

Cutting Type and IBA Treatment Duration Affect *Teucrium fruticans* Adventitious Root Quality

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Abstract

Root development of stem cuttings of Silver Germander (*Teucrium fruticans*) was investigated in relation to cutting type and indole-3-butyric acid (IBA) treatment. Terminal cuttings of a clone grown in Sicily were trimmed to three types: terminal cuttings with apex (TWA), terminal cuttings without apex (TWOA) or sub-terminal cuttings (ST). To verify the cutting response to exogenous auxin, cuttings were dipped to a 2.0 cm depth in a 0.5% indole-3-butyric acid solution for 0, 5 or 7 minutes. Overall percent survival was 97 to 98%. Rooting percent, root number and root length were affected by cutting type and indole-3-butyric acid treatment. In general, TWA cuttings demonstrated a higher capacity to form roots than cuttings without apex (TWOA and ST cuttings). In absence of indole-3-butyric acid treatment, TWA and ST cuttings gave higher rooting percentages than TWOA cuttings. Exposing cuttings to indole-3-butyric acid improved percent rooting, number of roots per cutting and root length. The best results in terms of rooting percentage and root number per cuttings were obtained with TWA cuttings in combination with 7 min indole-3-butyric acid basal dip. However, cuttings taken farther down the stem, such as sub-terminal cuttings gave satisfactory rooting performance as well. We suggest that the use of all cutting types tested associated to indole-3-butyric acid basal dip for 5 or 7 min may be beneficial to propagators wishing to produce *T. fruticans* rooted cuttings with well-developed root system.

Keywords: basal dip, growth regulators, Labiatae, native plants, rooting

Introduction

The genus *Teucrium* belongs to the family Labiatae and contains more than 300 perennial herbs, shrubs and sub-shrubs mainly native to Mediterranean region and south-western Asia (Bryant, 2003). *Teucrium fruticans*, also known as Silver Germander, is a shrub native to Southern Europe and North Africa (Graham, 1992). Cultivated for its attractive blue/violet flowers and evergreen foliage, gray-green above and silver-white beneath, *T. fruticans* is high drought tolerant and suitable for landscaping in areas with Mediterranean-type environment (Cassaniti *et al.*, 2009; La Mantia *et al.*, 2012; Lopez *et al.*, 2006). Four-year old plants (sold in 22-cm-diameter pots) obtained by rooted cuttings are generally used for landscaping. *Teucrium fruticans* propagation by seed is difficult because of poor germination (Bryant, 2003) and high percentage of male sterility (Ojeda and Diez, 1992); as a result, plant propagation via seed is unsatisfactory for the commercial production (Frabetti *et al.*, 2009). The species has been also characterized as difficult to propagate by cutting in certain periods of the year (Frangi and Nicola, 2004). To circumvent these problems micropropagation has been recommended (Frabetti *et al.*, 2009). Vegetative propagation by stem

cuttings has been successfully applied to other *Teucrium* species (*T. divaricatum*, *T. polium*, *T. chamaedrys*, *T. flavum*, *T. scorodonia*) (Dirr and Heuser, 2006; Iapichino *et al.*, 2006; MacDonald, 1987; Maloupa *et al.*, 2008). Although the effects of exogenous auxin on adventitious root formation in *Teucrium* species has been documented (Dirr and Heuser 1987; Frangi and Nicola 2004; Maloupa *et al.*, 2008) no research on the influence of the morphological characteristics of the cuttings on rooting of *T. fruticans* has been reported. The aim of our study was to examine rooting of stem cuttings in relation to cutting type and IBA treatment duration in a *T. fruticans* clone grown in Sicily.

Materials and methods

Plant material

The research was conducted at the University of Palermo, Experimental Station in the Northern coast of Sicily (Italy) (longitude 13° 19' E, latitude 38° 9' N). Terminal 30 cm long stem cuttings were harvested on 23 April 2012 from actively growing stock plants. Plants were 5 years old and from a single Sicilian unnamed clone. Cuttings were stored overnight at 6 °C. The next day, the cuttings were trimmed to three types:

softwood stem-tip cuttings in which the tip was retained (terminal cuttings with apex, TWA), softwood stem cuttings in which the tip was removed (terminal cuttings without apex, TWOA) or semi-hardwood cuttings taken farther down the stem (sub-terminal cuttings, ST) (Fig. 1).



Fig. 1. *Teucrium frutescens* cutting types tested; from left, terminal with apex, terminal without apex and sub-terminal

All cuttings were trinodal with the lower cut below a node at the base of each cutting. Leaves were removed from the base of the stem; length and diameter of the cuttings were 12-14 cm and 0.4 cm, respectively.

Auxin treatment

To verify the cutting response to exogenous auxin, cuttings were dipped to a 2.0 cm depth in a 0.5% indole-3-butyric acid (IBA) solution (Frangi and Nicola, 2004) for five or seven minutes. Reagent grade IBA (Sigma n. I 5386) was dissolved in 90% ethanol, then brought to final concentration of 6% ethanol 94% distilled water (v/v). Untreated cuttings were dipped in distilled water (control= 0 minutes).



Fig. 2. *Teucrium frutescens* cuttings in the rooting bench

Rooting conditions

Propagation was performed on 24 April 2012 in an unheated greenhouse covered with clear polyethylene (PE) and external 50% shade-cloth. Air temperature in the greenhouse was 18-22 °C during the day and 14-16 °C during the night. Cuttings were inserted 4 cm deep in a bottom heated bench containing perlite. Basal heat was provided at constant temperature of 25 ± 2 °C. Benches were covered with clear PE to maintain high relative humidity. Intermittent mist operated daily 30 sec every 2 hours from 8:30 AM to 6:00 PM. Ventilation of the cuttings was increased with time by increasing size of the holes made in the plastic.

Experimental design, data collection and analysis

The design was a randomized complete block (RCB) with three replications, nine treatments [three cutting types x three IBA exposure time duration (0, 5 or 7 minutes)] and 20 cuttings per replication. An IBA cutting exposition of a few seconds (quick dip method) was not included in our experimental design because in a preliminary experiment (performed in the same season) we did not find any significant increase in rooting capacity as compared to the untreated control. After 4 and 6 weeks, cuttings were evaluated for percent survival, percent rooting, number of roots, length of the six longest roots. Cuttings with non-withered stems (with and without roots) were considered to have survived. Cuttings with necrotic tissue without roots were classified as dead. Percentage data were subjected to arcsin transformation before ANOVA analysis. Mean separation was performed by Fisher least-significant-difference test at $p \leq 0.01$ (Petersen, 1985).

Plant establishment

Single plants were transferred to each plastic pot (diameter 16 cm) containing a peat-perlite mixture 1:1 (v/v) and kept for five weeks in a lath house covered with 70% shade-cloth. Acclimatized plants were thereafter transferred in the open field.

The experiment was repeated a second year using the same stock plants and obtaining similar results.

Results

Cutting survival and rooting percentage

Overall percent survival was 97 to 98%; no significant effect of cutting type and IBA treatment duration was found on cutting survival (Tab. 1). Rooting percent was affected by cutting type and IBA treatment (Tab. 1). In absence of IBA treatment, TWA and ST cuttings gave higher rooting percentages than TWOA cuttings. Percent rooting in TWA cuttings increased from 70% in absence of IBA treatment to 100 and 96% in the presence of IBA for 5 and 7 min, respectively; percent rooting in TWOA cuttings also increased from 56% in untreated control to 100% in cuttings receiving either 5 or 7 min IBA basal dips. Percent data recorded on ST cuttings paralleled data recorded on TWA and TWOA cuttings exposed to IBA giving higher performance than control.

Root number

The number of roots was affected by cutting type and IBA treatment. In general, TWA cuttings demonstrated a

higher capacity to form roots than cuttings without apex (TWOA and ST cuttings). Exposing cuttings to IBA improved the number of roots per cutting (Fig. 2). Data collected 4 weeks after planting revealed that formed roots on TWA cuttings increased from 1.0 in untreated control to 14 and 18 roots on stem cuttings receiving 5 and 7 min

IBA basal dips, respectively. Root count in TWOA cuttings also increased from 1.5 roots in absence of IBA to 11 roots at 5 min duration and to 20 roots at 7 min duration. Data collected on ST cuttings supported the trend established for the other two cutting types with a maximum of 13 roots for the cuttings receiving 7 min IBA basal dip. Data collected

Tab. 1. Effects of cutting type and IBA treatment on *Teucrium fruticans* rooting

Treatments		Roots per cutting (n)		Root length (mm)		Rooting (%)	Survival (%)
Cutting type	IBA (min.)	Weeks after planting					
		4	6	4	6	6	6
TWA	0	1.0 ± 0.8 e	11.4 ± 4.2 d	3.5 ± 1.6 de	6.8 ± 2.5 e	70 ± 0.3d	97.0 ± 1.3 a
TWA	5	14.7 ± 3.8 b	18.4 ± 3.9 bc	5.9 ± 0.5 ab	10.1 ± 3.9 abc	100 a	98.0 ± 2.8 a
TWA	7	18.5 ± 3.3 a	24.0 ± 3.6 a	4.7 ± 1.8 bcd	11.5 ± 4.1a	96 ± 2.8ab	97.1 ± 2.5 a
TWOA	0	1.5 ± 0.7 e	4.0 ± 2.4 e	2.5 ± 0.8 e	5.3 ± 2.8 f	56 ± 2.1e	98.0 ± 2.1 a
TWOA	5	11.0 ± 4.2 cd	14.3 ± 2.5 d	7.0 ± 1.0 a	10.5 ± 4.1 ab	100 a	98.0 ± 1.8 a
TWOA	7	20.1 ± 1.5 a	20.2 ± 2.1ab	5.0 ± 2.1 abc	9.3 ± 2.1bcd	100 a	97.6 ± 2.8 a
ST	0	1.9 ± 0.6 e	6.1 ± 2.6 e	2.6 ± 1.2 e	5.0 ± 2.7 f	81 ± 3.4cd	98.0 ± 3.6 a
ST	5	9.7 ± 2.9 d	13.1 ± 4.7 d	3.2 ± 0.9 de	7.8 ± 3.2 e	100 a	98.0 ± 2.0 a
ST	7	13.0 ± 1.8 bc	15.0 ± 2.2 d	4.2 ± 1.6 cd	7.7 ± 3.1 e	92 ± 1.7abc	97.6 ± 2.5 a

Values represent mean ± standard error. In each column, means followed by the same letters are not significantly different at the $p \leq 0.01\%$ level by Fisher's protected least significant difference test.

TWA = terminal cuttings with apex; TWOA = terminal cuttings without apex; ST = sub-terminal cuttings.



Fig. 3. *Teucrium fruticans* sub-terminal cuttings, exposed to 7 min IBA basal dip, showing a well developed rooting system

after 6 weeks again revealed that rooting TWA, TWOA and ST cuttings in the presence of IBA substantially increased the number of roots per cutting as compared to untreated cuttings. All cutting types generally produced higher number of roots per cutting with 7 min IBA basal dip as compared with 5 min IBA basal dip. Among the treatments tested, TWA cuttings in combination with 7 min IBA basal dip was the most effective in terms of number of adventitious roots produced.

Root length

Root length also varied by cutting type and IBA treatment. Generally, TWA cuttings formed longer roots than cuttings without apex (TWOA and ST cuttings). Data collected four and six weeks after planting, revealed that root length increased from the untreated control to stem cuttings receiving IBA basal dips. However, no significant differences were found between 5 and 7 min IBA basal dip.

Plant establishment and performance

In the present study, propagated plants grew vigorously, were morphological normal and began to flower during spring 2013.

Discussion

Our results are similar to those of Krisantini *et al.* (2003) who report tip cuttings having a higher capacity to form roots than decapitated cuttings in *Grevillea* and are consistent with those of Marks (1996) who found inhibition of rooting following tip removal in *Quercus* and *Daphne* cuttings. The higher rooting percentage obtained using tip cuttings rather than stem (decapitated) cuttings

has been hypothesized as due to production of a factor (presumably, IAA basipetally transferred from the meristems) that from the apex has an important role in adventitious root formation even though exogenous auxin is applied. In our study, the slightly higher, but not statistically different percentage rooting observed in ST cuttings as compared to TWA cuttings could be attributed to the fact that IAA was supplied from quiescent axillary buds. However, our study also demonstrated that the number of roots formed in cuttings with apices was superior to that observed in those without apices even in the presence of exogenous auxin.

In our study, the application of IBA improved rooting percentage and root number as compared to untreated control. IBA has been reported to markedly increase adventitious root formation in many species (Blazich, 1988; De Klerk *et al.*, 1999; Hartmann *et al.*, 1997). Our results are only partially in agreement with those obtained by Frangi and Nicola *et al.* (2004) who found IBA treatments enhancing rooting in *T. fruticans* stem tip cuttings. These authors report a low rooting response during spring (40% rooting) even applying exogenous auxin. Our higher response in the same season could be related to diverse genotype studied or different environmental conditions under which the stock plants were grown. Our results are in accord with the results obtained by Carpenter and Cornell (1992) who, investigating the adventitious root proliferation response of three *Hibiscus rosa-sinensis* cultivars, found that rooting is highly influenced by IBA application duration and concentration. Our results are also consistent with those of Telgen *et al.* (2007) who reported that diluted IBA-solutions and longer dipping times resulted in more uniform cuttings and higher root number than quick dips in *Rosa hybrida* 'First Red'.

In the present study, the best results in terms of rooting percentage and root number per cuttings were obtained with TWA cuttings. However, this propagation method yields fewer cuttings per plant compared with methods using cuttings taken farther down the stem, such as sub-terminal cuttings which, in our study, also performed well. Therefore, we suggest that all cutting types tested and exposed to an IBA basal dip of 5 or 7 min may be successfully used by propagators to produce *T. fruticans* rooted cuttings with well-developed root system.

This is an important information for nursery growers since there is little knowledge about cultural techniques of this species of increasing importance in the landscape and nursery trade.

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