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IN VITRO CULTURE OF *PISTACIA VERA* L. EMBRYOS AND AGED TREES EXPLANTS

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INTRODUCTION

An increasing rate of pistachio (*Pistacia vera* L.) new orchards has been detected in the mediterranean areas of the World, where the interest of growers for this nut crop is currently crescent (Monastra et al., 1988). Altough the seedling rootstock production doesn't present any special problem (Casini y Conticini, 1979; Frutos y Barone, 1988), the grafting or budding propagation doesn't look so easy to do (Borisova-Velkova, 1984; Avanzato et al., 1988; Romero et al., 1988); in spite of it, topworking of *P. vera* cultivars on *P. terebintus* and *P. kinjuk* adult trees (Kaska and Bilgen, 1988), and graft nursery production on *P. atlantica* (Needs and Alexander, 1982) have been noticed. Rooting *P. vera* softwood cuttings under mist treated with very high auxins concentration during some few seconds (Al Barazi and Schwabe, 1982), and *P. chinensis* hardwood cuttings (Morgan and Maika, 1984) look possible, but in both cases poor results were yielded.

In vitro micropropagation of pistachio species seems full of suggestions because of its classic propagation troubles above reviewed. At present some few micropropagation works have been noticed (Barghchi and Alderson, 1983 and 1985; Martinelli, 1988), although its results, that have been useful to initiate several experiments, don't seem to bring into general use because its genome and/or age of the micropropagated plant materials look related to its different in vitro growth behaviour.

MATERIAL AND METHODS

P. vera seeds from adult seedlings grown in Torreblanca (Murcia) where harvested, disinfected without endocarp, first into a 70 p.100 ethanol solution for 45 seconds, then into a 30 p.100 commercial bleach solution of 40 g/l of active chlorine for 10 minutes, and later three times rinsed with sterile distilled water. After that the embryos were isolated and sown on an aseptic Murashige and Skoog (1962) half strengthed medium (MS/2) without vitamins and without hormones, supplemen-

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ted with sucrose (30 g/l) and agar (8 g/l), and adjusted to pH 5.7. The in vitro embryos cultures were put into a climatic chamber at 24±1°C, 5.000 lux and 16:8 hours light: dark photoperiod. 5 weeks later, plantlets supplied with 4-5 leaflets where availables. Four micropropagation experiments to improve the medium were then established: 1) MS supplemented with 1.0 and 4.0 mg/l of 6-benzylaminopurine (BAP) in the above mentioned photoperiod and in the dark; 2) MS without vitamins, supplemented with BAP (2.0 and 4.0 mg/l) and with 0, 0.5 and 2.0 mg/l of gibberellic acid (GA3); 3) MS nitrate half strengthed, without vitamines and supplied with BAP (4.0 mg/l); and 4.0) Mc Cown and Lloyd´s (1981) Woody Plant Medium (WPM) supplemented with BAP (4.0 mg/l) and GA3 (0 and 0.5 mg/l). In all the tested media, sucrose (30 g/l) and agar (8 g/l) were also added.

For every one of the prior treatments, two kinds of explant were used: a) shoot segments with 1-2 buds from proliferated embryos as above mentioned, and b) one bud shoot pieces out of adult trees. In this case, some dormant limbs in the field were chosen and disinfected with ethanol (97 p.100) and captafol (2 g/l), then were bagged with paper and several weeks later, the springshoots out of the chosen dormant limbs were excised, defoliated, disinfected into a 70 p.100 ethanol solution for 45 seconds followed by a 30 p.100 bleach solution (40 g/l of active chlorine) for 10 minutes, three times rinsed with sterile distilled water, segmented and used as showed in point b).

The shoots from the best proliferation treatments were used for rooting experiment. In this fase the next treatments were tried: 1) MS supplemented with 0, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/l of indole-3-butyric acid (IBA); 2) MS supplemented with 0, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/l of naphthyleneacetic acid (NAA); 3) MS without vitamins, supplemented with IBA (0, 0.5, 1.0, 2.5, 5.0 and 7.5 mg/l) and with BAP (0, 0.1, 0.25 and 0.5 mg/l); 4) MS without vitamins supplemented with NAA (0, 0.5, 1.0, 2.5, 5.0 and 7.5 mg/l) and BAP (0, 0.1, 0.25 and 0.5 mg/l); 5) WPM supplemented with IBA (0, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/l); 6) WPM supplemented with NAA (0, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/l); 7) Driver and Kuniyuki (1984) medium (DKW) supplemented with IBA (0, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/l); and 8) DKW supplemented with NAA (0, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/l). All the rooting media tested were supplemented with sucrose (30 g/l) and agar (8 g/l) and adjusted to pH 5.7.

The four precedent experiments were placed in 3.000 lux, 24±1°C and 16:8 hours light: dark environmental conditions. A last rooting experiment on MS medium without vitamins supplemented with IBA (2.5 mg/l) were placed in the dark for the first week, and then in the above refered climatic conditions. In all cases the rooting shoots were recultured every two weeks on fresh medium.

RESULTS AND DISCUSSION

Stages I and II: establishment and clonal propagation of *P. vera* in aseptic tissue culture.

a) Explants from in vitro cul+ured embryos plantlets.

When the embryos culture were started on MS medium supplemented with 4.0 mg/l of BAP a good propagation rate were observed, but 3-4 weeks later, independently of light or dark conditons, a vascular necrosis were observed and shoots died. To solve this trouble a vitaminless MS medium were tried, and then the vascular necrosis appeared any more. However, several weeks later, shoots vitrifiction came up. Vitrification problems were corrected when WPM supplemented with 4.0 mg/l of BAP and 0.5 mg/l of GA3 were used. For a start, the in vitro plant material for clonal propagation consisted on shoot segments removed from in vitro cultured embryos plantlets. The behaviour of these materials were very different in the last medium according to the mother plantlet. Therefore, a certain relation could exist between the genotipe and the growth rate of the micropropagated plant materials. So, for we to avoid this different behaviour on the same medium, all the next trials were established by using clonal material from the same mother plantlet.

b) Adult trees explants.

To start the clonal propagation of adult bud explants was only usefull the WPM supplemented with BAP (4.0 mg/l) and GA3 (0.5 mg/l) to break its dormant state. But when MS medium were used, the dormant buds died before sprouting and the translucent and clean color of the MS medium become darkish and blurred.

The results a) and b) seem differents of those got by Bargchi and Alderson (1983 and 1985) and by Martinelli (1988), when they make reference to MS as a good medium for pistachio species micropropagation. On the other hand, it has been observed that the leaflets from embryos plantlets are singles while these from adult trees are composed. A certain correspondence could be consider between the juvenile state for singles and adult state for composed leaflets, respectively.

Stage III: Rooting of micropropagated shoot cuttings.

The rooting trials were carried out only with clonal shoot cuttings from seed. At present the results are not definitive, but can be advanced that roots emerge when MS without vitamins supplemented with IBA (2.5 mg/l) was used. Whether during the first week the culture were kept in the dark, 50 p.100 of rooting shoots were noted, while when rooting were promoted since the begining in light: dark 16:8 hours, only 5 p.100 rooting were observed.

CONCLUSIONS

The best results for clonal propagation of *P. vera* were produced when a WPM supplemented with BAP (4 mg/l) and GA3 (0,5 mg/l) were used so much for seed plantlets as for adult explants.

Even considering a not definitive results for rooting, it looks possible to promote the root formation on juvenile plantlets from seeds when MS medium without vitamins is used and during the first week the rooting material is kept in the dark and then is growed in the climatic chamber.

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TISSUES CULTURE AND REGENERATION IN JOJOBA

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INTRODUCTION

Up to now very few studies were carried on tissue cultures of Jojoba (Simmondsia chinensis Link). A few years ago, ROST and HINCHEE (1) proposed a technique for node propagation and a method in order to obtain undifferentiated callus tissue. WANG and JANICK (2) and LEE and THOMAS (3) reported successful somatic embryogenesis from very immature zygotic embryos. Never embryogenesis nor organogenesis were obtained from any vegetative part of the plant. The present report concerns these possibilities. The preliminary work consists to compare the embryogenic and organogenic capacities of different juvenile parts of the plant : immature and mature ovules and ovaries, immature embryos.

MATERIAL AND METHODS

. The flower buds were harvested from male and female plants growing in an orchard located near Bastia (Corse). They were sterilized by dipping for fifteen minutes in 0.4 W/V Benlate ((butylcarbamoyl-1 benzimidazolyl-2) carbamate de methyle) and for ten minutes in calcium hypochloride (4%W/V). Then they were rinsed four times in sterile water. Sepals, embryos, ovaries, ovules were excised and transfered to petri dishes (60mm diameter). The basal medium for all cultures contained the following substances : inorganic salts according to MURASHIGE and SKOOG (modified 1962) (4), vitamins of B5 medium (GAMBORG) (5), casein (0.1W/V), polyvinylpyrolidone (0.05W/V), myo-inositol (1 mM), sucrose (0.08 M), aminoacid supplementation according to CHU and HILL (6) and agar (0.65W/V). PH was ajusted to 6.5 with KOH. The growing factors were used at different concentrations and combinations : Naphthylacetic acid (N.A.A.) (3 to 6 µM), Benzylaminopurine (B.A.P.) (1 to 20 µM), 2-4Dichlorophenoxyacetic acid (2-4D) (2 to 3 µM), Zeatin (1 to 7 µM). The cultured material was maintained under continuous low red light, the temperature was 24ºC. For histological studies the explants were fixed in F.A.A., embedded in resin, sectionned and colored with pyronin.

RESULTS

A - Organogenesis from flower explants

We first examined callogenic capacities of different flower explants

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