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Micropropagation of Juvenile and Adult Flowering Ash

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Abstract. **A protocol for in vitro propagation in flowering ash (*Fraxinus ornus* L.) has been developed. Shoot apices or nodal segments from aseptically grown seedings or shoot apices from adult trees were used as initial explants. Highest shoot multiplication rates were obtained when the explants were cultured for 30 days in liquid Rugini induction medium supplemented with BA followed by 30 days on solidified Rugini multiplication medium without growth regulators. Regenerated shoots were rooted on Heller medium containing auxins alone or in combination with BA. Rooting percentages up to 71% (juvenile material) or 50% (adult material) were obtained in the presence of NAA and BA, and were not improved by treating the basal end of the shoots with concentrated NAA solutions. Following conventional procedures, regenerated plants were transferred to soil with more than 80% success. Chemical names used: N-(phenylmethyl)-1H-purin-6-amine (BA); 1-naphthaleneacetic acid (NAA).**

Flowering ash is a deciduous, temperate ash indigenous to southern Europe and western Asia that has been introduced as an ornamental in North America. Due to its timber and ornamental qualities, the species is used for a variety of wood products and has also become popular for urban planting.

Conventional vegetative or sexual methods for propagation of flowering ash are beset with numerous problems that hinder mass multiplication (Bonner, 1974; Hartmann et al., 1990). Thus, tissue culture techniques may constitute an alternative approach for efficient propagation of this species. Although these techniques have been successfully employed with several *Fraxinus* spp. (Browne and Hicks, 1983; Chalupa, 1983, 1990; Doley and Leyton, 1970; Leforestier et al., 1990; Navarrete et al., 1989; Preece et al., 1987, 1989; Wolter and Skoog, 1966), we found no studies reporting their application to flowering ash. Therefore, the objective of this research was to develop methods for in vitro propagation of flowering ash from juvenile and adult trees.

Materials and Methods

Plant material

Juvenile explants. Shoot apices and nodal segments with one axillary bud (0.3 cm long) from 30-day-old seedlings of flowering ash were used as initial explants. Seedlings were grown from mature embryos germinated under sterile conditions. Before embryo isolation, seeds (harvested from field-grown plants) were immersed in 70% ethanol for 30 sec followed by 20 min in 3% NaOCl and rinsed with sterile distilled water. Seeds were kept for 24 h at 26 ± 2C in a sterile solution of 100 mg·liter^{-1} each of citric and absorbic acids. Following imbibition, seeds were again surface-sterilized, but with 1% NaOCl for 15 min, and the embryos were then excised and transferred to glass tubes (one embryo per tube) containing a Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 3% sucrose and 0.7% Difco-Bacto agar (pH 5.8).

Adult explants. Actively growing shoots (4 cm long) were collected in June from several 30-year-old trees growing outdoors (Fuente del Enebro, Valencia, Spain). After the leaves were removed, stems were rinsed for 12 h in running tap water, surface-sterilized consecutively in 70% ethanol for 30 sec and 3% NaOCl containing 0.1% Tween-80 for 15 min, and finally they were rinsed three times with sterile distilled water. Initial explants (1 to 2 mm long), consisting of a meristematic dome plus one or two pairs of leaf primordia, were isolated under sterile conditions using a stereoscopic microscope. To avoid browning, explants were initially placed into petri dishes containing a 3% sucrose solution solidified with 0.7% agar; they were transferred daily, for 5 days, to fresh medium.

Shoot multiplication and rooting procedures

Juvenile explant cultures. In a first experiment, explants (shoot tips or nodal segments) were placed in glass tubes (one explant per tube) containing 25 ml of culture medium. Three nutrient media were tested: MSM (Murashige and Skoog, 1962); SHM (Schenck and Hildebrandt, 1972); and HM (Heller, 1953) modified by adding 1 mM $(NH_4)_2SO_4$. All media included MS minor salts (Murashige and Skoog, 1962), vitamins described by Vieitez et al. (1985), 100 mg·liter^{-1} each of citric and ascorbic acids, 3% sucrose and 0.7% Difco-Bacto agar; pH was adjusted to 5.6 before autoclaving for 20 min at 120C. All media were supplemented with BA (0.0, 0.4, 4.4, 22.2, or 44.4 µM). Shoot tips were also cultured on media containing these same BA concentrations plus 0.5 µM NAA. Each treatment contained 12 replicates and culture time was 60 days.

In a second experiment, explants were initially cultured for 30 days in liquid or agar-solidified Rugini 1 or Rugini 2 medium (Rugini, 1986) or HM supplemented with 22.2 µM BA. Subsequently, explants were transferred for another 30 days of culture to their respective solidified media without BA. Initial Rugini 1 cultures were also subcultured to solidified Rugini 2 medium without growth regulators. Eighteen shoot apices or nodal segments were cultured for each treatment. In both experiments, sprouting percentage, number of shoots per explant, and shoot elongation (length of the highest shoot) were recorded.

To induce rooting, shoots > 1 cm long were excised and cultured for 30 days on solidified HM containing 0.0, 0.5, 2.5, or

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Abbreviations: HM, Heller major salts; MSM, MS major salts; SHM, SH major salts.

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5.2 μM NAA, 1H-indole-3-acetic acid (IAA) or 1H-indole-3-butyric acid (IBA) alone or in combination with 0.4 μM BA. Subsequently, shoots were transferred to the same medium without growth regulators. Alternatively, the basal ends of the shoots (≈ 3 mm) were dipped in a sterile aqueous 1.0 mM NAA solution for 0.5, 2, or 5 min and then transferred to auxin-free solidified HM. Each treatment included 14 replicates. Rooting percentage and the number of roots per rooted shoot were evaluated after 60 days. A shoot having one or more macroscopically visible roots >0.5 cm long was considered rooted.

Mature explant cultures. Apical buds were cultured initially on agar-solidified SHM without growth regulators. After 30 days, shoots >2 cm long, which had developed from these cultures, were sectioned into shoot tips and nodal segments and then subcultured onto the same medium. This procedure was repeated three times. Shoot apices isolated from these proliferating cultures were placed in liquid and agar-solidified Rugini 1 medium or HM supplemented with 22.2 or 44.4 μM BA. After 30 days, explants were respectively subcultured onto agar-solidified Rugini 2 medium or HM without growth regulators. Total culture time was 60 days. Twelve shoot apices were cultured for each treatment. Sprouting percentage, number of shoots per explant, and shoot elongation (length of the highest shoot) were recorded.

For rooting, excised shoots were cultured on solidified HM without growth regulators or containing IAA or NAA (2.5 or 5.2 μM) and 0.4 μM BA for 30 days, then transferred to the same medium without growth regulators. In an alternative experiment, shoots were dipped in a 1.0 mM NAA solution for 0.5 min and cultured on HM without growth regulators. In both cases, each treatment consisted of 12 replicates. Rooting percentage and the number of roots per rooted shoot were recorded after 60 days.

Rooted shoots from juvenile and adult explants were transplanted individually to 100-ml pots containing a medium of 1 vermiculite : 1 peat (v/v). Plantlets were adapted to growth chamber conditions (see below) with gradual exposure to reduced relative humidity by progressively removing a glass cover during 2 to 3 weeks. Once acclimatization was accomplished, plants were transferred to the greenhouse.

Culture conditions

All cultures were incubated in growth chambers at 26 ± 2C with a 16-h photoperiod of 20 $W \cdot m^{-2}$ provided by cool-white fluorescent lamps (Sylvania GTE Gro-lux, F36W/GRO, Germany). Liquid cultures were maintained on an orbital shaker at 90 rpm.

Statistical analysis

Significance of treatment effects was determined using analysis of variance (ANOVA), employing a completely random design. Data from sprouting and rooting percentages were subjected to arcsin transformation before analysis. Variations among treatment means were analyzed using Tukey's (1953) procedure. All experiments were conducted at least twice.

Results

Shoot multiplication from juvenile explants

In a first experiment, apical or nodal explants were cultured on one of three agar-solidified basal media (MSM, SHM, or HM) with or without BA. Frequency of explants forming shoots depended mainly on the basal medium. SHM and HM gave superior results (92% to 100% successful) compared with MSM, particularly when high BA levels were employed (50%). Moreover, MSM without growth regulators drastically reduced the sprouting percentage from cultured nodal segments (33%). ANOVA of the sprouting response data indicated significant effects of the nutritive medium as well as an interaction among the three main factors (data not presented).

Flowering ash exhibited strong apical dominance with little tendency to branch. After 60 days of culture, most of the responding explants (apical or nodal) produced only a single shoot. Neither BA concentration nor basal medium significantly affected this response (data not presented). The average length of the shoots depended on the type of explant, the basal medium, and BA concentration (Table 1). Shoots originating from apical explants were longer than those derived from nodal segments. Maximal shoot elongation in apical and nodal segment cultures occurred on SHM and HM, respectively. Finally, optimal BA concentrations varied with the explant, being 22.2 μM for shoot apices and 22.2 μM or 44.4 μM for nodal segments. The pressence of NAA in the medium did not significantly affect the response promoted by BA in shoot tip cultures (data not presented). Nevertheless, we frequently observed root induction and development while shoots were maintained on HM supplemented with 0.4 μM BA and 0.5 μM NAA.

In an effort to break apical dominance and promote formation of axillary shoots, an experiment was conducted using new methods of exposure to growth regulators (liquid or agar-solidified induction medium with 22.2 μM BA) and new nutritive formulations (Rugini 1, Rugini 2, or HM). Explants initially cultured on Rugini 2 medium turned necrotic and died within 15 to 20 days. Treatments that included only Rugini 1 nutrients were also deleterious for the ash explants. Although the explants (apical or nodal) proliferated when cultured in BA-supplemented Rugini 1 medium, subsequent transfer to the same medium without BA caused shoot tip necrosis and a generalized chlorosis of the newly formed shoots. As a result, only those results obtained with explants initially cultured on Rugini 1 medium or HM and then transferred to Rugini 2 medium or HM, respectively, are reported.

Sprouting percentages after 60 days of culture ranged from 83% to 100%, with shoot tips responding significantly better than nodal segments (data not presented). Significant effects of induction medium consistency (liquid or agar-solidified) and the particular salt formulation used and their interaction were observed for the mean number of shoots formed per apical or nodal explant (Table 2A). Thus, axillary shoot proliferation was only observed on explants initially cultured in liquid induction medium, and better results were obtained when either explant type was first maintained in BA-supplemented Rugini 1 medium and subsequently transferred to Rugini 2 medium without growth regulators. This treatment also favored maximum shoot development (Table 2B). Shoots were vitrified, but vitrification disappeared following transfer to agar-solidified medium.

Shoot multiplication from adult explants

Initial cultures were established by placing apical buds of adult material on agar-solidified SHM without growth regulators. After 30 days, 90% to 100% of the buds produced a single shoot with strong apical dominance. This type of growth was not modified when apical or nodal segments excised from these shoots were subcultured on the same medium. Nevertheless,

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Table 1. Effect of salt formulation and BA concentration on shoot length (cm) of regenerated shoots from shoot tip and nodal segment cultures of juvenile flowering ash. Values are combined means ± SD from two independent experiments of 12 observations each. (Culture time was 60 days.)

Salt formulation	Explant type	BA concn (μM) 0.0	0.4	4.4	22.2	44.4
				cm		
MSM	Apical	0.7 ± 0.1	1.3 ± 0.3	1.1 ± 0.1	2.6 ± 0.3	0.7 ± 0.4
	Nodal	0.3 ± 0.01	0.4 ± 0.4	1.1 ± 0.2	1.3 ± 0.5	2.3 ± 0.3
SHM	Apical	2.4 ± 0.6	2.6 ± 0.1	3.5 ± 0.1	4.9 ± 0.6	4.1 ± 0.2
	Nodal	0.2 ± 0.03	0.5 ± 0.1	1.1 ± 0.2	2.5 ± 0.8	2.7 ± 0.6
HM	Apical	2.0 ± 0.3	1.3 ± 0.6	2.4 ± 0.3	4.1 ± 0.1	3.1 ± 0.06
	Nodal	1.4 ± 0.3	1.2 ± 0.3	2.9 ± 0.4	3.2 ± 0.1	2.9 ± 0.1

Table 2A. Effect of salt formulation, induction medium consistency, and explant type on shoot proliferation (number of shoots/explant) from juvenile explants of flowering ash. Shoot tips and nodal segments were cultured initially for 30 days on Rugini 1 medium or HM, supplemented with 22.2 μM BA, and then transferred to Rugini 2 medium or HM without growth regulators. Each value is based on 18 observations. (Culture time was 60 days.)

Salt formulation	Induction medium consistency	Explant type: Apical	Nodal	Mean[z]
			No.	
Rugini 1/Rugini 2	Agar-solidified	1.1	1.4	1.2 c
	Liquid	6.6	6.4	6.5 a
HM/HM	Agar-solidified	1.2	0.9	1.1 c
	Liquid	3.0	2.1	2.5 b

[z]Interaction of salt formulation with induction medium consistency. Means separation by Tukey's test, $P = 0.05$.

Table 2B. Effect of salt formulation, induction medium consistency, and explant type on shoot length (cm) of regenerated shoots from explant cultures of juvenile flowering ash. Shoot tips and nodal segments were cultured initially for 30 days on Rugini 1 medium or HM, supplemented with 22.2 μM BA, and then transferred to Rugini 2 medium or HM without growth regulators. Values are combined means ± SD from two independent experiments of 18 observations each. (Culture time was 60 days.)

Salt formulation	Induction medium consistency	Explant type: Apical	Nodal
		cm	
Rugini 1/Rugini 2	Agar-solidified	2.1 ± 0.6	2.0 ± 0.6
	Liquid	5.0 ± 0.7	4.7 ± 1.7
HM/HM	Agar-solidified	1.7 ± 0.6	1.4 ± 0.3
	Liquid	3.6 ± 0.4	1.2 ± 0.5

shoots produced by the recultured explants exhibited more juvenile characteristics; particularly, leaves were less lobed than those of the first culture. In the multiplication stage, shoot apicies isolated from the initial cultures were used as explants. Several methods of exposure to BA and various salt formulations were examined. The percentage of sprouting (85% to 100%) was not significantly affected by induction medium consistency, BA concentrations, or salt formulations (data not presented). Table 3A summarizes the influence of these factors on the mean shoot multiplication rate after 60 days of culture. Results obtained were similar to those for juvenile apical explants (Table 2A). Axillary bud development, resulting in multiple shoot cultures, was only observed when the explants were incubated ini-

Table 3A. Effect of salt formulation, induction medium consistency, and BA level on shoot proliferation (number of shoots/explant) from shoot tips of adult flowering ash. Explants were cultured initially for 30 days on Rugini 1 medium or HM, supplemented with BA, and then transferred to Rugini 2 medium of HM without growth regulators. Each value is based on 12 observations. (Culture time was 60 days.)

Salt formulation	Induction medium consistency	BA concn in induction medium (μM): 22.2	44.4	Mean[z]
			No.	
Rugini 1/Rugini 2	Agar-solidified	0.8	0.8	0.8 c
	Liquid	6.2	4.4	5.3 a
HM/HM	Agar-solidified	1.1	0.8	0.9 c
	Liquid	3.2	3.5	3.3 b

[z]Interaction of salt formulation with induction medium consistency. Means separation by Tukey's test, $P = 0.05$.

Thus, the highest mean number of shoots per explant was obtained when shoot apices, cultured in BA supplemented with liquid Rugini 1 medium, were transferred to agar-solidified Rugini 2 medium without BA (Table 3A and Fig. 1). In the same way, the highest shoot elongation rate was recorded in cultures established in liquid Rugini 1 medium (Table 3B).

Rooting and plant development

To induce rooting, growth regulators were included in the medium or used as a pulse. For greater uniformity, only shoots from cultures established in liquid Rugini 1 medium were used in these experiments. Irrespective of the shoot origin (juvenile or adult), root primordia began to emerge after 15 to 20 days. The number of roots per rooted shoot was highly variable (one to eight); therefore, only data from the percentage of shoots forming roots were subjected to ANOVA.

Rooting percentages of shoots of juvenile origin cultured on agar-solidified medium with or without growth regulators were significantly affected by auxin concentration and the presence of BA (Table 4). The effect of BA varied with the auxin. While BA did not modify IAA- or IBA-induced rooting, it had a significant effect on rhizogenesis induced by NAA. The highest rooting percentage (71%) was realized with 2.5 μM NAA and 0.4 μM BA. Also, 21% of the shoots rooted when cultured on HM without growth regulators (Table 4). Pulse treatments of excised shoots with auxin followed by root growth in HM without growth regulators did not improve rooting. Rooting of 46%, 33%, and 29% was attained using a 1.0-mM NAA dip treatment for 0.5, 2, or 5 min, respectively.

Treatments employed in rooting experiments of adult material

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Fig. 1. Effect of induction medium consistency on shoot proliferation from shoot tip explants of adult *Fraxinus ornus*. **(A, B)** liquid Rugini 1 medium or HM, respectively; **(C, D)** agar-solidified Rugini 1 medium or HM, respectively.

Table 3B. Effect of salt formulation, induction medium consistency, and BA concentration on shoot length (cm) of regenerated shoots from shoot tip cultures of adult flowering ash. Explants were cultured initially for 30 days on Rugini 1 medium or HM, supplemented with BA, and then transferred to Rugini 2 medium or HM without growth regulators. Values are combined means ±SD from two independent experiments of 12 observations each. (Culture time was 60 days.)

Salt formulation	Induction medium consistency	BA concn in induction medium (μM)	
		22.2	44.4
		cm	
Rugini 1/Rugini 2	Agar-solidified	2.0 ± 0.2	2.5 ± 0.5
	Liquid	4.6 ± 0.4	4.4 ± 1.1
HM/HM	Agar-solidified	1.6 ± 0.2	1.8 ± 0.3
	Liquid	2.7 ± 0.2	2.2 ± 0.5

were those determined to be most suitable for shoots of juvenile origin. When different auxin : BA ratios were supplied in the rooting medium, the frequency of rooted shoots was significantly affected by the type and concentration of auxin. NAA/BA combinations were superior to those including IAA, and maximum rooting (53%) was obtained with 5.2 μM NAA and 0.4 μM BA (Table 5). Pulse treatment in a 1.0-mM NAA solution for 0.5 min resulted in 50% rooting.

When transferred to soil and placed in a greenhouse, plantlets of both origins exhibited normal development compared with seedlings. The plants first were adapted to growth chamber conditions with gradual exposure to reduced relative humidity over 2 to 3 weeks. Survival rate was 80% to 85%.

Discussion

Results herein demonstrate successful micropropagation of flowering ash using explants from juvenile and adult trees. Based on these data, we infer that consistency of the induction medium (liquid or agar-solidified) and salt formulation of induction and proliferation media are important factors affecting axillary branching of cultured flowering ash. Liquid induction media with a low salt concentration (HM or Rugini 1) effectively overcame the strong apical dominance of this species, promoting multiple shoot formation from juvenile or adult explants. Nevertheless, maximum shoot multiplication rate required an additional transfer to high salt concentration Rugini 2 medium (Tables 2A and 3A). Rugini 1 and Rugini 2 formulations (Rugini, 1986) have been successfully employed for micropropagation of olive *(Olea europaea* L.), another member of the Oleaceae. The favorable effects of liquid medium have been associated with increased availability of nutrients and growth regulators or to a more effective diffusion of toxic metabolites (George and Sherrington, 1984). Preece et al. (1987) also found that liquid medium cultures promoted multiple shoot formation in white ash *(F. americana* L.). In contrast, axillary branching in cultures of European ash *(F. excelsior* L.) was achieved on agar-solidified medium supplemented with 1 to 5 mg BA/liter (Chalupa, 1983; Leforestier et al., 1990). A solidified medium containing BA, N-phenyl-N'-1,2,3-thidiazol-5-ylurea (thidiazuron) and IBA also stimulated axillary shoot proliferation from juvenile explants of white ash (Navarrete et al., 1989) and European ash (Chalupa, 1990).

In the shoot multiplication stage, the response of cultures from adult flowering ash was similar to that of juvenile material. In contrast, rooting capacity of shoots from mature explants was somewhat lower. Age-dependent differences in the ease of rooting also have been reported in several micropropagated forest trees (Evers et al., 1988; Németh, 1986). To date, no research

Table 4. Rooting percentage of shoots obtained from juvenile cultures of flowering ash.[z] Percentage data are based on 14 observations. Values in brackets are the mean number of roots per rooted shoot ± SD. (Culture time was 60 days.)

Auxin	BA (μM)	Auxin concn (μM) 0.5	2.5	5.2	Mean[y]
			Percent (no.)		
IAA	0.0	14 (2.0 ± 1.4)	36 (1.4 ± 0.9)	29 (2.0 ± 1.4)	26 ab
	0.4	54 (1.9 ± 0.7)	21 (1.0 ± 0.0)	57 (3.5 ± 1.9)	44 ab
IBA	0.0	29 (2.7 ± 2.2)	43 (2.3 ± 1.2)	43 (2.3 ± 1.2)	38 ab
	0.4	7 (3.0 ± 0.0)	21 (1.7 ± 0.6)	50 (2.7 ± 1.7)	26 ab
NAA	0.0	7 (3.0 ± 0.0)	14 (1.0 ± 0.0)	36 (2.6 ± 1.5)	19 b
	0.4	27 (2.0 ± 0.0)	71 (2.5 ± 1.3)	62 (3.3 ± 2.2)	53 a

[z]Rooting percentage of control (medium without growth regulators) = 21 (2.3 ± 1.1).
[y]Interaction of auxin with BA concentration. Means separation by Tukey's test, $P = 0.05$.

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Table 5. Rooting percentage of shoots obtained from adult cultures of flowering ash. Percentage data are based on 12 observations. Values in brackets are the mean number of roots/rooted shoot ± SD. (Culture time was 60 days.)

	Auxin/BA concn (μM)			
Auxin/BA	0.0/0.0	2.5/0.4	5.2/0.4	Mean[z]
		Percent (no.)		
IAA/BA	6 (1.0 ± 0.0)	6 (1.0 ± 0.0)	25 (1.3 ± 0.6)	12 b
NAA/BA	6 (1.0 ± 0.0)	25 (1.7 ± 1.0)	53 (2.2 ± 2.0)	28 a
Mean[z]	6 b	15 ab	39 a	

[z]Effect of the main factors on rooting percentages. Means separation by Tukey's test, $P = 0.05$.

has been reported on the rooting capacity of regenerated shoots from other *Fraxinum* spp. in the adult growth stage.

In conclusion, clonal propagation of flowering ash has been accomplished by axillary shoot multiplication from explants of seedlings and trees in the adult growth phase. With few exceptions, techniques for micropropagation of forest trees in the adult growth phase are still limited (Thorpe and Harry, 1990). Procedures described herein may provide a useful tool for large-scale regeneration of selected clones of flowering ash. However, further research is necessary to optimize rooting of in vitro-produced microshoots.

Literature Cited

Bonner, F.T. 1974. *Fraxinus* L. ash, p. 411-416. In: C.S. Schopmeyer (technol. coordinator). Seeds of woody plants in the United States. U.S. Dept. Agr. For. Serv. Agr. Hdbk. 450.

Browne, R. and G. Hicks. 1983. Development in vitro of white ash buds. Ann. Bot. 52:101-104.

Chalupa, V. 1983. Micropropagation of conifer and broadleaved forest trees. Commun. Inst. For Cechosloveniae 13:7-39.

Chalupa, V. 1990. Micropropagation of hornbeam *(Carpinus betulus* L.) and Ash *(Fraxinus excelsior* L.). Biol. Plant. 32:332-338.

Doley, D. and L. Leyton. 1970. Effects of growth regulating substances and water potential on the development of wound callus in *Fraxinus.* New Phytol. 69:87-102.

Evers, P.W., J. Donkers, A. Prat, and E. Vermeer. 1988. Micropropagation of forest trees through tissue culture. Pudoc, Wageningen, The Netherlands.

George, E.F. and P.D. Sherrington. 1984. Plant progagation by tissue culture. Exegetics Ltd., Everseley, Basingstoke, London.

Hartmann, H.T., D.E. Kester, and F.T. Davies, Jr. 1990. Plant propagation, principles and practices. 5th ed. Prentice-Hall, London.

Heller, R. 1953. Recherches sur la nutrition minerale des tissus végétaux cultivés in vitro. Ann. Sci. Nat. Bot. Biol. Veg. 14:1-223.

Loforestier, F., M. Gras, and C. Joseph. 1990. Micropropagation of *Fraxinus excelsior.* VIIth Intl. Congr. Plant Tissue and Cell Cult. 24-29 June 1990, Amsterdam. A3-117. (Abstr.)

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Navarrete, N.E., J.W. Van Sambeek, J.E. Preece, and G.R. Gaffney. 1989. Improved micropropagation of white ash *(Fraxinus americana* L.), p. 146-149. In: G. Rink, and C.A. Budelsky (eds.). Proc. VIIth Central Hardwood For. Conf. 5-8 Mar. 1989, Southern Illinois Univ., Carbondale.

Németh, J. 1986. Induction of rooting, p. 49-64. In: Y.P.S. Bajaj (ed.). Biotechnology in agriculture and forestry. vol. 1. Trees I. Springer-Verlag, Berlin.

Preece, J.E., P.H. Christ, L. Ensenberger, and J. Zhao. 1987. Micropropagation of ash *(Fraxinus).* Proc. Intl. Plant Prop. Soc. 37:366-372.

Preece, J.E., J. Zhao, and F.H. Kung. 1989. Callus production and somatic embryogenesis from white ash. HortScience 24:377-380.

Rugini, E. 1986. Olive *(Olea europaea* L.), p. 253-267. In: Y.P.S. Bajaj (ed.). Biotechnology in agriculture and forestry. vol. 1. Trees I. Springer-Verlag, Berlin.

Schenck, R.V. and A.C. Hildebrandt. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 50:199-204.

Thorpe, T.A. and I.S. Harry. 1990. Special problems and prospects in the propagation of woody species, p. 67-74. In: R. Rodriguez, R. Sanchez Tameés, and D.J. Durzan (eds.). Plant aging: Basic and applied approaches. Plenum, New York.

Tukey, J.W. 1953. Some selected quick and easy methods of statistical analysis. Trans. N.Y. Acad. Sci. Ser. II 16:88-97.

Vieitez, A.M., M.C. San-Jose, and E. Vieitez. 1985. In vitro plantlet regeneration from juvenile and mature *Quercus robur* L. J. Hort. Sci. 60:99-106.

Wolter, K.E. and F. Skoog. 1966. Nutritional requirements of *Fraxinus* callus cultures. Amer. J. Bot. 53:263-269.

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