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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’

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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 $AgNO_3$, 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 $AgNO_3$ 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.

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

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

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; Serek and Sisler, 2001; Sisler et al., 2009).

The aim of this study was to evaluate the effect of IBA (Indole-3-butyric acid) treatments at two concentrations (500 and 1000 mgL^{-1}) and the use of two ethylene inhibitors, 1-MCP **(**1-Methylcyclopropene) and $AgNO_3$, combined with IBA1000 mgL^{-1} treatment, on rooting and bud retention of semi-hardwood hazelnut cuttings from cultivar ‘Tonda Gentile delle Langhe’.

2. Materials and methods

2.1 Plant material

The experiment was carried out in 2010 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 July from 'Tonda Gentile delle Langhe' cultivar when the nut had attained full size, just before seed growth. Semi-hardwood shoots were chosen as propagation material following literature (Lagerstedt, 1982; Ercisli and Read, 2001). Shoots were collected, sprayed with water and maintained wet overnight in white plastic bags at 4°C; the following day the material was treated and placed in the greenhouse of the Dipartimento di Colture Arboree, of the University of Torino.

The terminal portion of shoots was discarded; 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 4.5±0.8 mm and mean length of 16.1±2.4 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 trial.

2.2 Treatment of plant material

Experiment 1. Effect of two IBA levels

Two different growth regulator treatments were tested: IBA 500 mgL^{-1} and IBA 1000 mgL^{-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 1N afterwards brought to a volume of 1L with distilled water. The basal portion (3 cm) of each cutting was dipped in the hormone solution for 1 minute. Untreated cuttings were used as control (Control 1).

Experiment 2. Effect of 1-MCP treatment in combination with IBA 1000 mgL^{-1} treatment

Two sets of cuttings dipped in tap water were placed in a gas-tight 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 mgL^{-1} solution for 1 minute.

Experiment 3. Effect of $AgNO_3$ treatment in combination with IBA 1000 mgL^{-1} treatment

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

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

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 sprinkler.

After 2 months, cuttings were removed and classifiedas: rooted, callused, living unrooted, and dead.

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

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

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

3. Results

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

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

Treatment with IBA 500 mgL^{-1} resulted in the highest amount of rooted cuttings with living buds (56.3%) with a significant difference to IBA 1000 mgL^{-1} treatment and the Control 1. The Control 1 retained the highest number of living buds per cutting (1.8), considering only cuttings with at least one retained bud, but showed the lowest number of roots/cutting and the shortest roots. 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 for bud retention (Table 2). With 1-MCP treatment a significantly higher percentage of buds retention was observed on treated cuttings, yielding 43.8% cuttings having both rooting and at least one living bud. The $AgNO_3$ treatment significantly promoted bud retention in rooted cuttings yielding 45.0% of rooted cuttings with at least one bud retained.

4. Discussion

The response of semi-hardwood hazelnut cuttings of ‘Tonda Gentile delle Langhe’ following application of two IBA concentrations (500-1000 mgL^{-1}) and the supply of volatile (1-MCP) and non-volatile ($AgNO_3$) inhibitors of ethylene action in combination with IBA1000 mgL^{-1} treatment were investigated.

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

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 (Rungruchkanont et al., 2007; Sun and Bassuk, 1993; Zhao and Hasenstein, 2009). Arteca and Arteca (2008) demonstrated that in *Arabidopsis thaliana* L. inflorescence stalks and leaves treated with high level of exogenous IAA exhibited an increase in ethylene production 2 h following treatment initiation. They also showed that the highest rates of ethylene production are found in actively dividing cells as in case of younger leaves and root tips.

The effect of ethylene inhibitors on bud retention has already been tested and showed a positive effect on preservation of cut flowers (Seglie, et al. 2010; Sun and Bassuk, 1993). Cuttings of ‘Tonda Gentile delle Langhe’ responded to the application of ethylene inhibitors improving bud retention in rooted cuttings. 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 and had not a negative influence on adventitious root formation of cuttings.

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 yield obtained make this cutting protocol interesting and suitable for the propagation of hazelnut. The higher bud retention following the use of ethylene inhibitors indicates the involvement of the hormone in the process of bud abscission. The effect of ethylene inhibitors should be further investigated in combination with IBA 500 mgL^{-1} in cultivars exhibiting good rooting capacity, such as 'Tonda di Giffoni' and 'Tonda Gentile delle Langhe', and in cultivars recalcitrant to rooting, in this case associated with higher doses of IBA.

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

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

Table 2. Effect of IBA1000 treatment in combination with two ethylene inhibitors (1-MCP and $AgNO_3$) 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
p	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+AgNO3	57.5	6.3	11.3	25.0	15.4	5.0	62.5	1.2	45.0
p	ns	ns	ns	ns	ns	ns	ns	ns	*