Micropropagation of Yellow Passionfruit by Axillary Bud Proliferation

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Abstract. A protocol for in vitro propagation in yellow passionfruit (*Passiflora edulis* F. *flavicarpa* Deg) has been developed. Shoot apices from aseptically grown seedlings were used as initial explants. Multiple shoot formation was obtained by placing the explants on solidified Murashige and Skoog medium containing BA. Regenerated shoots were rooted on media without growth regulators. Following conventional procedures, plantlets were transferred to soil with more than 90% success. Chemical name used: *N*-(phenylmethyl)-1*H*-purin-6-amine (BA).

Passiflora edulis F. *flavicarpa* Deg (yellow passionfruit) is valued for its edible fruits and ornamental qualities. Several studies have indicated that various juvenile tissues from *Passiflora* spp. can be used as explants to obtain adventitious shoots (Dornelas and Vieira, 1994; Kantharajah and Dodd, 1990; Kawata et al., 1995). However, the plants obtained may exhibit somaclonal variation. Meristem and shoot tip cultures, in which shoot proliferation results from axillary branching, is less prone to the risk of genetic instability and is therefore still the method of choice for in vitro mass propagation (Nehra and Kartha, 1994). However, axillary bud proliferation in *Passifloraceae* still presents technical difficulties. In fact, published work reports only shoot elongation with no multiplication (Dornelas and Vieira, 1994; Drew, 1991). The present study describes an efficient protocol for mass propagation of yellow passionfruit from axillary buds.

Materials and Methods

Yellow passionfruit seedlings were obtained from seeds germinated under sterile conditions (Faria and Segura, 1997). Shoot apices (0.3 cm long) from 40-day-old seedlings were used in the experiments. The basal medium tested included MS (Murashige and Skoog, 1962) nutrients, 3% sucrose, and 0.8% Difco Bacto-agar (pH 5.7). Growth regulators were added to the medium before autoclaving (20 min at 120 °C, 1×10^5 Pa). Explants were cultured in 150 × 25-mm glass tubes, closed

with polypropylene closures (Bellco), containing 30 mL of nutrient medium. Cultures were maintained in a growth chamber at 26 ± 2 °C and a 16-h photoperiod with light supplied by Sylvania (GTE Gro-lux, F36W/GRO, Erlangen, Germany) fluorescent tubes (80 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ irradiance at culture level). In all experiments, cultures were examined for sprouting percentage, number of shoots per explant, and shoot length. Twelve explants were cultured per treatment, and all experiments were conducted at least twice.

In a first experiment, apical explants were initially cultured for 45 d on MS medium with 0, 2, 5, or 20 μM BA. Subsequently, explants were transferred for another 45 d of culture to the same medium without BA. The effect of 2 μM 1*H*-indole-3-acetic acid (IAA) on culture establishment was examined in apical explants cultured on the same medium supplemented with 2, 5, or 20 μM BA.

In a second experiment, apical explants were cultured for 45 d on a modified MS medium supplemented with 5 μM BA and 2 μM IAA. Nitrate : ammonium ratio was modified using the following $\text{NO}_3^- : \text{NH}_4^+$ concentrations (mM): 3.5:56.5; 12:48; 30:30; 40:20; 48:12; and 56.5:3.5. Inorganic N sources were KNO_3 and NH_4Cl . A control treatment including the original MS salt formulation ($\text{NO}_3^- : \text{NH}_4^+$, 40:20) was also tested.

Rooting experiments were carried out with shoots longer than 1 cm excised from proliferating cultures. A minimum of 16 shoots per initial treatment were excised and transferred individually to tubes containing basal MS medium. Rooting percentage and the number of roots per shoot were evaluated after 30 d. The experiment was repeated. Plantlets were transplanted individually to 100-mL pots containing a medium of 2 peat : 1 perlite and maintained for 20–30 d in a growth chamber at 75% relative humidity, 25 ± 2 °C, and an 18-h photoperiod (90 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ irradiance at culture level). Before transplanting, all the leaves were removed and the roots pruned to 3 cm from the base. Subsequently, plants were

kept in a greenhouse (2–3 months) and then transferred to field conditions.

Significance of treatment effects was determined using analysis of variance (ANOVA) (Statgraphics program, version 6, Manugistics, Rockville, Md.). Percentage data were subjected to arcsin transformation before analysis. Variation among treatment means was analyzed using Tukey's (1953) procedure. Data sets were combined before ANOVA.

Results and Discussion

All explants formed shoots on MS medium with or without BA. Callus formation was occasionally observed at the base of explants grown in the presence of 20 μM BA, but shoot proliferation occurred via axillary branching from buds present in the original explants. Axillary bud development, resulting in multiple shoot cultures, took place only when the explants were initially cultured in the presence of BA (effect of BA significant at $P = 0.05$), but there were no significant differences among the effects of the BA concentrations tested (1.1, 4.0, 4.2, and 3.4 shoots per explant at 0, 2, 5, and 20 μM BA). The presence of 2 μM IAA in the induction medium did not affect sprouting percentage or the mean number of shoots per explant (data not shown).

The changes in the concentration and source of ammonium and nitrate only limited shoot proliferation from apical explants when the ratio of $\text{NO}_3^- : \text{NH}_4^+$ was very low (3.5:56.5 mM) (Table 1).

The final length of regenerated shoots ranged from 2 to 3 cm and none of the treatments affected shoot length appreciably. Rooting of yellow passionfruit shoots was easily induced (70%) in a medium without auxins, corroborating previous investigations with this genus (Dornelas and Vieira, 1994; Kantharajah and Dodd, 1990; Kawata et al., 1995). Rooted leafless plants acclimatized readily to growth chamber conditions. This contrasts with the results obtained by Dornelas and Vieira (1994), who reported that successful acclimatization of *Passiflora* plants regenerated in vitro was dependent on the presence of leaflets. These authors also observed that survival rates were drastically reduced when plants were not protected by a plastic bag. The establishment of in vitro-grown plants in soil was easily achieved, and survival rates were 95% to 100%. After

Table 1. Effect of $\text{NO}_3^- : \text{NH}_4^+$ ratio on shoot proliferation from shoot tips of juvenile yellow passionfruit after 45 d in culture on 5 μM BA and 2 μM IAA.

NO_3^- (mM)	NH_4^+ (mM)	$\text{NO}_3^- : \text{NH}_4^+$ ratio	No. shoots/ explant
3.5	56.5	1:16	2.0 b ²
12.0	48.0	1:4	3.2 a
30.0	30.0	1:1	3.2 a
40.0	20.0	2:1 ³	3.2 a
40.0	20.0	2:1	3.7 a
48.0	12.0	4:1	3.3 a
56.5	3.5	16:1	3.4 a

²Mean separation by Tukey's test at $P = 0.05$.

³Control MS medium (20 mM KNO_3 and 20 mM NH_4NO_3)

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their acclimatization, the plants were transferred to a greenhouse and, finally, to the field, where they exhibited normal development, blossoming abundantly.

To date, no references specifically dealing with axillary shoot proliferation in cultured explants of *Passiflora* spp. have been published. Most published works report only on the elongation of the apical buds (Dornelas and Vieira, 1994) or on adventitious budding from nodal segments, isolated from shoots originating from cultured apical buds (Drew, 1991). Our results demonstrate, however, that axillary bud proliferation, resulting in multiple shoot cultures, is feasible by using explants from juvenile *P. edulis* F. *flavicarpa*. The procedure can be of great usefulness for

mass multiplication and improvement of this economically important plant species.

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