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The Effect of Severance Date on Rooting Ability of Chestnut Cuttings and Associated Changes in Phenolic Content During Adventitious Root Formation

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With 1 Figure

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Summary

OSTERC G., ŠTEFANČIČ M., SOLAR A. & ŠTAMPAR F. 2007. The effect of severance date on rooting ability of chestnut cuttings and associated changes in phenolic content during adventitious root formation. – Phyton (Horn, Austria) 46 (2): 285–294, 1 figure. – English with German summary.

The relation between the date of cutting severance (May 30th, June 12th, June 27th and July 18th) and the rooting ability of two different hybrid (*Castanea crenata* × *Castanea sativa*) chestnut clones ('Marsol' and 'Maraval') was studied in relation to the accumulation of different phenolics in basal parts and leaves of the cuttings during the first days after cutting preparation. The 'Maraval' clone had a significantly higher rooting ability than the 'Marsol' clone. The cuttings collected on the earliest date (May 30th) tended to the best rooting results (high rooting ratio, low callus formation) for both clones. The concentrations of caffeic and sinapic acid in the basal cutting parts of the 'Maraval' clone decreased on the first day after cutting severance; later increased amounts were observed. The cutting leaves contained, on the first day after severance, the highest concentrations of chlorogenic and ellagic acid, when cuttings were prepared in May.

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Zusammenfassung

OSTERC G., ŠTEFANČIČ M., SOLAR A. & ŠTAMPAR F. 2007. Der Einfluss des Steckzeitpunktes auf die Bewurzelung und die Polyphenoldynamik während der adventiven Wurzelbildung bei der Esskastanie. – Phyton (Horn, Austria) 46 (2): 285–294, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Der Zusammenhang zwischen dem Steckzeitpunkt (30. Mai, 12. Juni, 27. Juni und 18. Juli) und der Bewurzelung in Abhängigkeit von der Einlagerung verschiedener Phenolstoffe in der Stecklingsbasis und in den Stecklingsblättern während der ersten Tagen nach dem Stecken bei den zwei verschiedenen Esskastaniehybridklonen (*Castanea crenata* × *Castanea sativa*; 'Marsol' und 'Maraval') wurde geprüft. Die 'Maraval' Stecklinge ließen sich signifikant besser bewurzeln als die 'Marsol' Stecklinge. Die Stecklinge, die am ersten Stecktermin (30. Mai) geschnitten worden sind, wiesen unabhängig vom Klon tendenziell die besten Vermehrungsergebnisse (größte Bewurzelungsanteile, kleinste Kallusanteile) auf. Die Kaffeesäure- und Sinapinsäuregehalte in der Stecklingsbasis zeigten beim Klon 'Maraval' am ersten Tag nach dem Stecken einen abnehmenden Trend, später nahmen die Werte zu. Es konnten die höchsten Chlorogensäure- und Ellagsäuregehalte in den Stecklingsblättern am ersten Tag nach dem Stecken beim ersten Stecktermin gemessen werden.

Introduction

Adventitious root formation in plants is one of the most important processes in horticultural science (DE KLERK & al. 1999, HARTMANN & al. 1997). Different phenolic compounds, in conjunction with auxin metabolism, may be partially responsible for root formation capacity in some plants (DE KLERK & al. 1999, HARTMANN & al. 1997). Several phenolic compounds might be involved in rooting. HARTMANN & al. 1997 hypothesised that (iso)chlorogenic acid and different terpenoids can promote IAA (indole-3-acetic acid) action during the rooting process in plants. The problem of juvenility in some woody plants may be related to special phenolics like crenatin, which inhibit IAA oxidation (MATO & al. 1994). In chestnut cuttings, it was suggested that several phenolics, such as tannic, gallic and ellagic acid, quercetin-3D-rhamnoside, and quercetin-3D-galactoside can affect the rooting process (OSTERC & al. 2004). In tip cuttings, the decreasing curve of tannic acid at the basal portion of cuttings during the first months after severance could probably have a positive effect on the rooting process. Regarding involvement of different phenolics in the root formation metabolism FAIVRE-RAMPANT & al. 2002 reported chlorogenic acid as auxin antioxidant compound in tobacco mutants. Therefore, the IAA-pool in the cuttings stayed high and the root formation was blocked. Additionally, chlorogenic acid is confirmed to be a substance which helps in the defence mechanism against stress (DIXON & PAIVA 1995). This defence mechanism helps cuttings to overcome the stress caused by cutting, i. e. the removal from mother plants. Chlorogenic acid concentration showed a sharp increase in chestnut cuttings in the first days after

removal (our unpublished results). A similar role was also ascribed to ellagic acid in the same study. One of the most important factors for successful rooting is the season in which the cuttings are taken (GASPAR & COUMANS 1987, SPETHMANN 1997). Probably the polyphenol status at the time of cutting preparation represents the first parameter affecting rooting response. Later changes in the polyphenol status also appear to be important for the rooting response. In the present study, we followed changes in the polyphenol concentration in relation to adventitious rooting of chestnut cuttings taken in different seasons. The aim was to evaluate the relationship between concentrations of various polyphenolics, the severance date and the rooting ability of cuttings of two chestnut varieties showing contrasting rooting rates. Chestnut is an interesting material as it is considered as a difficult-to-root species.

Material and Methods

Experimental Design and Plant Material

The experiment was carried out in 2002 in an unheated plastic greenhouse, under a fog system at the experimental field of the Biotechnical Faculty in Ljubljana (Slovenia). The fog system assured constant high relative humidity in the greenhouse. Due to the high humidity, extremely high temperature conditions in the greenhouse – daytime 50 °C, at night between 18 and 20 °C – did not damage the plants. Two different *Castanea crenata* × *Castanea sativa* hybrid clones ('Marsol' and 'Maraval') and four different severance times were tested in the experiment with 4 replications and 40 cuttings (additionally, 6 extra cuttings were inserted in each replicate for further polyphenol analyses, see below) per replication in a completely randomised design. Leafy cuttings (12 cm long) were cut in 2002 at the Faculty experimental station in Maribor, Slovenia (46.22° N, 14.48° E, north hemisphere) on 30th May, on 12th June, on 27th June and on 18th July. Six-year-old in-vitro derived plants grown in the open were used as mother plants. The cuttings were treated for 2 sec. with 0.5 % (5000 mg L^{-1}) indole-3-butyric acid (IBA) with 10 % Euparen on talcum basis and then placed (5 cm depth) in the rooting substrate (peat/sand in a 3:1 v/v ratio). Before inserting the cuttings, the substrate was fertilized (mixing into the substrate) with 2.0 g L^{-1} slow-release fertilizer Osmocote Exact Standard 3-4 M (15+11+13+3 = N+P+K+Mg, Scotts International B.V., Heerlen, The Netherlands), the pH value of the substrate was adjusted to 4.0 by adding 1.5 g L^{-1} lime. The cutting final evaluation (visual evaluation of the presence or absence of roots and callus) was made at the end of February 2003 after the first winter period (222 – 270 days after insertion in the substrate).

Extraction and Analyses of Phenolic Compounds

The material for polyphenol analyses was harvested at the time of excision (day 0) and one, four and six days after the establishment of cuttings. The cuttings gathered for further polyphenol analyses (two cuttings per replicate, 64 cuttings for each date) were divided into a basal part (2 cm) and leaves. Plant samples were immediately frozen in liquid nitrogen. Fifty mg lyophilised material was extracted with

10 ml acetone-water (80:20, v/v) solution containing Triton X-100 (0.4 %) for 30 min or 10 days (used for cutting basal parts) at 4 °C (USENIK & ŠTAMPAR 2000, FERNANDEZ-LORENZO & al. 1999). After the extraction, the solvent was centrifuged and finally evaporated to dryness in a speed-vac at 40 °C. The residue was dissolved in methanol (2 ml). The samples were clarified by filtration through 0.45 µm pore size membranes (MN, Chromafil 25 mm) and stored at –20 °C until the HPLC analyses. Polyphenols were determined by reversed phase HPLC of Thermo Separation Products (TSP) with the WellChrom K-2500 detector and Thermo Finigan Surveyor and PDA detector, using a Varian Chromsep column (SS 250 × 4.6 mm, Hypersil 5 ODS). The mobile phase was dependent on the material which was analysed. For leaf analyses, for detection of chlorogenic and ellagic acid, the mobile phase consisted in a gradient formed from solvent A (water : acetic acid, 1%) and solvent B (methanol : acetonitrile; 1:1, v/v) as follows: 0 – 10 min, 15% B in A; 10 – 50 min, 15 – 40% B in A; 50 – 60 min, 40 – 60% B in A; 60 – 80 min, 60 – 100% B (FERNANDEZ-LORENZO & al. 1999) with a flow rate of 0.5 ml min^{-1}. For analyses of bases, for detection of caffeic, sinapic, ferulic and p-coumaric acid the solvent A (water : methanol : phosphoric acid, 940:50:1) and solvent B (methanol : phosphoric acid, 990:1) were used, with the following gradient elution: 30% B in A; 0 – 40 min, 30 – 60% B in A; 40 – 80 min, 60 – 90% B in A; 80 – 120 min (SCALBERT & al. 1998). The flow rate was 1.0 ml min^{-1} and the injection volume was in both cases 5 µl. The individual phenolic compounds were identified by comparing their retention time and UV (absorbance) spectra with those of reference compounds.

Statistical Analyses

The experiment was evaluated as a three-factorial experiment (clone, date of severance, date of sampling) by ANOVA, and the analyses of means were carried out with the Tukey-test at $p = 0.05$. The statistic program Statgraphics Plus 4.0 (Manugistics, Statistical Graphics Corp.) was used.

Results

Propagation Results

The data collected at the end of the propagation season showed significant differences in rooting response between the clones ($p < 0.001$). The 'Maraval' cuttings rooted better than 'Marsol' cuttings. The date of cutting preparation did not show any significant effects on rooting ($p = 0.185$). However, the cuttings taken from the mother plant on the last date (July 18) exhibited significant callus formation ($p = 0.001$, evident in 'Maraval' cuttings) (Fig. 1).

Phenolic Concentrations during the Root Initiation Phase

At the time of excision, the basal parts of the cuttings contained several phenolic acids: caffeic, sinapic, ferulic and p-coumaric acid. For the four severance dates, the phenolic showing the greatest concentration in the basal parts was that of sinapic acid (0.56 to 1.85 mg g^{-1} dry weight [DW]). There was no difference in sinapic, ferulic and p-coumaric acid

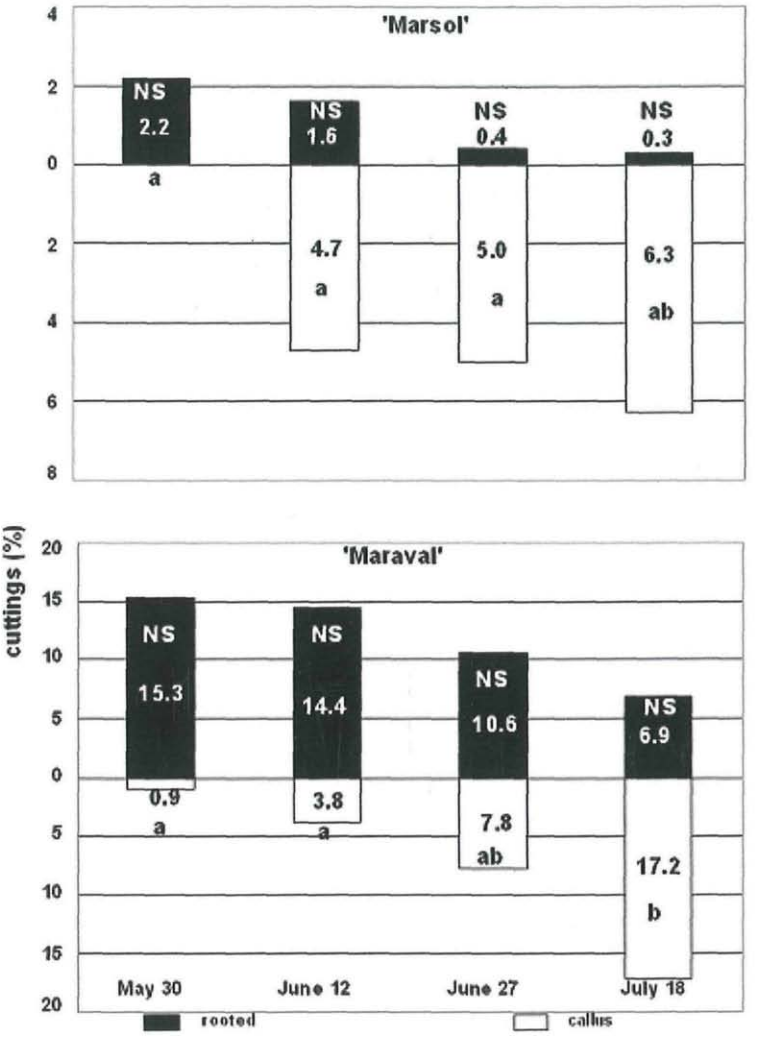

Fig. 1. Rooting rate and callus formation (222 – 270 days after insertion in substrate) regarding different dates of cutting collection in two chestnut hybrid clones 'Marsol' and 'Maraval'. Statistical data correspond to all dates for each clone. Each value is the mean of four replicates of 40 cuttings each. Means with different letters are statistically significant at $p \leqslant 0.05$, NS = Nonsignificant.

concentrations with regard to cutting preparation date. Very important is the phenolic acid dynamics in these first days after severance. Our attention was especially focused on caffeic and sinapic acid (Table 1), although ferulic and p-coumaric acid showed similar fluctuations. The changes in phenolic contents in cutting basal parts at that time differed strongly between 'Marsol' and 'Maraval'. The differences were particularly notable on the first date of cutting preparation when the contents of caffeic and sinapic acid in 'Maraval' cutting basal parts decreased (the decrease in sinapic acid was statistically significant, see Table 1). Later, until the fourth day after severance the contents of caffeic and sinapic acid increased (the increase in caffeic acid was statistically significant, see Table 1). In contrast, for 'Marsol' cutting basal parts a significant accumulation (sinapic acid, see Table 1) was measured on the first day after severance, and later a decreasing trend was noticed. Among substances measured in leaves, the chlorogenic and ellagic acid were followed (Table 1). The contents of both acids differed significantly between 'Marsol' and 'Maraval'. The 'Maraval' cutting leaves contained significantly more phenolics than the 'Marsol' cutting leaves. In both clones, the accumulation of both phenolics in cutting leaves was noticed immediately after the severance. Thereafter, a decreasing trend was detected. The accumulation time-course was especially evident for the earliest date of cutting preparation (May 30th) (Table 1).

Discussion

Successful adventitious root formation in woody plants, especially in difficult-to-root species is often associated with the optimal period of cutting collection (Spethmann 1997, Hartmann & al. 1997). Unfavourable days of cutting collection lead to poor rooting of the cuttings, intense callus formation, small number of main roots and low root quality. Root quality is mainly characterised by the speed of root formation (Spethmann 1985, 1986, 1997). These results were supported by our data on chestnut cuttings. With the progress of the suboptimal date of severance, the rooting success was tending to decrease, and the callus formation increased. This relationship was obvious in both 'Marsol' and 'Maraval' clones, although there was a clear difference in rooting capacity between both clones. The optimal date of cutting severance is reported to be one of the most important factors that influence successful rooting. This is the reason why this relationship can be observed regardless of species or climate conditions in different years (Spethmann 1997).

The accumulation of different phenolic compounds is often associated with the rooting process (Gaspar & Coumans 1987, de Klerk & al. 1999). Our attention focused on different phenolic acids which were suggested to be involved in the rooting process (Gaspar & Coumans 1987, Dixon & Paiva 1995, Faivre-Rampant & al. 2002, Osterc & al. 2004, Trobec & al. 2005).

Table 1. Time-course of caffeic and sinapic acid content in cutting basal parts and of chlorogenic and ellagic acid content in leaves of cuttings of two chestnut hybrid (*Castanea crenata* × *Castanea sativa*) clones, 'Marsol' and 'Maraval' collected at different dates. Statistical data correspond to both clones and all dates. Each value is the mean of four replicates of 2 cuttings each (64 cuttings for each date for both clones). DW dry weight. Asterisks indicate statistically different means at $p \leq 0.05$, NS = Nonsignificant.

Clone	Severance date	Sampling time (days)	Caffeic acid (mg g^{-1} DW)	Sinapic acid (mg g^{-1} DW)	Chlorogenic acid (mg g^{-1} DW)	Ellagic acid (mg g^{-1} DW)
'Marsol'	May 30th	Cutting time	0.10 ± 0.06	0.56 ± 0.51	2.90 ± 0.70	1.86 ± 0.88
		1	0.18 ± 0.12	1.85 ± 1.47	6.20 ± 5.74	7.14 ± 6.81
		4	0.06 ± 0.04	1.00 ± 0.73	3.46 ± 1.05	4.59 ± 1.10
		6	0.01 ± 0.004	0.11 ± 0.16	0.85 ± 0.44	2.83 ± 2.35
	June 12th	Cutting time	0.29 ± 0.08	0.90 ± 0.25	3.73 ± 2.40	2.77 ± 1.19
		1	0.17 ± 0.10	1.16 ± 0.43	3.76 ± 1.59	3.65 ± 1.27
		4	0.07 ± 0.06	0.36 ± 0.30	3.29 ± 1.25	3.33 ± 3.48
		6	0.09 ± 0.09	0.28 ± 0.36	3.74 ± 1.78	2.94 ± 1.30
	June 27th	Cutting time	0.16 ± 0.11	0.62 ± 0.36	2.29 ± 1.37	1.82 ± 1.10
		1	0.15 ± 0.09	0.90 ± 0.80	1.93 ± 0.49	3.93 ± 1.46
		4	0.11 ± 0.05	1.16 ± 0.60	2.72 ± 0.59	4.14 ± 2.03
		6	0.16 ± 0.19	1.11 ± 0.54	1.49 ± 0.72	2.72 ± 1.53
	July 18th	Cutting time	0.19 ± 0.13	1.22 ± 0.69	4.95 ± 3.16	10.44 ± 4.71
		1	0.14 ± 0.08	1.11 ± 0.33	2.19 ± 1.11	3.67 ± 4.80
		4	0.15 ± 0.08	1.04 ± 0.48	1.96 ± 1.41	3.13 ± 1.45
		6	0.16 ± 0.17	0.81 ± 0.56	2.43 ± 0.99	4.42 ± 2.82
'Maraval'	May 30th	Cutting time	0.13 ± 0.08	1.23 ± 0.33	39.77 ± 15.59	24.09 ± 13.80
		1	0.05 ± 0.05	0.57 ± 0.56	43.29 ± 18.79	43.34 ± 27.50
		4	0.63 ± 0.76	1.33 ± 1.30	6.64 ± 5.07	9.10 ± 1.95
		6	0.23 ± 0.13	0.84 ± 0.53	5.14 ± 2.47	8.14 ± 3.50
	June 12th	Cutting time	0.37 ± 0.14	1.31 ± 0.34	29.09 ± 10.26	16.98 ± 14.32
		1	0.34 ± 0.14	1.37 ± 0.72	42.89 ± 22.17	31.62 ± 29.76
		4	0.09 ± 0.03	0.89 ± 0.18	47.31 ± 14.55	50.06 ± 73.94
		6	0.17 ± 0.10	0.65 ± 0.57	39.64 ± 6.83	21.44 ± 8.38
	June 27th	Cutting time	0.26 ± 0.12	1.26 ± 0.62	4.66 ± 2.11	6.37 ± 3.05
		1	0.19 ± 0.07	1.29 ± 0.62	30.81 ± 8.72	29.50 ± 19.70
		4	0.12 ± 0.05	1.00 ± 0.54	40.40 ± 15.16	38.06 ± 16.42
		6	0.14 ± 0.11	1.00 ± 0.50	25.40 ± 2.96	35.08 ± 20.48
	July 18th	Cutting time	0.58 ± 0.71	1.42 ± 0.67	35.66 ± 12.67	33.42 ± 15.17
		1	0.19 ± 0.08	1.04 ± 0.45	23.65 ± 18.32	17.77 ± 12.45
		4	0.44 ± 0.50	1.25 ± 0.84	10.11 ± 8.60	12.53 ± 13.04
		6	0.22 ± 0.14	1.25 ± 0.68	28.89 ± 11.68	29.70 ± 19.06

Factor				
Clone	NS	NS	NS	NS
Severance date	NS	NS	NS	NS
Sampling time	NS	NS	NS	NS
Clone × Severance date	NS	NS	NS	NS
Clone × sampling time	NS	NS	NS	NS
Severance date × Sampling time	NS	NS	NS	NS
Clone × Severance date × Sampling time	*	*	*	*

The contents of these substances at the severance time in the cutting basal parts, which are the sites of root development, did not indicate a clear relation with the subsequent root induction. The best rooting tended to correspond with the first date of cutting severance, when phenolic contents in the cutting basal parts were low. The majority of measured phenol substances showed the highest concentrations on the fourth date of severance, when the worst rooting results were observed.

However, it seems unlikely that only the accumulation of different phenolic acids inhibited the rooting process. As reported by different authors (DE KLERK & al. 1999, HARTMANN & al. 1997, NAG & al. 2001), the first days after cutting preparation are the most active time for the root induction and initiation. The main factor responsible for successful rooting at this time is surely auxin, but the participation of different phenolic substances as rooting promoters is also necessary (HARTMANN & al. 1997). The role of different phenolic acids has been widely discussed in the literature. DAVIES 1995 reported about diphenols and o-monophenols, such as caffeic and sinapic acid, which enhance the rooting process. VOLPERT & al. 1995 divided different phenolic acids in two groups regarding their involvement in adventitious rooting. Caffeic, sinapic and ferulic acid were considered rooting promoters, whereas p-coumaric and vanilic acid showed an inhibitory effect on rooting. In our experiment, we could detect a decrease in concentration of caffeic and sinapic acid in 'Maraval' cuttings on the first day after severance, and later significant increases in phenolic amounts toward the fourth day after severance in the cutting basal parts on the first date of cutting collection. The same trend was observed for ferulic, p-coumaric and vanilic acid (data not shown). Similar results examining sinapic and vanilic acid were also observed in cuttings of the cherry rootstock 'Gisela 5' (TROBEC & al. 2005). Therefore, successful rooting appears to be connected with lowering concentrations of these phenolic acids in the early phase of adventitious rooting. These results are different from those found by NAG & al. 2001, suspecting that the adventitious root formation in chestnut is similar to that in mung bean. In contrast to the assumption of NAG & al. 2001 the phenolic acid concentration in the cutting bases of the variants with the best rooting results (best rooting, absence of callus formation) increased immediately after severance. These results clearly support the idea that the phenolic acid action should be different from that assumed by NAG & al. 2001 for different phenolics. These differences could be due to herbaceous versus woody and/or easy-to-root versus recalcitrant species characteristics. Additionally, the low initial concentration of phenolic acids can be explained via other factors, such as wounding reaction or such as the fact of excision from the mother plant.

The results of the chlorogenic and ellagic acid in leaves support in some way the previously mentioned data. The high concentrations of both

acids were noticed on the first day after severance in cutting leaves, when the cuttings were prepared on the first date (May 30). This accumulation trend was not so significant for later severance dates. This was the case in both clones regardless of the great difference in accumulation of phenolics between them. These results suggest that chlorogenic and ellagic acids are involved in a defence mechanism against stress, caused by cutting excision from the mother plant (DIXON & PAIVA 1995), perhaps acting as antioxidants at the wound site (DE KLERK & al. 1999). Different days of cutting preparation lead to differences in the intensity of this defence mechanism.

Conlusions

The day of cutting excision did not cause any significant difference in rooting but partially ('Maraval' clone) affected stronger callus formation in the suboptimal days. The clone effect on rooting response was very pronounced. The relationship between auxins and different phenolic compounds in the process of root formation can only be clarified through detailed analyses of different aspects (auxin metabolism, metabolism of special phenolics). The further experiments shall be orientated in the clarification of the observed decreasing tendency of rooting with the later severance dates. It seems important to compare the time-course content of different phenolics. The variants with the best rooting reaction ('Maraval' clone, first day of cutting preparation) showed a decrease in the initial phenolic acid concentration in the cutting bases. On the contrary, the concentration of different phenolic acids (chlorogenic, ellagic acid) increased in the leaves of cuttings with the best rooting reaction during the first days after the cutting excision. Therefore, the biochemical analyses on the role of the days of cutting preparation provide useful information on the functioning of the rooting process and may clarify the role of phenolics in adventitious root formation.

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References

DAVIES P.J. 1995. Plant hormones. Physiology, biochemistry and molecular biology, pp. 833, 2nd edition. – Kluwer Publishers, Netherland.

DE KLERK G.-J., KRIEKEN W. & DE JONG J.C. 1999. Review: The formation of adventitous roots: new concepts, new possibilities. In vitro cell dev. – Biol. Plant. 35: 189199.

DIXON R.A. & PAIVA N.L. 1995. Stress-induced phenylpropanoid metabolism. – Plant Cell 7: 1085–1097.

FAIVRE-RAMPANT O., CHARPENTIER J.-P., KEVERS C., DOMMES J., VAN ONCKELEN H., JAY-ALLEMAND C. & GASPAR T. 2002. Cuttings of the non-rooting rac tobacco mutant overaccumulate phenolic compounds. – Funct. Plant Biol. 29: 63–71.

FERNANDEZ-LORENZO J.L., RIGUEIRO A. & BALLESTER A. 1999. Polyphenols as potential markers to differentiate juvenile and mature chestnut shoot cultures. – Tree Physiol. 19: 461–466.

GASPAR T. & COUMANS M. 1987. Root formation. – In: BONGA J.M. & DURZAN D.J. (Eds.), Cell and tissue culture in forestry, pp. 202–217. – Martinus Nijhoff, The Hague.

HARTMANN H.T., KESTER D.E., DAVIES F.T. JR. & GENEVE R L. 1997. Plant propagation – Principles and practices, pp. 770, 6th edition. – Prentice Hall, Englewood Cliffs, N.J., US.

MATO M.C., MENDEZ J. & VAZQUEZ A. 1994. Polyphenolic auxin protectors in buds of juvenile and adult chestnut. – Physiol. Plant. 91: 23–26.

NAG S., SAHA K. & CHOUDHURI M.A. 2001. Role of auxin and polyamines in adventitious root formation in relation to changes in compounds involved in rooting. – J. Plant Growth Regul. 20: 182–194.

OSTERC G., TROBEC M., USENIK V., SOLAR A. & ŠTAMPAR F. 2004. Changes in polyphenols in leafy cuttings during the root initiation phase regarding various cutting types at *Castanea*. – Phyton 44: 109–119.

SCALBERT A., MONTIES B. & FAVRE J.-M. 1998. Polyphenols of *Quercus robur*: adult tree and in vitro grown calli and shoots. – Phytochemistry 27: 3483–3488.

SPETHMANN W. 1985. Propagation of *Prunus avium* by summer and winter cuttings and survival after different storage treatments. – Acta Hort. 169: 353–363.

— 1986. Stecklingsvermehrung von Stiel- und Traubeneiche (*Quercus robur* L. und *Quercus petraea* [Matt.] Liebl.). – Schriften Forstl. Fak. Univ. Göttingen u. d. Nieders. Forstl. Versuchsanstalt 86: 1–99.

— 1997. Autovegetative Gehölzvermehrung. – In: KRÜSSMANN G. (Eds.), Die Baumschule, pp. 382–449. Parey Verlag, Berlin.

TROBEC M., VEBERIČ R., ŠTAMPAR F. & OSTERC G. 2005. Influence of certain monophenols on the rooting of 'Gisela 5' leafy cuttings. – J. Plant Physiol. 162: 589–597.

USENIK V. & ŠTAMPAR F. 2000. Influence of various rootstocks for cherries on p-coumaric acid, genistein and prunin content and their involvement in the incompatibility process. – Gartenbauwissenschaft 65: 245–250.

VOLPERT R., OSSWALD W. & ELSTNER E. F. 1995. Effects of cinnamic acid derivates on indole acetic acid oxidatio by peroxidase. – Phytochemistry 38: 19–22.