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Factors influencing *in vitro* rooting of chestnut

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Abstract
Riboflavin (2mg/l) and ascorbic acid (50 mg/l) were added to the basal medium during the rooting stage of chestnut (*Castanea crenata x Castanea sativa,* cv. Maraval) microcuttings in order to improve adventitious root formation. The addition of riboflavin in the culture medium did not improve the rooting ability of leafy shoots which showed apical necrosis phenomenon and callus formation. There was, however, a positive effect when ascorbic acid was added, especially on the quality of rooted plants where there was a marked reduction in the occurence of the apical necrosis phenomenon.

Keywords: *Castanea*, *in vitro* rooting, riboflavin, ascorbic acid, apical necrosis, callus formation

1 Introduction

Vitamins have long been found to promote root formation in numerous plant species (BOSE *et al*. 1982, HATI *et al*. 1990, DREW *et al*. 1991, CHEE 1995). The aim of this study was to examine the influence of riboflavin and ascorbic acid on the development of the root system in chestnut (cv. Maraval) microcuttings.

2 Material and methods

Plant Material: Mother stock cultures of the chestnut cultivar Maraval *(C. crenata x C. sativa)* were cultivated on a basal Driver Kuniyuki-Walnut medium (DKW) (DRIVER and KUNIYUKI 1984), supplemented with 0.2 mg/l 6-Benzylaminopurine (BAP). The cultures were kept in plastic containers in a growth room at 22 °C with a 16 h photoperiod at an irradiance intensity of 55 $\mu mol.s^{-1}.m^{-2}$ using cool-white fluorescent tubes.

Culture conditions: Shoot explants (2–3 cm in length) taken from mother stock cultures were induced to root according to the BdR-technique/Ctifl-Balandran, France (Bourrain and Navatel 2000). It consists of treating microcuttings with 2 mg/l 3-indolylbutyric acid (IBA) for seven days in semi-darkness and transferring them subsequently to a hormone-free medium containing MURASHIGE and SKOOG (1962) basal salt and vermiculite in order to allow root emergence in light. To examine the influence of vitamins on adventitious root formation, microcuttings were treated with either 2 mg/l riboflavin or 50 mg/l ascorbic acid. The pH was adjusted to 5.6 prior to autoclaving at 121 °C (1.1 kg/cm^2) for 15 minutes. The cultures were kept at 22 °C in complete darkness for the first 7 days and then at a light period of 16 h for the root emergence phase.

Each treatment was tested on 20 microcuttings, and the experiments were repeated at least three times. The number of roots produced per shoot, the percentage of rooted shoots and the appearance of apical necrosis were recorded after three weeks in culture.

3 Results and discussion

Effect of riboflavin
There were significant differences in the rooting response when riboflavin was added to the basal medium compared to the control or when ascorbic acid was added (Fig. 1). Riboflavin reduced the rooting rate and caused a significant decrease in the number of roots produced

per shoot as well. A similar response has been reported by DREW *et al.* (1991) with *Carica papaya* when a high concentration of riboflavin (3.76 mg/l) was combined with IBA. Our finding contrasted with previous results with chestnut obtained by BOURRAIN and NAVATEL (2000), who reported a superior rooting response in terms of plant quality with the same concentration of riboflavin. The inhibitory effect of riboflavin noted in our experiments may have resulted from the rapid degradation of IBA due to the oxidation of auxin in the presence of riboflavin in the medium (GORST and DE FOSSARD 1983). This would prevent auxin treatment continuing for long enough to promote root formation, as reported by MILLER *et al.* (1982) for the rooting of *Prunus persica*.

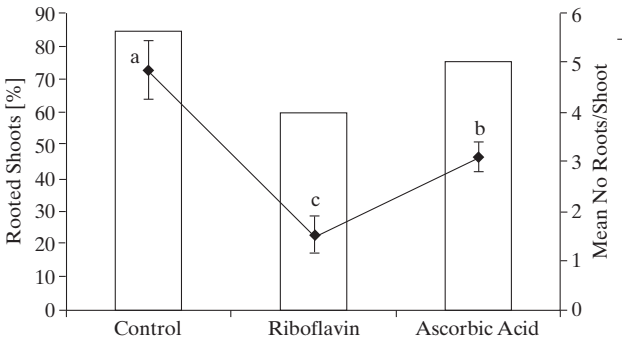

Fig. 1. Influence of riboflavin and ascorbic acid on the adventitious root development in microcuttings of chestnut (cv. Maraval) after three weeks in culture. Means with different letters are significantly different at 5% level. Vertical bars indicate ± SE (Standard Error).

Effect of ascorbic acid

The incorporation of ascorbic acid into the basal medium affected the capacity of rooting in chestnut microcuttings. As shown in Figure 1, ascorbic acid was considerably superior in terms of rooting percentage and the number of roots produced per shoot than to riboflavin, but was inferior compared to the control treatment (without riboflavin or ascorbic acid). Ascorbic acid did, however, have a positive effect on the quality of rooted plants with a marked reduction in the apical necrosis phenomenon (Fig. 2). But, callus formation was promoted, as reported by BHARDWAJ and RAI (1987). *In vitro* it has been shown that ascorbic acid used as an antioxidant agent can reduce browning resulting from the phenolic oxidation of the plant, and, therefore, prevent necrosis, which is usually associated with the death of shoots in spruce (RUMARY and THORPE 1984) and bananas (GUPTA 1986). In addition, it has also been reported that: the application of ascorbic acid in combination with an auxin (IBA) promotes rooting in terms of number of roots/cutting in various plant species (BOSE *et al.* 1982), makes rooting earlier and improves the quality of roots compared to those treated with an auxin alone (SHARMA and RAI 1993).

In the present study, the beneficial effect of ascorbic acid applied in combination with growth regulators has important implications in terms of the viable plants capable of recovering growth after being established in soil, in spite of the callus formation. This is because the growth of rooted plantlets with apical necrosis was inhibited during acclimatization and most of them failed to develop further. In this respect, VIEITEZ *et al.* (1989) have also observed that apical necrosis halts the growth of shoots in chestnut and oak, and found that callus formation acts as a sink for nutrients due to its large absorbing surface area and seems to promote a better shoot development.

Further experiments are needed to determine the optimal concentration of ascorbic acid to promote root growth and development so as to ensure successful acclimatization of chestnut plantlets.

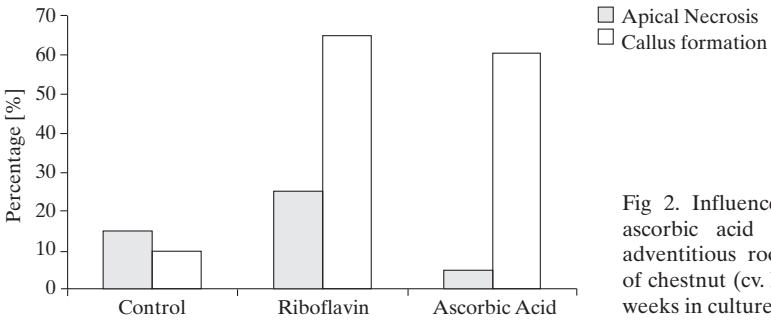

Fig 2. Influence of riboflavin and ascorbic acid on the quality of adventitious roots in microcuttings of chestnut (cv. Maraval) after three weeks in culture.

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