Available online at www.sciencedirect.com
SCIENCE DIRECT
Scientia Horticulturae 100 (2004) 75–82
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Growing season and hydrogen peroxide effects on root induction and development in *Olea europaea* L. (cvs 'Frantoio' and 'Gentile di Larino') cuttings

L. Sebastiani [a,*], R. Tognetti [b]

[a] *Scuola Superiore "Sant'Anna" di Studi Universitari e di Perfezionamento, Piazza Martiri della Libertà, 33, 56127 Pisa, Italy*

[b] *Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università del Molise, Via De Sanctis, 86100 Campobasso, Italy*

Accepted 18 August 2003

Abstract

Semi-hardwood cuttings of 'Frantoio' (high rooting ability) and 'Gentile di Larino' (low rooting ability) cultivars were obtained from 1-year-old olive shoots sampled in mid-August during the 2000 and 2001 growing season. Semi-hardwood cuttings were dipped in IBA 4000 ppm and H_2O_2 (0%-control or 3.5% w/v) solutions before rooting in greenhouse equipped with an automatic mist system. At 57 and 88 days after the beginning of rooting treatments cuttings were scored for the presence of callus, roots and roots number. In both cultivars and years, the IBA 4000 + H_2O_2 treatment significantly modified rooting of cuttings at 57 days in comparison with IBA 4000 treatment. The positive effects of H_2O_2 on rooting were gradually smoothed after 88 days. At the end of the propagation cycle (88th days), cuttings treated with IBA 4000 + H_2O_2 had significantly higher root number in comparison with those treated with IBA 4000 alone.

Keywords: Auxins; Hydrogen peroxide; Olive; Rooting ability; Semi-hardwood cuttings

1. Introduction

The propagation of olive (*Olea europaea* L.) cultivars requires grafting or budding on seedling rootstocks or propagation of self-rooted cultivars from 1 or 2 years old woody cuttings (Hartmann et al., 1990). Although self-rooted cultivars can be very interesting in establishing new olive orchard, the low rooting ability, the unsatisfactory viability and the low rooting quality of cuttings in some cultivars represent limiting factors (Wiesman

* Corresponding author. Tel.: +39-050-883070; fax: +39-050-883495.
E-mail address: l.sebastiani@sssup.it (L. Sebastiani).

0304-4238/$ – see front matter
doi:10.1016/j.scienta.2003.08.008

and Epstein, 1987; Wiesman and Lavee, 1994). The overcoming of rooting inability or the increase in rooting efficiency will, therefore, strongly enhance the commercial benefits of olive cutting use. Cutting propagation could in fact speed up the olive nursery production cycle and the new plants bear fruit in fewer years.

Many plant and environmental factors, including genotypes, nutritional status, phenological stage and environmental conditions determine seasonal variations in rooting ability of woody cuttings (Loreti and Pisani, 1982; Hartmann et al., 1990). Cuttings from species such as quince and poplar root well at any time of the year, while others obtained from species like cherry and olive root successfully only at particular times of the year (Hartmann and Brooks, 1958; Hartmann and Loreti, 1965). In olive, Mancuso (1998) showed a marked seasonal variation in rooting ability of cuttings, achieving the highest (80%) rooting in spring–summer and the lowest (20–30%) rooting in winter. Moreover, high variability in olive rooting has been observed between cultivars, ranging from easy-to- to difficult-to-root cultivars (Rugini and Fedeli, 1990; Sarmiento et al., 1990; Ozkaya and Celik, 1999).

Adventitious root formation is mediated by multiple changes in plant metabolism (Davis and Haissig, 1994; Altman and Waisel, 1997) and it is controlled by successive and interdependent physiological phases (Gaspar et al., 1992, 1994). Adventitious root formation can be stimulated by auxins, but their role in rooting is not exclusive and others compounds are involved (Gaspar et al., 1997).

In olive cuttings exogenous application of indole-3-butyric acid (IBA), polyamine (putrescine) and H_2O_2 has been shown to promote early rooting (Ozkaya et al., 1993; Rugini et al., 1990, 1997). Moreover, in real nursery propagation condition H_2O_2 in combination with IBA treatments significantly improved rooting both in cultivars with high (‘Frantoio’) and low (‘Gentile di Larino’) rooting ability (Sebastiani et al., 2002).

In this work, the interactions between treatment (H_2O_2 and IBA), genotype (olive mother plants) and sampling year on rooting of semi-hardwood cuttings was tested in a commercial nursery. To this purpose, semi-hardwood cuttings were obtained from olive cultivars with high (‘Frantoio’) or low (‘Gentile di Larino’) rooting ability in two different growing seasons and easy-to-assess rooting quality parameters monitored.

2. Materials and methods

2.1. Plant material

One-year-old olive shoots (50–70 cm in length) were collected on 16th (year 2000) and 15th (year 2001) of August from 10-year-old olive mother plants. The cultivars were ‘Frantoio’, classified as “high rooting ability cultivar” and ‘Gentile di Larino’, classified as “low rooting ability cultivar” (FAO, 1996). Mother plants were trained at ‘*monocono*’ in southern Italy (Larino, Campobasso). Meteorological data were collected by an automatic weather station located near the mother plant field. Seasonal courses of rainfall and mean air temperature were recorded in the years 1999, 2000 and 2001.

Shoots were collected and maintained wet in black plastic bags. Two-three semi-hardwood cuttings with 2–4 leaves and 15–20 cm in length were obtained from each shoot the day after their arrival to the nursery (one day after collection).

2.2. Cutting treatments

Indole-3-butyric acid solution at 4000 ppm was freshly prepared dissolving IBA powder (Sigma, St Louis, MO, USA) in an alcohol/water/glycerol (2.5:6.5:1 v/v/v) solution. Hydrogen peroxide 3.5% (w/v) solution was prepared diluting a 35% (w/v) H_2O_2 stock solution (Sigma, St Louis, MO, USA) in distilled water.

Semi-hardwood cuttings were immediately dipped in IBA and H_2O_2 solutions wetting 2 cm of their basal end. Rooting treatments were as follows:

(A) 10 s dipping in IBA 4000 ppm;
(B) 30 s dipping in 3.5% (w/v) H_2O_2 + 10 s dipping in IBA 4000 ppm.

Semi-hardwood cuttings were placed 5 cm deep in perlite on a bench of a rooting greenhouse equipped with an automatic mist system.

2.3. Sampling scheme and statistical analysis

Sampling of semi-hardwood cuttings was performed at 57 and 88 days after the beginning of rooting treatments and each cutting was scored for the presence of callus, roots and roots number.

Fifty cuttings for each cultivar and treatment combination were measured at each sampling date. Cuttings were arranged in a completely randomised block design using sampling date as blocking factor. At each sampling date, data were subjected to χ^2-test to statistically examine the effects of rooting treatment within each cultivar. Analysis of variance (ANOVA) was performed on root number data at the last sampling date to statistically examine the effects of year, rooting treatments, cultivar and their interactions.

3. Results and discussion

Physiological status of mother plant is an important prerequisite in achieving a homogeneous rooting of cuttings (Hartmann et al., 1990). Beside the number of parameters that determine mother plant and cutting physiological status, the meteorological conditions in the months preceding sampling of cuttings or in the year before are also important factors. Meteorological data reported showed little variation in climatic parameters in the months (March–July) preceding the 1-year-old olive shoots sampling, both in the years 2000 and 2001. Averaged values for mean daily temperatures in the period March–July were, in fact, 18 °C (S.D. = ±6) versus 18 °C (S.D. = ±5) in the years 2000 and 2001, respectively. Similarly, the cumulative precipitation values in the period March–July remained stable: 134 mm versus 138 mm in the years 2000 and 2001, respectively.

In the year 2000 (year before the 2001 sampling) mother plants had to withstand more severe drought conditions than in the year 1999 (year before the 2000 sampling): 409 mm versus 821 mm yearly cumulative precipitation values, respectively.

Rooting process was monitored after 57 (October) and 88 (November) days from the beginning of the experiments scoring semi-hardwood cuttings for the presence of callus and roots. Results showed significant changes in semi-hardwood cuttings frequency distribution

Table 1
Frequencies (%) of rooted, no-rooted and no-rooted with callus semi-hardwood cuttings in *O. europaea* L. cultivars Gentile di Larino and Frantoio in October (57th day) and November (88th day) 2000 and 2001[a]

Cultivar/season	Cutting score						*P*-values
	Callus (%)		No-rooted (%)		Rooted (%)		
	IBA	IBA + H_2O_2	IBA	IBA + H_2O_2	IBA	IBA + H_2O_2	
Gentile di Larino							
2000							
October	52	30	90	78	10	22	0.008
November	12	12	82	80	18	20	0.937
2001							
October	23	35	77	51	0	14	0.000
November	26	20	72	79	2	2	0.576
Frantoio							
2000							
October	4	2	44	23	56	77	0.005
November	26	6	48	43	52	57	0.003
2001							
October	32	14	24	41	44	45	0.005
November	20	14	36	40	45	47	0.557

[a] Cuttings were treated with hydrogen peroxide (3.5% w/v) and IBA at 4000 ppm (IBA + H_2O_2) or only with IBA (IBA) at 4000 ppm. Fifty cuttings for each cultivar and treatment combination were scored for rooting at each sampling date and were subjected to χ^2-test to statistically examine the effects of rooting treatment within each cultivar and sampling date. *P*-values are also given.

within these three classes (Table 1). In both cultivars and years, the IBA 4000 + H_2O_2 treatment significantly modified frequency distribution at 57 days in comparison with IBA 4000 treatment. In particular, the relative frequency of the class of rooted cuttings was higher in IBA 4000 + H_2O_2 treatment in comparison with IBA 4000 treatment: 22% versus 10% (year 2000) and 14% versus 0% (year 2001) in Gentile di Larino and 77% versus 56% (year 2000) and 45% versus 44% (year 2001) in Frantoio, respectively.

According to Rugini et al. (1997), H_2O_2 treatment of olive cuttings at the beginning of the rooting process promotes early rooting and increases rooting percentage. The positive effects of H_2O_2 on rooting were gradually smoothed after 88 days, when the frequency of cuttings distribution in IBA 4000 + H_2O_2 treatment in comparison with IBA 4000 treatment did not differ anymore.

Successful rooting is determined not only by rooting percentage, but also by the number of roots formed (Hartmann et al., 1990). Rugini et al. (1997) found a small but significant increase in root number in in vitro olive explants treated with putrescine (putrescine degradation through Δ'-pyrroline pathway is accompanied by the formation of H_2O_2) in the initial phase of rooting. Data reported in Fig. 1, show the mean value of roots per cutting at the end of the rooting period (88th day). Cuttings treated with IBA 4000 + H_2O_2 had significantly ($P = 0.0087$) higher root number in comparison with those treated with IBA 4000 alone: 2.6 versus 1.8 roots/cutting, respectively.

Highly significant year ($P = 0.0032$) and cultivar ($P = 0.0000$) effects were also observed, while the interactions between the three factors (year, treatment and cultivar)

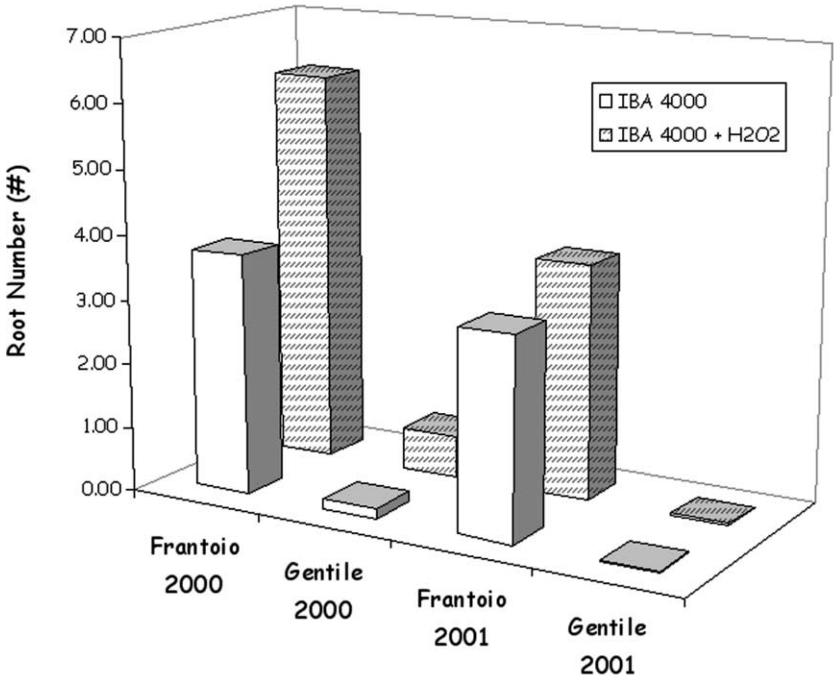

Fig. 1. Number of roots per cuttings in *O. europaea* L. cultivars Gentile di Larino and Frantoio at the end of the rooting period (88th day) for 2000 and 2001 growing season. Cuttings were treated with hydrogen peroxide (3.5% w/v) and IBA at 4000 ppm (IBA 4000 + H_2O_2) or only with IBA (IBA) at 4000 ppm. Fifty cuttings for each cultivar and treatment combination were scored and subjected to ANOVA. Hydrogen peroxide treatment ($P \leq 0.01$), cultivar ($P \leq 0.01$), and year ($P \leq 0.01$) were significantly different. There were no significant interactions between treatment, cultivar and growing seasons.

always remained not significant. In particular, the mean number of root in the year 2000 was higher than in the year 2001 (2.7 versus 1.7 roots/cutting, respectively), suggesting that the physiological conditions of cuttings in the year 2001 were not the same as for the year 2000. Although a simple explanation of these results is not easy, it is possible that the more severe drought conditions experienced by mother plants in the year before sampling (2000) has determined the production of cuttings with lower rooting ability.

The mean number of root in ‘Frantoio’ was higher than in ‘Gentile di Larino’: 4.2 versus 0.2 roots/cutting, respectively or 6.5 versus 2.4 roots/cutting, when calculated on effectively rooted cuttings. When compared to ‘Frantoio’, the lower rooting responses of ‘Gentile di Larino’ was therefore both quantitative than qualitative, although ‘Gentile di Larino’ rooting quality could be significantly improved by IBA 4000 + H_2O_2 in comparison to IBA 4000 alone: 0.3 versus 0.1 roots/cutting, respectively or 2.8 versus 1.5 roots/cutting, when calculated on effectively rooted cuttings.

In order to better describe rooting quality at the 57th and 88th day, semi-hardwood cuttings were also ranked in three rooting classes (Table 2): (1) cuttings without roots; (2) cuttings with 1–2 roots (low quality); (3) cuttings with three or more than three roots (higher quality). Results showed significant changes in frequency distribution of semi-hardwood

Table 2
Frequencies (%) of rooting per class of roots: (1) cuttings without roots—no-roots; (2) cuttings with 1–2 roots (low quality); (3) cuttings with ≥3 roots (higher quality) in semi-hardwood cutting in October (57th day) and November (88th days) 2000 and 2001 in *O. europaea* L. cultivars Gentile di Larino and Frantoio[a]

Cultivar/season	Rooting class						*P*-values
	No-roots (%)		1–2 roots (%)		≥3 roots (%)		
	IBA	IBA + H_2O_2	IBA	IBA + H_2O_2	IBA	IBA + H_2O_2	
Gentile di Larino							
2000							
October	90	78	4	10	6	12	0.257
November	90	78	10	10	0	12	0.040
2001							
October	100	85	0	15	0	0	n.d.
November	98	98	2	2	0	0	n.d.
Frantoio							
2000							
October	44	20	12	14	44	66	0.034
November	42	20	12	14	46	66	0.056
2001							
October	56	56	8	6	36	39	0.865
November	55	54	7	4	38	43	0.643

[a] Cuttings were treated with hydrogen peroxide (3.5% w/v) and IBA at 4000 ppm (IBA + H_2O_2) or only with IBA (IBA) at 4000 ppm. Fifty cuttings for each cultivar and treatment combination were scored for rooting. Data were subjected to χ^2-test to statistically examine the effects of rooting treatment within each cultivar and year. *P*-values are also given, n.d.: not determined.

cuttings within these three classes, but they were subject to sampling year variability. In both cultivars, the frequency distribution in the year 2001 was not modified by treatments, while in the year 2000 the frequency distribution at the 88th day showed better performances for cuttings treated with IBA 4000 + H_2O_2.

The H_2O_2-induced changes in rooting percentage and in roots number and distribution both in 'Frantoio' and 'Gentile di Larino' suggest that olive rooting in nursery propagation cycles might be significantly improved by this simple and inexpensive treatment, especially when growing conditions of mother plants ensure high cutting quality at sampling.

4. Conclusions

During the nursery propagation cycle, the degree and the quality of rooting in olive cuttings were strongly conditioned by the genotype and the great differences existing between 'Frantoio' and 'Gentile di Larino' in their ability to regenerate roots from semi-hardwood cuttings were not removed by hydrogen peroxide treatments. However, rooting can be significantly improved by this simple and inexpensive addition of hydrogen peroxide to IBA treatment both in cultivars with high ('Frantoio') and low ('Gentile di Larino') rooting ability. In this sense, it must be pointed out that the economic threshold for cutting propagation is estimated around 20% rooting (Wiesman and Lavee, 1995). Further improvement in rooting induction could be probably obtained ensuring optimal growing conditions of mother plants.

Acknowledgements

We sincerely thank the assistance of the 'Società Pesciatina di Orticoltura' of Pescia, for the very effective assistance in the maintenance of olive cuttings. We also acknowledge Teresa D'Uva and the 'Azienda Zeoli' of Larino for providing the mother plants of 'Frantoio' and 'Gentile di Larino' used for sampling semi-hardwood cuttings. This work was supported by a grant (A6001LS) by Scuola Superiore 'S. Anna'—Pisa.

References

Altman, A., Waisel, Y., 1997. Biology of Root Formation and Development. Plenum Press, New York.

Davis, T.D., Haissig, B.E., 1994. Biology of Adventitious Root Formation. Plenum Press, New York.

FAO, 1996. Publications Division, Food and Agriculture Organization of the United Nations, 1996. http://www.fao.org/waicent/faoinfo/agricult/agp/agps/seed/oliv.htm

Gaspar, T., Kevers, C., Hausman, J.F., Berthon, L.X., Ripetti, V., 1992. Practical uses of peroxidase activity as a predictive marker of rooting performance of micropropagated shoots. Agronomie 12, 757–765.

Gaspar, T., Kevers, C., Hausnian, J.F., Ripetti, V., 1994. Peroxidase activity and endogenous free auxin during adventitious root formation. In: Lumsden, P.J., Nicholas, J.R., Davis, W.J. (Eds.), Physiology, Growth and Development of Plants in Culture. Kluwer Academic Publisher, Dordrecht.

Gaspar, T., Kevers, C., Hausman, J.F., 1997. Indissociable chief factors in the inductive phase of adventitious rooting. In: Altman, A., Waisel, Y. (Eds.), Biology of Root Formation and Development. Plenum Press, New York, pp. 55–63.

Hartmann, H.T., Brooks, R.M., 1958. Propagation of Stockton Morello cherry rootstock by softwood cuttings. Annual Report. E. Malling Research Station, pp. 101–108.

Hartmann, H.T., Loreti, F., 1965. Seasonal variation in the rooting of olive cuttings. Proc. Am. Soc. Hort. Sci. 87, 194–198.

Hartmann, H.T., Kester, D.E., Davies Jr., J.T., 1990. Plant Propagation. Principle and Practices, 5th ed. Prentice-Hall, New Jersey.

Loreti, F., Pisani, P.L.P., 1982. Physiological and technical factors affecting rooting in woody species. In: Proceedings of the 21st Horticultural Congress, Hamburg, pp. 294–309.

Mancuso, S., 1998. Seasonal dynamics of electrical impedance parameters in shoots and leaves relate to rooting ability of olive (*Olea europaea*) cuttings. Tree Physiol. 19, 95–101.

Ozkaya, M.T., Celik, M., 1999. The effects of various treatments on endogenous carbohydrate content of cuttings in easy-to-root and hard-to-root olive cultivars. Acta Hort. 474, 51–54.

Ozkaya, M.T., Celik, M., Lavee, S., 1993. The effect of rooting environment and combination of auxin polyamine on the rooting ability of Turkish olive cultivars Gemlik and Domat. Acta Hort. 356, 31–34.

Rugini, E., Fedeli, E., 1990. Olive (*Olea europaea* L.) as an oilseed crop. In: Bajaj, J.P.S. (Ed.), Biotechnology in Agriculture and Forestry, vol. 10: Legumes and Oilseed Crops I. Springer, Berlin.

Rugini, E., Politi, V., Bignami, C., Grego, S., De Agazio, M., 1990. Effect of polyamine treatments on rooting cuttings of three olive cultivars. Acta Hort. 286, 97–100.

Rugini, E., Di Francesco, G., Muganu, M., Astolfi, S., Caricato, G., 1997. The effects of polyamines and hydrogen peroxide on root formation in olive and the role of polyamines as an early marker for rooting ability. In: Altman, A., Waisel, Y. (Eds.), Biology of Root Formation and Development. Plenum Press, New York, pp. 65–73.

Sarmiento, R., Garcia, J.L., Mazuelos, C., 1990. Free amino acids in easy- and difficult-to-root olive varieties. Acta Hort. 286, 105–108.

Sebastiani, L., Tognetti, R., Di Paolo, P., Vitagliano, C., 2002. Hydrogen peroxide and indole-3-butyric acid effects on root induction and development in cuttings of *Olea europaea* L. (cv. Frantoio and Gentile di Larino). Adv. Hort. Sci. 1, 7–12.
Wiesman, Z., Epstein, E., 1987. Metabolism and transport of 5-^{3}H-indole-butyric acid in cuttings of olive. Olea 18, 29–33.
Wiesman, Z., Lavee, S., 1994. Vegetative growth-retardation, improved rooting and viability of olive cuttings in response to application of growth-retardants. Plant Growth Regul. 14, 83–90.
Wiesman, Z., Lavee, S., 1995. Enhancement of IBA stimulatory effect on rooting of olive cultivar stem cuttings. Sci. Hortic. 62, 189–198.