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Original Article

Effect of Cutting Size and Basal Heat on Rooting of *Micromeria fruticulosa* Stem Cuttings

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Abstract

Micromeria fruticulosa (Bertol.) Grande is a small pulviniform shrub, belonging to the Labiates, and characterized by twisted stems and pink-purple flowers. Endemic to Campania and Sicily (Italy), *M. fruticulosa* grows from sea level to 600 m altitude. This specie might be considered an excellent native plant for landscape purposes in Mediterranean areas because of its long flowering period, extending from November to June, and its tolerance to heat and drought. The exploitation of wild plants for ornamental purposes implies knowledge on the factors influencing the propagation methods. Root development of stem cuttings of *M. fruticulosa* was investigated in relation to basal heat and cutting size. Softwood terminal cuttings of a clone grown in Sicily were trimmed to two sizes: short (3 cm) or long (6 cm) length. Propagation was performed in unheated greenhouse covered with clear polyethylene and external 70% shade-cloth. To verify the rooting response to basal heat, half of the cuttings were placed on a basal heated bench (22 ± 2 °C constant temperature) while the remaining were placed on an unheated bench. Acclimatized rooted cuttings were thereafter transplanted into each plastic pot (diameter 16 cm). Plants were thereafter transplanted in the open field and were evaluated for their ornamental value. Basal heat promoted earlier rooting and positively affected adventitious root formation. Six cm long cuttings exposed to basal heat exhibited the best development in terms of number and length of adventitious roots. Flowering plants derived from 6 cm long cuttings exposed to basal heat showed the highest number of flowering branches and flowers per plant.

Keywords: adventitious root**,** basal heat, cutting, ornamental plant, flowering

Introduction

The Mediterranean wild flora includes several species, which might be exploited as ornamental outdoor plants in order to realize healthy and long lived gardens (Gildemeister, 2002). In particular, the use of native species belonging to the Labiatae family as garden plants is of increasing interest due to their adaptation to the Mediterranean environmental conditions characterized by high summer temperatures, dry alkaline soils, frequent high-velocity and desiccating winds (Lopez *et al.*, 2007; Toscano *et al.*, 2014). The genus *Micromeria* (family Labiatae) comprises approximately 78 species distributed over Asia, Europe, Africa and North America (Bräuchler *et al.*, 2008). *Micromeria fruticulosa* (Bertol.) Grande is a small shrub endemic to Campania and Sicily growing from sea level to 600 m altitude (Pignatti, 2003). For its appealing pink-purple flowers blooming from November to June (Pignatti, 2003) and its tolerance to heat and drought of the Mediterranean region (Giardina *et al.*, 2007), *M. fruticulosa* could be promoted for use both in landscapes and in the bedding plant industry. Investigation of factors controlling the propagation of a species is a fundamental prerequisite to the exploitation of native plants with potential for commercial ornamental nursery involved in urban landscape (Romano, 2004). *Micromeria fruticulosa* regenerates naturally by seed and 80and 63% germination at 24 and 20 °C, respectively, has been reported under laboratory conditions (Iapichino *et al.*, 2006a).Vegetative propagation by cutting could be an alternative and effective method for rapid mass propagation of selected clones of *M. fruticulosa* and would also preserve wild plants from irresponsible cutting collection. Iapichino *et al.* (2006a) in a preliminary study report that *M. fruticulosa* propagation by stem cutting is feasible and cuttings collected in December were responsive to exogenous IBA application. However, no information are available on other factors which might affect adventitious rooting in *M. fruticulosa* such as rooting medium temperatures (heated bench vs. unheated bench), cutting length and size. In the present study, we investigate root development of stem cuttings of *M. fruticulosa* in relation to basal heat and cutting length.

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Materials and Methods

Location and biological material

The research was conducted at the Department of Agriculture, Food and Forest Sciences (SAAF) of the University of Palermo, in the northern coast of Sicily (Italy). Softwood terminal cuttings ~12 cm in length were collected on December 27, 2015 from a selected clone of *M. fruticulosa* located in the landscape at the Horticulture and Floriculture experimental farm of the SAAF Department near Palermo (long. 13° 19' E, lat. 38° 9' N).

Experimental procedures

Cuttings were stored over night at 10 °C in polyethylene bags. The next day, prior to planting, the bases of cuttings were trimmed to two lengths: short (3 cm) or long (6 cm) length and the leaves removed from the basal end (Fig. 1a).

Node number and average stem diameter ranged from 7-8 to 14-16 nodes and from 0.5 to 1.0 mm of short and long cuttings, respectively. Propagation was performed in an unheated greenhouse covered with clear polyethylene and external 70% shade cloth. Air temperature in the greenhouse was 10-12 °C during the night and 16-22 °C during the day (Fig. 2). During rooting the light level inside the greenhouse was recorded using a quantum light meter[LI-190 quantum sensor (Licor, Lincoln NE)] and the average daily photosynthetic light integral (DLI) was calculated (Fig. 3). Cuttings were inserted to a 1-cm depth in plastic trays containing a peat-perlite mixture 1 : 1 (v/v) at a 5 cm spacing. To verify the cutting rooting response to different rooting medium temperatures, trays were either placed on a basal heated bench or on an unheated bench. Basal heat was provided at constant temperature of (22 ± 2 °C). Medium temperatures in the unheated bench were 8-10 °C during the night and 14-16 °C during the day. The medium was watered and the trays covered with clear plastics to maintain cutting turgidity. Ventilation of the cuttings was increased with time by increasing size of the holes made in the plastic. A 2 × 2 [two cutting lengths (3 or 6 cm) × two rooting medium temperatures (heated bench vs. unheated bench)] factorial set of treatments within a complete randomized block design was used with 3 blocks per treatment and 20 cuttings per block. Three weeks after cutting insertion in the rooting medium, cuttings were evaluated for number of roots per cutting and average root length. Rooting percent and cutting survival (expressed as percentage of rooted and unrooted survived cuttings) were evaluated after three and six weeks. After three weeks acclimatized rooted cuttings were transplanted into each plastic pot (diameter 16 cm) containing the same growing mix; all plants were pinched leaving 4 nodes on each and kept for five weeks in a lath-house covered with 70% shade-cloth. The propagation treatment was repeated two more times obtaining similar results.

Qualitative analysis and statistical procedures

Plants were thereafter transplanted in the open field and were evaluated for their ornamental performances. To verify the effects of the different length of the cuttings and of the two rooting conditions (heated bench vs. unheated bench) on plant visual quality at the flowering stage we opted to measure the following flowering-related parameters: number of flowering branches and number of flowers per plant at December 20. A 2 × 2 (plants derived from 3 or 6 cm long cuttings × plants derived from cuttings rooted on heated or unheated bench) factorial set of treatments within a complete randomized block design was used with three blocks per treatment and 20 plants per block. Percentage data were subjected to arcsin transformation before ANOVA analysis. Mean separation was performed by Duncan Multiple Range Test. All the statistical analysis were performed using SPSS software version 14.0 (StatSoft, Inc., Chicago, USA).

Results

Basal heat in the rooting medium dramatically affected rooting of cuttings. Ninety-six rooting percent was accomplished after three weeks at constant temperature of 22 ± 2 °C. Rooting dropped to 1.7% when cuttings were placed in the unheated bench. Six weeks after cutting insertion in the rooting medium, 63% and 100% rooting was accomplished in absence and in the presence of basal heat, respectively. Rooting percent was not affected by cutting length (Table 1).

Table 1. Effects of basal heat and cutting length on *Micromeria fruticulosa* cutting survival, rooting characteristics and flowering performances

Treatment	Survival after 3 wks (%)		Survival after 6 wks (%)		Rooting after 3 wks (%)		Rooting after 6 wks (%)		Roots (No.)		Root length (cm)		Flowering branches (No.)		Flowers (No.)	
Cutting length (L)																
3 cm	99.1	NS	97.4	NS	49.0	NS	81.3	NS	8.3	b	1.0	NS	16.5	NS	40.8	NS
6 cm	99.3		97.7		49.4		82.1		13.7	a	1.0		16.9		40.2	
Basal heat (H)																
yes	100.0	NS	98.3	NS	96.7	A	100.0	a	18.7	a	1.6	A	19.1	a	44.9	a
no	98.3		96.7		1.7	B	63.3	b	3.3	b	0.4	B	14.3	b	36.1	b
Significance (F value)																
L	3.7^{NS}		1.9^{NS}		1.9^{NS}		2.6^{NS}		100.0^{***}		0.8^{NS}		1.1^{NS}		0.5^{NS}	
H	7.6^{NS}		36.7^{NS}		31854.1^{***}		6518.2^{***}		830.2^{***}		396.8^{***}		245.1^{***}		102.7^{***}	
LxH	3.7^{NS}		3.4^{NS}		2.5^{NS}		0.4^{NS}		100.0^{***}		0.8^{NS}		9.7^{**}		2.0^{NS}	

In each column and for each fixed factor, values followed by same letters are not statistically different according to Duncan test ($P \le 0.05$). The significance is designated by asterisks as follows: *, statistically significant differences at *P*-value below 0.05; **, statistically significant differences at *P*-value below 0.01; ***, statistically significant differences at *P*-value below 0.001. NS=not significant.

Fig. 1. a) Softwood terminal cuttings of *Micromeria fruticulosa* trimmed to 3 cm (below) and 6 cm (above) length. b) *Micromeria fruticulosa* plantlet with adventitious root system regenerated in basal heated bench. c) All plants of *Micromeria fruticulosa* obtained from softwood terminal cuttings flowered profusely twelve months after propagation

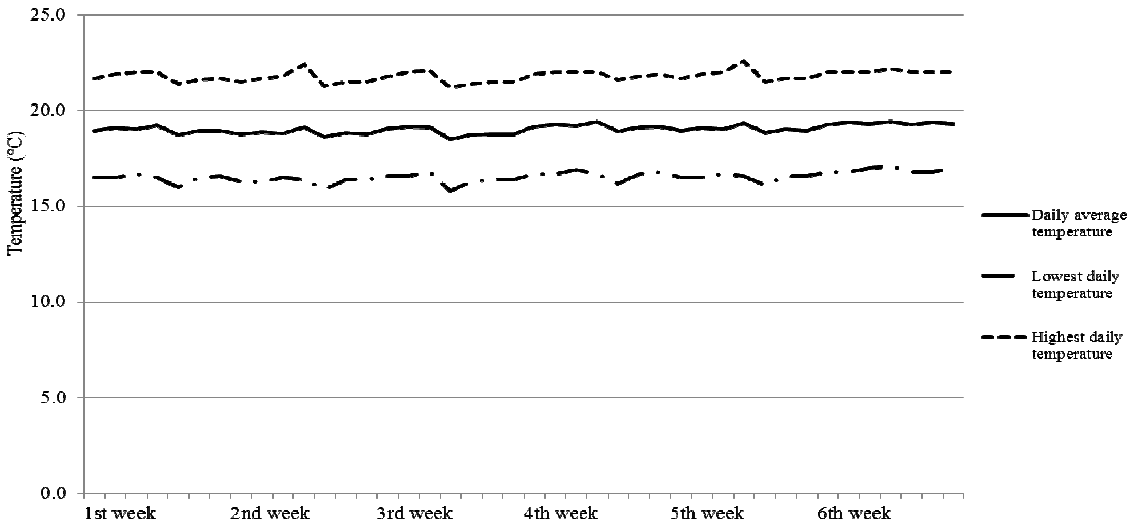

Fig. 2. Average, lowest and highest daily temperatures in Palermo, Sicily during the rooting period in an unheated greenhouse covered with clear polyethylene and external 70% shade cloth

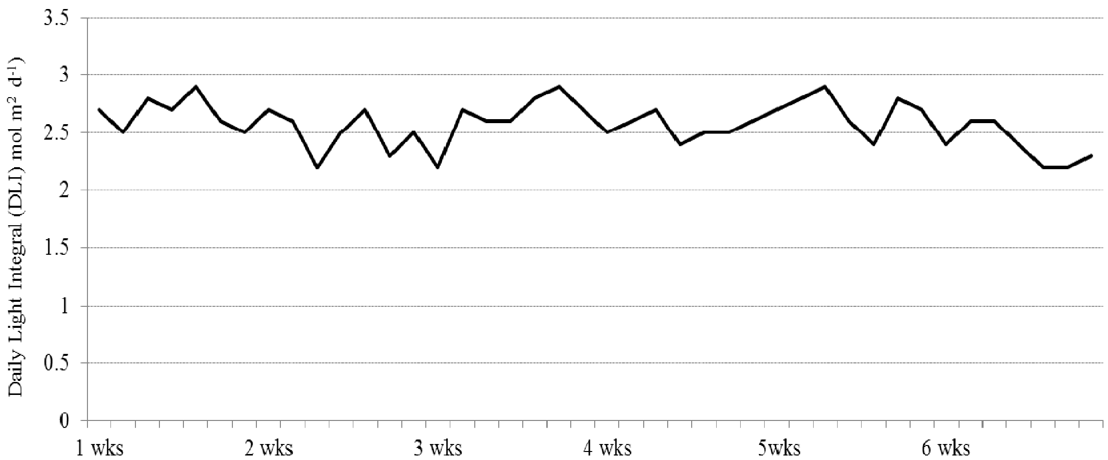

Fig. 3. The average daily light integrals during the rooting period in Palermo, Sicily in an unheated greenhouse covered with clear polyethylene and external 70% shade cloth

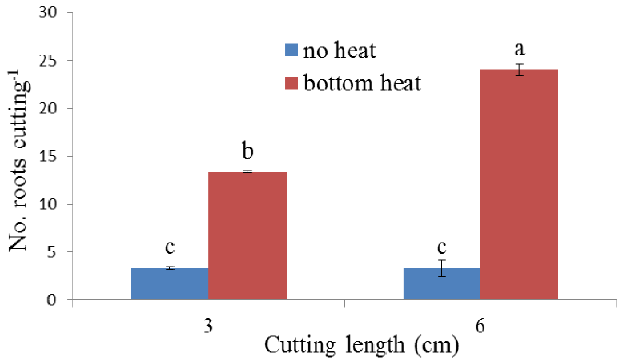

Fig. 4. Effect of cutting length and basal heat on *Micromeria fruticulosa* number of roots per cutting after three weeks from planting. Bars with different letters are significant by Duncan Multiple Range Test (P<0.001)

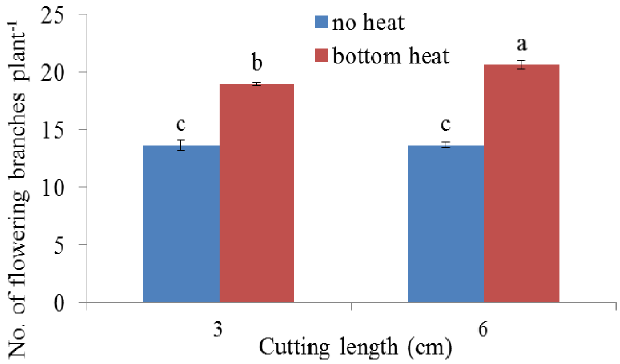

Fig. 5. Effect of cutting length and basal heat on *Micromeria fruticulosa* number of flowering branches per plant. Bars with different letters are significant by Duncan Multiple Range Test (P<0.01)

Cutting survival was not affected by basal heat and cutting length. Regardless of cutting length, cutting survival ranged from 100% after three weeks in the presence of basal heat to 97% after six weeks in the absence of basal heat (Table 1).

Roots were evenly distributed at the cut basal portion of the cutting. Cutting length and basal heat significantly affected the number of roots per cutting (Figs. 1b and 4).

Three weeks after cutting insertion in the rooting medium, root number per cutting ranged from 3.1 when 3 cm long cuttings were placed in the unheated bench to an optimum of 24 roots for 6 cm long cuttings rooted in basal-heated bench. Average root length was affected by the temperature of the rooting medium averaging 1.5 cm in the basal heated bench and 0.4 cm in the unheated bench (Table 1). However, root length was unaffected by cutting length and there was no significant interaction between basal heat and cutting length.

All plants flowered profusely in December 2016. The number of flowering branches per plant was significantly affected by basal heat and cutting length. Plants derived from cuttings rooted in the unheated bench displayed fewer flowering branches compared to those obtained from cuttings exposed to basal heat (14.3 and 19.1 flowering branches per plant, respectively) (Fig. 5).

Regardless of the cutting length, plants derived from cuttings rooted in the heated bench were also superior to plants originated from cuttings rooted in the unheated bench in terms of number of flowers per plant (44.8 and 36.0 number of flowers per plant, respectively) (Table 1 and Fig. 1c).

Discussion

Our study demonstrates that proper control of root zone temperature is critical for rooting of *M. fruticulosa* stem cuttings and that 6 cm long cuttings were more responsive to basal heat than shorter cuttings. Basal heat has been often demonstrated beneficial for rooting of stem cuttings in several species (Loach, 1988; Blanchard *et al.*, 2006; Greer, 2006; Iapichino *et al.* 2006 a,b; Iapichino and Airò, 2008; Iapichino and Bertolino, 2009; Sabatino *et al.* 2014). Rooting medium temperatures of 18-25 °C are considered optimal for most cool-season species, whereas suboptimal temperatures are known to inhibit or limit adventitious root formation because the cuttings will not metabolize at a sufficiently rapid rate for optimum rooting (Preece, 1993).

Synergistic effects of long cutting and basal heat has been also reported by Caruso and Iapichino (2014) in *Plumeria rubra*. Our results are also in accord with to those obtained by Smalley and Dirr (1987), Henry *et al.* (1992) and Hinesley *et al.* (1994) who reported that cutting length positively affected root count in *Acer rubrum*, *Juniperus virginiana* and *Chamaecyparis thyoides*, respectively.

Conclusions

Much Mediterranean garden design in the past has used a wide range of ornamental plants, often repetitive and inappropriate (Kingsbury, 2004). However, nowadays, there is a growing interest and consciousness among gardeners and landscape professionals in their native flora and wild landscapes (Oudolf and Kingsbury, 2013). Locally native plants have the capacity to adapt and survive to adverse environmental conditions and may play a major role not only in landscaping, but also in replacing lost habitats and in vegetation recovery projects (Iapichino *et al.*, 2009). Proper plant selection and nursery production of ornamental plants is critical to sustainable urban landscapes and particularly in semi-arid environments (Franco *et al.*, 2006). On this respect, we suggest the inclusion of *M. fruticulosa* in Mediterranean garden planting designs especially on those situations where support of biodiversity, sustainability and exploitation of native flora are major objectives. We also suggest that in the Mediterranean region the use of basal heat and of 6 cm long cuttings may be beneficial to propagators wishing to produce *M. fruticulosa* rooted cuttings with well-developed root system and able to support profuse flowering in mature plants.

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