



PROPAGATION OF *ARTEMISIA ARBORESCENS* L. BY STEM-CUTTING: ADVENTITIOUS ROOT FORMATION UNDER DIFFERENT CONDITIONS

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Abstract

Artemisia arborescens L. has gained a strong importance worldwide due to its many industrial uses and it has been recently considered as ornamental plant. A major constraint to its widespread cultivation is represented, by far, by the scarce availability of high-quality plant material for field establishment; hence, development of a fast and effective methods for its vegetative propagation is needed. An experiment was conducted to assess the effects of different harvest periods, NAA, and rooting substrates on rooting of stem cuttings of *A. arborescens*. Semi hardwood cuttings were collected from wild plants in February, April, and November. Half of the material was treated with 0.4% NAA and placed on different mixtures of sphagnum peat and perlite (2 : 1, 1 : 1, and 1 : 2 v/v) under mist. After 40 days the percentage of rooted cuttings was significantly influenced by the harvest period as cuttings collected in February showed the highest rooting rate, and numerous alive but not rooted cuttings evidenced callus formation. In contrast, the use of different rooting substrates as well as NAA addition did not show any significant effect on rooting capacity. The best results, in terms of root number (4.2) and root length (8.8 cm), were achieved on cuttings grown in a 1 : 1 v/v sphagnum peat : perlite mixture, without NAA application.

Key words: harvest period, naphthalene acetic acid, rooting substrates, Tree wormwood.

INTRODUCTION

Artemisia arborescens L. (Asteraceae) is one of the numerous species of genus *Artemisia* and is typical for the wild flora of southern Mediterranean area. It is a woody perennial shrub with green-silvery leaves and yellow flowers appearing throughout the late spring until summer depending on the environmental conditions. The species has a large number of uses in ethno-medicine (Palmese et al. 2001, Vicidomini 2007) as antispasmodic, antipyretic, anti-inflammatory and abortifacient (Dessi et al. 2001), as antiherpesvirus (Saddi et al. 2007), and as active antimicrobial agent against *Listeria monocytogenes* (Militello et al. 2011). Moreover, as aridoresistant and nitrophilous plant, *A. arborescens* has a great importance due to its potential use in environmental engineering (Cella and Collu 2004); due to its hardiness, it may be strongly considered for ornamental purposes as other related species of the same genus (Deng et al. 2012) as hedge plant in low-maintenance gardens.

Seed germination and micropropagation techniques were studied in different *Artemisia* species (Liu et al.

2004, Govindaraj and Bollipo 2007, Zia et al. 2007, Sujatha and Ranjitha Kumari 2008, Shatnawi 2011), but only few studies are available on the vegetative propagation of this genus, as in *A. ludoviciana* Nutt. (Bradley Rowe and Cregg 2002), *A. tridentata* Nutt. (Alvarez-Cordero and McKell 1979) and *A. vulgaris* L. 'Variegata' (Deng et al. 2012).

Micropropagation and seed multiplication are largely utilized practices, but it is well known that they are not always suitable to all conditions. As a matter of fact, the first method is expensive (Saranga and Cameron 2006) and time-consuming in order to obtain a good adaptation from *in vitro* culture to open field conditions, whereas the second one is characterized by high genetic variability and scarcely homogeneous production. Hence, propagation by stem cuttings remains a very appropriate method in order to obtain a rapid propagation with reduced costs.

Rooting capacity in cuttings is influenced by internal factors, as genotype, nutritional status or phenological stage, and external factors, as temperature and light intensity (Hartmann et al. 1997, Agbo and Obi 2008,

Priadjati et al. 2001). Internal factors are strictly related to the amount of plant growth regulators that are physiologically necessary for the rooting phase (Guo et al. 2009, Amri et al. 2010, Zobolo 2010). For such reason, it is also important to know the favorable growing conditions for plants (external factors) in order to select suitable rooting substrates (Ofori et al. 1996, Mesh et al. 1997, Tchoundjeu et al. 2002) and environmental parameters.

Adventitious root formation is mediated by multiple changes in plant metabolism and is controlled by interdependent physiological phases where auxins play an important role stimulating the root formation. Exogenous auxins are commonly used to improve natural rooting efficiency in stem cuttings, but it was demonstrated in various plant species that relatively high auxin concentrations are required only during the induction phase, while during development these plant growth regulators become inhibitory (Hartmann et al. 1997). The effects of different auxins and concentrations on adventitious rooting of many plant species were studied previously (Moura-Costa and Lundoh 1994, Aminah et al. 1995, Copes and Mandel 2000).

Root formation of stem-cuttings is also affected by physical and chemical characteristics of rooting substrates (bulk density, porosity, water-holding capacity, pH, etc.) which allow promoting or inhibiting root growth (Hartmann et al. 1997). Numerous researches were already conducted on the effects of different substrates on rooting of stem-cuttings in different ornamental species (Pivetta et al. 1999, Lee et al. 2000, Rifaki et al. 2001).

Moreover, adventitious rooting is often related to the season in which cuttings are collected, as the availability of internal auxin as well as nutrients content of plant tissue may determine significant variations in root capacity of cuttings (Rosier et al. 2004).

Hence, taking into account the scarce availability of researches conducted about *in vivo* propagation in *Artemisia* species (Pridham 1963, Weglarz and Zalecki 1984); the aim of this work was to define an effective and low-cost propagation protocol of *Artemisia arborescens* L. by means of stem-cuttings method, investigating the effects of different harvest periods, auxin (NAA) application and rooting substrates.

MATERIALS AND METHODS

Plant material and experimental design

The trial was carried out in 2010/11, in an East-West oriented greenhouse (200 m²) with steel structure and polymethacrylate methyl cover, located at the Research Unit for Mediterranean Flower Species near Palermo (38°05'25.22" N, 13°31'18.15" E, 23 m altitude), on the coastal area of North Western Sicily.

Semi hardwood cuttings 15 cm-long were harvested from basal and lateral stems of donor plants

of a wild population (38°06'47.74" N, 13°31'16.38" E, 31 m altitude) of *Artemisia arborescens* growing on rocky slopes located in alkaline and nitrophilous substrates.

In order to evaluate the effect of harvest period on rooting capacity, cuttings were collected in February, April, and November 2010 during bud break, active elongation and resting stage, respectively. For each harvest period, a factorial experimental design (three rooting substrates with or without NAA application), with three replications per treatment, was used. Each replication consisted of 22 cuttings.

Auxin application and rooting substrates

In 50% of the harvested stem cuttings, after apex and basal leaves removal, the basal end was treated with 0.4% of α -naphthaleneacetic acid (NAA) powder (Germon, Gerlach GmbH, Lubbecke, Germany), whereas the remaining cuttings were left untreated. Both NAA-treated and untreated cuttings were then inserted to a 5-cm depth in 66-cell polystyrene seed trays. Each tray contained a different combinations (2 : 1, 1 : 1, 1 : 2 v/v) of sphagnum peat (TECNIC - Free Peat B.V., Sluiskade, Vriezenveen, Netherlands) and perlite Ø 2-5 mm (AGRIPAN 100, Perlite Italiana s.r.l., Corsico (MI), Italy). Organic matter and organic nitrogen content of sphagnum peat were 46% and 0.5%, respectively. The seed trays were placed into a bottom-heated bench (6 m × 1.2 m) covered with a polyethylene film and equipped with a mist nebulization system under natural photoperiod. Substrate temperatures ranged from 18°C to 24°C throughout the trial, and basal heating was necessary only during the winter; relative humidity of the bench was maintained at approximately 75% for each harvest period by varying number and duration of irrigations.

Measurement and data analysis

In each treatment, data on cuttings survival rate (%), rooting rate (%) of survived cuttings, mean number of roots/cutting, and mean length (cm) of roots of each cutting were collected forty days after the beginning of the trial. The cuttings were considered rooted when they had a root at least 0.2 cm long.

All collected data were subjected to univariate analysis of variance (ANOVA) by means of the package "Statistica" (V. 6.0 for Windows, Statsoft Inc., Tulsa, OK) to determine the effect of treatments. When *F* was significant, means were separated by means of the LSD protected Fisher's test at $p \leq 0.05$. Prior to statistical analysis, ANOVA assumptions about the homogeneity of variances and the normality of data distribution were verified in all studied parameters. Before ANOVA, cuttings survival and rooting percentages were converted in angular values (arcsin transformation) (Gomez and Gomez 1984).

RESULTS

Rooting percentage of *Artemisia arborescens* cuttings was affected by the different treatments: in two groups of cuttings (dead and alive non rooted) in the different harvest collection periods determined highly significant ($p \leq 0.01$) differences while in the remaining group (rooted) significant ($p \leq 0.05$) differences were recorded (Table 1).

In particular, mortality rate of cuttings, decreased sharply from 44.4% (February) to 26.6% and to 5.3%, respectively in April and November (Table 1).

A clear increase throughout collecting dates (from 7.8 in February to 48.1 and 62.0% in April and November, respectively) was observed in the number of cuttings that, although alive, did not produce roots but formed callus (Table 1).

The counting of rooted cuttings first decreased from 47.8% to 25.3% (in cuttings collected in February and April, respectively) then increased to 32.7% (November) (Table 1).

Addition of exogenous auxin (NAA) did not influence the percentage of dead cuttings (mean 27.0% and 24.0% in cuttings treated with NAA 0 and 0.4%, respectively) as well as the number of alive but non rooted cuttings (36.5% and 42.0% for treated and untreated cuttings, respectively) (Table 1).

In the same way, rooting rate was not affected by NAA treatment as mean values of 36.5% and 34.0%

rooted cuttings were recorded irrespective of auxin application (Table 1).

The adoption of different rooting substrates did not affect cuttings survival and dormancy: in fact, the use of a lower amount of sphagnum peat in the growing mixture (as in the 1 : 2 v/v substrate), although bringing a moderate increase of dormant cuttings (alive but not rooted, +18% with respect to the 2 : 1 v/v peat : perlite), did not exert any evident effect on the mortality rate (-4% compared to the 2 : 1 substrate) (Table 1).

Moreover, the rooting ability of cuttings (R%) was not influenced by the substrates as mean rooting rates of 40.6%, 38.8% and 26.4% were recorded for 2 : 1 v/v, 1 : 1 v/v and 1 : 2 v/v peat : perlite, respectively, with no significant difference among treatments (Table 1).

Harvest date seemed to influence the number of roots of cuttings, as higher values were recorded in the cuttings harvested in February (4.7 roots/cutting) in comparison to those obtained in November (2.6 roots/cutting) (Fig. 1). Rooting substrate also affected root emission as higher amount was observed on cuttings grown in 1 : 1 v/v peat : perlite (4.2 roots/cutting) whereas lower values were measured on cuttings rooted in 1 : 2 v/v peat : perlite (2.6). In contrast, the use of exogenous auxin did not have any effect on the number of roots (mean 3.5 roots/cutting) (Fig. 1).

The harvest period and the rooting substrate appeared to have some effect on the mean length of roots,

Table 1. Effect of harvest periods, NAA application and rooting substrates on rooting rates of *Artemisia arborescens* cuttings.

Rooting rates in the three harvest periods (mean values \pm SE across NAA application and rooting substrates)			
Harvest periods	Dead (%)	Alive non rooted (%)	Rooted (%)
February	44.4 \pm 4.5 a	7.8 \pm 4.0 b	47.8 \pm 5.5 a
April	26.6 \pm 3.5 b	48.1 \pm 4.0 a	25.3 \pm 2.7 b
November	5.3 \pm 2.3 c	62.0 \pm 2.7 a	32.7 \pm 1.6 ab
Significance of <i>F</i> test	**	**	*
Rooting rates with or without NAA application (mean values \pm SE across harvest dates and rooting substrates)			
NAA	Dead (%)	Alive non rooted (%)	Rooted (%)
0.4%	27.0 \pm 5.0	36.5 \pm 6.5	36.5 \pm 4.0
0%	24.0 \pm 5.0	42.0 \pm 7.4	34.0 \pm 4.5
Significance of <i>F</i> test	n.s.	n.s.	n.s.
Rooting rates on different rooting substrates (mean values \pm SE across harvest dates and NAA application)			
Rooting substrates	Dead (%)	Alive non rooted (%)	Rooted (%)
peat : perlite 2 : 1	28.2 \pm 6.0	31.2 \pm 8.0	40.6 \pm 5.5
peat : perlite 1 : 1	23.9 \pm 5.5	37.3 \pm 6.4	38.8 \pm 5.0
peat : perlite 1 : 2	24.2 \pm 6.5	49.3 \pm 5.2	26.5 \pm 2.6
Significance of <i>F</i> test	n.s.	n.s.	n.s.

The means (\pm SE) within a column followed by different letters are significantly different according to one-way ANOVA and separated using LSD test.

**,* = significant at $p \leq 0.01$ and $p \leq 0.05$, respectively.

n.s. = non significant (according to one-way ANOVA).

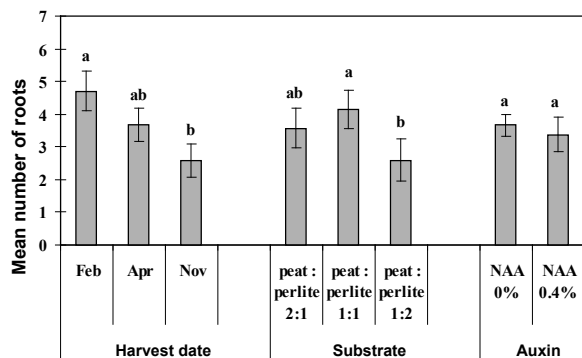


Fig. 1. Mean number of roots of *Artemisia arborescens* cuttings according to harvest date, rooting substrate and NAA application.

Values are means ± SE. For each treatment, histograms with different letters are significantly different at $p \leq 0.05$ (LSD test).

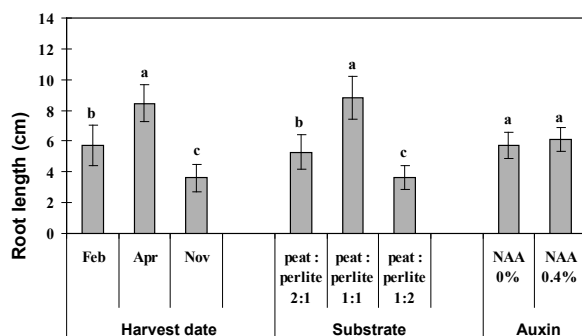


Fig. 2. Mean length of roots of *Artemisia arborescens* cuttings according to harvest date, rooting substrate and NAA application.

Values are means ± SE. For each treatment, histograms with different letters are significantly different at $p \leq 0.05$ (LSD test).

as cuttings collected in November and rooted in the 1 : 2 v/v peat : perlite showed shorter roots (less than 4 cm) than those harvested in April and rooted in the 1 : 1 v/v peat : perlite substrate (longer than 8 cm) (Fig. 2). NAA application did not affect significantly the root length as a mean value of 5.9 cm was recorded irrespective of auxin presence (Fig. 2).

DISCUSSION

Harvest period

A progressive decrease of rooting rate of *Artemisia arborescens* cuttings was recorded in the present experiment from the first harvest date (February) to the other investigated periods (April and November). This outcome agrees with that reported by other authors (Alvarez-Cordero and McKell 1979) who observed that cuttings of *Artemisia tridentata* obtained in winter

showed a greater rooting activity than those collected in other periods.

The influence of harvest period on rooting efficiency of cuttings was previously reported for several plant species (Hartmann et al. 1997, Sharma and Aier 1989). Karami and Salehi (2010) reported that the rooting ability of Rohida (*Tecomella undulata* (Sm) Seem) was seasonal as the rate recorded in late winter was higher than that of cuttings harvested in late autumn. Akoumianaki-Ioannidou et al. (2010a), during a year-round propagation trial of *Eleagnus × ebbingei*, observed that cuttings taken in autumn had the highest rate of rooting irrespective of auxin concentration. Similarly, in an experiment on vegetative multiplication of *Pterocephalus perennis* (Akoumianaki-Ioannidou et al. 2010b), the highest rooting success was recorded in cuttings planted at spring with respect to those planted in autumn and summer. Iliev et al. (2010) reported that in cuttings of *Chamaecyparis lawsoniana* collected in March, the rooting potential was highest in comparison with the cuttings harvested in July and November. Finally, Guo et al. (2009) referred that cuttings of *Paeonia* collected in three different periods did not vary in percent mortality and number of roots but significantly differed in rooting percentage as well as in root length.

These differences in rooting performance are generally attributed to initial physiological characteristics of the plant material, like nutrient reserves such as carbohydrate and nitrogen in the plant tissues at the time of picking (Hambrick et al. 1991, Tsipouridis et al. 2006). In particular, with regard to the nutritional status of propagation material, it was previously observed that high carbohydrate and low nitrogen content of cuttings contribute mainly to the root formation (Rowe et al. 1999, Rapaka et al. 2005).

Therefore, the observed differences among the three harvest periods regarding the mortality and rooting rate of *Artemisia arborescens* cuttings might be related to a specific level of nutrient reserves and/or to the interactions between the endogenous auxins.

Auxin application

During the present experiment, *Artemisia arborescens* cuttings treated with NAA showed similar rooting rates than those observed on non-treated cuttings. This result concurs with the outcomes from a study on southernwood (*A. abrotanum* L.) propagation (Schroeder and Le Duc 1996), where no significant difference was recorded between untreated cuttings and these treated with auxin.

The inefficacy of auxin treatments on rooting response of *A. arborescens* cuttings seem to confirm earlier results recorded on different *Platanus* species (Myers and Still 1979, Dirr and Heuser 1987, Panetos et al. 1994) where no significant difference was recorded between cuttings treated and untreated with

auxin, especially when cuttings were characterized by a natural high rooting potential. Opposite results were obtained by Alvarez-Cordero and McKell (1979) who referred that indole-3-butyric acid (IBA) treatment in cuttings of *A. tridentata* increased root formation as a function of increased auxin concentration. The different behavior of the studied species could suggest that some propagation characteristics may be genotype-dependent, as observed in *Grevillea* (Krisantini et al. 2006) and *Magnolia* (Sharma et al. 2006), with a high variability in rooting capacity of the cuttings.

In the current study auxin treatments did not increase root length of *Artemisia* cuttings. This result is supported by recent findings about some *Euphorbia* species: Fascella et al. (2008), after an experiment on *in vivo* propagation of *Euphorbia milii* × *lophogona* hybrids did not observe any differences between non-treated and NAA-treated cuttings as concerned cuttings viability, root length and plant establishment. Ibáñez-Torres (2004) observed that auxin treatment (IBA and NAA) did not influence root length of *Euphorbia lagascae* cuttings after one month compared to control, but differences were evident only after two months, a time that is often considered too long for the nursery industry.

Rooting substrates

Results obtained in the present experiment showed that rooting rate of *Artemisia arborescens* cuttings was not influenced by rooting substrates. In the same way, Fascella and Zizzo (2009) previously observed that a progressive reduction of the peat percentage in the rooting substrates did not significantly affect growth performance of rooted cuttings of *Euphorbia* × *lomi* Thai hybrids.

Rooting substrates investigated in our study seemed to influence only root number and length of *Artemisia* cuttings as higher values were recorded on 1 : 1 v/v peat : perlite. Likewise, Ercişli et al. (2005) reported that the highest root number of *Rosa dumalis* hardwood cuttings was obtained in peat/perlite substrate. King et al. (2011) reported that softwood baldcypress cuttings should be grown in a substrate with intermediate water-holding capacity (peat : perlite 1 : 1 v/v) to achieve an acceptable balance between rooting percentage and rooted cutting quality. A balanced composition of a substrate based on peat and perlite seems to be a frequent option in order to obtain high rooting success in cuttings of ornamental plants (Akoumianaki-Ioannidou et al. 2010a,b, Collado et al. 2010), probably due to an adequate equilibrium between air and moisture content.

With the present research a vegetative propagation protocol allowing a fast (40 days) and low-cost production of plants of *Artemisia arborescens* L. was defined.

In particular, February should be the best period for harvesting semi hardwood cuttings suitable for rooting and cuttings showed the best growing aptitude (in terms

of number and length of roots) in the substrate containing sphagnum peat and perlite at a 1 : 1.

Acknowledgements: The authors would like to thanks Dr. Zizzo G.V. from Agricultural Research Council for his kind collaboration.

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