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Decreasing the concentration of IBA or combination with ethylene inhibitors improve bud retention in semi-hardwood cuttings of hazelnut cultivar 'Tonda Gentile delle Langhe'

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/133161> since

Published version:

DOI:10.1016/j.scienta.2011.09.029

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8
9 Cecilia Contessa, Nadia Valentini, Roberto Botta. 2012. Decreasing the concentration of IBA or
10 combination with ethylene inhibitors improve bud retention in semi-hardwood cuttings of hazelnut
11 cultivar 'Tonda Gentile delle Langhe'. *Scientia Horticulturae*, Volume 131, 22 November 2011, Pages
12 103–106. DOI:10.1016/j.scienta.2011.09.029

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28 **Decreasing the concentration of IBA or combination with ethylene inhibitors**
29 **improve bud retention in semi-hardwood cuttings of hazelnut cultivar ‘Tonda**
30 **Gentile delle Langhe’**

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34 **Cecilia Contessa^{*}, Nadia Valentini, Roberto Botta**

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38 Dipartimento di Colture Arboree, Università degli Studi di Torino,

39 Via Leonardo Da Vinci, 44 10095 Grugliasco (TO), Italy

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43 *Corresponding author: Tel.: +39 011 6708816; Fax: +39 011 6708658

44 e-mail address: cecilia.contessa@unito.it

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Abstract

The effect of two concentrations (500 and 1000 mgL⁻¹) of indole-3-butyric acid (IBA) and the combination of IBA treatments (1000 mgL⁻¹) with two ethylene inhibitors, 1-MCP (1-Methylcyclopropene) and AgNO₃, on adventitious root formation and bud retention of semi-hardwood cuttings were investigated in hazelnut (*Corylus avellana* L.) cultivar ‘Tonda Gentile delle Langhe’.

The IBA 500 mgL⁻¹ treatment promoted percentages of rooting (70.0%) similar to IBA 1000 mgL⁻¹ treatment but reduced bud abscission resulting in 56.3% of rooted cuttings with at least one bud retained. The use of 1-MCP and AgNO₃ in combination with IBA 1000 mgL⁻¹ treatment reduced bud abscission without modifying the rooting response.

Keywords: *Corylus avellana*; bud abscission; indole-3-butyric acid; 1-MCP; silver nitrate

1. Introduction

The most common techniques of propagation of hazelnut are by stool layering and rooted suckers. Micropropagation is the safest and most productive form of propagation, but in hazelnut it still shows low yield due to contamination during culture establishment and the limited adaptability of the explants to *in vitro* conditions (Bacchetta et al., 2008; Yu and Reed, 1993).

The propagation by cutting can be considered an alternative, rapid and relatively economic method but, in spite of the numerous studies conducted for the hazelnut, the technique has not yet been transferred to an industrial scale due to poor rooting ability and cutting survival of most cultivars.

76 In some plant species, root formation initiates without any treatment, while in others it
77 requires the application of growth regulators, usually auxins (Syros et al., 2004). The hazelnut
78 hardly roots simply by cutting and treatments with auxins are required, as reported by several
79 researchers (Cristofori et al., 2010; Ercisli and Read, 2001; Kantarci and Ayfer, 1994). The
80 rooting ability of cuttings is strongly influenced by collection time, age of the cutting and
81 genotype (Cristofori et al., 2010). Although most authors were able to obtain rooting in
82 several hazelnut cultivars, less information is available on the bud retention. As first reported
83 by Lagerstedt (1982) bud abscission is a limiting factor to propagation of hazelnut stem
84 cuttings, even though the rooting percentage may be acceptable (Bassil et al., 1991;
85 Proebsting and Reihls, 1991).

86 It is known that auxin can affect the ethylene production (Abeles et al., 1992; Ecker,
87 1995; Wei et al., 2000). In ornamental species it was observed that ethylene, produced
88 following a stress, has an effect on leaf drop, bud abortion and bud abscission, senescence and
89 physiological disorders of vegetative and generative organs (Reid, 1985; Reid and Wu, 1992;
90 Serek et al., 2006).

91 Several investigations have been reported on the use of ethylene inhibitors such as silver
92 salt (silver thiosulfate and silver nitrate) 1-MCP (1-Methylcyclopropene) and N,N-dipropyl(1-
93 cyclopropenylmethyl)amine (DPCA) to prevent ethylene action at the receptor level (Seglie et
94 al., 2010; Serek and Sisler, 2001; Sisler et al., 2009).

95 The aim of this study was to evaluate the effect of IBA (Indole-3-butyric acid)
96 treatments at two concentrations (500 and 1000 mgL⁻¹) and the use of two ethylene inhibitors,
97 1-MCP (1-Methylcyclopropene) and AgNO₃, combined with IBA1000 mgL⁻¹ treatment, on
98 rooting and bud retention of semi-hardwood hazelnut cuttings from cultivar ‘Tonda Gentile
99 delle Langhe’.

100 101 **2. Materials and methods**

102 **2.1 Plant material**

103 The experiment was carried out in 2010 on cuttings collected from twelve years old
104 plants grown in Cravanzana (Piedmont, NW Italy) in the Langhe District (latitude 44°34',
105 longitude 8°07', altitude 550 m a.s.l.).

106 Semi-hardwood shoots, collected from the canopy, were harvested on 13th July from
107 'Tonda Gentile delle Langhe' cultivar when the nut had attained full size, just before seed
108 growth. Semi-hardwood shoots were chosen as propagation material following literature
109 (Lagerstedt, 1982; Ercisli and Read, 2001). Shoots were collected, sprayed with water and
110 maintained wet overnight in white plastic bags at 4°C; the following day the material was
111 treated and placed in the greenhouse of the Dipartimento di Colture Arboree, of the
112 University of Torino.

113 The terminal portion of shoots was discarded; the sub-terminal portion (Proebsting and
114 Reihls, 1991) was cut every third node producing 2 buds cuttings (the basal third bud was
115 buried). Cuttings had a mean diameter of 4.5±0.8 mm and mean length of 16.1±2.4 cm. The
116 basal leaf was removed whereas the highest one was cut at half length. Four replicates of 20
117 cuttings per treatment were used for the trial.

118

119 **2.2 Treatment of plant material**

120 *Experiment 1. Effect of two IBA levels*

121 Two different growth regulator treatments were tested: IBA 500 mgL⁻¹ and IBA 1000
122 mgL⁻¹. Indole-3-butyric acid (Sigma, St. Louis, MO, USA) solutions were freshly prepared
123 dissolving the IBA powder in 3.75 ml and 7.5 ml of NaOH 1N afterwards brought to a
124 volume of 1L with distilled water. The basal portion (3 cm) of each cutting was dipped in the
125 hormone solution for 1 minute. Untreated cuttings were used as control (Control 1).

126

127 *Experiment 2. Effect of 1-MCP treatment in combination with IBA 1000 mgL⁻¹ treatment*

128 Two sets of cuttings dipped in tap water were placed in a gas-tight cabinet (40 L) at
129 21°C for 6 h; the first set was exposed to 500 ppb 1-MCP (EthylBloc[®], Rohm & Haas
130 Company, USA) while the second one was not treated and used as control (Control 2).
131 Afterwards, the basal portion of cuttings of both sets was dipped in IBA 1000 mgL⁻¹ solution
132 for 1 minute.

133

134 *Experiment 3. Effect of AgNO₃ treatment in combination with IBA 1000 mgL⁻¹ treatment*

135 The basal portion of cuttings was dipped in IBA 1000 mgL⁻¹ solution for 1 minute;
136 cuttings were transferred into the planting bench and sprayed with AgNO₃ 250 mgL⁻¹ (Sigma,
137 St. Louis, MO, USA). Cuttings treated with IBA 1000 mgL⁻¹ in experiment 1 were used as
138 control (Control 3).

139

140 All treated cuttings were planted in a growing bench filled with a mixed perlite and
141 vermiculite substrate (ratio 1:1) under a glass greenhouse covered with a shading net (60%)
142 where temperature ranged between 26 and 28°C and relative humidity was 80-90%. The
143 experimental design was completely randomised.

144 Irrigation was supplied using a RRS-1 mist system (Netafim, Tel Aviv, Israel) with
145 sprinkler lines under the control of a mist propagation controller. A modified wet rain sensor
146 was used as an artificial leaf to activate the sprinkler.

147 After 2 months, cuttings were removed and classified as: rooted, callused, living
148 unrooted, and dead.

149 The number of cuttings with retained buds was counted. The percentage of cuttings with
150 retained buds was calculated across all living (rooted, callused and unrooted) cuttings (Living
151 cuttings with retained buds) and over rooted cuttings (Rooted cuttings with retained buds). The
152 mean number of retained buds was calculated as the number of cuttings with at least one
153 retained buds (Number of retained buds per cutting).

154 The quality of rooting was evaluated counting roots and calculating the number of roots
155 per rooted cutting, and measuring root length (Root length per rooted cutting), using a ruler.

156 Data were statistically analysed by ANOVA and Tukey's test using the SPSS software
157 Inc. (Chicago, USA).

159 **3. Results**

160 The auxin treatments tested in experiment 1 produced a highly significant effect on
161 rooting in comparison with the control (Table 1). With regard to the percentage of rooting, no
162 significant differences were found between the IBA treatments with percentages of rooting of
163 70.0% for IBA 500 mgL⁻¹ and 72.5% for IBA 1000 mgL⁻¹. The highest presence of callusing
164 was observed in Control 1 (60.0%). No significant difference was detected as concerns cutting
165 mortality (7.5-16.3%).

166 Considering all the living cuttings, including those without roots or callus, the control
167 showed the highest percentage of cuttings with living buds (91.3%); this percentage was
168 significantly different from the value recorded in the IBA 1000 mgL⁻¹ treatment.

169 Treatment with IBA 500 mgL⁻¹ resulted in the highest amount of rooted cuttings with
170 living buds (56.3%) with a significant difference to IBA 1000 mgL⁻¹ treatment and the Control
171 1. The Control 1 retained the highest number of living buds per cutting (1.8), considering only
172 cuttings with at least one retained bud, but showed the lowest number of roots/cutting and the
173 shortest roots. No significant differences of root development were found between IBA
174 treatments.

175 Ethylene inhibitors in combination with IBA 1000 mgL⁻¹ had no significant effects on
176 any parameters except for bud retention (Table 2). With 1-MCP treatment a significantly
177 higher percentage of buds retention was observed on treated cuttings, yielding 43.8% cuttings
178 having both rooting and at least one living bud. The AgNO₃ treatment significantly promoted

179 bud retention in rooted cuttings yielding 45.0% of rooted cuttings with at least one bud
180 retained.

181

182 **4. Discussion**

183 The response of semi-hardwood hazelnut cuttings of ‘Tonda Gentile delle Langhe’
184 following application of two IBA concentrations (500-1000 mgL⁻¹) and the supply of volatile
185 (1-MCP) and non-volatile (AgNO₃) inhibitors of ethylene action in combination with
186 IBA1000 mgL⁻¹ treatment were investigated.

187 Our results showed that IBA treatments were effective in promoting rooting. The relation
188 between IBA concentration and bud death in semi-hardwood hazelnut cuttings was confirmed,
189 in agreement with the results by Bassil et al. (1991) in which treatments with IBA at 1000 to
190 2500 mgL⁻¹ caused almost complete bud abscission.

191 Data obtained in our study support the hypothesis that the application of exogenous auxin
192 affect bud abscission, probably due to ethylene production, in agreement with the results
193 reported for different species of ornamental plants and cut flowers (Rungruchkanont et al.,
194 2007; Sun and Bassuk, 1993; Zhao and Hasenstein, 2009). Arteca and Arteca (2008)
195 demonstrated that in *Arabidopsis thaliana* L. inflorescence stalks and leaves treated with high
196 level of exogenous IAA exhibited an increase in ethylene production 2 h following treatment
197 initiation. They also showed that the highest rates of ethylene production are found in actively
198 dividing cells as in case of younger leaves and root tips.

199 The effect of ethylene inhibitors on bud retention has already been tested and showed a
200 positive effect on preservation of cut flowers (Seglie, et al. 2010; Sun and Bassuk, 1993).
201 Cuttings of ‘Tonda Gentile delle Langhe’ responded to the application of ethylene inhibitors
202 improving bud retention in rooted cuttings. This indicates that ethylene action is actually at
203 least one of the factors that causes bud abscission following application of IBA. 1-MCP and

204 AgNO₃ provided a significant protection against ethylene preventing bud drop and had not a
205 negative influence on adventitious root formation of cuttings.

206

207 In conclusion, our results showed that the use of low IBA concentration (500 mgL⁻¹) in
208 ‘Tonda Gentile delle Langhe’ promotes an adequate rooting and reduce bud abscission in
209 comparison with higher IBA concentrations. The yield obtained make this cutting protocol
210 interesting and suitable for the propagation of hazelnut. The higher bud retention following the
211 use of ethylene inhibitors indicates the involvement of the hormone in the process of bud
212 abscission. The effect of ethylene inhibitors should be further investigated in combination with
213 IBA 500 mgL⁻¹ in cultivars exhibiting good rooting capacity, such as ‘Tonda di Giffoni’ and
214 ‘Tonda Gentile delle Langhe’, and in cultivars recalcitrant to rooting, in this case associated
215 with higher doses of IBA.

216

217 **Acknowledgements**

218 The research was funded by Regione Piemonte (Project: “Filiera nocciolo: aspetti agronomici
219 e tecnologici per il miglioramento delle produzioni piemontesi” - CORIFIL).

220

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271

272 Table 1. Effect of IBA treatment on cuttings of 'Tonda Gentile delle Langhe' after 60 days. Means
 273 followed by the same letter are not statistically different at $p \leq 0.05$ (small) or $p \leq 0.01$ (capital).
 274

Treatments	Rooted (%)	Callused (%)	Living unrooted (%)	Dead (%)	Number of roots per rooted cutting	Root length per rooted cutting (cm)	Living cuttings with retained buds (%)	Number of retained buds per cutting	Rooted cuttings with retained buds (%)
Experiment 1									
Control 1 (Untreated)	7.5 B	60.0 A	25.0 A	7.5	1.2 B	1.4 B	91.3 A	1.8 a	7.5 c
IBA 500	70.0 A	2.5 B	16.2 AB	11.3	19.2 A	7.4 A	73.8 AB	1.1 b	56.3 a
IBA 1000	72.5 A	6.3 B	4.9 B	16.3	18.5 A	5.2 A	41.3 B	1.2 b	30.0 b

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Table 2. Effect of IBA1000 treatment in combination with two ethylene inhibitors (1-MCP and AgNO₃) on cuttings of ‘Tonda Gentile delle Langhe’ 60 days after the application. *significantly different at p≤0.05.

Treatments	Rooted (%)	Callused (%)	Living unrooted (%)	Dead (%)	Number of roots per rooted cutting	Root length per rooted cutting (cm)	Living cuttings with retained buds (%)	Number of retained buds per cutting	Rooted cuttings with retained buds (%)
Experiment 2									
Control 2 (IBA 1000)	61.3	8.8	15.0	15.0	15.1	5.1	47.5	1.1	31.3
IBA1000+1-MCP	61.3	7.5	16.3	15.0	13.2	5.0	61.3	1.1	43.8
<i>p</i>	ns	ns	ns	ns	ns	ns	*	ns	*
Experiment 3									
Control 3 (IBA 1000)	72.5	6.3	5.0	16.3	18.5	5.2	41.3	1.2	30.0
IBA 1000+AgNO ₃	57.5	6.3	11.3	25.0	15.4	5.0	62.5	1.2	45.0
<i>p</i>	ns	ns	ns	ns	ns	ns	ns	ns	*

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