IN VITRO CULTURE OF MATURE COMMERCIAL *PISTACIA VERA* L. CULTIVARS

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INTRODUCTION

The *in vitro* micropropagation of *Pistacia* species (1) and commercial *Pistacia vera* cultivars from nodal segments taken from seedlings up to 2 years of age (3) has been previously reported. This paper presents results of a study which examined the *in vitro* culture of *Pistacia vera* cultivars from mature tissue.

MATERIALS AND METHODS

The mature *P. vera* cultivars included in this study were: 'Kerman' (female), 'Peters' (male), 'Lambertin' (male and female), 'NAZ' (male), 'Red Aleppo' (male), 'ASK' (male), and 'Rashti' (male). Nodal axillary/apical bud segments or meristem tips (containing 2 to 3 leaf primordia) taken from mature, fruitbearing trees, were cultured *in vitro*. Sterilization, medium preparation, and incubation conditions were as reported previously (2).

The following combination of treatments was used to establish mature explants in culture:

(a). Pre-soak of explants prior to culture for 0, 10, and 20 min in 100 mg/l malonic acid (MA), with or without the same treatment between each subsequent subculture. Alternatively, MA was supplemented to the medium at 0, 50, and 100 mg/l in combination with the pre-soak treatments;

(b). Murashige and Skoog (MS) or Woody Plant Medium (WPM), supplemented either with 0.3 mg/l 6-benzylaminopurine (BAP) plus 0.05 mg/l indolebutyric acid (IBA), or with 2 mg/l BAP;

(c). Removal or reduction of ferrous sulphate to one-half concentration in the MS medium;

(d). The concentration of agar was increased from 7 g/l to either 10 or 14 g/l in the MS medium;

(e). Attempts were made to select rapid, vigorously growing material for cultures or, alternatively, to rejuvenate the mature mother plants prior to selecting material for culture by: (i) severe pruning; (ii) applying a foliage spray of either 500

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mg/l gibberellic acid (GA3) or 200 mg/l BAP; (iii) repeatedly grafting mature scion material onto a vigorous juvenile rootstock *(P. vera)*; (iv). *in vitro* micrografting of a mature scion meristem tip (containing only a few leaf primordia) onto a juvenile *P. vera* rootstock.

RESULTS

Unless specified the results are only for the 'Kerman' cultivar.

Establishment of nodal segment explants in culture was very difficult due to severe browning and high contamination. Meristem tip explants dissected after the standard sterilization had very little contamination and transfer of explants in the same jar once the browning was apparent improved the establishment of cultures in all cultivars.

Pre-soak of the explants in MA for up to 20 min and the presence of MA in the medium at 50 mg/l delayed browning only marginally. From the earlier tests no difference was noted between the performance of explants in either WPM or MS medium, so the latter was used throughout this study. Browning of explants and medium was more severe on MS medium at 2.0 mg/l BAP than the medium supplemented was 0.3 mg/l BAP, with or without 0.5 mg/l IBA. Browning was almost eliminated when ferrous sulphate was excluded. However, it was included in the medium for later subcultures to prevent the development of iron deficiency; browning was not severe at these later stages. Increased concentration of agar was inhibitory to the growth of the explants.

Pruning, grafting, BAP and GA_3 spray treatments [(i) to (iii) above] stimulated new growth of shoots on the mature plants and these shoots appeared to be more suitable for culture. However, plants which had been sprayed with GA_3 produced material which subsequently showed poor establishment in culture. Gibberellic acid supplements added to the culture medium was previously reported not to be beneficial to growth of seedling material explants in culture (2). *In vitro* micrografting [(iv) above] was tried in an attempt to obtain results from the repeated grafting approach at a shorter time period. *In vitro* micrografting of mature scion material onto a juvenile rootstock was carried out on an MS medium containing 0.3 mg/l BAP and 0.05 mg/l IBA. Most of the failures in the micrografting were due to desiccation of the scion explant, its small size, or its poor contact at the cambium region with the rootstock. Placing the scion explant on a flat cut surface of the rootstock resulted in a greater number of successful grafts than insertion of the scion in a vertical cut made on the decapitated rootstock

shoot. Although a successful method for micrografting of *P. vera* was established, the growth of the scion was very slow and application of GA_3 to the medium did not stimulate elongation of the grafted scion. A good callus was made at the union point of the scion and rootstock, but the vascular connection between the two was not invested. The mature scion on a successful micrograft could be cut off from the rootstock and cultured independently and such material had very good subsequent growth and establishment in culture. Only one generation of *in vitro* micrografting has been completed to date.

When shoots from mature explants were treated with MA, or when rejuvenation of the mature plant had been attempted using severe pruning, GA_3 spraying or repeated grafting, and where there were a number of frequent subcultures at an early stage of incubation in the presence of 0.3 mg/l BAP, there were some cultures which produced multiple shoots. Most of the explants producing multiple shoots became vitrified and the subsequent growth of individual shoots was reduced.

DISCUSSION

This study has shown that results obtained from procedures used successfully with seedling material can be usefully adapted to the establishment of mature material *in vitro*. However, at maturation the physiological and biochemical status of a plant appears to change, so that a mature explant does not respond in the same way as a seedling explant to the various treatments. Culture establishment was improved in this study through treatments that conditioned the mother plant, although none of the treatments produced rejuvenation. The results indicate that more improvement can be anticipated from further conditioning and possible rejuvenation of mother plants. Repeated grafting will need to go through a number of generations before its effectiveness can be assessed.

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LITERATURE CITED

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