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Research Article

An investigation on rooting of *Juglans regia* L. Hardwood cuttings

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Abstract: The rootinf of *Juglans regia L.* (*Juglandeceae*) hardwood cuttings was investigated. The cuttings were treated with a solution of 100 and 1000 ppm IBA concentrations. One and two year old shoot cuttings were used and carbohydrate changes were recorded during rooting. No rooting was observed in any cutting, 32% callus formation was observed on the basal parts of the cuttings. The flower buds burst earlier than the vegetative buds and developed male or female flowers. Callus formation rate or bud burst was not significantly affected by the treatment of IBA. Carbohydrate changes occurred during the culture, but there was no correlation between carbohydrate levels and callusing or bud burst. Callus formation was only observed on the cuttings with vegetative buds.

Key Words: Juglans, walnut, rooting, cutting

Juglans regia L.'nin Çelik Köklenmesi Üzerine Bir Araştırma

Özet: Bu çalışmada, *Juglans regia* L.'nin (*Juglandaceae*) sert odunlu çeliklerinin köklenme durumları incelenmiştir. Çelikler 100 ve 1000 ppm'lik IBA ile muamele edilmiştir. Denemelerde 1 ve 2 yıllık sürgün çelikleri kullanılmış ve köklenme sırasında Karbohidrat değişimleri araştırılmıştır. Hiç bir çelikte köklenme saptanmamış, %32 oranında kallus oluşumu gözlenmiştir. Çiçek tomurcukları sürgün tomurcuklarına göre daha önce patlamış ve erkek veya dişi çiçekler oluşturmuşlardır. Sürgün tomurcukları daha geç patlamış ve kallus oluşumu sadece bu çeliklerde gözlenmiştir. Köklenme sırasında Karbohidrat düzeyleri değişmiş olmasına karşın Karbohidrat düzeyi ile kallus oluşumu veya tomurcuk patlaması arasında bir ilişki kurulamamıştır.

Anahtar Sözcükler: Juglans, ceviz, köklenme, çelikleme

Introduction

Juglans regia L. is a commercially important species because of its high quality wood, nutritious nuts and pharmacological leaves. Vegetative propagation of walnut trees has been studied intensively for many years. However, the vegetative propagation of *Juglans regia* has not been totally perfected for efficient commercial applications in spite of recent progress and important technical improvements (1-3(. Some promising results have also been obtained with *J. regia* (4) and *J. hindsii* X *J. regia* (5), all of which sugessts a significant potential for organogenesis in Juglans. On the other hand, some authors (6, 7) recently concluded that walnut propagation is still an unsolved problem and the main reasons are irregular and often low rooting rates and high mortalities of rooted plants during acclimatization. Earlier investigations (8, 9) suggested that the continuity of the sclerenchymatous cylinder encircling the phloem inhibits rooting or root emergence. Jay-Allemand et al. (10) suggeseted that Juglone is a major internal factor with a role in adventitious root induction during early stages of rhizogenesis and there is a positive correlation between Juglone content and the rooting capacity of microcuttings. Many treatments on difficult-to-root Juglans species have been studied in order to improve rooting efficiency (11-13), but expected results have not been obtained.

In the present study, walnut (*Juglans regia* cv. Krı) was selected as an important commercial species with poor rooting ability. The main goal of this paper is to describe the morphological characteristics, carbohydrate levels and rooting ability of one and two year old shoot cuttings.

Materials and Methods

One or two year old shoot cuttings of Juglans regia L. cv. Kr1, taken in January, February, November and December 1997 from Çimerli koyü, Boztepe-Kırşehir were used (600 in number). The cuttings were prepared, 14-16 cm in length with 3-4 buds and placed in distilled water or basally-dipped in solution of 100 and 1000 ppm IBA concentration for 24 h, in a beaker at room temperature. The cuttings were transferred to rooting medium in darkness at 25°C (Perlit was used as rooting medium). The callus formation and bud burst were recorded daily. One centimeter sections from the basal part of the cuttings were taken for carbohydrate analysis.

Carbohydrate extraction and analysis: Soluble sugar extraction and analysis procedures were adapted from Ebell (14). Dry samples of 100 mg were weighed and extracted in a Soxhlet for 4 h with 20 ml of 80% ethanol. Ethanol wes evaporated from the extracts under vacuum at 55°C. The fraction was solubilized in bidistilled water (20 ml). The extracts were deproteinized with 1 ml saturated, neutral lead acetate and excess lead removed with 2 ml of saturated disodium phosphate. The extracts were docolorized with approximately 200 mg of powdered charcoal and centrifuged at 8000 x g for 20 min. The supernatants were filtered and made up to 200 ml final volume with bidistilled water.

After extraction of the soluble sugars, the solid starch containing residue harvested upon centrifugation was incubated with NOH (0.02 N) in a water bath at 90°C for 60 min to solubilize the starch. After cooling and addition of 2.5 ml acetate buffer (2 M), the starch was hydrolyzed with amyloglucosidase (E.C 3.2-13) for 2 h at 60°C. The glucose liberated by hydrolysis was then quantified by colorimetry at 540 nm according to the method described by Lloyd and Whelan (15). Measuremenets were made using a Shimathsu 1201 spectrophotometer. The results of the above experiments were analysed statistically using Snedesor's F-test (16) for analysis of variance and to determine statistically significant differences between means the "multiple range test" (17) was applied.

Results

Rooting was not observed in any of the cuttings and there were no considerable differences between the cuttings collected in different months. The results were determined according to January's cuttings, because these cuttings had better callus morphology. Callus formation was observed on the basal parts of the cuttings, calli appeared by the 14th day (in cuttings treated with IBA) or the 18th day (in untrated cuttings) and the rate reached 32-33% on the 22nd day. Callusing time was correlated with age of shoots and treatment of IBA. The older the shoot, the later the callusing and the treated cuttings had earlier callusing). However, the final callus rate was not significantly affected by the age or IBA treatment. The texture of the calli was different due to treatment of IBA. Calli on treated cuttings were yellow-brown, smooth and well developed, and the calli on untreated cuttings were white, compact and small. Callus formation only occurred in the cuttings without flower buds. After 28 days of culture, callus senescence was initiated and the cuttings started to decay from basal parts.

An interesting result of the study is the bud burst and reproductive development of the cuttings. The cuttings, prepared from one or two year old shoots had a female flower bud at the tip of the cuttings (Figure 2) and the cuttings from two year old shoots had numerous male flower buds located laterally (figures 3, 4) and some of the cuttings from 1 or 2 year old shoots had vegetative buds (figure 5). Flower buds burst earlier (12-14 days) and developed male or female flowers (figures 2, 3, 5) and no calli formed on these cuttings. Vegetative buds burst in 20-22 days and all the calli occurred on these cuttings. There was no considerable effect of IBA treatment.

Carbohydrate levels changed according to callus formation or bud burst. At the beginning, starch concentration was highest in all cuttings and a continuous decrease was noted after day 6 (table 1, figure 1). Soluble sugar concentrations in the cuttings followed irregular changes, with a rise before or during the callus formation, a fall after callus formation and rapid decline after bud burst (table 1). Such variation also occurred in the absence of callusing. Finally, a decrease in concentrations of both starch and soluble sugars for all types of cuttings (with or without callus) was recorded. However, considerable changes occurred during callus formation or bud burst. Carbohydrate levels were not affected by treatment of IBA.

Table 1. Starch and Soluble Sugar levels (mg/g DW±Standard error). In each column values with the same letter are not significantly different at a probability level of 0.05

	Control			100 ppm IBA			1000 ppm IBA		
Days	Soluble Sugar	Starch	Callus %	Soluble Sugar	Starch	Callus %	Soluble Sugar	Starch	Callus %
2	38.2±0.18a	74.0±0.20a	--	37.6±0.37a	76.6±	--	39.1±0.05a	69.3±0.75a	--
4	38.6±0.14a	73.9±0.43a	--	39.4±0.62a	75.2±	--	38.6±0.08a	71.0±0.14a	--
6	40.0±1.24a	70.7±0.91a	--	40.3±0.88a	65.3±	--	38.7±0.64a	70.8±1.08a	--
8	41.2±1.52a	56.2±1.40b	--	48.0±1.29b	61.2±	--	46.9±0.95b	63.6±0.70b	--
10	43.8±0.15a	47.8±0.85b	--	56.7±0.47b	51.4±	--	57.0±1.02c	50.1±0.43c	--
12	54.5±1.00b	46.9±1.24b	--	57.2±0.50b	47.0±	--	61.2±1.67c	42.3±1.78c	--
14	58.3±0.95b	41.8±2.28c	--	61.5±0.33c	38.5±	4.2±0.12d	63.4±0.78c	31.8±1.12d	6.2±0.10
16	56.2±0.70b	34.2±0.64d	--	62.1±0.77c	30.3±	16.1±0.50	61.3±0.95c	27.4±0.55d	21.3±0.08
18	45.0±1.24b	30.5±0.85d	17.8±0.05	53.8±2.18b	24.7±	30.2±1.02	60.8±1.20c	25.2±0.08d	27.4±0.54
20	41.9±0.16a	26.7±0.43d	26.0±0.85	44.4±0.62a	20.8±	30.0±0.95	51.2±0.54bc	21.0±0.05cd	30.8±0.72
22	36.4±0.05a	25.0±0.05cd	33.2±0.76	38.0±0.08a	213±	33.4±0.05	36.5±0.45a	20.4±0.75cd	32.4±0.08
24	30.7±0.24c	20.3±0.72cd	33.2±0.76	38.0±0.08a	20.1±	33.4±0.05	28.4±0.30e	19.9±0.12cd	32.4±0.95

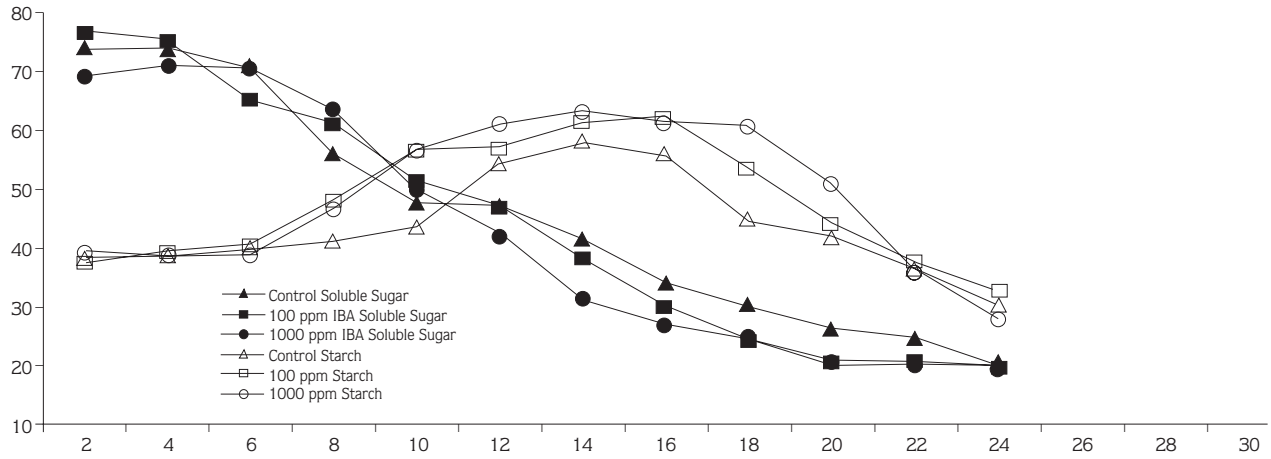

Figure 1. Starch and Soluble Sugar changes in cuttings

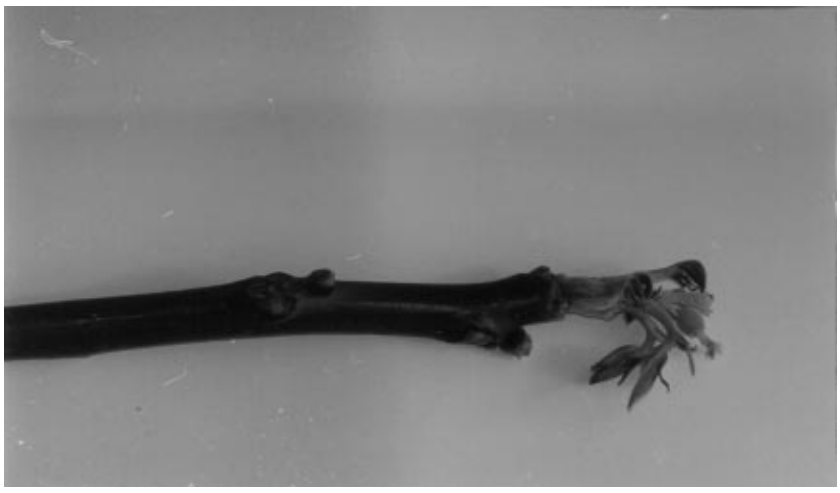

Figure 2. Female flower at the tip of the cutting.

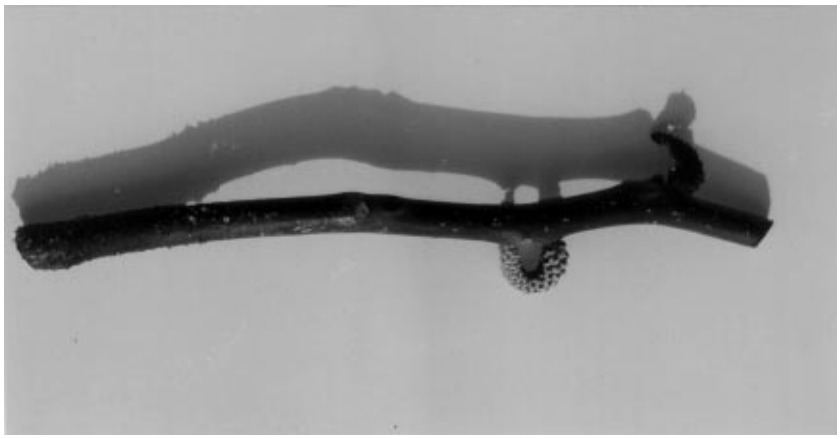

Figure 3. Male flowers located laterally

Figure 4. Development of vegetative shoots and male flower.

Figure 5. Development of vegetative shoot, male and female flowers.

Discusssion

Vegetative propagation of *J. regia* has been studied for many years, and many treatments have been made to improve the rooting efficiency of hardwood cuttings (3, 11, 18). Despite intensive studies, the difficulties have not been eliminated. Therefore, alternative micropropagation methods have been developed for walnut propagation (12, 19-21). In the rooting studies with cuttings, one or two year old shoots are often used as the cutting source (22). In our study one and two year old shoot cuttings were used, and the cuttings had vegetative or reproductive buds. Most of the cuttings developed male or female flowers. There was a negative

correlation between reproductive and vegetative development, and we claim that the reproductive development inhibits rooting, because the callusing only occurred on the cuttings with vegetative (shoot) buds, but these cuttings also did not develop roots. Endogenous factors probably inhibit rooting. Some authors (3, 9, 10) have suggested that endogenous juglone, poliamines or continuity of the scleranchymatous cylinder inhibits root formation or root emergence. These findings support our conclusion. A decrease in concentration of both starch and soluble sugars for all types of cutting was recorded after callusing or bud burst and no apparent correlation was found between callusing and carbohydrate level. Chelawant et al. (11) suggested that exogenous sucrose treatment increased rooting percentage and root number. However, in our study, there was no rooting, although the carbohydrate level was sufficient. Stephens et al. (23) claimed that shoot cuttings only rooted when treated with 1-1.5% IBA and the percentage was 12% of these. We also treated cuttings with 100-1000 ppm IBA, but no rooting was observed. We claim that IBA is not effective on root formation, probably it increases the rate of rooting.

In conclusion, one and two year old shoot cuttings are not useful for vegetative propagation of walnut, because these cuttings have reproductive buds and they inhibits rooting. Perhaps removal of the buds from shoots or using new greening cuttings ensures rooting. IBA does not affect root formation, perhaps it stimulates the rooting rate, and the carbohydrate level is not related to rooting. Further studies must be carried out for explanations.

References

1. Rosanglura, S. and Sharma, S.D. Clonal Propagation of Walnut. Agr. Sci. Digest Karnal. 11(4) 185-189 (1991).
2. Driver, J.A. and Kuniyuki, A.H. In vitro Propagation of paradox Walnut rootstock. Hortscience, 19: 507-509. (1984).
3. Rugini, E., Jacoboni, A. and Luppino, M. Role of basal shoot darkening and exogenous Putrescine treatments on in vitro rooting and on endogenous Polyamine changes in difficult-to-root woody species. Scientia Horticulturae, 53: 63-72. (1993).
4. McGranahan, G., Leslie, C.A. and Driver, J. In vitro Propagation of mature Persian walnut cultivars. Hortscience, 53: 63-72. (1988).
5. Berros, B., Astorga, R., Rey, M., Peneuela, R. and Rodriguez, R. Rooting studies on "in vitro" Walnut tissues: aging effect. Acta Horticulturae, 311: 105-116. (1993).
6. Rodriguez, R., Revilla, A., Albuerne, M., and Perez, C. Walnut (Juglans sp.) in Y.P.S. Bajaj (Ed.), Biotecnology in Agriculture and Forestry. Vol. 5. Tree II Springer-Verlag, Berlin, Heidelberg pp. 100-126. (1989).
7. Chenevard. D., Frossard, J.S. and Jay-Allemand, C. Carbohydrate reserves and CO2 balance of Hybrid walnut (Juglans nigra no. 23X Juglans regia) Plantlets during acclimatisation. Scientia Horticulturae 68: 207-217. (1997).
8. Claudet, A.C., Drauet A and Jay-Allemand C. Tissue distribution of phenolic compounds in annual shoots from adult and rejuvenated hybrid walnut trees. Plant Physiol. and Biochem. 30(5) 565-572. (1992).
9. Yalçın, İ. Juglans regia L. sürgün çeliklerinin kök oluşturmasında anatomik engeller ve kök oluşumu üzerine bir araştırma. C.Ü. Fen Bil. Der. 15(16) 63-80. (1993a).
10. Jay-Allemand, C., Doumas, P., Sotta, B., Tranvan, H., Niginiac., E., Sandermann, H., Bonnet-Masimbert, M. Juvenility and Physiology of Rhizogenesis in two woody species (Sequoia sempervirens and Juglans regia X Juglans nigra). Contr. to forest tree physiol. Final W.S. No-76, 48 ref. Dourdan-France. (1995).
11. Chelawant, D; Jay-Allemand, C; Gendraud, M; Frossard, J.S. The effect of Sucrose on the development of hybrid walnut microcuttigns (Juglans regia X Juglans nigra). Consequences on their survival during acclimatization. Ann. des scie. Forest. 52(2) 147-156. (1995).
12. Stephans, L.C; Krell, S.L.; and Domoto P.A. in vitro propagation of Juglans regia. 81 sf. Ann. report of Northern Nut growers. 9 ref. 122-126 Hamden, Connecticut USA. (1990).
13. Heloir, M.C; Kevers, C; Hausman, J.F; Gaspar, T. Changes in the Concentrations of auxins and Polyamines during rooting of in vitro propagated walnut shoots. Tree Physiology 16(5) 515-519.(1996).
14. Ebell, L.F. Variation in total Soluble Sugars of Conifer Tissues with method of analysis. Phytochemistry, 8: 227-233. (1969).
15. Lloyd, J.B; and Whelan W.J. An improved for enzymatic determination of glucose in the presence of maltose. Ann. Bioch. 30: 467-469. (1968).
16. Snedecor, G.W. and Cochron, W.G. Statistical Methods. Iowa State Univ. Press Ames. (1980).
17. Duncan, D.B. Multiple Range and Multiple F-test. Biometrics, 11, 1-41. (1955).
18. Kantarcı, M; and Jacob H. Ceviz odun çeliklerinin köklenmesinde uyartıcı bazı işlemlerin ve büyüme düzenleyici maddelerin etkileri. A.Ü. Ziraat Fak. Yıllığı, 39: 1-12 (1991).

19. Ripetti, V; Kevers, C; and Gaspar, T. Two successive media for the rooting of walnut shoots in vitro. Changes in peroxidase activity and in ethylene production. Adv. in Hort. Sci. 8(1) 29-32. (1994).

20. Jay-Allemand, C; Capelli, P; and Cornu, D. Root development of in vitro hybrid walnut microcuttings in a vermiculite-containing gelrite medium. Sciente Hort. 51: 335-342. (1992).

21. Barbas, E; Chaillou, S; Cornu, D; Doumas, P; Jay-Allemand, C; and Lamaze, T. Ortho-phosphate nutrition of in vitro propagated hybrid walnut (Juglans nigra X Juglans regia) trees: Pi(Pi) uptake and transport in relation to callus and shoot development. Plant Physiol. Biochem. 31(1), 41-49. (1993).

22. Hartman, H.A. and Kestler, D.E. Plant Propagation Principles and Practices. 3rd ed. Prentice-Hall. Inc. New Jersey, pp. 211-270. (1975).