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Investigation on Effects of IBA Treatments and Ethylene Inhibitors on the Rooting and Bud Retention of Semi-hardwood Cuttings from 'Tonda Gentile delle Langhe' Hazelnut Cultivar

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The most common techniques of propagation of hazelnut are by stool layering and rooted suckers. The propagation by cutting can be considered an alternative, rapid and relatively economic method but the technique has not yet been transferred to an industrial scale due to poor rooting ability and cutting survival of most cultivars. The effect of IBA (indole-3-butyric acid) treatments at two concentrations (500 and 1000 mg L^{-1}) on the rooting and bud retention of semi-hardwood hazelnut cuttings from cultivar 'Tonda Gentile delle Langhe' was investigated in 2010 and 2011. In addition, the application of two ethylene inhibitors, 1-MCP (1-Methylcyclopropene) and $AgNO_3$, combined with IBA 1000 treatment, and the use of two different substrates (perlite and vermiculite; perlite and loam) were tested. The plant material was collected in July and, following the treatments, was placed on a planting bench under mist. 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. The IBA treatments promoted high percentages of rooting (75.6% for IBA 500 and 76.9% for IBA 1000, means 2010-2011) but IBA 500 reduced bud abscission in comparison with IBA 1000 (64.4 and 48.1% of rooted cuttings with retained buds, respectively). The use of 1-MCP and $AgNO_3$ in combination with IBA 1000 reduced bud abscission without modifying the rooting response. The perlite and loam substrate gave a lower percentage of rooting in comparison with the perlite and vermiculite one. For perlite and loam substrate the IBA 1000 treatment showed the best results both for rooting and bud retention. 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 by cutting.

INTRODUCTION

Hazelnut (*Corylus avellana* L.) is commonly vegetatively propagated for orchard establishment. Propagation by cutting could be a valid method to quickly obtain large numbers of plants at low costs; however, the use of this technique presents some difficulties either due to poor root initiation or abscission of the vegetative buds (Contessa et al., 2011b). Both the ease of rooting

and the aptitude to bud abscission appear to be under genetic control (Lagerstedt, 1982; Cristofori et al., 2010; Contessa et al., 2011b), but are also influenced by the method used for the propagation.

The hazelnut hardly roots simply by cutting and treatments with auxins are required, as reported by several researchers (Contessa et al., 2011a; Ercisli and Read, 2001; Kantarci and Ayfer, 1994). It is known that auxin can affect the ethylene production (Ecker, 1995). In ornamental species it was observed that ethylene, produced following a stress, has an effect on leaf drop, bud abortion and bud abscission (Serek et al., 2006). Bud abscission is a limiting factor to propagation of hazelnut stem cuttings (Lagerstedt 1982; Bassil et al., 1991; Proebsting and Reihs, 1991; Contessa et al., 2011a). Several investigations have been reported on the use of ethylene inhibitors such as silver salt (silver thiosulfate and silver nitrate), 1-MCP (1-Methylcyclopropene) and N,N-dipropyl(1- cyclopropenylmethyl)amine (DPCA) to prevent ethylene action at the receptor level (Seglie et al., 2010).

The aim of this study was to evaluate the effect on rooting and bud retention of semi-hardwood hazelnut cuttings from cultivar 'Tonda Gentile delle Langhe' of: i) treatments with different concentrations (500 and 1000 mg L^{-1}) of IBA (indole-3-butyric acid); ii) treatment with two ethylene inhibitors, 1-MCP (1- Methylcyclopropene) and $AgNO_3$ combined with IBA1000 treatment; iii) the use of different substrates (vermiculite/perlite and perlite/loam).

MATERIALS AND METHODS

Plant material

The experiment was carried out on cuttings collected from twelve years old plants grown 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 harvested on 13th and 26th July in 2010 and 2011, respectively, from 'Tonda Gentile delle Langhe' cultivar when the nut had attained full size, just at onset of seed growth. Semi-hardwood shoots were chosen as propagation material following literature (Lagerstedt, 1982; Ercisli and Read, 2001). The terminal portion of shoots was discarded and the sub-terminal portion (Proebsting and Reihs, 1991) was cut every third node producing 2 buds cuttings (the basal third bud was buried). Cuttings had a mean diameter of 4.3 ± 0.3 mm and mean length of 18.2 ± 2.3 cm. The basal leaf was removed whereas the highest one was cut at half length. Four replicates of 20 cuttings per treatment were used for the trials.

Treatment of plant material

Experiment 1. Two different growth regulator treatments were tested: IBA 500 mg L^{-1} and IBA 1000 mg L^{-1}. Indole-3-butyric acid (Sigma, St. Louis, MO, USA) solutions were freshly prepared dissolving the IBA powder in 3.75 ml and 7.5 ml of NaOH 1 N afterwards brought to a volume of 1 L with distilled water. The basal portion (3 cm) of each cutting was dipped in the IBA solution for 1 min. All cuttings were planted in a growing bench filled with a mixed perlite and vermiculite substrate (ratio 1:1). Untreated cuttings were used as control (Control 1). The experiment was performed in 2010 and 2011 years.

Experiment 2. Two sets of cuttings dipped in tap water were placed in a gastight cabinet (40 L) at 21°C for 6 h; the first set was exposed to 500 ppb 1-MCP (EthylBloc®, Rohm & Haas Company, USA) while the second one was not treated and used as control (Control 2). Afterwards, the basal portion of cuttings of both sets was dipped in IBA 1000 mg L^{-1} solution for 1 min. The experiment was performed in 2010.

Experiment 3. The basal portion of cuttings was dipped in IBA 1000 mg L^{-1} solution for 1 min; cuttings were transferred into the planting bench and sprayed with $AgNO_3$ 250 mg L^{-1} (Sigma, St. Louis, MO, USA). Cuttings treated with IBA 1000 mg L^{-1} in experiment 1 were used as control (Control 1). The experiment was performed in 2010.

Experiment 4. Cuttings treated with two different IBA concentrations 500 mg L^{-1} and 1000 mg L^{-1} were planted in a growing bench with two different substrates: mixed perlite and vermiculite (ratio 1:1, substrate S1) and perlite and loam (ratio 1.4:1, substrate S2). Untreated cuttings were used as control for the IBA treatments (Control 4). The experiment was performed in 2011.

The cuttings were planted in a growing bench under a glass greenhouse of the Dipartimento di Colture Arboree, of the University of Torino, covered with a shading net (60%). The experimental design was completely randomised. Mist irrigation was supplied using a RRS-1 mist system (Netafim, Tel Aviv, Israel) with sprinkler lines under the control of a mist propagation controller. A modified wet rain sensor was used as an artificial leaf to activate the sprinklers. After 2 months, cuttings were removed and classified as: rooted, callused, living unrooted and dead. The percentages of rooted cuttings with at least one retained bud was also detected. The quality of rooting was evaluated counting roots, calculating the number of roots per rooted cutting, and measuring root length (root length per rooted cutting). Data were statistically analysed by ANOVA and Tukey's test using the SPSS software Inc. (Chicago, USA). The effect of substrate, treatment and interaction substrate x treatment (experiment 4) was also analysed.

RESULTS

The auxin treatments tested in experiment 1 produced a significant effect on rooting in comparison with the Control 1 (Table 1). With regard to the percentage of rooting, no significant differences were found between the IBA treatments with percentages of rooting of 75.6% for IBA 500 mgL^{-1} and 76.9% for IBA 1000 mgL^{-1}. The highest presence of callusing and cutting mortality was observed in Control 1 (55.1% and 27.5%, respectively). Treatment with IBA 500 mgL^{-1} resulted in the highest amount of rooted cuttings with living buds (64.4%), despite the difference with IBA 1000 mgL^{-1} (48.1%) was not statistically significant. No significant differences of root development were found between IBA treatments.

Ethylene inhibitors in combination with IBA 1000 mgL^{-1} had no significant effects on any parameters except bud retention (Table 2). A significantly higher percentage of rooted cuttings with retained buds (43.8%) was observed with 1-MCP treatment in comparison with the Control 2. The $AgNO_3$ treatment significantly promoted bud retention in rooted cuttings yielding 45.0% of rooted cuttings with at least one bud retained.

The effect of substrate was statistically significant for rooting, callusing, cutting mortality, percentage of rooted cuttings with retained buds, and number of roots per rooted cutting (Table 3). The treatment significantly influenced all the considered parameters, except the cutting mortality and the percentage of living unrooted cutting. A significant interaction between substrate and treatment was revealed only for the number of roots per rooted cutting.

The effects of the use of vermiculite/perlite and loam/perlite substrates are shown in Table 4. The vermiculite/perlite substrate was more effective in promoting rooting and bud retention in comparison to the loam/perlite (81.2% and 55.0% of rooted cutting and 69.4% and 51.9% of rooted cuttings with retained bud, respectively). The vermiculite/perlite substrate reduced callusing, and increased the number of roots per cutting with highly significant statistical differences in comparison with the loam/perlite substrate (13.4 and 7.4 roots/cuttings respectively) while no significant effect on the length of roots per cutting was observed.

No statistical differences were observed between IBA 500 mgL^{-1} and IBA 1000 mgL^{-1} treatments for the considered parameters, confirming the results of the experiment 1 (Table 5). On the contrary, Control 4 showed statistical differences with the IBA treatments for all parameters except for the living unrooted and the dead cuttings.

DISCUSSION

The results have shown that the application of IBA treatments are effective to determine high percentages of rooting in cuttings of the hazelnut cultivar 'Tonda Gentile delle Langhe'. The two concentration tested demonstrated that low concentration of IBA (500 mg L^{-1}) are able to promote good rooting without determine high level of bud abscission. The percentage of bud retention decreased at increasing IBA concentration, in agreement with the results of Bassil et al. (1991) and Contessa et al, 2011a.

Data obtained in our study support the hypothesis that the application of exogenous auxin affect bud abscission, probably due to ethylene production, in agreement with the results reported for different species of ornamental plants and cut flowers (Sun and Bassuk, 1993; Zhao and Hasenstein, 2009).

Cuttings of 'Tonda Gentile delle Langhe' responded to the application of ethylene inhibitors improving bud retention in rooted cuttings without changing the rooting capacity. This indicates that ethylene action is actually at least one of the factors that causes bud abscission following application of IBA. 1-MCP and $AgNO_3$ provided a significant protection against ethylene preventing bud drop. The applications of ethylene inhibitors can be transferred to other cultivars with low rooting capacity that requires the application of high level of IBA (Cristofori et al., 2010; Contessa et al., 2011b).

The comparison between two substrates showed that the vermiculite/perlite substrate lead to a higher root formation in respect to the loam/perlite one. This results suggest that a light and porous substrate increases the rooting, probably due to a greater availability of oxygen at the base of the cutting during root differentiation.

CONCLUSIONS

In conclusion, our results showed that the use of low IBA concentration (500 mgL^{-1}) in 'Tonda Gentile delle Langhe' promotes an adequate rooting and reduce bud abscission in comparison with higher IBA concentrations. The higher bud retention following the use of ethylene inhibitors indicates the involvement of the hormone in the process of bud abscission. The use of a light and aerated substrate such as the vermiculite/perlite mix has allowed to obtain a better rooting. The use of a lower percentage of loam with vermiculite and perlite could still be considered to provide a light and aerated substrate able to supply e nutrients and avoid the need of repotting at the and of the rooting period.

Table 1. Effect of IBA treatments on cuttings of 'Tonda Gentile delle Langhe' after 60 days (mean values 2010-2011)

Treatments	Rooted (%)	Callused (%)	Living unrooted (%)	Dead (%)	Rooted cuttings with retained buds (%)	Number of roots per rooted cutting	Root length per rooted cutting (cm)
Experiment 1							
Control 1	13.7b	55.1a	3.7b	27.5a	13.7b	2.1b	2.3b
IBA 500	75.6a	1.3b	8.7ab	14.4ab	64.4a	15.7a	5.9a
IBA 1000	76.9a	3.8b	13.1a	6.2b	48.1a	16.5a	5.6a

Means followed by the same letter are not statistically different at $p \leq 0.05$.

Table 2. Effect of IBA1000 treatment in combination with two ethylene inhibitors (1-MCP and $AgNO_3$) on cuttings of 'Tonda Gentile delle Langhe' after 60 days (mean values 2010)

Treatments	Rooted (%)	Callused (%)	Living unrooted (%)	Dead (%)	Rooted cuttings with retained buds (%)	Number of roots per rooted cutting	Root length per rooted cutting (cm)
Experiment 2							
Control 2 (IBA 1000)	61.3	8.8	15.0	15.0	31.3	15.1	5.1
IBA1000+1-MCP	61.3	7.5	16.3	15.0	43.8	13.2	5.0
p	ns	ns	ns	ns	*	ns	ns
Experiment 3							
Control 3 (IBA 1000)	72.5	6.3	5.0	16.3	30.0	18.5	5.2
IBA 1000+AgNO3	57.5	6.3	11.3	25.0	45.0	15.4	5.0
p	ns	ns	ns	ns	*	ns	ns

*significantly different at $p \leq 0.05$ or ** $p \leq 0.01$. ns=not significant

Table 3. Effect of substrate type, treatment and interaction substrate x treatment on cutting of 'Tonda Gentile delle Langhe'.

	Rooting	Callusing	Living unrooted cutting	Cutting mortality	Rooted cuttings with retained buds	Number of roots per rooted cutting	Root length per rooted cutting
Substrate	0.006	0.043	ns	0.023	0.033	0	ns
Treatment	0.000	0.002	ns	ns	0.000	0.000	0.000
Substrate x Treatment	ns	ns	ns	ns	ns	0.043	ns

ns=not significant at $p \leq 0.05$

Table 4. Effect of vermiculite/perlite (S1) and loam/perlite (S2) substrates on cutting of 'Tonda Gentile delle Langhe' treated with IBA after 60 days

Treatments	Rooted (%)	Callused (%)	Living unrooted (%)	Dead (%)	Rooted cuttings with retained buds (%)	Number of roots per rooted cutting	Root length per rooted cutting (cm)
Experiment 4							
S1	81.2	0.6	10.0	8.1	69.4	13.4	5.8
S2	55.0	18.7	13.1	13.1	51.9	7.4	5.3
p	*	*	ns	ns	*	*	ns

*significantly different at p≤0.05 or ** p≤0.01. ns=not significant

Table 5. Effect of treatments with IBA 500 and IBA 1000 on cutting of 'Tonda Gentile delle Langhe' in S1 and S2 substrates after 60 days

Treatments	Rooted (%)	Callused (%)	Living unrooted (%)	Dead (%)	Rooted cuttings with retained buds (%)	Number of roots per rooted cutting	Root length per rooted cutting (cm)
Experiment 4							
Control 4	17.5b	50.6a	25.6	6.2	16.9b	2.2b	2.2b
IBA 500	65.6a	12.5b	14.4	7.5	59.4a	8.6a	5.2a
IBA 1000	70.6a	6.9b	8.7	13.7	61.9a	12.1a	5.9a

Means followed by the same letter are not statistically different at p≤0.05.

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