Tissue Culture of Passionfruit

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A protocol has been developed for micropropagation of the passionfruit hybrid 'Super Sweet' (*Passiflora edulis* × *P. edulis* f. *flavicarpa*). Apical shoot tips were initiated on MS medium plus 10 µM kinetin and grown to apically dominant shoots. They were dissected into nodal sections and again cultured on MS medium plus 10 µM kinetin. Shoots that developed from axillary buds were dissected and rooted as micro-cuttings by short exposure to 1/2 MS medium containing either 10 µM NAA or 10 µM IBA before transfer to hormone free medium. Ninety percent rooting was obtained after 7 days exposure to medium containing NAA. Exposure to either auxin for >5 days resulted in increased callus production and reduced shoot growth.

INTRODUCTION

The genus *Passiflora* has been disseminated throughout tropical and sub-tropical areas of the world and is known for both its fruit and its colourful flowers. Of the hundreds of species contained in the genus approximately 50 to 60 bear edible fruit (Martin and Nakasone, 1970), the production of which is strongly influenced by species and climate.

On the east coast of Australia *P. edulis* (purple passionfruit) produces a heavy summer crop but few fruit in winter, while *P. edulis* f. *flavicarpa* (yellow passionfruit) produces fruit from late summer to early winter (Beal and Farlow, 1984). To achieve a more even yield over an extended period the Australian passionfruit industry is based on hybrids between *P. edulis* and *P. edulis* f. *flavicarpa*. Because seedlings from these hybrids are highly variable, scions are cleft-grafted onto rootstocks when they are 30 to 40 cm high and planted in the field after a further 4 to 6 weeks. Grafting is preferred to rooted cuttings as it takes advantage of the disease resistance of *P. edulis* f. *flavicarpa* when used as a rootstock. Micropropagation of passionfruit would be useful both for clonal multiplication of elite scion material and for multiplication of superior rootstocks, as self-incompatibility occurs in some clones of *P. edulis* f. *flavicarpa*.

Micropropagation systems that include a callus or adventitious budding phase are prone to production of genetic off-types. To avoid this complication, clonal propagation has been achieved for other species via production of microcuttings from axillary buds of apically dominant shoots. This technique has been used successfully in vitro for *Carica papaya* (Drew, 1992), *Azadirachta indica* (Drew, 1993), and *Coffea arabica* (Drew, 1991b). This paper reports experiments in the development of a similar protocol for passionfruit.

MATERIALS AND METHODS

Plants were grown in a glasshouse from seed of the passionfruit hybrid 'Super Sweet'. Apical tips were removed and disinfested for 15 min in a vacuum with 1% sodium hypochlorite solution containing a few drops of 7× detergent. After three

rinses in autoclaved water, basal stem sections were trimmed and explants placed on three culture media. Media treatments were MS, MS plus 10 μM kinetin, and MS plus 10 μM kinetin and 5 μM IAA. At the conclusion of this experiment, apically dominant plants were dissected into nodal sections and placed on either MS medium, MS plus 10 μM kinetin, or MS plus 5 μM 2iP. These concentrations of the two cytokinins were optimal in a previous experiment. In both experiments, shoot growth was assessed after 4 weeks.

In the rooting experiments, nodal sections from apically dominant shoots produced in the above experiments were cultured on MS medium containing 10 μM kinetin. After 6 weeks axillary shoots were dissected from the nodal sections and cultured on half-strength (1/2) MS medium containing 10 μM NAA. At various intervals 20 replicates were removed—10 were transferred to 1/2 MS medium and 10 to 1/2 MS plus 10 μM riboflavin. As a control treatment, plants were placed on the latter two media at day 0. In a second rooting experiment, axillary shoots from nodal sections were placed on 1/2 MS medium plus 10 μM IBA for various intervals before 20 replicates were transferred to 1/2 MS medium plus 10 μM riboflavin. After 30 days shoot height and root number were measured and callus growth at the base of the shoot was rated according to size (0 = no callus, 1,2, and 3 = increasing callus production). Root initiation was assessed daily for 30 days and then weekly thereafter.

Media contained 30 g litre^{-1} sucrose for shoot growth experiments and 20 g litre^{-1} sucrose for rooting experiments. All media contained 8 g litre^{-1} Difco bacto-agar and had pH adjusted to 5.6 with 0.1 M KOH before autoclaving at 121C for 15 min. Cultures were incubated at 25±1C with cool white fluorescent tubes providing a light irradiance of 55 mmol m^{-2} s^{-1} for a 16-h photoperiod.

RESULTS

Shoot growth was measured in terms of the percentage of explants with actively growing shoots, and the mean shoot height in cm. Shoot growth was good on hormone-free MS medium [90%, 1.7 cm] and on MS medium containing 10 μM kinetin [90%, 1.6 cm] compared with explants on medium containing 10 μM kinetin and 5 μM IAA [50%, 1.1 cm]. In the second experiment best growth of axillary buds from nodal segments in terms of shoot quality and length occurred on medium containing 10 μM kinetin.

In the rooting experiments, NAA was better than IBA for root initiation. Seven days of exposure to 10 μM NAA before transfer to hormone free medium was optimum in terms of percentage of shoots that initiated roots, however, more than 5 days exposure to NAA significantly increased callus production on the base of the shoot and reduced shoot height per rooted shoot. Similar results were observed with IBA treatments where more than 5 days exposure to 10 μM IBA resulted in increased callus production and reduced shoot height per rooted shoot. Shoot quality was poor after 30 days exposure to both auxins.

DISCUSSION

There are many reports of organogenesis from leaf discs and from petiole and stem sections of *Passiflora* species, however, there are few reports of shoot cultures from bud explants of *P. edulis* and *P. edulis* f. *flavicarpa* (Dornelas and Vieira, 1994; Drew, 1991a; Kantharajah and Dodd, 1990). Although IAA has been useful for establish-

ing adult and juvenile shoots in vitro (Drew, 1991a) and in shoot multiplication systems (Carvalho and Segura, 1994), optimum shoot growth was obtained in these experiments without IAA. In both the shoot and rooting experiments auxin reduced shoot growth as measured by shoot height (Tables 1 and 2). To stimulate growth of axillary buds from nodal sections, kinetin was superior to 2iP which was previously shown to be the best cytokinin for growth of adult shoots (Drew, 1991a).

Table 1. Effect of duration of exposure to 10 μM naphthalene acetic acid on rooting of passionfruit micro-cuttings in vitro.

Duration of exposure to NAA (days)	Percent rooted after 30 days #	Percent rooted after 30 days*	Percent rooted after 42 days*	Callus rating	Mean root number per shoot	Mean shoot height per rooted shoot (cm)**	Mean shoot height per unrooted shoot (cm)**
0	0	10	10	0.1 a	1	1	0.44
1	0	0	0	0.1 a	-	-	0.43
3	20	0	0	0.1 a	-	-	0.58 a
5	60	20	20	0.4 a	1.5	1.2	0.57 a
7	50	60	90	1.5 b	1.5	0.9	0.68 a
10	30	50	50	2.1 b	2.2	0.8	0.42
30	30	40	50	3.0 c	1.8	0.3	0.26 b

\# = transferred to hormone free MS medium.
* = transferred to hormone free MS medium containing 10 μM riboflavin.
\+ = callus at base of shoot.
** = after 30 days.
a, b, and c differ significantly at $P<0.01$

As auxin is essential to initiate adventitious roots but is inhibitory to root growth and development, root initiation and growth in vitro for other species has been optimised by controlling exposure to auxin (Drew, 1991b). In these experiments this principle has been applied to in vitro rooting of passionfruit shoots. NAA was more effective than IBA for rooting passionfruit shoots and this is consistent with the findings of Kantharajah and Dodd (1990) for *P. edulis* shoots. Seven days was the optimum duration of exposure to NAA and IBA in terms of the percentage of shoots that rooted. If shoots were exposed to either auxin for more than 5 days there was a significant increase in callus at the base of the explant and reduction in shoot growth of both rooted and unrooted shoots (Tables 1 and 2). Reduced shoot growth limits further multiplication and large amounts of callus at the base of a shoot can make acclimatisation difficult.

In these experiments it was apparent that some auxin was transferred with shoot explants during subculture and continued to affect rooting. When shoots were transferred to hormone-free MS medium containing 10 μM riboflavin, maximum rooting percentage occurred with the 7 days of NAA treatment compared to 5 days

Table 2. Effect of duration of exposure to 10 μM indolebutyric acid on rooting of passionfruit micro-cuttings *in vitro*.

Duration of exposure to IBA (days)	Percent rooted after 30 days #	Percent rooted after 56 days#	Callus rating*	Mean root number per shoot	Mean shoot height per rooted shoot (cm)**	Mean shoot height per unrooted shoot (cm)**
0	0	0	0 a	-	-	0.55 a
1	5	5	0.35 a	1	0.9	0.60 ac
3	5	25	1.0 b	2	1	0.57 ac
5	5	10	1.5 b	1	1.6	0.58 ac
7	15	55	2.6 c	1	0.7	0.93 b
10	20	50	2.5 c	1.5	0.8	0.52 a
15	20	50	2.6 c	1.5	0.6	0.50 a
30	40	50	2.2 c	1.9	0.4	0.42 ad

= transferred to hormone free MS medium containing 10 μM riboflavin.
* = callus at base of shoot.
+ = after 30 days.
Means followed by different letters differ significantly at $P<0.01$.

Figure 1. Growth of rooted passionfruit micro-cuttings into apically dominant shoots 1, 3, and 5 weeks (left to right) after transfer to hormone-free medium after exposure to medium containing 10 μM IBA for 7 days.

for shoots that were transferred to MS medium without riboflavin. Riboflavin rapidly photooxidises IBA in tissue culture medium (Drew et al., 1991) and has been shown to photooxidise NAA (Gortner and Kent, 1953). Consequently riboflavin would cause rapid breakdown of any auxin that was transferred from the previous medium with the shoot during subculture. The use of riboflavin in hormone-free medium following culture in medium containing IBA prevented "carry-over" effects of auxin on papaw root quality and callus production (Drew et al., 1993).

These experiments have shown that passionfruit can be micropropagated via rooting of microcuttings produced from nodal sections of apically dominant plants.

Plants of cultivar 'Super Sweet' have been subcultured for 18 months without loss of vigour using these protocols (Fig. 1). Plantlets have been acclimatised in a glasshouse without difficulty.

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