

## Short communication

# Organogenesis potential for kiwifruit (*Actinidia deliciosa*) cuttings and pedicel treated with Agromil-Plus®

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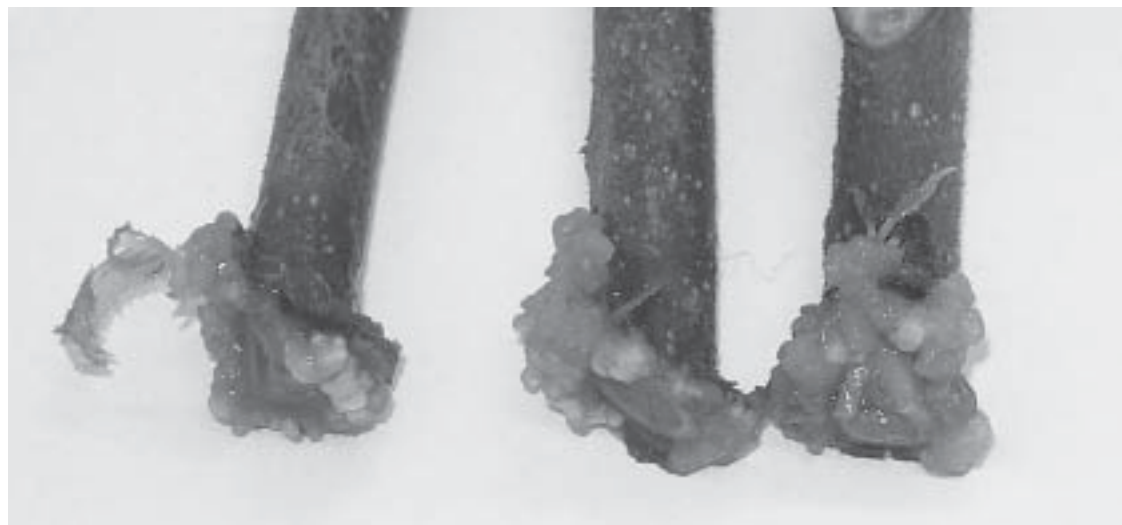
**Abstract** A preliminary evaluation was made of the organogenesis capability of kiwifruit (*Actinidia deliciosa*) pedicels *in vivo* and orchard cuttings *in vitro*, using Agromil-Plus® (Agroenzymas Laboratories, Tlanepantla, Mexico) which is a mixture of CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea) with natural bioregulators. 'Hayward' kiwifruit (*A. deliciosa* var. *deliciosa*) cuttings were submerged in a solution with a final concentration of 10 µl/litre CPPU plus natural bioregulators. Leafy shoot formation from callus was observed in 10% and 30% of the cuttings treated with Agromil-Plus® on the distal and proximal ends, respectively. For pedicel treatments, after the fruitlet was removed, the pedicel was inserted in a vial filled with Agromil-Plus®. Leafy shoot formation from callus was recorded in 20% of the treated pedicels. Some cuttings from field-grown plants and pedicels were capable of producing shoots when they were treated with CPPU plus natural plant growth regulators.

**Keywords** callus production; cytokinins; diphenylurea; indirect organogenesis

## INTRODUCTION

CPPU or forchlorfenuron (N-(2-chloro-4-pyridyl)-N'-phenylurea) is a synthetic cytokinin-active phenylurea with physiological activity exceeding that of zeatin (Mok & Mok 2001). The formation of organs from plant callus depends on several factors such as the type of inoculum, the medium, and the environmental conditions (Dodds & Roberts 1985). Kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa*) organogenesis *in vitro* has resulted from callus derived from leaves (Hopping 1994), buds (Lionakis & Zirari 1991), internodes (Shimura et al. 1990), petioles and roots (Famiani et al. 1997). There are no reports of shoot regeneration from pedicel tissue in the literature.

Shoot organogenesis provoked by the inclusion of CPPU in culture media has occurred in callus of internodes (Shimura et al. 1990) and leaves (Matsuta et al. 1993) of *A. deliciosa*, and leaves of *A. eriantha* (Zhang & Qian 1994), cotyledons of apple (Liu et al. 1994), and internodes of raspberry (Millan-Mendoza & Graham 1999). This type of organogenesis frequently produces *in vitro* variations (Demarly 1986). Physiological plant processes such as the promotion of fruit (Woolley et al. 1992) and vegetative growth (Cruz-Castillo 1998) have been manipulated through field applications of CPPU. The fruit growth response to this compound has been enhanced when applied with other bioregulators such as 2,4-dichlorophenoxyacetic acid and gibberellic acid (Cruz-Castillo et al. 1993). In the production of regenerated plants from callus, the knowledge of the organogenesis ability of different tissues is useful to exploit mutagenesis and select new genotypes via somoclonal variations. The aim of this work was to obtain a preliminary evaluation of the organogenesis capability of kiwifruit pedicels *in vivo* and orchard cuttings *in vitro* using Agromil-Plus® that contains a mixture of CPPU with natural hormones.



**Fig. 1** Leafy shoots from callus produced at the base of 'Hayward' kiwifruit (*Actinidia deliciosa*) stem cuttings.

## MATERIALS AND METHODS

### Experiment 1

Canes were excised from five adjacent 8-year-old 'Hayward' (*A. deliciosa* var. *deliciosa*) vines established in the Fruit Crops Unit of Massey University, Palmerston North, New Zealand. Plant material was collected at 0900 h on the 2 February 2002, and immediately divided into 12-cm lengths of 12–16 mm diameter. Thirty two-node cuttings without any buds on the base were randomly selected and completely submerged for 6 h in a diluted solution of the bioregulator formulation Agromil-Plus® (Agroenzymas Laboratories, Tlanepantla, Mexico). This product contains 2000 µl/litre CPPU plus natural auxins, gibberellins, cytokinins, and vitamins from plant extracts. Agromil-Plus® was diluted with reverse-osmosis purified water to give a treatment of 10 µl/litre CPPU plus natural bioregulators. Another 30 two-node cuttings were treated with purified water as controls. Each group of cuttings after treatment was submerged in a solution of Bravo™ fungicide (500 g/litre chlorothalonil) 350 ml/litre for 15 min. The cuttings were placed in jars with the proximal end and one-third of the length of each cutting immersed in purified water. Water was replaced every 2 days for the first 6 days and afterwards every week. Cuttings were maintained at room temperatures of 21–25°C in natural daylight. Jars with cuttings were arranged

in a completely randomised design. The number of cuttings that callused and/or had apical or basal leafy shoot regeneration was recorded. The Wilcoxon rank-sum non-parametric test was used to determine differences between the two treatments. This test is the non-parametric equivalent of the independent or two-sample *t*-test (McCall 1996). The procedure Npar1way of the SAS-8e version was used to perform this test.

### Experiment 2

The experiment was carried out on five adjacent vines in the same kiwifruit orchard as Experiment 1. Twelve inflorescences per vine representing one terminal, and one or two lateral fruit were selected (see fig. 1 in Antognozzi et al. 1991) to establish experimental inflorescences with only one terminal and one lateral fruit. The lateral fruit on each of the 60 inflorescences was removed 30 days after full bloom in 2001, and the pedicels inserted into empty vials partially covered with parafilm™. The vials were attached by two rubber bands to the pedicel of the terminal fruit. Each vial with the pedicel inside was filled with 2.5 ml of purified water or 2.5 ml of Agromil-Plus® at a final concentration of 5 µl/litre CPPU plus natural bioregulators. Treatments were repeated on 11 and 23 January 2002, 49 and 61 days after full bloom, respectively. The two treatments were arranged in a randomised complete block design where the vines were considered as blocks.

Leafy shoot formation from callus was counted on the pedicels studied, and the standard errors considering the vines as replicates were determined.

## RESULTS AND DISCUSSION

A significantly ( $P \leq 0.01$ ) larger number of cuttings collected in the orchard and submerged in 10  $\mu\text{l/litre}$  CPPU plus natural bioregulators for 6 h produced callus in comparison to untreated cuttings. Callus production was observed on the cut surfaces on the bottom (in water) and top of the cuttings. Visible callus production started 2 weeks after treatment with 100% of treated cuttings having basal callus compared to 50% for the controls. Corresponding figures for the top of the cuttings were 70% and 20%. Callus on both cutting ends treated with Agromil-Plus<sup>®</sup> was green and of a disorganised type (Atwell et al. 1999). Leafy shoot formation in 30% of the cuttings treated with Agromil-Plus<sup>®</sup> collected in the orchard was recorded on the basal callus (Fig. 1), and only 10% in the top callus. Visible leaves appeared on callus at the bottom and top of the cuttings, after 50 and 60 days of the Agromil-Plus<sup>®</sup> treatment, respectively. Control cuttings produced callus only.

Pedicels inserted in 5  $\mu\text{l/litre}$  CPPU plus natural bioregulators were thicker than those observed in kiwifruit dipping applications of CPPU (Woolley et al. 1992). Some of them were deformed and thick, and the green callus totally covered the cut surface where the fruit had been removed. Leafy shoot formation from callus (Fig. 2) was recorded in 12 treated pedicels (20% total) that corresponded to  $2.4 \pm 0.6$  leafy shoot formations per vine. Leaves appeared 70 days after the first Agromil-Plus<sup>®</sup> application.

In previous studies, no callus organogenesis was obtained by application of kinetin, gibberellic acid, indolebutyric acid, thiourea, and abscisic acid to field kiwifruit rootless cuttings *in vitro* (Lionakis & Schwabe 1984). In the present work, a mixture of CPPU with natural bioregulators promoted shoot regeneration through callus formation on vegetative material of that type. *In vitro* organogenesis mediated by CPPU in plant tissue culture on media has been observed (Liu et al. 1994). In kiwifruit, Shimura et al. (1990) and Matsuta et al. (1993) found shoot primordia on callus derived from kiwifruit internodes and leaf, respectively, after culture on a media supplied with CPPU as the only growth regulator.



**Fig. 2** Leafy shoot from callus produced by a lateral pedicel (arrow). Pedicel inserted into a vial filled with Agromil-Plus<sup>®</sup> attached to the terminal fruit pedicel using rubber bands (right).

Shoot organogenesis from pedicel-cultured callus has been found in some crops (Mohamed et al. 1993; Eapen & George 1997) but this is the first report regarding *in vivo* shoot formation on pedicel ends in kiwifruit. The *in vivo* organogenesis found in the pedicels may be used to explore mutation in the field.

Conventional procedures for rooting (Lawes 1990) the pedicel and orchard cuttings showing shoot organogenesis would facilitate the study of regenerated plants avoiding laborious steps of subculturing and acclimatisation.

Both CPPU and natural cytokinins have been found to promote organogenesis in various kiwifruit tissues (Shimura et al. 1990; Matsuta et al. 1993). Therefore, it is not clear in the present work whether the effect of Agromil-Plus<sup>®</sup> was due to CPPU, natural hormones, or an interaction between the two, as hormonal balance has been shown to be important in kiwifruit organogenesis *in vitro* (Famiani et al. 1997).

Our findings show that kiwifruit pedicels *in vivo* and orchard cuttings *in vitro* treated with Agromil-Plus<sup>®</sup> had a reasonable high capacity to produce indirect organogenesis.

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