c. Combined growth chamber analysis

Source of Variation*	Expected mean squares
Chamber (CH)	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K5\sigma^2_{CHC} + K7\sigma^2_{CHP} + K8\phi_{CHM} + K11\phi_{CH}$
Population (P)	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K3\sigma^2_{MC} + K4\sigma^2_{MP} + K5\sigma^2_{CHC} + K6\sigma^2_{C} + K7\sigma^2_{CHP} + K9\sigma^2_{P}$
Clone(P) (C)	$\sigma^2 + K1\sigma^2_{CHMC} + K3\sigma^2_{MC} + K5\sigma^2_{CHC} + K6\sigma^2_{C}$
CHP	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K5\sigma^2_{CHC} + K7\sigma^2_{CHP}$
CHC	$\sigma^2 + K1\sigma^2_{CHMC} + K5\sigma^2_{CHC}$
M	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K3\sigma^2_{MC} + K4\sigma^2_{MP} + K8\phi_{CHM} + K10\phi_{M}$
CHM	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K8\phi_{CHM}$
MP	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP} + K3\sigma^2_{MC} + K4\sigma^2_{MP}$
MC	$\sigma^2 + K1\sigma^2_{CHMC} + K3\sigma^2_{MC}$
CHMP	$\sigma^2 + K1\sigma^2_{CHMC} + K2\sigma^2_{CHMP}$
CHMC	$\sigma^2 + K1\sigma^2_{CHMC}$
Error	σ^2

*) Source of variation as described previously in text.

The Ki values varied due to missing data but were equal to or less than the following: K1 = 3, K2 = 17, K3 = 6, K4 = 33, K5 = 6, K6 = 12, K7 = 32, K9 = 64. For THOMAS et al., 1997b: K1 = 2, K2 = 8, K3 = 4, K4 = 16, K5 = 4, K6 = 8, K7 = 16, K9 = 32.

Effect of Genotype on Micropropagation of Walnut Trees (*Juglans regia*)

By A. SCALTSOYIANNES, P. TSOULPHA, K. P. PANETSOS and D. MOULALIS

Laboratory of Forest Genetics and Plant Breeding, Department of Forestry and Natural Environment, University of Thessaloniki, 54006 Thessaloniki, Greece

(Received 30th January 1997)

Summary

Embryos of *Juglans regia* originating from elite trees, selected for their wood quality were cultured on DKW medium supplemented with 4.44 µM 6-benzylaminopurine (BA) and 0.005 µM indole-3-butyric acid (IBA). Results on the effects of cytokinin (BA) and auxin (IBA) on shoot development indicated that elongation is enhanced with 2.22 µM BA while the formation of new axillary shoots is favored by 4.44 µM BA and 0.005 µM IBA. It was, also, observed that different genotypes had different requirements for growth regulators.

Significant differences were observed among the multiplication rates of twelve different clones of *J. regia*. The effect of genotype is obvious in the rooting phase, as well. Some clones exhibit high rooting ability (95%) and some low (5%).

Key words: *Juglans regia*, micropropagation, genotype, multiplication, rooting.

FDC: 165.44; 176.1 *Juglans regia*.

Abbreviations: BA: 6-benzylaminopurine
IBA: indole-3-butyric acid
DKW: DRIVER and KUNIYUKI Walnut medium

Introduction

Persian walnut (*Juglans regia*) is a valuable cultivated forest species of economic importance both for the production of nuts and wood and according to MALVOLTI *et al.*, (1994), great variation, has been observed, in morphological and biochemical characters among distinct geographic ecotypes. At present, although, the tree improvement programmes for *Juglans* species have made some progress (e.g. new promising clones and varieties have been produced) problems in vegetative propagation of selected walnut trees are holding back the improvement of the species and thereby their wider use (ZOBEL and TALBERT, 1984).

Generally, the most popular way of walnut vegetative propagation is that of grafting which is, however, labor-intensive, time-consuming and costly. On the other hand, propagation by cuttings is very difficult due to their low rooting ability (MCGRANAHAN *et al.*, 1988; LAND and CUNNINGHAM, 1994).

In the last decade, other more sophisticated techniques (micropropagation, embryogenesis etc.) have been investigated for the successful large scale propagation of walnut. RODRIGUEZ and SANCHEZ-TAMES (1981) and RODRIGUEZ (1982a, b) were among the first to report establishment of walnut cultures *in vitro* and to describe the development ol shoots or roots from cultured walnut embryos. Later, a large amount of work was conducted on different walnut species using different types of explants, media, culture conditions and rooting techniques, with encouraging results (JAY-ALLEMAND and CORNU, 1986; GRUSELLE *et al.*, 1987; CORNU and JAY-ALLEMAND, 1989; JAY-ALLEMAND *et al.,* 1992).

Most of the above work was based on a medium developed by DRIVER and KUNIYUKI (1984) (DKW medium) for the *in vitro* culture of *Juglans* spp..

As it is well known, genotype plays a major role in all phases of vegetative propagation (BROWN, 1981; HARTMAN and KESTER, 1983; PANETSOS *et al*., 1987, HAISSIG and RIEMENSCHNEIDER, 1988; FOSTER, 1990; ZSUFFA *et al*., 1993; LEAKEY *et al*., 1994; LAND and CUNNINGHAM, 1994). In particular, for micropropagation HORGAN (1987), emphasised the fact that different clones of *P. radiata* react differently to the various steps of micropropagation (establishment, proliferation, rooting) and SCALTSOYIANNES *et al*., (1994) reported that genotype determines the rooting ability of microshoots of the pine hybrid *Pinus brutia* (TEN.) x *Pinus halepensis* (MILL.). Also, as for hardwoods, AHUJA and MUHS (1982), AHUJA (1983), ERNST (1993), COLEMAN and ERNST (1989) reported that shoot regeneration ability of different *Populus* clones, is genotype dependent. CORNU and CHAIX (1981) found a positive correlation between rooting ability of microshoots and clone of *Prunus avium*.

The present work investigates the effect of genotype on shoot regeneration and rooting ability among different clones of *Juglans regia* (persian walnut) *in vitro*.

Materials and Methods

Origin of plant material: open pollinated nuts from two individuals (Plemiana 1 and Plemiana 2 named by the prefixes P and S, respectively), selected for their wood quality were collected in the autumn of 1993 from west Crete and stored at room temperature.

Surface sterilisation: the nuts were cracked and the kernels were immersed in NaOCl (1.0 % w/v) for 5 min followed by 75% v/v EtOH for another 5 min and were rinsed three times in sterile distilled water. Finally embryos were carefully isolated from the kernels.

For establishment, multiplication and rooting, the protocol described by JAY-ALLEMAND *et al*. (1992) was followed. Thus, embryos were established on DKW medium supplemented with 4.44 µM BA and were incubated in a growth chamber in darkness. After three weeks germinated embryos were transferred to DKW medium supplemented with 4.44 µM BA and 0.005 µM IBA for multiplication, through axillary shoot production. Cultures were incubated at 27 °C ± 1°C with 16 h photoperiod under cool white fluorescent lamps (55 to 65 $\mu E.m^{-2}.s^{-1}$). Subculturing occurred every four weeks.

Rooting procedure consisted of two phases: i) root induction phase: microshoots (4 cm to 5 cm) were established on DKW medium (1/4 macroelements) supplemented with 24.6 µM IBA and 40 $g.1^{-1}$ sucrose (JAY-ALLEMAND *et al*., 1992) and were kept in darkness for 6 days at 24 °C ± 1 °C for 16 hours and 21 °C ± 1 °C for the remainig 8 hours and ii) initiation phase: the pretreated shoots were transferred to sterilized vermiculite in which gelified DKW medium (1/4 macroelements) free of hormones, was added. Culture conditions were maintained as in multiplication phase. Rooting percentage and total root length were recorded after three weeks and then the plantlets were transferred for acclimatization.

In our experiments DKW medium supplemented with 4.44 µM BA and 0.005 µM IBA was used unless otherwise stated. When adequate material (microshoots 2 cm in length) was available, the following experiments were conducted:

a) effect of growth regulators on the production of axillary shoots and elongation of shoots.

The effects of cytokinin (BA) and auxin (IBA) on shoot growth of *J. regia* (mixed clones derived from embryos of Plemiana 1) were assessed in a 5 x 2 factorial design with 12 replicates in each treatment. Cytokinin (BA) was examined at 0.0 µM, 2.22 µM, 4.44 µM, 8.87 µM and 17.7 µM in combination with auxin (IBA) at 0.0 µM and 0.005 µM. Elongation, was measured by the increase in the shoot length during a four-week period.

b) interaction of genotype and growth regulators.

Because in the previous experiment we had some indications of the interaction between growth regulators and clone we decided to repeat the previous experiment on P_3 and P_7 *J. regia* clones. Five replicates in each treatment were used for each clone.

c) effect of genotype on the multiplication rate of shoots.

Multiplication rates of twelve different *J. regia* (Plemiana 1) clones were estimated at the end of the fourth subculture according to the formula described by BEKKAOUI (1986):

multiplication rate % = $[(a_{n+1} - a_n)/a_n] \times 100$

a_{n+1}: the number of the newly formed axillary shoots of the subculture n + 1

a_n: the number of the newly formed shoots of the subculture n.

d) effect of exposure to auxin on root induction phase.

Twenty microshoots (4 cm to 5 cm) of each of two *J. regia* clones (P_3, P_7) were transferred for rooting after their pretreatment for 3 and 6 days in darkness as previously described in the root induction phase. Then the microshoots were transferred to the initiation phase.

e) effect of vermiculite size-type on rooting.

Microshoots (4 cm to 5 cm) of the above clones (P_3, P_7) after the induction phase were transferred to the rooting medium using two size-types of vermiculite (medium-type I and small-type II). In each treatment, 20 microshoots were used.

f) effect of genotype on rooting ability.

Twenty microshoots (4 cm to 5 cm) of each of ten clones of *J. regia*, eight originating from Plemiana 1 plus tree (P_2, P_3, P_4, P_6, P_7, P_8, P_{10}, P_{11}) and two from Plemiana 2 plus tree (S_2, S_8), were tested for rooting.

Results

The surface sterilization procedure followed was proved to be efficient and the success of disinfection ranged from 41% to 100%. After three weeks in darkness at 27 °C ± 1 °C the disinfected embryos germinated (35% to 50%). It is worth to notice that both germination and disinfection seemed to be family dependent. In our case, although the best reaction was observed for the nuts originating from Plemiana 2 plus tree, only two clones remained in culture due to an unexpected accident in our facilities. Hypocotyls were swollen and the obtained adventitious shoots were transferred for multiplication.

a) In *figure 1* the results on the production of new axillary shoots and elongation of *J. regia* from Plemiana 1 are shown. Regarding the number of the newly formed axillary shoots, the best combination was that of 4.44 µM BA and 0.005 µM IBA that produced an average of 4.17 new axillary shoots per explant in a period of four weeks.

Athough, higher concentrations of BA, i.e. 8.87 µM and 17.7 µM BA in conjunction or not with 0.005 µM IBA gave similar or better response, vitrified shoots appeared and their subsequent development was problematic.

As for the elongation, it seems that the best combinations of growth regulators were those of 2.22 µM BA alone or in

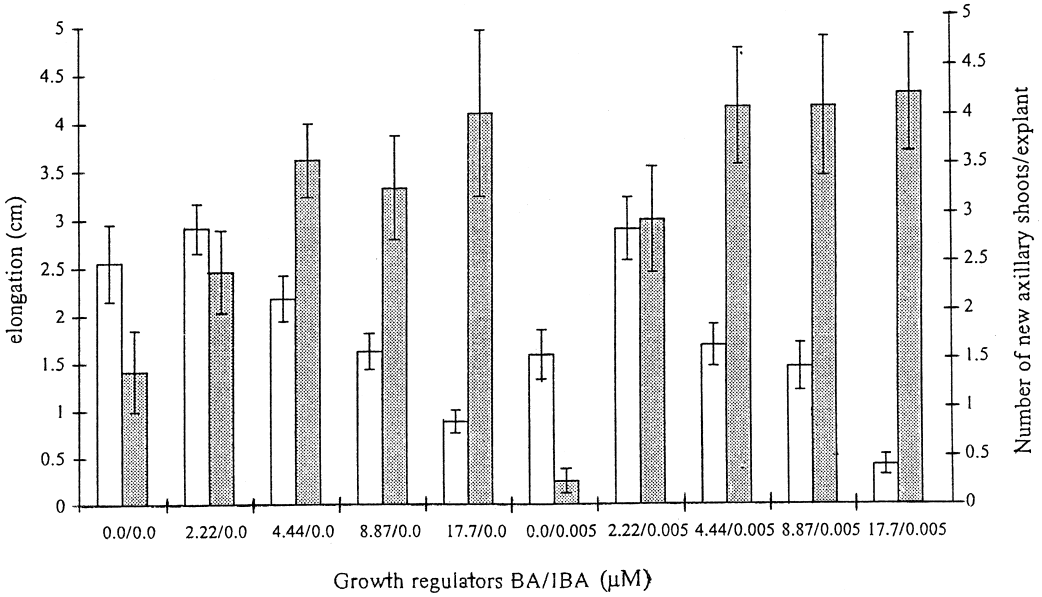


Figure 1. – Effect of growth regulators on elongation and production of new axillary shoots/explant of mixed *Juglans regia* clones (Plemiana 1) in a period of 4 weeks on DKW medium. □ elongation (cm) ■ number of new axillary shoots/explant.

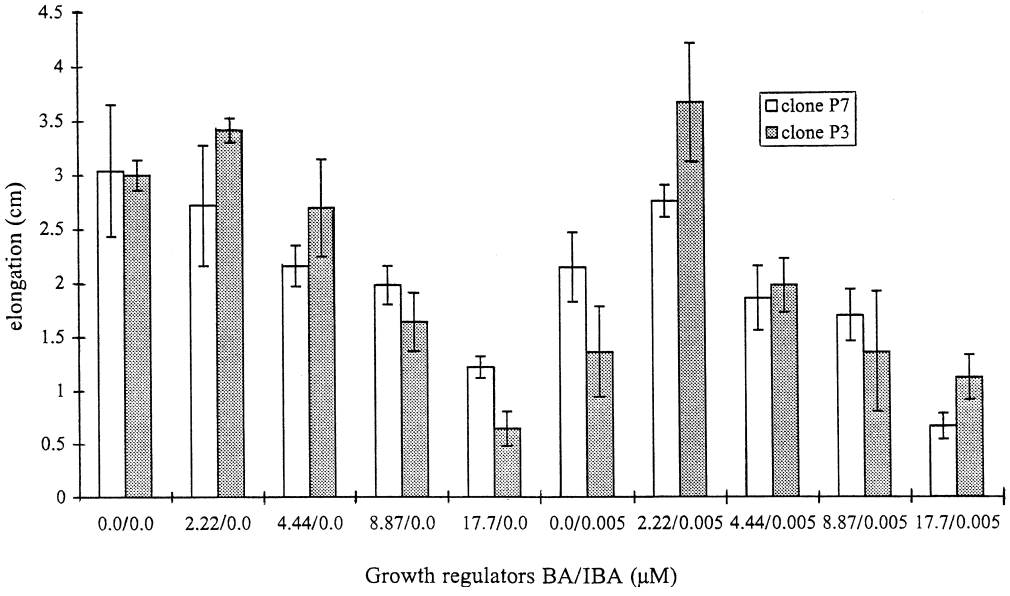


Figure 2. – Effect of growth regulators on elongation of two *Juglans regia* clones (P_7, P_3) of Plemiana 1 in a period of 4 weeks on DKW medium.

conjunction with 0.005 μM IBA that resulted in 2.91 cm and 2.9 cm increase in length, respectively.

b) In *figures 2* and *3* the effect of genotype on elongation and production of newly formed shoots is presented. In particular, the most elongation for clone P_3 (3.68 cm) was obtained when the medium was supplemented with the combination of 2.22 μM BA and 0.005 μM IBA whereas for clone P_7 (3.04 cm) in a hormone-free medium. As for the production of axillary shoots, for clone P_3 the highest production was achieved with the combination of 8.88 μM BA and 0.0 μM IBA while for clone P_7 with that of 4.44 μM BA and 0.005 μM IBA (4.0 and 5.4 new axillary shoots per explant, respectively).

c) The different clone reaction in a specific combination of growth regulators was, also, confirmed by the results of the multiplication rate experiment on different *J. regia* clones, originating from Crete (Plemiana 1) which are shown in *figure 4.* Among the twelve clones, studied, significant differences were observed for their multiplication rates. Although most of them ranged from 150 to 460 axillary shoots per 100 explants, there was one, P_5, with a low multiplication rate and another, P_6, with a high rate (*Fig. 5*).

d) Different response in rooting of the two clones (P_3, P_7) tested, was observed for 3 and 6 day pretreatment in auxin. These results indicated that the exposure time of the micro-shoots to auxin (IBA) is very crucial for their subsequent response to rooting since for both clones, rooting and secondary root formation were enhanced with the six day auxin treatment (*Table 1*).

e) Between the two size-types of vermiculite (medium-type I and small-type II) that were tested, the medium size promoted both microshoot rooting percentage and the quality of the root system as it is shown in *table 2*.

f) Great variation on rooting percentage, and total root length was observed among 10 *J. regia* clones. Although the average rooting ability was moderate (44%) some clones showed low rooting ability, e. g. P_4 (5%) whereas others such as P_3 showed high rooting ability (95%) (*Fig. 7*).

It should be mentioned that clones with low rooting ability developed fewer but longer roots compared with the high rooting ability clones which formed many main and secondary roots.

Discussion

Juglans regia clones originating from two half-sib families, (Plemiana 1 and Plemiana 2 plus trees) were tested for establishment, multiplication and rooting *in vitro*. Both plus trees were selected for their excellent performance in desired characteristics such as fast growth, wood quality, etc. and due to the fact that both of them seem to posess high general combining ability in the field test (unpublised data) we decided to investigate the behaviour of their families *in vitro*. Because both trees were surrounded by other individuals we assumed that selfing was restricted.

As it is indicated by the results of *figure 1* for elongation and production of new axillary shoots the best combination of

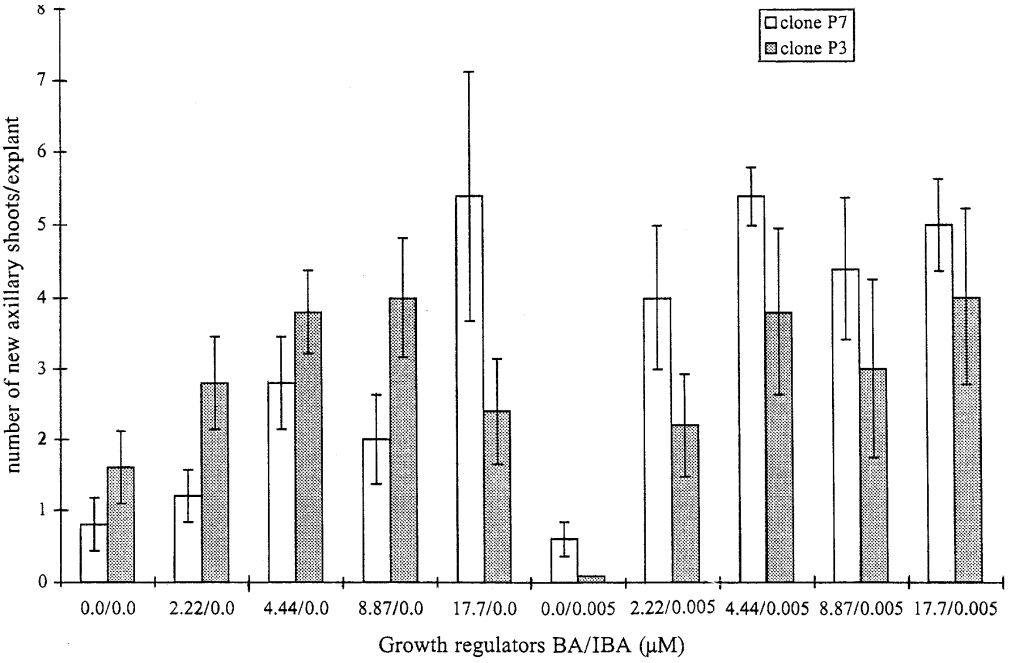
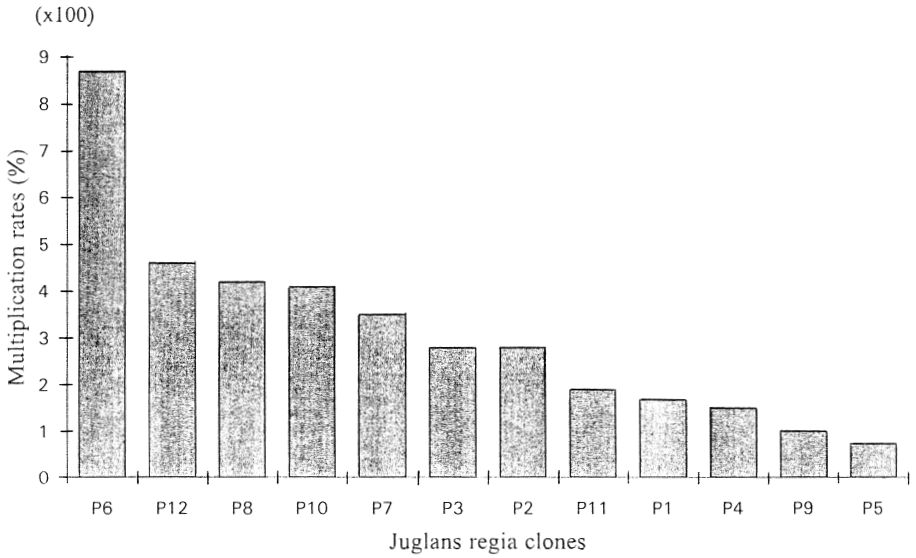


Figure 3. – Effect of growth regulators on production of new axillary shoots of two *Juglans regia* clones (P_7, P_3) of Plemiana 1 in a period of 4 weeks on DKW medium.



*) Multiplication rate according to BEKKAOUI, (1986): the increase of new axillary shoots/100 explants in 2 successive subcultures.

Figure 4. – Multiplication rates*) of 12 *J. regia* clones (Plemiana 1) at the end of the fourth subculture cultured on DKW medium supplemented with 4.44 µM BA and 0.005 µM IBA.

Figure 5. – A clone (P_6) with a high multiplication rate *in vitro* on DKW medium supplemented with 4.44 µM BA and 0.005 µM IBA.

growth regulators was that of 4.44 µM BA and 0.005 µM IBA. These results are in agreement with those of GRUSELLE and BOXUS (1990), who reported that the optimum shoot regeneration *in vitro* was achieved on DKW medium supplemented with BA (3.55 µM to 4.44 µM). The same authors, also, stated that the proliferation rates of *J. regia* remain relatively stable in the first four subcultures. In our case, high concentrations of BA (> 4.44 µM) caused the appearance of vitrified shoots. Similarly, REVILLA *et al.*, (1989) reported vitrification problems *in vitro* on *J. regia* shoots, when high concentrations of BA (> 8.87 µM) were applied.

Although, the clones that were tested for multiplication originated from one family (Plemiana 1) great variation was observed (*figure 4*). The above seem to verify the findings of JAY-ALLEMAND (personal communication), who, also, noticed different multiplication rates for various genotypes of the hybrid *J. nigra* x *J. regia*. Other workers (AHUYA and MUHS, 1982; Ahuja, 1983; ERNST, 1993; COLEMAN and ERNST, 1989) reported that shoot regeneration ability of different *Populus* clones, is genotype depended, as well.

Great differences among clones were noticed in rooting. Some clones exhibited high rooting percentage (P_3 95%) and

Table 1. – Effect of duration of exposure to auxin (3 and 6 days, in darkness) on subsequent rooting of *J. regia* clones (P_3, P_7) cultured on DKW medium (1/4 macroelements) supplemented with 24.6 µM IBA and 40g. l^{-1} sucrose.

Clone	Rooting (%)	Total root length (cm)	Secondary roots
P_3 (3 days)	35±11	7.36±2.7	few
P_3 (6 days)	90±6.7	6.01±1.13	many
P_7 (3 days)	50±11.2	5.4±2.42	few
P_7 (6 days)	65±114	7.72±2.63	many

Mean number ± SE. Sample size 20.

Table 2. – Effect of vermiculite size-type (I, II) on rooting of P_3 and P_7 *J. regia* clones cultured on DKW medium (1/4 macroelements) free of hormones.

Clone	Rooting (%)	Total root length (cm)	Secondary roots
P_3 (II)	40±10.9	6.04±1.13	few
P_3 (I)	95±4.9	17.6±2.71	many
P_7 (II)	55±11.1	5.14±2.08	few
P_7 (I)	70±10.2	7.41±1.99	many

Mean number ± SE. Sample size 20.

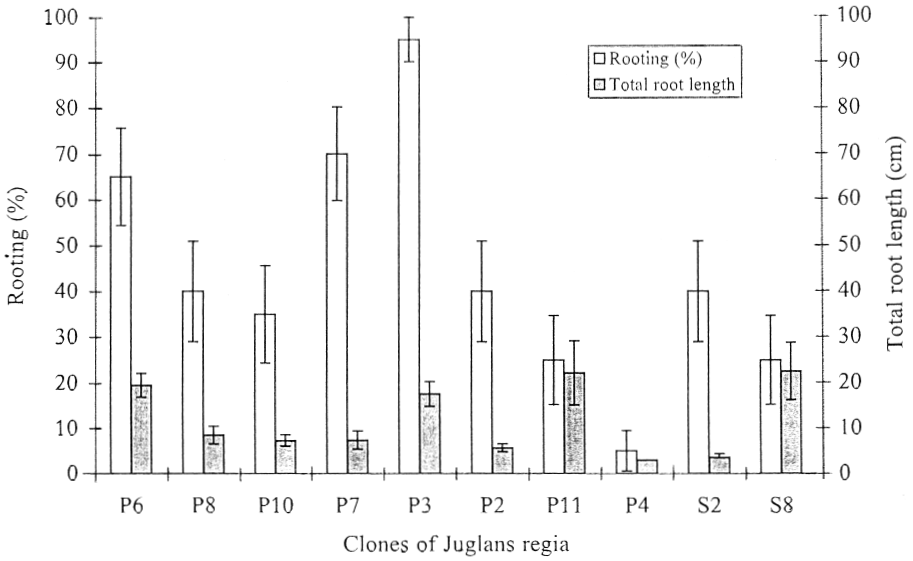


Figure 6. – Rooting percentage and total root lenght of 10 *Juglans regia* clones (Plemiana 1) cultured for 3 weeks on vermiculite (type I) gelified with DKW medium (1/4 macroelements) free of hormones.

Figure 7. – Plantlets of *J. regia* (clone P_3) with good quality rooting system rooted on vermiculite (type I) gelified with DKW medium.

some others low (P_4 5%). The effect of genotype on rooting was, also, reported by other researchers in different plant species (conifers and hardwoods) (CORNU and CHAIX, 1981: Cornu *et al.*, 1981, EVERS *et al.*, 1988; SCALTSOYIANNES *et al.*, 1994).

Walnut is known to accept a high selfing rate besides outbreeding. In the present work, although, most of the tested clones belong to the same family great variation was observed in, both, shoot regeneration and rooting fact that seems to support our initial assumption that in our case selfing is restricted.

Concerning the rooting substrate the positive effect of the medium size vermiculite in rooting was, also, found by JAY-ALLEMAND *et al.*, (1992), who stated that this is probably due to the improved (better) aeration of the rooting system achieved by this type of vermiculite. The same authors noticed that the pretreatment the hybrid microcuttings (*J. nigra* x *J. regia*) with 24.6 µM IBA for 5 days in darkness is essential for rooting induction. In our case, the 6-day pretreatment with the same concentration of IBA was found to enhance subsequent rooting.

As the level of endogenous hormones and peroxidase activity proved to be useful predictive markers of the rooting performance (GASPAR *et al.*, 1992, 1994) of micropropagated shoots, it is in our perspectives to study the possible correlation between rootability of *J. regia* clones and the above factors.

The results of the present study and other results on acclimatisation and the behaviour of new walnut clones *in vitro* (data are not presented here) indicate that genotype plays a crucial role in micropropagation of *Juglans regia*. Thus, clones that exhibit good reaction in shoot regeneration and rooting could be exploited in large-scale propagation.

Acknowledgements

This work is been financially supported by the EEC in the framework of the AIR3-CT92-0142 programme under the title of "European development of walnut trees for wood and fruit production as an alternative and extensive system to agricultural crops".
Note: Parts of this work are portions of the Doctorate thesis of co-author TSOULPHA PARTHENA.

References

AHUJA, M. R.: Somatic cell differentiation and rapid clonal propagation of aspen. Silvae Genetica **32**: 3–4 (1983). — AHUJA, M. R. and MUHS, H. J.: Control of growth and differentiation in tissues and protoplast derived callus in different genotypes of aspen. In: Proc. 5th Int. Cong. Plant Tissue and Cell Culture. Plant Tissue Culture 1982. pp. 177–178 (1982). — BEKKAOUI, F: Microbouturage *in vitro* et culture de meristemes de Douglas (*Pseudotsuga menziesii*); problemes physiologiques lies a l' age et au milieu de culture. These de Doctorat de L' Universite P. et M. Curie (Paris VI). pp. 173 (1986). — BROWN, C. L.: Application of tissue culture technology to production of woody biomass. National Swedish Board for Energy Source Development. IEA Report: NE 1981–18 (1981). — COLEMAN, G. D. and ERNST, S. G.: *In vitro* shoot regeneration of *Populus deltoides*: effect of cytokinin and genotype. Plant Cell Reports 8: 459–462 (1989). — CORNU, D. and CHAIX, C.: Multiplication par culture *in vitro* de merisiers adultes (*Prunus avium*): application a un large eventail de clones. In: Proc. IUFRO sect. S2 01 5. Int. Workshop "in vitro" for tree species. Fontainebleau, France, pp. 71–79 (1981). — CORNU, D. and JAY-ALLEMAND, C.: Micropropagation of hybrid walnut trees (*J. nigra* x *J. regia*) through culture and multiplication of embryos. Ann. Sci For. **46** (suppl.) 113s–116s (1989). — CORNU, D., RIFFAUD, J. L. and CAPELLI, P.: *In vitro* propagation of wild cherry tree (*Prunus avium* L.). In: Proc. IUFRO sect. S2 01 5. Int. Workshop "in vitro" for tree species. Fontainebleau, France, pp. 133–134 (1981). — DRIVER, J. and KUNIYUKI, A. H.: *In vitro* propagation of Paradox walnut rootstock. HortScience **19**(4) 507–509 (1984). — ERNST, S. G: *In vitro* culture of pure species non-aspen poplars. In: "Micropropagation of woody plants". AHUJA, M. R. (ed). Kluwer Academic Publishers. pp. 195–207 (1993). — EVERS, P. W., DONKERS, J. PRAT, A. and VERMEER, E.: Micropropagation of forest trees through tissue culture. EVERS, P. W., DONKERS, J., PRAT, A. and VERMEER, E. (eds). Pudoc, Wageningen (1988). — FOSTER, G. S.: Genetic control of rooting ability of stem cuttings from loblolly pine. Can. J. For. Res. **20**, 1361–1367 (1990). — GASPAR, T., KEVERS, C., HAUSMAN, J. F., BERTHON, J. Y. and RIPETTI, V.: Practical use of peroxidase activity as a predictive marker of rooting performance of micropropagated shoots. Agronomie **12**, 757–765 (1992). — GASPAR, T., KEVERS, C., HAUSMAN, J. F., BERTHON, J. Y. and RIPETTI, V.: Peroxidase activity and endogenous free auxin during adventitious root formation. In: "Physiology, Growth and Development of plants in culture". P. J. LUMSDEN, J. R. NICHOLAS and W. J. DAVIES (eds). Kluwer Academic Publishers, Netherlands (1994). — GRUSELLE, R., BADIA, N. and BOXUS, PH.: Walnut micropropagation: First results. Acta Horticulturae **212** (1987). — GRUSELLE, R. and BOXUS, P.: Walnut micropropagation. Acta Horticulturae **284:** 45–52 (1990). — HARTMANN, H. T. and KESTER, D. E.: Plant propagation. Principles and practices. Prentice Hall, Inc. Englewood Cliffs, New Jersey (1983). — HAISSIG, B. E. and RIEMENSCHNEIDER, D. E.: Genetic effects on adventitious rooting. In: "Adventitious root formation in cuttings". DAVIS, T. D., HAISSIG, B. E. and SANKHLA, N. (eds). Dioscorides Press, Portland, Oregon. pp. 47–60 (1988). — HORGAN, K.: *Pinus radiata*. In: "Cell and tissue culture in forestry". Vol 3. Case histories: Gymnosperms, Angiosperms and Palms. BONGA, J. M. and D. J. DURZAN (eds.). Martinus Nijhoff Publ. pp. 128–145 (1987). — JAY-ALLEMAND, C., CAPELLI, P. and CORNU, D.: Root development of *in vitro* hybrid walnut microcuttings in a vermiculite containing gelrite medium. Scientia Horticulturae **51**: 335–342 (1992). — JAY-ALLEMAND, C. and CORNU, D.: Culture *in vitro* d'embryons isoles de noyer commun (*Juglans regia* L.). Ann. Sci. For. **43**: 189–198 (1986). — LAND, S. B. and CUNNINGHAM, M.: Rooted cutting macropropagation of hardwoods. In: "Applications of vegetative propagation in forestry". Proc. of the Southern regional information exchange group biennial symposium on forest genetics. FOSTER, G. S. and A. M. DINER (eds.). Published by Southern Forest Experiment Station New Orleans, Louisiana. pp 75–96 (1994). — LEAKEY, R. R. B., NEWTON, A. C. and MCP. DICK, J.: Capture of genetic variation by vegetative propagation processes determining success. In: "Tropical trees: the potential for domestication and the rebuilding of forest resources". LEAKEY, R. R. B. and NEWTON, A. C. (eds.). London, HMSO. pp. 72–83 (1994). — MALVOTI, M. E., FINESCHI, S. and PIGLIUCCI, M.: Morphological integration and genetic variability in *Juglans regia* L.. J. Heredity **85**: 389–394 (1994). — MCGRANAHAN, G., LESLIE, C. A. and DRIVER, J. A.: In vitro propagation of mature persian walnut cultivars. Hortscience **23**(1): 220 (1988). — PANETSOS, K. P., ECONOMOU, A. and SCALTSOYIANNES, A.: Propagation *in vitro* of Aspen hybrid *Populus spartiatica* x *Populus tremula* from mature trees. Agricultural Research **11**: 449–459f (1987). — REVILLA, M. A., MAJADA, J. and RODRIGUEZ, R.: Walnut (*Juglans regia* L.) micropropagation. Ann. Sci. For. **46** suppl.: 149s–151s (1989). — RODRIGUEZ, R.: Callus initiation and root formation from *in vitro* culture of walnut cotyledons. HortScience **17**(2): 195–196 (1982a). — RODRIGUEZ, R.: Stimulation of multiple shoot-bud in walnut seeds. HortScience **17**(4): 592 (1982b). — RODRIGUEZ, R. and SANCHEZ-TAMES, R.: Cultivo detejitos y differentiation en nogal. Rev. Fac. Cienc. Univ. Oviedo (Ser Biologia) **22**: 21–28 (1981). — SCALTSOYIANNES, A., PANETSOS, K. P., ECONOMOU, A. and TSOULPHA, P.: Micropropagation of the pine hybrid *Pinus brutia* (TEN) x *P. halepensis* (MILL.) by culturing fascicle shoots. Ann. Sci. For. **51,** 175–182 (1994). — ZOBEL, B. J. and TALBERT, J. T.: In: "Applied Forest Tree Improvement". John Wiley and Sons Inc. (publ), New York, Toronto (1984). — ZSUFFA, L., SENNERBY-FORSSE, L., WEISBERGER, H. and HALL, R. B.: Clonal forestry. Vol. II. AHUJA, M. R. and LIBBY, W. J. (eds.). Springer Verlag. pp. 91–119 (1993).

Height Growth Variation in a Comprehensive Eurasian Provenance Experiment of (*Pinus sylvestris* L.)

By A. M. SHUTYAEV[1]) and M. GIERTYCH[2])

[1]) Research Institute of Forest Genetics and Breeding, Lomonosova 105, 394043 Voronyezh, Russia
[2]) Institute of Dendrology, 62-035 Kórnik, Poland

(Received 26th May 1997)

Summary

In the years 1974 to 1976, on the initiative of the Forest Research Institute in Pushkino, near Moscow, a major Scots pine experiment was established with 113 provenances over 33 planting sites, well scattered over the whole former USSR. Basing on reports from co-operating institutions information is compiled on the provenances used, on the planting sites and on the mean tree height at latest measurement. Interaction parameters are calculated and the data on tree heights, converted to units of standard deviation from location means, is plotted onto maps of the locations demonstrating the extent of genotype environment interaction. The range of the species in the former USSR can be divided into regions (Northwestern, Baltic, Western Continental, Northern Russia, Central European Russia, Middle Volga, Central Trans-Urals, Southern fringe, Eastern Siberia), that have characteristic for them responses to seed transfer in terms of height growth performance at various locations. Western populations (Baltic