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Propagation of *Corylus avellana* L. by Means of Semi-hardwood Cutting: Rooting and Bud Retention in Four Italian Cultivars

C. Contessa, N. Valentini, M. Caviglione and R. Botta
(Dipartimento di Colture Arboree, Università degli Studi di Torino, Grugliasco (TO), Italy)

Summary

The effects of the application of indole-3-butyric acid (IBA) and 1,4-diaminobutane (putrescine) on the rooting and bud retention of cuttings from 'Tonda di Giffoni', 'Tonda Gentile delle Langhe', 'Daria' and 'Tonda Romana' Italian hazelnut cultivars has been investigated. Semi-hardwood stems were collected in July from the canopy and cut at every third node; the cuttings from the apical and basal portions of the stem were treated separately with IBA 1000 mg L^{-1}, IBA 1000 mg L^{-1} combined with putrescine 1600 mg L^{-1}, IBA 1500 mg L^{-1}, IBA 2000 mg L^{-1}. The cuttings were then placed on a planting bench for 60 d under mist. The presence of callus and the rooting, bud retention, number of roots per rooted cutting and mean root length were recorded. The dead cuttings and live cuttings without roots were also counted. The rooting ability resulted to be influenced by genotype, hormonal dosage and cutting diameter. The application of IBA 1000 mg L^{-1}, IBA 1000 mg L^{-1} with putrescine and IBA 1500 mg L^{-1} increased the rooting percentage in 'Tonda di Giffoni', 'Tonda Gentile delle Langhe' and 'Daria', but different responses and percentages of bud retention were observed. 'Tonda di Giffoni' showed the highest percentages of rooting and bud retention, while 'Tonda Gentile Romana' showed poor rooting, even under the hormone treatments. The results have shown that IBA promotes rooting in hazelnut cuttings, but there is also a significant effect on bud retention in relation to the hormone concentration. In general, the use of basal cuttings from vigorous stems, together with treatments with the lowest dose of IBA able to induce rooting, is recommended to reduce bud abscission.

Key words. 1,4-diaminobutane – bud abscission – hazelnut – indole-3-butyric acid – mist

Introduction

Hazelnut is an important nut crop and the world's production is mainly obtained from Turkey (604,358 t), Italy (114,992 t), the USA (33,494 t) and Azerbaijan (27,650 t) (Faostat 2010, values for 2005–2009). In recent years, Georgia, Chile, Iran, China and Poland have been planting hazelnut orchards, and an interesting growth in production has been observed. Overall, the diffusion of the species has caused an increase in the total cultivated area, which reached 602,264 ha in 2009, 8.3 % higher than in 2005 (Faostat 2010). The high demand for hazelnut plants from valuable cultivars (Nas and Read 2004) requires the development of efficient propagation techniques that are able to provide nursery material of good quality at low costs.

Hazelnut is commonly vegetatively propagated by rooted suckers, layering and grafting, but none of these methods is completely satisfactory because of the low yields and high costs (Ercisli and Read 2001). The micropropagation of hazelnut from mature tissues is still limited due to contamination during culture establishment, low adaptability of the explants to in vitro conditions and the lack of appropriate culture media (Diaz-Sala et al. 1990; Yu and Reed 1993; Nas and Read 2004; Damiano et al. 2005; Bacchetta et al. 2008).

Propagation by cutting could be a valid and alternative method to quickly obtain large numbers of plants at low costs. Hazelnut can root by softwood, semi-hardwood or hardwood cutting (Ercisli and Read 2001). However, hazelnut rooting through cutting is difficult without the inducing effect of growth regulators (Tombesi 1967; Roversi 1969; Kantarci and Ayfer 1994; Ercisli and Read 2001; Tajbakhsh et al. 2009; Cristofori et al. 2010). Softwood cuttings root quickly under mist with basal heating and a hormonal treatment, but the development of the buds stops between the 6th and 10th week of rooting (Lagerstedt 1983). Winter survival and acclimatization problems have been observed for hardwood cuttings. The best rooting has been obtained from semi-hardwood cuttings taken from mid-June to mid-July. Before this period, cuttings are too herbaceous and survive with difficulty on benches.

The most commonly used root-promoting chemical is indole-3-butyric acid (IBA). The synthetic auxins that are added exogenously are involved in the process of adventitious root formation, and the physiological stages of the rooting process are associated with changes in endogenous auxin concentrations (Nag et al. 2001; George et al. 2008).

Polyamines (PAs), mainly diamine putrescine (Put), triamine spermidine (Spd), and tetra-amine spermine (Spm),

are implicated in the regulation of the cell cycle and plant growth (Rey et al. 1994), and can induce rooting (Rey et al. 1993; Nag et al. 2001), flowering and pollination (Bibi et al. 2010). Neves et al. 2002 observed that Put, Spd and Spm are the major polyamines found in the root meristematic region in grapevine and cork oak.

Although several authors have been able to obtain rooted cuttings, some of them reported that bud abscission is a limiting factor to the propagation of hazelnut stem cuttings, even though the rooting percentage may be acceptable (Lagerstedt 1982; Bassil et al. 1991). Both the ease of rooting and the aptitude to bud abscission appear to be under genetic control (Lagerstedt 1982), but are also influenced by the method used for the propagation. It is thus possible to propagate hazelnut by cutting by adoptius the appropriate choice of clones, type of material, plant growth regulators and environmental conditions.

The aim of this study was to determine the effects of IBA and IBA associated with putrescine on the rooting and bud retention of different cutting portions from four Italian hazelnut cultivars in order to improve the nursery plant production method and favour the spread of new cultivars and selected material.

Materials and Methods

Plant material

The present study has investigated the effect of indole-3-butyric acid (IBA) and putrescine application on the rooting and bud abscission of semi-hardwood cuttings of Italian hazelnut cultivars. The experiment was carried out using cuttings taken from twenty year old plants cultivated in Cravanzana (Piedmont, NW Italy) in the Langhe District (latitude 44°34', longitude 8°07', altitude 550 m a.s.l.).

Semi-hardwood shoots, collected from the canopy, were taken on 9th July from four cultivars: 'Tonda di Giffoni', 'Tonda Gentile delle Langhe', 'Daria' ('Tonda Gentile delle Langhe' × 'Cosford') and 'Tonda Gentile Romana'. The picking time was chosen on the basis of trials and observations conducted in previous years. Stems were collected and maintained wet in white plastic bags at 4 °C (overnight); the following morning, the material was treated and placed in the greenhouse of the Dipartimento di Colture Arboree, at the University of Torino.

The portion of each stem with the terminal bud was eliminated; the following lower portion with three buds was cut and treated separately as 'apical'; the remaining portion was cut just below each third bud and the cuttings were labelled as 'basal'. Four replicates of 10 cuttings were used for each treatment and for the control. The leaves were removed, except the highest one, which was cut at half length.

The 'apical' cuttings had a mean diameter of 3.0 ± 0.4 mm and a mean length of 12.3 ± 1.7 cm, while the 'basal' cuttings had a mean diameter of 4.4 ± 0.4 mm and a length of 13.7 ± 2.2 cm.

Cutting treatments

Four different hormone treatments were tested: IBA 1000 mg L^{-1}; IBA 1000 mg L^{-1} combined with putrescine 1600 mg L^{-1} (IBA 1000+P); IBA 1500 mg L^{-1}; IBA 2000 mg L^{-1}. An untreated control was used as the reference.

Indole-3-butyric acid (Sigma, St. Louis, MO, USA) and indole-3-butyric acid, combined with putrescine (Sigma, St. Louis, MO, USA) solutions were freshly prepared by dissolving the IBA powder in 7.5–12.5 ml of 1 N NaOH, and then were brought to a volume of 1 L with distilled water. The basal portion (3 cm) of each cutting was dipped in the hormone solution for 5 min.

The cuttings were placed on a planting bench filled with a mixed perlite and vermiculite substrate (ratio 1:1) under a glass greenhouse covered with a shading net. The average temperature was 29 °C and the relative humidity was 80–85 %. The experimental design was completely randomised.

Water was supplied using an RRS-1 mist system (Netafim, Tel Aviv, Israel) with sprinkler lines controlled by means of a mist propagator. A modified wet rain sensor was used to activate the sprinklers and ensure that the leaves were kept moist. A fungicide solution (COPRANTOL® WG 32 %, Syngenta, Basel, Switzerland) was applied after 20 d.

After 60 d, the cuttings were removed and divided into categories: rooted, callused, dead and living without roots. The percentage of bud retention was calculated for all the living cuttings (rooted, callused and unrooted). The quality of rooting was evaluated by counting the roots and calculating the number of roots per rooted cutting, and by measuring the root length (root length per rooted cutting).

Statistics

The results were subjected to analysis of variance (ANOVA), which was performed on data pertaining to the individual cultivar and on all the data, in order to estimate the overall effect of the different factors: treatment, genotype and type of cutting ('apical' or 'basal'). The means were compared using the Student-Newman-Keuls Test (SNK test). The data were elaborated using the SPSS software Inc. (Chicago, USA).

Results

Effect of treatment and genotype

The effect of treatment was statistically highly significant for rooting, bud retention, cutting mortality and number of roots per cutting (Table 1). The genotype significantly influenced all the considered parameters, except the number of roots/cutting. A significant interaction between treatment and genotype was revealed for rooting, callusing and mortality percentage, and for number of roots per cutting.

IBA 1000 mg L^{-1} and IBA 1000 mg L^{-1} with putrescine promoted the highest percentage of rooting (Table 2), whereas IBA 1500 mg L^{-1} induced rooting, but was less efficient. The control reported the highest percentage of callusing, but no significant differences were found for this parameter between the treatments. IBA 2000 mg L^{-1} caused the highest percentage of dead cuttings and the lowest bud retention. The lowest percentage of dead cuttings and the highest proportion of retained buds were obtained in the control.

Plant genotype significantly affected rooting aptitude and bud retention (Table 3). The 'Tonda di Giffoni' in fact

Table 1. Effect of treatment and genotype and interaction treatment × genotype on aptitude to rooting, callusing, bud survival, cutting mortality, number of roots per cutting and root length.

	Rooting	Callusing	Bud retention	Dead cuttings	No. roots per cutting	Root mean length
Treatment	0.000	ns	0.000	0.000	0.000	ns
Genotype	0.000	0.002	0.000	0.002	ns	0.002
Treatment × Genotype	0.029	0.000	ns	0.022	0.002	ns

ns = not significant at $P \leq 0.05$

Table 2. Effect of IBA and putrescine concentration on rooting, callus formation, bud retention and cutting mortality 60 d after the hormone treatments for the 4 considered cultivars.

Treatments (mg L^{-1})	Rooting (%)	Callusing (%)	Bud retention (%)	Dead cuttings (%)
Control	12.2 c	39.5 a	18.1 a	27.4 c
IBA 1000	45.4 a	9.2 b	13.1 a	36.6 bc
IBA 1000+P	47.0 a	6.0 b	8.4 ab	39.4 bc
IBA 1500	38.3 ab	9.1 b	9.6 ab	43.6 b
IBA 2000	28.4 b	6.7 b	2.5 b	61.9 a

P = putrescine 1600 mg L^{-1}. Values followed by the same letter in a column are not significantly different at $P \leq 0.05$.

Table 3. Aptitude to rooting, callusing, bud retention and mortality of 4 hazelnut cultivars after hormonal treatment (mean data of the 4 treatments).

Cultivar	Rooting (%)	Callusing (%)	Bud retention (%)	Dead cuttings (%)
'Tonda di Giffoni'	52.1 a	8.5 b	21.7 a	31.4 b
'Tonda Gentile delle Langhe'	37.1 b	8.0 b	6.5 b	46.4 a
'Daria'	31.2 b	22.3 a	8.7 b	42.8 a
'Tonda Gentile Romana'	16.8 c	17.7 ab	4.0 b	46.4 a

Values followed by the same letter in a column are not significantly different at $P \leq 0.05$.

showed significantly higher mean percentage values fot the rooted cuttings (52.1 %) and bud retention (21.7 %) than the other cultivars. In addition, 'Tonda di Giffoni' had the lowest percentage of cutting mortality. 'Tonda Gentile Romana' was the cultivar with the lowest aptitude to rooting (16.8 %), while 'Tonda Gentile delle Langhe' and 'Daria' showed intermediate rooting values. 'Daria' had the highest value (22.3 %) regarding callus formation. No significant differences were found for cutting mortality or bud retention for 'Tonda Gentile delle Langhe', 'Daria' and 'Tonda Gentile Romana'.

Effect of treatments on cultivars

The data were analysed using the SNK test in order to define the best treatment for each cultivar. (Table 4).

'Tonda di Giffoni' showed the highest rooting formation for cuttings treated with IBA 1500 mg L^{-1} (76.7 %). The treatment produced an average number of roots of 14.4, with no significant differences with IBA 1000 mg L^{-1} (63.7 %) and IBA 1000 mg L^{-1} with putrescine (62.5 %). IBA 2000 mg L^{-1} yielded the worst rooting result (34.3 %), which, however, was not significantly different from the control value. The treated cuttings showed a low callus formation without any significant differences between the treatments. The highest value of dead cuttings was observed for the IBA 2000 mg L^{-1} treatment (53.4 %), while no significant differences were found for the other treatments, including the control. The best results, concerning bud retention, were obtained in the control (32.5 %) and with the IBA 1000 mg L^{-1} treatment (31.2 %), although the statistics were not significant, and this was followed by the treatments with IBA 1500 mg L^{-1} (23.7 %) and IBA 1000 mg L^{-1} with putrescine (17.5 %). The worst bud retention was found in the material treated with IBA 2000 mg L^{-1} (3.7 %). Rooting quality, evaluated as the number of roots/cutting and mean root length, was good for all the treatments.

Table 4. Effect of different IBA concentrations and putrescine on rooting and response of cuttings 60 d after the hormone treatments in 'Tonda di Giffoni', 'Tonda Gentile delle Langhe', 'Daria' and 'Tonda Gentile Romana'.

Cultivar	Treatments	Rooting (%)	Callusing (%)	Dead cuttings (%)	Living cuttings without roots (%)	Bud retention (%)	No. roots per rooted cutting	Root length (cm)
'Tonda di Giffoni'	Control	23.1 b	32.6 a	24.2 b	20.1 a	32.5 a	5.5 c	5.0 ab
	IBA 1000	63.7 a	0.0 b	33.7 b	2.5 b	31.2 a	15.8 a	6.7 a
	IBA 1000+P	62.5 a	0.0 b	28.7 b	8.7 ab	17.5 ab	14.6 ab	5.5 ab
	IBA 1500	76.7 a	6.2 b	17.1 b	0.0 b	23.7 ab	14.4 ab	6.6 a
	IBA 2000	34.3 b	3.8 b	53.4 a	8.5 ab	3.7 b	12.0 b	4.3 b
'Tonda Gentile delle Langhe'	Control	13.7 c	33.7 a	28.7 b	23.7 a	12.5	5.3 b	4.3
	IBA 1000	48.7 ab	5.0 b	41.2 b	5.0 b	5.0	14.4 a	5.6
	IBA 1000+P	58.2 a	1.2 b	36.8 b	3.7 b	10.0	12.3 a	5.2
	IBA 1500	38.5 abc	0.0 b	56.5 ab	5.0 b	3.8	13.4 a	5.0
	IBA 2000	26.4 bc	0.0 b	69.8 a	3.8 b	1.3	11.5 a	4.2
'Daria'	Control	10.8 c	57.5 a	19.2 c	12.5 a	13.8	1.5 c	3.9 b
	IBA 1000	53.2 a	13.9 c	27.9 bc	5.0 ab	15.0	11.5 a	8.5 a
	IBA 1000+P	33.4 b	7.1 c	58.2 a	1.2 b	2.5	9.6 ab	7.5 a
	IBA 1500	31.5 b	27.8 b	39.4 b	1.2 b	7.5	7.3 ab	6.5 ab
	IBA 2000	25.3 b	5.1 c	68.4 a	1.2 b	5.0	6.1 b	6.2 ab
'Tonda Gentile Romana'	Control	1.2 d	34.3 a	37.4	27.1 a	13.7 a	1.0 b	1.0 b
	IBA 1000	16.1 bc	18.1 ab	43.6	22.2 ab	1.2 b	2.3 a	3.5 ab
	IBA 1000+P	34.1 a	15.6 ab	33.7	16.6 ab	3.7 b	7.5 a	4.6 ab
	IBA 1500	6.4 c d	2.5 b	61.4	29.7 a	1.2 b	7.8 a	6.8 a
	IBA 2000	26.0 ab	17.0 ab	57.0	0.0 b	0.0 b	10.6 a	5.0 ab

Values followed by the same letter in a column for each cultivar are not statistically different at $P \leq 0.05$.

The 'Tonda Gentile delle Langhe' cuttings showed fairly good rooting when treated with IBA 1000 mg L^{-1} with putrescine (58.2 %) and IBA 1000 mg L^{-1} (48.7 %). No significant differences were found among the treatments regarding the percentages of callused cuttings and living cuttings without roots. The IBA 2000 mg L^{-1} and IBA 1500 mg L^{-1} treatments had a negative effect on cutting survival, with values of mortality of 69.8 % and 56.5 %, respectively. Considering bud retention, no significant differences were found among the treatments, and lower values than 10 % were observed. No significant effect on the number or length of roots per cutting was observed using the different treatments.

'Daria' showed the best rooting with the IBA 1000 mg L^{-1} treatment, reaching 53.2 % of rooted cuttings. The highest callus formation was observed in the control (57.5 %), and this was followed by IBA 1500 mg L^{-1}. The IBA 2000 mg L^{-1} and IBA 1000 mg L^{-1} with putrescine treatments let to the highest percentages of dead cuttings, reaching values of 68.4 % and 58.2 %, respectively. As far as bud retention is concerned, no significant difference was found between the treatments and the control. All the treatments showed a significant difference with the control as far as the number of roots per cutting is concerned. The application of IBA 1000 mg L^{-1} and IBA 1000 mg L^{-1} with putrescine improved the root length in comparison to the control.

The treatments with IBA 1000 mg L^{-1} with putrescine and with IBA 2000 mg L^{-1} in 'Tonda Gentile Romana' induced the highest percentages of rooting (26.0 % and 34.1 %, respectively), which were significantly different from the control. The highest presence of callusing was observed in the control (34.3 %). No significant difference was detected as far as cutting mortality is concerned. The highest values for bud retention were found for the control, while no significant differences were found for the treatments. The number of roots per cutting was high in the rooted cuttings stimulated with IBA 2000 mg L^{-1}, IBA 1500 mg L^{-1} and IBA 1000 mg L^{-1} with putrescine.

Aptitude to rooting and bud retention in the 'apical' and 'basal' cuttings

Considering all the data, the 'basal' cuttings showed the best performances as far as both the percentage of rooting and bud retention are concerned (Table 5).

The percentage of rooting was only significantly higher in the 'basal' cuttings than in the 'apical' cuttings in 'Tonda Gentile delle Langhe'. Instead, bud retention was significantly higher in the 'basal' cuttings than in the 'apical'

Table 5. Effect of the type of cutting portion on the rooting and bud retention percentages.

	'Tonda di Giffoni'	'Tonda Gentile delle Langhe'	'Daria'	'Tonda Gentile Romana'	Total mean
Rooting (%)					
Apical	55.4	23.7	26.1	16.1	30.3
Basal	48.7	50.5	36.3	17.4	38.2
P	ns	*	ns	ns	*
Bud retention (%)					
Apical	8.5	2.0	3.0	4.5	4.5
Basal	35.0	11.0	14.5	3.5	16.0
p	*	*	*	ns	*

* Significant at $P \leq 0.05$; ns = not significant

ones in 'Tonda di Giffoni', 'Tonda Gentile delle Langhe' and 'Daria', but not in 'Tonda Gentile Romana'.

Discussion

The results have shown that the rooting of cuttings is promoted by IBA, with different responses, which depend on the genotype and IBA concentration.

'Tonda di Giffoni' obtained the highest rooting percentage of the considered cultivars, confirming its aptitude to be a relatively easy rooting variety. Conversely, 'Tonda Gentile Romana' resulted to be a hard rooting cultivar. Similar results were obtained by Falaschi and Loreti (1969) on 'Tonda Gentile Romana', and by Cristofori et al. (2010) on 'Tonda di Giffoni' and 'Tonda Gentile Romana'.

The observed differences in aptitude to root formation of the cultivars could be related to differences in plant vigour, the highest aptitude was observed in 'Tonda di Giffoni' and progressively becoming lower in 'Tonda Gentile delle Langhe', 'Daria' and 'Tonda Gentile Romana'. Plant vigour appears to be one of the factors that facilitates the vegetative propagation of hazelnut.

In general, the control showed a lower percentage of rooting than the hormonal treatments, in agreement with Ercisli and Read (2001), but it also demonstrated a higher bud retention than the material treated with exogenous auxin. Although the IBA treatments promoted rooting in hazelnut cuttings, there was also a significant effect on bud abscission, in agreement with the results reported by Paglietta (1965), Bassil et al. (1991), and Ercisli and Read (2001).

There are several possible reasons for the low bud retention of the propagated cuttings. Hartmann et al. (1997), reported that bud and cutting death during the winter following rooting could be due to the toxicity of the high IBA concentrations used on semi-hardwood material. It is interesting to note that inhibitor substances can be produced due to physiological stress and IBA treatments. Sun and Bassuk (1993) observed an ethylene production after an exogenous IBA application in rose cuttings, with physiological disorders of the vegetative organs.

The best results for 'Tonda di Giffoni' were obtained with the application of IBA 1000 mg L^{-1} and IBA 1500 mg L^{-1}, with a percentage of bud retention that was comparable with the control. The IBA application at 1000 mg L^{-1} also gave an acceptable rooting performance in 'Tonda Gentile delle Langhe' (with putrescine) and 'Daria'. 'Tonda Gentile Romana' can be considered a tough rooting cultivar, and the response was poor even with the treatments with IBA and putrescine.

On average, the use of putrescine substantially did not improve hazelnut rooting or the number of roots/cutting, in comparison with the use of IBA 1000 mg L^{-1} alone. However, the treatment with IBA 1000 mg L^{-1} + putrescine in 'Tonda Gentile Romana' stimulated more rooting than the other treatments, in agreement with Cristofori et al. (2010). The use of IBA, associated with putrescine, produced the highest rooting percentage in 'Tonda Gentile delle Langhe', although this percentage was not significantly different from the value obtained with the application of IBA 1000 mg L^{-1} alone.

Howard (1967) and Kantarci and Ayfer (1994) observed that callused cuttings eventually formed roots, and assumed that two months for rooting is an insufficient rooting time. In fact, the cultivars with low rooting capacity, such as 'Daria' and 'Tonda Gentile Romana', showed rooting activity in callused cuttings (22.5 % and 17.0 %, respectively) 20 d after removal from the substrate and transfer to pots under mist conditions. These observations suggest that a longer time under mist may be beneficial for the purpose of rooting, especially for cultivars that do not root easily, on condition that the substrate composition and watering are set appropriately.

Overall, the results of the research indicate that the 'basal' portions of cuttings are the best material for propagation and are in agreement with Proebsting and Reihs (1991) and Ughini and Roversi (2002). The stem portion, and consequently the diameter of the cutting, showed an effect on bud retention, confirming that the vigour and developmental stage of the plant material influence the rooting response.

In conclusion, the rooting aptitude in hazelnut cuttings is influenced to a great extent by the genotype, hormonal dosage and cutting diameter. Each examined cultivar responded in a different way to hormonal application, therefore it is not possible to define an unique treatment. In general, the lowest concentration of IBA able to induce

rooting is recommended to reduce bud abscission, the main factor that limits the propagation of hazelnut. An efficient solution to overcome the problem of bud drop still has to be found; the indications obtained from this work, which had the aim of improving the yield of rooted plants in commercial propagation, are to choose cuttings of appropriate diameter (> 4 mm) from vigorous stems and to optimise the concentration of IBA on the basis of the requirements of each cultivar.

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Addresses of authors: Cecilia Contessa (corresponding author), Nadia Valentini, Mauro Caviglione and Roberto Botta, Dipartimento di Colture Arboree, Università degli Studi di Torino, Via Leonardo Da Vinci, 44 10095 Grugliasco (TO), Italy, e-mail (corresponding author): cecilia.contessa@unito.it.