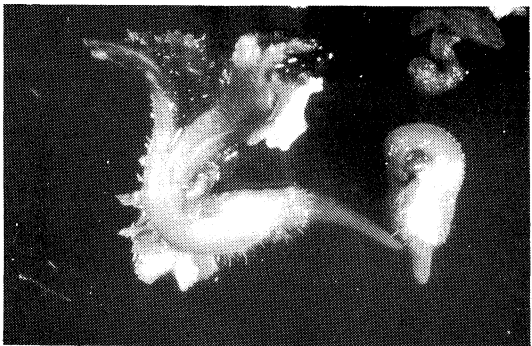


Figure 3. Somatic embryos (x 8).



Figure 4. Morphological variation in plants originating in tissue culture (x 0.25).

Anther wall tissue responds to the addition of  $1 \text{ mgm. l}^{-1}$  Zeatin plus  $1 \text{ mgm l}^{-1}$  IAA to the basic medium with the production of embryos (Fig. 3).

Regenerating cultures have been initiated from embryo and endosperm tissue excised from immature seeds. Plants of embryo origin have been successfully reared, but while endosperm callus has been induced to shoot, plantlets are slow growing and remain in culture.

Ploidy level investigation of tissue culture derived plants and their assessment in the field is continuing. Morphological variation such as leaf shape and size, and internodal length, and altered growth rates have been observed among plantlets (Fig. 4) but the usefulness of these variants cannot be assessed until the plants have reached maturity. To date, tissue culture derived plants retain the seedling character of a long juvenile phase. Further characterisation of the genome, and linkage and correlation of genes would be of help in assessing the value of plants.

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