

## Short communication

# Methods to improve the *in vitro* culture of GF677 (peach × almond) peach rootstock

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**Abstract** In this study, the effect of exogenous application of gibberellic acid (GA<sub>3</sub>) and the presence of both leaves and petioles on sprouting of the rosetting plantlets of GF677 (peach × almond hybrid) was examined. GA<sub>3</sub> caused a significant increase in sprouting of rosetting plantlets at application rates of 12.5 and 50 mg litre<sup>-1</sup>. The results showed that rosetting plantlets without leaves and petioles, or with only one petiole, sprouted significantly better compared with those with one-quarter of leaf, all petioles, or all petioles and leaves. In addition, Mg + Zn + B (trace) in Murashige & Skoog medium significantly increased the fresh weight and water content of plantlets.

**Keywords** propagation; plantlet; rooting; rosetting; sprouting

## INTRODUCTION

GF677 is a rootstock that was produced by Bernhard in France at the Grand Ferrad Research Station in 1965 (Stylianides et al. 1988). It is an interspecific hybrid between peach and almond and is important to the peach (*Prunus persica* (L.) Batsch) industry in the Mediterranean basin. GF677 is clonally propagated and is particularly useful to control

replant diseases. It is a good rootstock for peach orchards planted on moderate or poor fertility soils. GF677 is well suited to the cultural practices used in peach orchards in Greece and, therefore, has become the primary rootstock used for most peach cultivars in Greece. However, its vegetative propagation is quite difficult (Stylianides et al. 1988). Vegetative propagation of peach can be achieved by hardwood, semi-hardwood, and softwood cuttings, as well as by suckers, root cuttings, and also tissue culture (Loreti et al. 1985; Estaun et al. 1999).

*In vitro* culture of GF677 is usually based on the protocol published by Murashige (1962), but the cost of *in vitro* propagation of GF677 combined with some problems as a result of vitrification (during tissue culture), rosetting of plantlets (rooted plantlets after acclimation which did not grow or remained dormant), and low rooting percentages make such trees very expensive for high density planting.

In this study, the effect of gibberellic acid (GA<sub>3</sub>) and the presence of both leaves and petioles on sprouting of rosetting plantlets was examined. Also, the effect of the addition of a double dose of trace elements to Murashige & Skoog (MS) medium on the growth of plantlets was investigated.

## MATERIALS AND METHODS

### Effect of GA<sub>3</sub> on sprouting percentage of rosetting plantlets

The effect of exogenous application of GA<sub>3</sub> on sprouting of rosetting plantlets was examined in this experiment. A total of 420 rosetting plantlets was collected from acclimated (in a greenhouse) tissue-cultured plantlets on 15 May 1998 and repeated on the same date in 1999. Immediately after collection, the rosetting plantlets were sprayed with GA<sub>3</sub> at six concentrations (0, 12.5, 25, 50, 75, 100 mg litre<sup>-1</sup>) and kept in a greenhouse (at a temperature of 18–26°C). Measurements were taken 19 days later. The experimental design was randomised: 6 treatments × 7 replications of 10 plantlets each.

### Effect of leaf and petiole on sprouting percentage of rosetting plantlets

This experiment was designed to examine whether leaves and petioles (5–8) have any effect on sprouting of the rosetting plantlets. One hundred and eighty rosetting plantlets (chosen from acclimated plantlets as in previous experiment) were collected on 18 August 1998 and repeated on the same date in 1999. Five treatments were applied (untreated control, one petiole left, one-quarter of leaf left, all petioles left, no leaves or petioles left) in a randomised design: 5 treatments  $\times$  6 replicates of 6 plantlets each. Immediately after treatment, the plantlets were irrigated and transferred into the greenhouse (26°C during the day and 18°C during the night). Measurements were taken 53 days after treatment.

### Effect of modified MS media, on fresh and dry weight of GF677 tissue-cultured plantlets

A total of 180 jars were prepared for aseptic culture of GF677 shoot growth *in vitro* (growth medium) and divided between 18 treatments. In addition to MS medium, they contained a double dose of various trace elements alone or in combinations.

Shoots, 10–20 mm long, were isolated from sub-cultures, the leaf area was reduced to a minimum by excising several leaves from the shoots which were then placed in the jars (10 shoots per jar). After shoot establishment in the culture media, the jars were transferred into the growth room at 22°C, 80% relative humidity (RH), a photoperiod of 8 h, and light intensity of 40 W/m<sup>2</sup>. The statistical analysis was based on a randomised design: 18 treatments  $\times$  10 replications per treatment. Measurements (made on one plantlet from each replication) were taken 27

days later. This experiment was conducted on 30 April 1998 and repeated on the same date in 1999.

## RESULTS AND DISCUSSION

### Effect of GA<sub>3</sub> on sprouting percentage of rosetting plantlets

The results showed that there were significant differences among the GA<sub>3</sub> treatments. The 12.5 mg litre<sup>-1</sup> concentration of the GA<sub>3</sub> induced a significantly higher sprouting of rosetting plantlets than the untreated control (Table 1). Significant differences were also observed between 12.5 and 50 mg litre<sup>-1</sup> GA<sub>3</sub> concentrations. Although further increases of GA<sub>3</sub> concentration above 50 mg litre<sup>-1</sup> gave further increases in the rate of growth, these did not make sense (Table 1). Also, Bailiss & Hill (1971) reported that exogenously supplied gibberellin affects the growth of intact plants.

### Effect of leaf and petiole on sprouting percentage of rosetting plantlets

Sprouting was least in the untreated control, one-quarter of leaf, and petioles left and considerably higher in the treatments without leaves and petioles or with only one petiole (Table 2). During acclimation, environmental stress to some plantlets, for example, as a result of damage of some plantlets, caused temporal dormancy. This could increase the level of abscisic acid (ABA) (Wareing & Phillips 1975). Possibly, ABA, which is characterised by its ability to inhibit many growth processes, and its attribute to occur in the transport systems of plant (Lenton et al. 1968), is of particular relevance to any hypothesis that the formation of an inhibitory

**Table 1** Effect of exogenously applied gibberellic acid (GA<sub>3</sub>) on sprouting (%) of rosetting plantlets of GF677. Values followed by the same letters are not significantly different according to least significant difference (LSD) ( $P < 0.05$ ). (Values are the mean of two experiments, each with 70 cuttings; results were similar according to the Bartlett's test of homogeneity of variance, so data were combined.)

GA <sub>3</sub> (ppm)	Sprouting (%)
0	3.5 c
12.5	46.5 b
50	65 a
75	69.5 a
100	70 a

**Table 2** Effect of leaf and petiole on sprouting (%) of rosetting plantlets of GF677. Values followed by the same letters are not significantly different according to least significant difference (LSD) ( $P < 0.05$ ). (Values are the mean of two experiments, each with 36 replicates; results were similar according to the Bartlett's test of homogeneity of variance, so data were combined.)

Treatments	Sprouting (%)
One petiole	80 a
No leaves and petioles	78 a
All petioles	54 b
One-quarter leaf	52 bc
Control	42 c

**Table 3** Fresh and dry weight of tissue-cultured GF677 plantlets after 27 days in Murashige & Skoog (MS) media modified by different treatments (FW, fresh weight; DW, dry weight). Values followed by the same letters are not significantly different according to least significant difference (LSD) ( $P < 0.05$ ). (Values are the mean of two experiments, each with 10 plantlets; results were similar according to the Bartlett's test of homogeneity of variance, so data were combined.)

Treatments	FW/ per plantlet (g)	DW/ per plantlet (g)	FWDW × 100/DW
Zn	1.410 abc	0.095 ab	1.384 abc
B	1.063 bcde	0.071 ab	1.396 ab
Mn	1.087 bcde	0.102 ab	0.966 bc
Mg	1.087 bcde	0.092 ab	1.082 abc
Zn + B	1.262 bcd	0.099 ab	1.175 abc
Zn + Mn	0.936 de	0.089 ab	0.952 c
Zn + Mg	0.970 cde	0.081 ab	1.098 abc
B + Mn	1.338 abcd	0.095 ab	1.308 abc
B + Mg	1.424 ab	0.100 ab	1.324 abc
Mn + Mg	0.996 bcde	0.083 ab	1.100 abc
Zn + B + Mn	1.122 bcde	0.079 ab	1.320 abc
B + Mn + Mg	1.156 bcde	0.078 ab	1.382 abc
Mn + Mg + Zn	1.152 bcde	0.082 ab	1.305 abc
Mg + Zn + B	1.769 a	0.109 ab	1.523 a
Zn + B + Mn + Mg	0.992 bcde	0.076 ab	1.205 abc
Fe	0.709 e	0.067 b	0.958 bc
Mn + Fe	0.930 de	0.071 ab	1.210 abc
Control (MS)	1.062 bcde	0.093 ab	1.042 bc

hormone in leaves is translocated to the growth point. Generally, it seems that ABA is mainly significant by interacting with other internally or externally applied growth regulators than itself. When terminal buds are first formed, they can frequently be induced to resume growth by various treatments including defoliation (Wareing & Phillips 1975).

#### Effect of modified MS media, on fresh and dry weight of GF677 tissue-cultured plantlets

Only the combination of Mg + Zn + B significantly increased the fresh weight and relative water content of plantlets. No significant differences were observed between other treatments and the control (Table 3). The results suggest that the addition of Mg + Zn + B in MS media can improve them for *in vitro* culture of GF677 and reduce vitrification. Remotti & Löffler (1995) reported that the addition of micro-elements and vitamins in MS medium increased the induction of callus capable of plant regeneration. Ruzic et al. (2000) found that there is a relationship between the concentration of micro-elements, their uptake, and multiplication of cherry rootstock Gisela 5 *in vitro*. D'Angeli et al. (2001) reported the effect of macro- and micro-elements on adventitious shoot

formation from the callus originating from the vegetative shoot apice of apple. According to Shible et al. (1999), nutrient element imbalance can occur under saline conditions causing poor plant growth.

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