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## Development of mycorrhizal infection in in vitro- and in vivo-formed roots of woody fruit plants

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**Summary** — Micropropagated shoots of grapevine (*Vitis berlandieri* x *V riparia* Kober 5 BB), kiwifruit (*Actinidia deliciosa* cv Hayward) and apple (*Malus pumila* MM106) were rooted in media containing a vital staining in order to distinguish in vitro-formed from in vivo-formed roots. Presence of the dye reduced plant growth in apple only. At transfer into the soil, plants were inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae*. The dye did not prevent root colonization by the mycorrhizal fungus. After 4 to 5 weeks from transplant both in vitro- and in vivo-formed roots were colonized by the arbuscular mycorrhizal fungus.

**VA mycorrhiza / kiwifruit / grapevine / apple / micropropagation**

**Résumé** — Développement de l'infection mycorhizienne dans des racines formées in vitro et in vivo de plants fruitiers. Des vitroplants de vigne (*Vitis berlandieri* x *V riparia* Kober 5 BB), de kiwi (*Actinidia deliciosa* 'Hayward') et de pommier (*Malus pumila* MM106) ont été produits dans des milieux gélosés contenant un colorant vital pour différencier, après transplantation dans le sol, les racines développées in vitro de celles in vivo. La présence du colorant a réduit la croissance des plantes seulement dans le cas du pommier. Les vitroplants ont été inoculés, lors de la transplantation dans le sol, avec des spores du champignon mycorhizogène à arbuscules *Glomus mosseae*. La coloration des racines n'a pas empêché le développement de l'infection racinaire : aussi bien les racines développées in vitro que celles in vivo étaient mycorhizées après 4 à 5 semaines.

**mycorhize arbusculaire / kiwi / vigne / pommier / micropropagation**

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\* Correspondence and reprints

## INTRODUCTION

Acclimatization of micropropagated plants is more successful if the *in vitro*-formed root system is able to efficiently support the plant during hardening and growth after transplant. According to some authors, *in vitro*-formed roots degenerate and are mostly replaced by new roots during acclimatization (Maene and Debergh, 1983; McClelland et al, 1990); on the contrary, their survival after transfer to soil has been observed in Asian jasmine (Apter et al, 1993). In micropropagated grapevine, addition of the dye gentian violet to the rooting medium made it possible to distinguish between *in vitro*- and *in vivo*-formed root segments after acclimatization (Gribaudo et al, 1995): up to 80% of *in vitro*-originated roots survived the acclimatization phase. Gentian violet (crystal violet, methyl violet, basic violet 3) is a water-soluble, cationic triaryl-methane dye with low or no toxic effects on plants at low concentration (Kaufmann, 1968; Arnold and Young, 1990).

The aim of this research was to assess i) the efficacy of the staining system on different woody fruit species and ii) the capability of *in vitro*- and *in vivo*-formed roots to establish mycorrhizal symbiosis.

## MATERIALS AND METHODS

Three species were used in these experiments: the grapevine rootstock Kober 5 BB (*Vitis berlandieri* x

*V. riparia*), kiwifruit (*Actinidia deliciosa* cv Hayward) and the apple rootstock MM106 (*Malus pumila*). Rooting media were i) Murashige and Skoog (1962) modified as indicated by Gribaudo et al (1995) for grapevine and kiwifruit, and ii) Murashige and Skoog with 5  $\mu$ M indolebutyric acid (IBA) for apple; 0.15 mg·L<sup>-1</sup> gentian violet (sterile-filtered aqueous solution; Fluka Chemie AG, Switzerland) were added to the rooting media immediately after autoclaving at 120 °C for 10 min.

Shoots were excised from stock plants and cultured on the staining media or on a dye-free medium (control) for 3 (grapevine and kiwifruit) or 4 (apple) weeks. Cultures were kept in a culture chamber at 24 °C and 35  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux (PPF), 16 h photoperiod. Results were analyzed using the analysis of variance (ANOVA) test.

Rooted plantlets were transferred to a sterilized 1:1 mixture of soil (pH 7.4) and sand under a plastic tent, and acclimatized by gradually opening the tent during a 2-week period. Half of the plants were inoculated with the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* (Nicol and Gerd) Gerdemann and Trappe (BEG12), adding 1.5 g of inoculum to each 250 mL pot (= 20 propagules; Porter, 1979) at transplant. After 4 (kiwifruit) or 5 (grapevine) weeks, the plants were collected and the roots were cut and divided, according to the origin, into three groups: i) the basal part of old roots, ie root segments formed *in vitro*; ii) the apical part of old roots, which was formed after transfer to the soil (*in vivo*); iii) new roots entirely formed after transfer to the soil (*in vivo*).

For plants rooted on staining media, collected roots were assigned to a group on the basis of the colour of the roots themselves. For plants rooted in absence of the stain, roots were assigned to a group on the basis of the measurements (root number, length and position on the stem base) taken at the transplant.

**Table 1.** Shoot length and root number and length of micropropagated kiwifruit cv Hayward and apple rootstock MM106 rooted on media containing 0 or 0.15 mg·L<sup>-1</sup> gentian violet (30 kiwifruit and 60 apple plants/treatment).

	Shoot length (cm)	Root number	Root total length (cm)
<i>Kiwifruit cv Hayward</i>			
- Dye	2.8 <sup>a</sup>	2.7 <sup>a</sup>	5.7 <sup>a</sup>
+ Dye	3.0 <sup>a</sup>	2.4 <sup>a</sup>	4.0 <sup>b</sup>
<i>Apple rootstock MM106</i>			
- Dye	2.0 <sup>a</sup>	5.5 <sup>a</sup>	3.7 <sup>a</sup>
+ Dye	1.6 <sup>b</sup>	8.3 <sup>b</sup>	1.4 <sup>b</sup>

<sup>ab</sup> For each treatment and species, values followed by the same letter do not differ significantly at  $P = 0.01$ .

**Table II.** Mycorrhization percentages of the three types of roots or root segments, after 4 (kiwifruit) or 5 (grapevine) weeks from the transfer to soil.

	+ Dye + AM fungus	- Dye + AM fungus	+ Dye - AM fungus	- Dye - AM fungus
<i>Grapevine Kober 5BB</i>				
In vitro-formed segments of old roots	43	37	0	0
In vivo-formed segments of old roots	60	45	0	0
New roots formed entirely in vivo	60	75	0	0
<i>Kiwifruit cv Hayward</i>				
In vitro-formed segments of old roots	30	20	0	0
In vivo-formed segments of old roots	60	40	0	0
New roots formed entirely in vivo	40	50	0	0

The dye gentian violet ( $0.15 \text{ mg}\cdot\text{L}^{-1}$ ) was added to the rooting medium during in vitro culture. *Glomus mosseae* was inoculated at the transfer to soil (six grapevine and eight kiwifruit plants/treatment).

## RESULTS

In kiwifruit the presence of  $0.15 \text{ mg}\cdot\text{L}^{-1}$  gentian violet did not affect shoot growth and root number but slightly reduced root length (table I) after 3 weeks of culture in the rooting medium. Afterwards, plants were acclimatized and after 4 weeks in the soil no more significant differences were found between plants with stained roots and controls (data not shown).

In apple the dye initially reduced plant height and root length but increased root number.

In grapevine and kiwifruit, the dye in the rooting medium made the in vitro-formed roots easily recognizable after 4 or 5 weeks in soil. In contrast, in apple this dye concentration was insufficient to distinguish in vitro- and in vivo-formed roots after 4 weeks of culture in soil. The number of roots was too elevated to discriminate them on the basis of measurements taken at transplant. Consequently, AM infection of the various root types was assessed on grapevine and kiwifruit only.

In grapevine, in vitro-formed roots usually have a smaller diameter compared to in vivo-formed ones, as previously observed (Gribaudo et al, 1995).

All types of roots were colonized by the AM fungus, both in grapevine and in kiwifruit (table II). The presence of gentian violet in the rooting medium did not prevent root colonization by the AM fungus.

## CONCLUSION

The effect of gentian violet was previously evaluated on Kober 5BB, by adding  $0.15$  and  $0.3 \text{ mg}\cdot\text{L}^{-1}$  to the rooting medium (Gribaudo et al, 1995). Based on those results, only the lowest dye concentration ( $0.15 \text{ mg}\cdot\text{L}^{-1}$ ) was used in this experiment. The effects of dye on kiwifruit plant growth were similar to those reported for grapevine (Gribaudo et al, 1995) and Asian jasmine (Apter et al, 1993). Apple plantlets showed a different feature: in apple the dye induced a growth reduction, and the concentration used did not allow to individuate in vitro-formed roots after 4 weeks in soil. Solutions to this problem could be a shorter period in soil or higher stain concentration, although the latter could further depress root growth.

In grapevine and kiwifruit the use of the dye gentian violet at the concentration of  $0.15 \text{ g}\cdot\text{L}^{-1}$  in the rooting media confirmed its usefulness as a method for easy identification of in vitro-formed roots after acclimatization. The slight growth reduction effect weakens in the long run. Moreover, AM root colonization was not prevented by the dye.

In grapevine and kiwifruit, both in vitro- and in vivo-formed roots were colonized by the AM fungus *Glomus mosseae*. Our results show that in vitro-formed roots can successfully establish AM infection after transfer to soil.

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