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Micropropagation of hybrid walnut trees *(Juglans nigra x Juglans regia)* through culture and multiplication of embryos

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Introduction

Walnuts are very valuable trees for nuts and wood production, but we see a reduction in the number of logs available for veneer. Although Persian walnut *(Juglans regia)* and black walnut *((J. nigra)* can be used, hybrids between these two species have better growth and wider adaptability. Breeding programs are in progress at INRA (Institut National de la Recherche Agronomique, Bordeaux and Orléans) and they need efficient methods of vegetative propagation. Micropropagation was established in 1984 for 'Paradox' (Driver and Kuniyuki, 1984) and recently for Persian walnut (McGranahan *et al.,* 1988). Previous works have shown strong effects of ageing and rejuvenation (Jay-Allemand *et al.,* 1988), latent contamination, low reactivity of buds or meristems on the establishment of mature selected clones. On the contrary, with very juvenile material, such as embryos, it is possible to avoid these problems (Jay-Allemand and Cornu, 1986; Heile-Sudholt *et al.,* 1986).

The purpose of this study was: 1) to estimate the ability of hybrid progeny to be propagated by micropropagation, and 2) to improve culture factors acting on shoot development.

Materials and Methods

This study used half-sib hybrid nuts (*J. nigra* (no. 23) x *J. regia*) supplied by E. Germain (INRA, Bordeaux), collected in September 1987. Embryonic axes (48) were isolated under sterile conditions and then introduced *in vitro* into the medium defined by McGranahan *et al.* (1987). Three main steps have been determined: 1) elongation of epicotyls and buds during 3 wk of darkness followed by 2 wk of 16 h of light at 28°C; 2) multiplication by transferring nodes from elongated shoots or clusters of buds every 2 wk (16 h light, 28°C); 3) rooting (not presented in this paper).

Two kinds of solidifying compounds (Difco-Bacto agar, 6 g/l, and Gelrite, 2.3 g/l) in 750 ml jars were compared. Then, instead of one transfer onto a fresh medium after 2 wk of culture, the addition of about 2 cm of a new liquid medium without transfer was studied (double phase system, Viseur, 1987).

The number of shoots (>5 mm) was determined for each clone at the 3rd and 8th transfers, and the number of bud-clusters and elongated shoots (>15 mm) at the 10th, 11th, 12th and 13th transfers.

Results

The 48 clones which were cultivated under the same conditions show great variability in their bud-cluster development and shoot elongation. After 3 and 8 transfers, we obtained a normal distribution of clones (Fig. 1A and 1B). Ranking of some clones changed during this time but stabilized after the 8th transfer. Eight of the best clones were selected for bulk propagation. They were characterized by good development of leaves and elongation of shoots. The production of buds and shoots during 3 transfers is summarized in Table I. In 6 wk, the number of bud-clusters multiplied by 1.5. An average of 60 shoots, usable for rooting, were produced every 2 wk to 100 bud-clusters.

After 2 transfers, the development of clones and particularly callus formation increased significantly (1% level) in the Gelrite (Table II). If liquid medium did not increase the mean number of elongated shoots, the elongation of those shoots was significantly higher (Table III).

Discussion and Conclusion

The studies have shown that the micropropagation of juvenile walnut depends upon many factors. At a general level, characteristics of the medium are im-

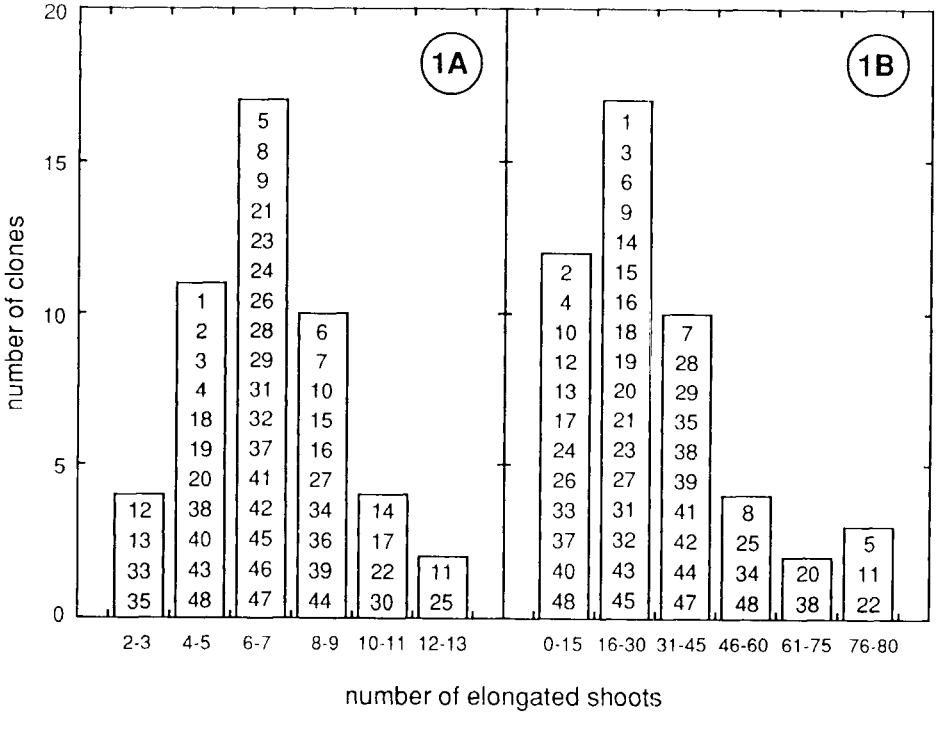

Fig. 1. Clonal variation of shoot production after 3 (**1A**) and 8 (**1B**) transfers.

Table I. Production of bud-clusters and elongated shoots for the 8 selected best clones after 6 weeks.

Transfer	*Bud-clusters*		*Elongated shoots (15 mm)*	
	total	*increase*	*total*	*%*
10	157			
11	178	1.13	99	63
12	224	1.26	109	61
13	233	1.04	135	60

Table II. Effect of solidifying compounds on the production of buds and shoot elongation.

	No. of buds	*Shoot elongation (mm)*	*Callus fwt (mg)*
Difco-Bacto agar 6 g/l	2	10.4	55
Gelrite 2.3 g/l	5.1	18.6	637

One clone, 48 repetitions per treatment (all differences significant at 1% level).

portant. Results obtained with Gelrite confirm our previous observations when we lost all material growing on agar (unpublished data). Many different hypotheses have been proposed to explain the influence of agar. These include the presence of inhibitors, rate of diffusion of molecules and variability in the availability of water. This last effect could be associated with the positive action observed with liquid medium. Chun *et al.* (1986) obtained better results with poplar in liquid medium than with a gelified one. Nevertheless, some cases of vitrification appear after long-term culture in liquid medium. On the contrary, Viseur (1987), avoided vitrification in pear and increased bud production by adding liquid medium. All these phenomena should be connected with the metabolism of phenolic compounds, lignification or ethylene. With our system on walnut, studies are and will be conducted in these fields to determine the more important medium factor.

McGranahan *et al.* (1988) recommended for Persian walnut micropropagation a 1 wk transfer interval for gelified medium. According to our results, and from a practical point of view, results presented here clearly illustrate that some of the very expensive transfer work can be avoided by adding liquid medium to cultures. Finally, the great variability between clones, also observed by Heile-Sudholt *et al.* (1986), could limit the interest of bulk micropropagation. Complementary research is needed to determine if the best clones for micropropagation are also the best for field plantations.

Table III. Effect of adding liquid medium on bud production and number and length of shoots.

Treatment	*Clones*	*No. of buds*	*No. of shoots (15 mm)*	*Mean length of shoots (mm)*
Gelrite	34	3.3	1	25
	108	4.3	1.1	24
Liquid medium	34	4.2	0.8	36
	108	7.9	2	31
Level of significance between treatments		5%	NS	1%

32 repetitions per clone and per treatment.

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