Table 2. Average root elongation of germinated lettuce seed measured in the second 24-hr period when subjected to various dilutions of aqueous and methanol extracts of sunflower hulls.[z]

Extract:[y] distilled water ratio (v/v)	Avg root elongation (cm)	
	Distilled water	Methanol
10:0	0.9	1.5
8:2	1.3	1.9
6:4	1.0	1.3
4:6	1.3	1.2
2:8	2.2	3.9
0:10	8.6	8.6
LSD 5%	0.8	2.9
1%	1.1	4.6

[z]5 seedlings on blotting paper moistened with 6-ml aliquot.

[y]Extract was 100 ml from 35 finely ground hulls soxhlet extracted for 24 hr. Both distilled water and methanol extracts were evaporated to dryness under vacuum and redissolved in distilled water.

blotting paper in Petri dishes. Four replications of 25 seeds were placed on the moist blotting paper, the Petri dishes were taped closed, and germination tests were conducted in the dark at a temperature of 22°C. Counts were made daily until germination was deemed complete. In the root elongation test, lettuce seed germinated on distilled water-moistened blotting paper until radicle protrusion was visible. Four replications of 5 germinated seeds were then transferred onto blotters moistened (6 ml) with the test solutions. The Petri dishes were again taped closed and placed in racks in a near vertical position in the dark at 22°. The test plants were allowed to stabilize for 24 hr, and then the primary root elongation during the next 24-hr period was measured.

Split-plot analyses indicated that there were no significant germination differences in the response to distilled water and methanol extracts. Using the water extract as an example (Table 1), there was a pronounced inhibitory effect on germination of both lettuce and radish, with lettuce being slower to germinate than radish, and the inhibitory effect more prolonged.

In the root elongation bioassay (Table 2), the distilled water and methanol extracts yielded significantly different results (split-plot analysis P = 5%) with the distilled water extract giving greater inhibition of root elongation. Both extracts and dilutions severely reduced primary root elongation compared to the control (0:10 ratio).

Wilson and Rice (2) have reported that certain associated species show reduced growth which was not due to competition when grown with sunflower (*H. annuus*) under field conditions. They also noted inhibitory effects of extracts from various parts of the sunflower plant on the germination of sunflower seeds and the seeds of numerous other plants. Although they showed inhibitory effects from extracts of the inflorescence, no specific mention was made of the seeds or hulls of sunflower. Later, Anderson et al. (1) reported similar allelopathic inhibition of growth on radish, *Raphanus sativus* L., and wheat, *Triticum aesativum* L., when associated with *Helianthus mollis* Lam.

Literature Cited

1. Anderson, R. C., A. J. Katz, and M. R. Anderson. 1978. Allelopathy as a factor in the success of *Helianthus mollis* Lam. J. Chem. Ecol. 4:9–16.
2. Wilson, R. and E. L. Rice. 1968. Allelopathy as expressed by *Helianthus annuus* and its role in old-field succession. Bul. Torrey Bot. Club 95:432–448.

HortScience 17(6):888–889. 1982.

In Vitro Propagation of *Castanea sativa* Mill. through Meristem-tip Culture[1]

Roberto Rodríguez[2, 3]

Department of Pomology, University of California, Davis, CA 95616

Additional index words. chestnut, micropropagation

Abstract. **Meristem tips of *Castanea sativa* Mill. from *in vitro* cultures derived from seedling explants initiated multiple shoot-buds and developed shoot-bud in the presence of 1 mg/liter 6-benzylamino purine (BA) + 0.01 mg/liter indolebutyric acid (IBA). Root formation was readily achieved within 20 to 25 days when excised single shoots were transferred onto a fresh medium supplemented with IBA (1 mg/liter).**

Castanea sativa, widely distributed throughout northern regions of Spain (2), is highly valued for its economical and ecological importance (3). Unfortunately, habitat has been decreasing in recent decades due to several fungal and viral diseases. Propagation of chestnut by tissue culture would be desirable since meristems from *in vitro* cultures are often disease-free and genetically stable.

Explants from chestnut seedlings were cultured on a basal medium: Cheng's mineral salts (1) and (per liter) thiamine 0.25 mg, inositol 0.25 g, sucrose 30 g, and bacto-agar 6 g plus 5 mg BA. All media were adjusted to pH 5.5 before autoclaving. Cultures were maintained at a constant temperature of 25°C and 18-hr photoperiod with a light intensity of 2.5 klx.

Explants formed multiple shoots from which meristem tips (0.3 × 0.2 mm) were excised and cultured for 2 weeks with 1 mg/liter BA + 0.1 mg/liter IBA and then transferred to various concentrations of IAA or IBA (0.01, 0.1, 1, or 10 mg/liter) alone and in combination with zeatin or BA (0.1, 1, 5, or 10 mg/liter). There were 5 cultures per treatment.

Meristem tips developed as a single shoot in the presence of 1 mg/liter IBA; however, with 1 mg/liter BA + 0.01 mg/liter IBA, several new shoots (3 to 5 per explant) originated directly from the meristem tip (Fig. 1). The new shoots formed could be transferred for rooting after 20 days in culture (Fig. 2). To increase the number of shoot-buds generated, the amount of BA applied was increased to 2 and 5 mg/liter (Fig. 3). The number of shoots initiated increased greatly, but callus also developed.

Single shoots longer than 2 cm were excised from proliferating cultures and explanted onto half-strength basal medium supplemented with 1 mg/liter IBA (Fig. 4a). Under these conditions, the single shoots rooted readily within 20 to 25 days; shoots grew normally showing perfect leaf enlargement. Although IBA at higher concentrations of 2, 5, and 10 mg/liter stimulated some rooting, more callus originated from the base of the excision zone along with roots. Furthermore, the shoots grew more slowly and frequently had necrosis and malformations on the shoot tips.

Plants were established in soil despite some transplanting shock and plants grew normally. These results indicate that large-scale micropropagation of chestnut trees is feasible.

[1]Received for publication on March 19, 1982.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[2]Permanent address: Departamento de Fisiología Vegetal, Facultad de Biología. Universidad de Oviedo, Oviedo, Spain.

[3]I thank A. Kuniyuki for advice and encouragement.

Fig. 1. Stimulation of multiple shoot-bud formation from meristem-tips cultured for 2 weeks in basal medium supplemented with 1 mg/liter BA plus 0.01 mg/liter IBA.

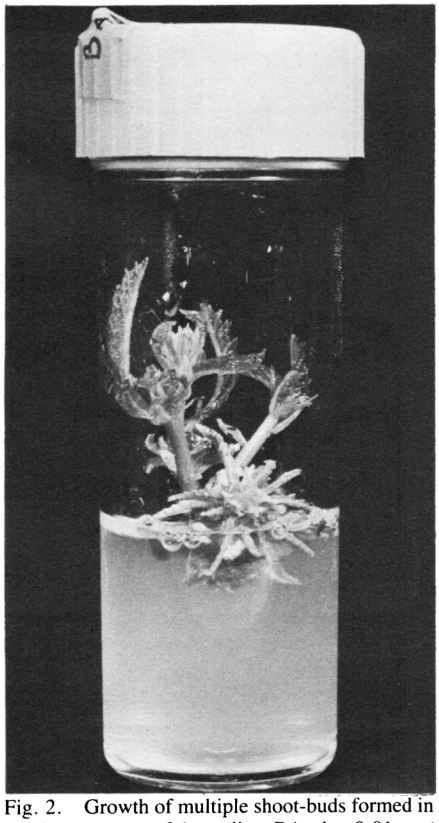

Fig. 2. Growth of multiple shoot-buds formed in the presence of 1 mg/liter BA plus 0.01 mg/liter IBA after 20 days in culture.

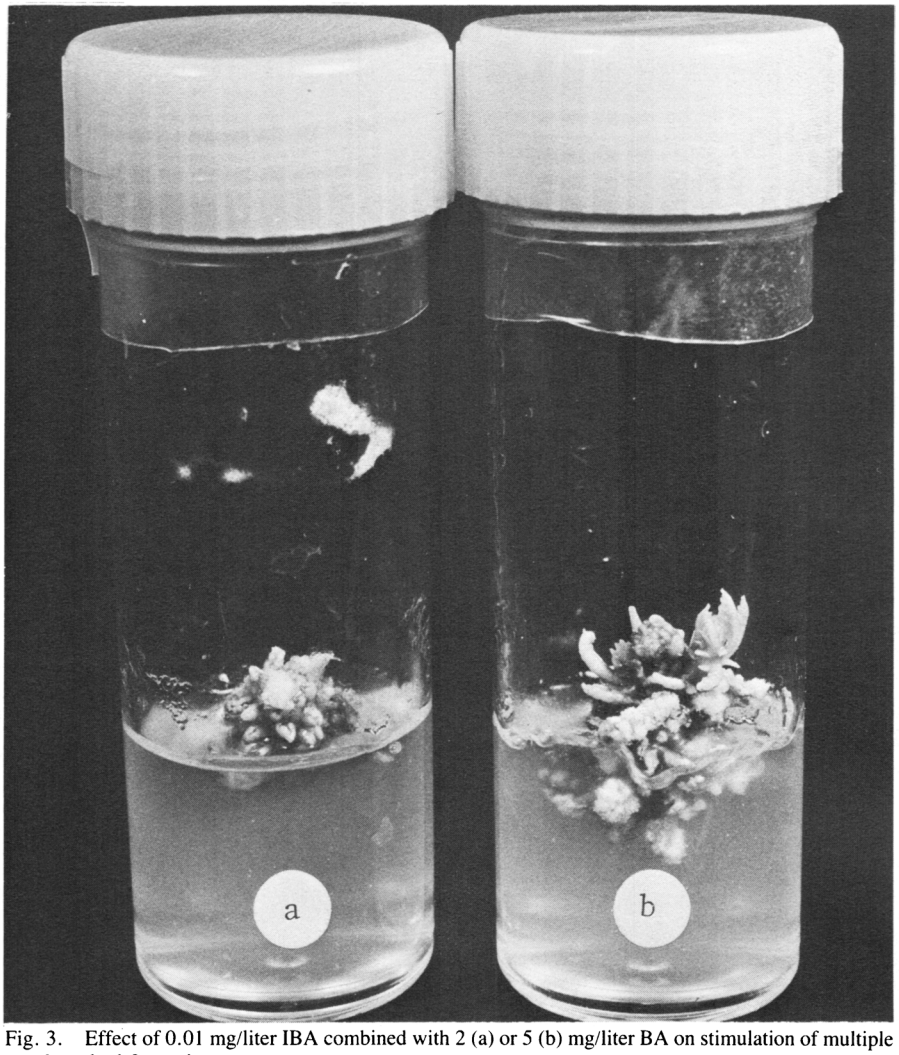

Fig. 3. Effect of 0.01 mg/liter IBA combined with 2 (a) or 5 (b) mg/liter BA on stimulation of multiple shoot-bud formation.

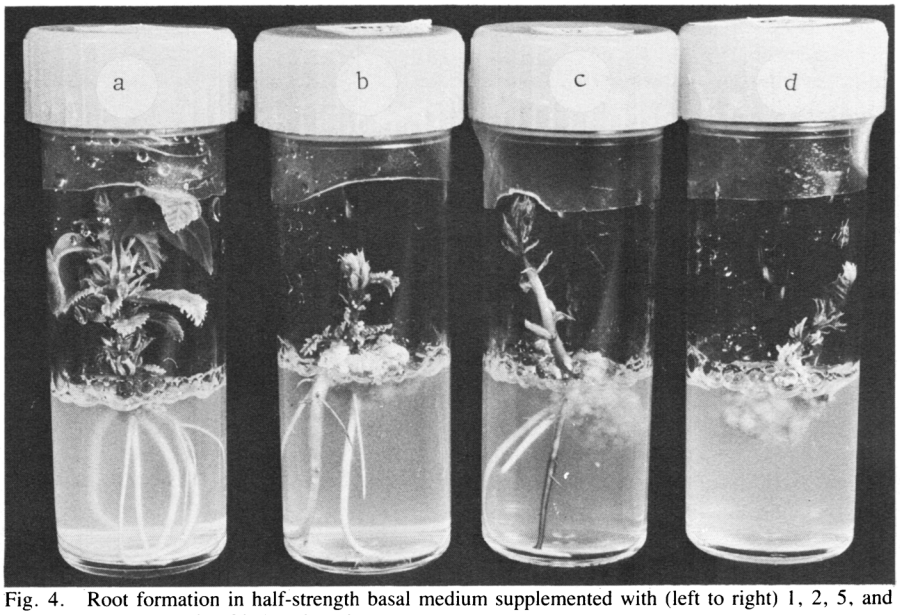

Fig. 4. Root formation in half-strength basal medium supplemented with (left to right) 1, 2, 5, and 10 mg/liter IBA after 20 days in culture.

Literature Cited

1. Cheng, T. Y. and T. H. Voqui. 1977. Regeneration of Douglas fir plantlets through tissue culture. Science 198:306–307.
2. González Vázquez, E. 1938. Castaño. In: Selvicultura, Tomo I. Los bosques Ibéricos. Instituto Forestal de Investigaciones y experiencias. Valencia, Spain.
3. Sander, I. L. 1974. *Castanea* Mill. Chestnut. p. 273–275. In: Seeds of woody plants in the United States. Forest Service, U.S. Dept. Agr., Agr. Handb. 450.