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Stimulation of Multiple Shoot-bud Formation in Walnut Seeds.[1]

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***Abstract. In vitro* initiation and development of shoot-buds of walnuts (*Juglans regia* L.) was obtained on a defined medium using seedlings as a primary explants. Benzylamino purine (BA) at 40 μM induced multiple shoot formation derived from an abnormal conical shoot. Periodic subculture on a fresh media with 0.4 μM of both indolebutyric acid (IBA) and BA resulted in shoots multiplication.**

Traditional nursery techniques for vegetative propagation of walnuts (*Juglans regia* L.) are relatively slow and often difficult (7). The potential significance of tissue culture for walnut has been described by Jacquiot (5). This study was undertaken to develop an approach for *in vitro* propagation of walnuts.

Walnut seeds were soaked in running tap water for 12 hr to remove phenolic compounds. Then seeds were surface-sterilized in 3 steps: 1) submersion in 95% ethanol for 3 min; 2) submersion in a 1% NaOCl solution for 30 min; and 3) several subsequent rinses in sterile deionized water. The media used [K(h)] consisted of T. Y. Cheng's mineral salts (1, 2), thiamine 0,25 mg/liter, inositol 0,25 g/liter, sucrose 30 g/liter and 6 g/liter bacto-agar as a solidified agent.

Seeds were sown directly in the half-strength K(h) medium to determine the effect of BA on seed germination and multiple shoot formation (Table 1). Seed germination remained high regardless of BA concentrations. Increasing concentration of BA increased axillary bud development and adventitious bud primordia.

BA at 40 μM (Fig. 1) was the optimum concentration to induce multiple shoot formation. These arose from an altered main axis which developed as an abnormal conical shoot during germination. Periodic subculture of these tissues onto the same BA concentrations continued producing shoot-buds but subsequent growth was inhibited.

In a 2nd experiment, various concentrations of IBA were tested with BA maintained at 40 μM (Table 2). Seed germination decreased from nearly 70 to 21% as IBA increased from 0 to 20 μM. The number of shoot-bud formations also decreased as the IBA concentrations were increased. Although a low concentration of IBA (Fig. 2) decreased the total generation of shoots, growth of these shoots was enhanced. To establish multiplying tissues, single shoots excised from seedlings treated with BA at 40 μM were cultured in the presence of different lower concentrations of BA.

The application of both IBA and BA (0.4 μM) proved to be optimal for supporting a good rate of shoot production in subcultured shoots. Under these conditions, development proceeded normally. When the shoots reached maturity the same morphological characteristics were evident as those derived from intact seeds.

Table 1. Effect of BA on initiation and development of shoot-buds in walnut seeds. Seeds were cultured on half-strength K(h) medium for 5–7 weeks supplemented with BA. The number of shoots originating was averaged from at least 12 replicates.

BA concn (μM)	Germination rate (%)	No. shoots/seed		
		>1 cm	<1 cm	Total
0	80	1	---	1
4	80	---	5	5
20	80	1	11	12
40	73	5	10	15

Table 2. Effect of IBA on development and initiation of shoot-buds. Seeds were cultured on half-strength K(h) medium containing a constant amount of BA (40 μM) and various concentrations of IBA. The number of shoots was determined from at least 12 replicates after 5 weeks in culture.

IBA concn (μM)	Germination rate (%)	No. shoots/seed		
		>1 cm	<1 cm	Total
0	80	5	10	15
0.4	70	7	2	9
4.0	36	1	---	1
20.0	12	---	1	1

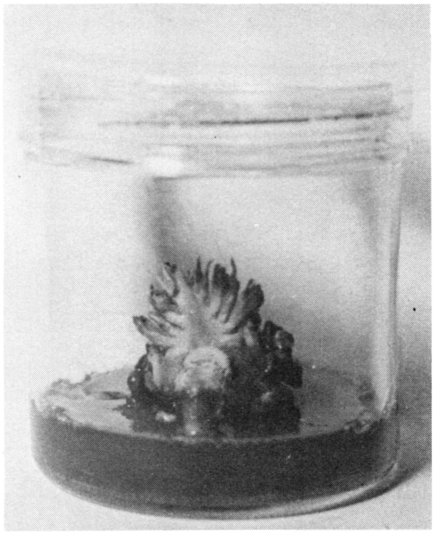

Fig. 1. Stimulation of shoot-bud in walnuts. Whole seeds were cultured during 5 weeks (without transfer), on a half-strength K(h) medium in the presence of BA at 40 μM.

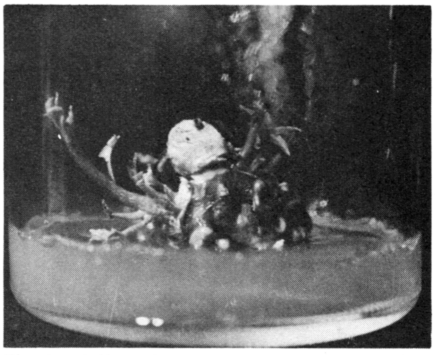

Fig. 2. Effect of IBA on stimulation of shoot bud formation. Walnut seeds were cultured on half-strength K(h) medium containing BA 40 μM and IBA at concentration 0.4 μM.

Literature Cited

1. Cheng, T. Y. 1975. Adventitious bud formations in culture of Douglas fir [*Pseudotsuga menziensii* (Mirb.) Franco]. Plant Sci. Lett. 5:97–102.
2. Cheng, T. Y. and T. H. Voqui-Dinh. 1977. Regeneration of Douglas fir plantlets through tissue culture. Science 198:306–307.
3. Hartmann, H. T. and D. E. Kester. 1975. Plant propagation: principles and practices. Prentice-Hall, Englewood, N.J.
4. Jacquiot, C. 1966. Plant tisue and excised organ cultures and their significance in forest research. J. Inst. Wood Sci. 16:22–34.
5. Wayne, R. W. and H. B. Lagerstedt. 1969. Plastic tents for early walnut grafting. Proc. Nut. Grow. Soc. Ore. & Wash. 55:98–102.

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