Collection season and size of apical cuttings in the vegetative propagation of *Origanum majorana* L.

Época de coleta e tamanho de estacas apicais na propagação vegetativa de *Origanum majorana* L.

Época de recolección y tamaño de estacas apicales en la propagación vegetativa de *Origanum majorana* L.

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Abstract

This study aimed to evaluate the vegetative propagation of marjoram according to the size of the apical cuttings collected in two periods of collection. The experimental design was completely randomized, with a factorial arrangement (4 x 2) composed of two collection periods (autumn and spring cuttings) and four sizes (3, 6, 9 and 12 cm), with four replications of 12 cuttings each. The cuttings were established in thick sand boxes washed and sanitized with sodium hypochlorite (10%) and implanted in the plant production sector, with 50% shading and daily irrigation. Cuttings collection times did not influence the rooting and

mortality of marjoram. The highest percentage of rooting was obtained with cuttings 3 cm long (98%), with linear reduction due to the increase in size. The spring cuttings showed higher fresh weight, dry mass of the aerial part, higher final height of the seedlings and additional growth than the autumn cuttings. The largest number of shoots was obtained with the largest cutting size, and the average percentage of inflorescences was 23.7%. Marjoram can be propagated with apical cuttings developed autumn and spring, preferably with cuttings of 3 and 6 cm to obtain the optimal rooting and initial formation of seedlings.

Keywords: Formation of seedlings; Medicinal and aromatic plant; Rooting.

Resumo

O estudo teve como objetivo avaliar a propagação vegetativa da manjerona de acordo com o tamanho das estacas apicais coletadas em dois períodos de coleta. O delineamento experimental foi inteiramente casualizado, com arranjo fatorial (4 x 2) composto por duas épocas de coleta (estacas de outono e primavera) e quatro tamanhos (3, 6, 9 e 12 cm), com quatro repetições de 12 estacas cada. As estacas foram estabelecidas em caixas de areia grossas lavadas e higienizadas com hipoclorito de sódio (10%) e implantadas no setor de produção vegetal, com sombreamento de 50% e irrigação diária. A época de coleta das estacas não influenciou no enraizamento e mortalidade da manjerona. A maior porcentagem de enraizamento foi obtida com estacas de 3 cm de comprimento (98%), com redução linear devido ao aumento do tamanho. As estacas de primavera apresentaram maior massa fresca, massa seca da parte aérea, maior altura final das mudas e crescimento adicional que as estacas de outono. O maior número de brotos foi obtido com o maior tamanho de estaca, e o percentual médio de inflorescências foi de 23,7%. A manjerona pode ser propagada com estacas apicais desenvolvidas no outono e na primavera, preferencialmente com estacas de 3 e 6 cm para se obter o enraizamento ideal e a formação inicial das mudas.

Palavras-chave: Formação de mudas; Planta medicinal e aromática; Enraizamento.

Resumen

Este estudio tuvo como objetivo evaluar la propagación vegetativa de la mejorana según el tamaño de las estacas apicales recolectadas en dos períodos de recolección. El diseño experimental fue completamente al azar, con arreglo factorial (4 x 2) compuesto por dos períodos de recolección (esquejes de otoño y primavera) y cuatro tamaños (3, 6, 9 y 12 cm), con cuatro repeticiones de 12 esquejes cada una. Los esquejes se establecieron en gruesos cajones de arena lavados y desinfectados con hipoclorito de sodio (10%) e implantados en el

sector de producción vegetal, con 50% de sombra y riego diario. Los tiempos de recolección de esquejes no influyeron en el enraizamiento y mortalidad de la mejorana. El mayor porcentaje de enraizamiento se obtuvo con esquejes de 3 cm de largo (98%), con reducción lineal por aumento de tamaño. Los esquejes de primavera presentaron mayor peso fresco, masa seca de la parte aérea, mayor altura final de las plántulas y crecimiento adicional que los esquejes de otoño. El mayor número de brotes se obtuvo con el mayor tamaño de estaca, y el porcentaje promedio de inflorescencias fue de 23,7%. La mejorana se puede propagar con esquejes apicales desarrollados en otoño y primavera, preferiblemente con esquejes de 3 y 6 cm para obtener el óptimo enraizamiento y formación inicial de las plántulas.

Palabras clave: Formación de plântulas; Planta medicinal y aromática; Enraizamiento.

1. Introduction

Origanum majorana L. is a cespitose perennial herb, 30 to 60 cm high, belonging to the Lamiaceae family, whose center of origin is the Mediterranean and Middle East region. Its stems are erect, square, and branchy. The leaves are simple, opposite, small oval or rounded-elliptical, grayish-green in color, hairy on the underside, and smooth on the upper. Its flowers are also small, with colors ranging from white to pink (Castro & Ramos, 2003) clustered together in inflorescences (Rupplelt, et al., 2015).

Popularly known as marjoram, sweet marjoram and real marjoram (Castro & Ramos, 2003; Ruppelt, et al., 2015), it belongs to the same family as other medicinal, aromatic, and condiment species, such as pennyroyal (*Mentha pulegium* L.), lemon balm (*Melissa officinalis*), mint (*Mentha* sp.), oregano (*Origanum vulgare*), and rosemary (*Rosmarinus officinalis*), among others (Lorenzi & Matos, 2002).

The interest in spices, aromatic and medicinal species is no longer limited to culinary use or the preparation of herbal medicines. Due to the different essential oils, *Origanum majorana* can be used for different purposes. Sales, et al., (2020) reports that the species is widely used in the treatment of diseases of the Central Nervous System (CNS), due to its antiepileptic and sedative effect.

The biological properties (antifungal and antibacterial) and antioxidants of *Origanum* extracts have aroused the interest of researchers and food industries alike, as they can act as microbial inhibitors as well as alternatives to additives, respectively. Accordingly, Freire, et al. (2011) found in in vitro tests that the essential oil of *Origanum majorana* L. has an inhibitory effect on the bacteria *Staphylococcus aureus* and *Escherichia coli*. Souza, et al.

(2010) observed that the essential oils of *Origanum* have the potential to control pathogenic fungi and the occurrence of mycoses. In addition, its bioactive compounds have been tested in post-harvest fruits, with the aim of controlling and reducing the occurrence of diseases (Cruz, et al., 2010).

Despite the wide array of functions associated with the plant extracts of *Origanum* sp., there is little scientific research focused on their propagation (Signor, et al., 2007) and cultivation (Corrêa, et al., 2010). However, these studies are specifically restricted to oregano (*Origanum vulgare* L.), highlighting the scarcity of references for research on marjoram. Much of the available technical information comes from popular knowledge from the experiences of farmers and extension workers (Amaro, et al., 2013), which indicates the possibility of propagation through seeds, division of clumps, and use of cuttings. However, there are no further guidelines regarding the collection, type, and, size of the stake.

Vegetative propagation is an important technique for plant multiplication as it helps maintain the desirable characteristics of the stock plant and greater homogeneity in the crops, in addition to reducing the juvenile period, which is an extremely important aspect for certain species (Scalon, et al., 2003).

The cutting technique is the most economically viable among the vegetative propagation methods, as it represents a fast and low-cost process, allowing the collection of a large number of seedlings from a few stock plants that are widely used for their fruiting, medicinal, and ornamental benefits (Hartmann, et al., 2002). Using cuttings as plant materials are an efficient and practical way of propagating new plants because it consists of removing segments from the matrix plant with at least one meristem callus capable of forming adventitious roots (Hartmann, et al., 2002; Signor, et al., 2007). However, the cutting rooting process is influenced by both endogenous (inherent characteristics of the species) and exogenous (environmental conditions) factors. The rhizogenic capacity of the stake varies between species and even between cultivars and is also dependent on endogenous interactions with the environment (Signor, et al., 2007).

When studying the propagation of oregano, Signor, et al. (2007) found that for this species the highest efficiency occurred with 3 cm long apical herbaceous cuttings. This study aimed to investigate the vegetative propagation of marjoram using different sizes of apical herbaceous cuttings collected in two developmental periods (autumn and spring cuttings).

2. Material and Methods

The experiment was carried out at the Federal Institute of Education, Science and Technology of Mato Grosso do Sul (IFMS), Campus Ponta Porã, located in the southwest region of the state. The climate, according to the Köppen classification, is Cfa (humid subtropical) with hot summers and average temperatures between 20 and 22 ° C (Alvares, et al., 2013).

The research used the quantitative method, with the establishment of an experiment and data collection (Pereira et al., 2018), carried out in two stages, using apical cuttings of marjoram obtained from adult stock plants collected in July 2015, whose stems underwent vegetative development during the autumn season, and cuttings collected in January 2016, which developed in the spring. After collection, the herbaceous stems were immediately placed in a moistened plastic container and stored in thermal packaging for transportation, where they remained until the preparation of treatments, to maximally reduce the risk of dehydration.

In the plant processing laboratory, the apical ends of the branches were sectioned to obtain different lengths of cuttings (3, 6, 9, and 12 cm), and immediately deposited after cutting into a container with water to reduce the risk of oxidation and dehydration.

The cuttings were established with a spacing of 2 cm between each cutting in a thick sandbox, washed and previously sanitized with 10% sodium hypochlorite, located inside a nursery under 50% shading. Irrigation was performed twice a day, manually, with abundant watering.

After 40 days, the following biometric characteristics were evaluated: the percentage of rooted and dead cuttings, length of the largest root (cm), percentage of additional growth (cm) obtained by the difference in the final cut height in relation to the initial height, green matter and stem dry matter (g), total number of shoots (autumn cuttings) and presence of inflorescences (spring cuttings), as indicated in the methodology of Signor et al., (2007), adapted of the present study.

To determine the fresh mass, the cuttings were separated from the root system and weighed on a semi-analytical balance. They were subsequently dried in an oven with forced air circulation, at 60 °C for 72 h, and then weighed to obtain the dry mass of the aerial part.

The design used was completely randomized, with a factorial arrangement consisting of two collection times and four cuttings (4 \times 2), with four replicates of 12 cuttings per experimental unit. To test for homogeneity of variances, the Bartlett test was used, following

which, factor analysis was applied to the data, and means of data were compared qualitatively by the Tukey test at 5% probability by ASSISTAT version 7.7 beta (Silva & Azevedo, 2006). Quantitative data were analyzed with polynomial regression and the figures were created using the SigmaPlot 11.0 program.

3. Results and Discussion

The percentages of rooting and mortality of apical cuttings of marjoram were not significantly influenced by the time of collection of the cuttings for propagation of the species (Table 1). However, the response to these variables according to the size of the cuttings was adjusted to that of linear regression (Figure 1). The maximum rooting of apical herbaceous cuttings of marjoram (98%) was obtained with cuttings of 3 cm in length (Figure 1 A). A reduction in their rooting capacity was observed with increasing size of the cuttings, with 86% for 12 cm cuttings.

Table 1 - Averages for rooting and mortality (%), length of largest root (cm), fresh matter of the aerial part (GMAP), and dry matter of the aerial part (DMAP) of marjoram cuttings (*Origanum majorana* L.) in the cuttings collection seasons.

Season	Rooting	Mortality	Length of the	FMAP	DMAP
	(%)	(%)	largest root (cm)	(g)	(g)
Autumn	90.6 ns	4.68 ns	10.0 a*	0.352 b	0.092 b
Spring	95.3	4.68	8.0 b	0.461 a	0.108 a

* followed by distinct letters in the column indicates that the value differs by Tukey's test with a 5% probability of error ($p \le 0.05$). ns: did not show significant differences in the column. Source: Authors.

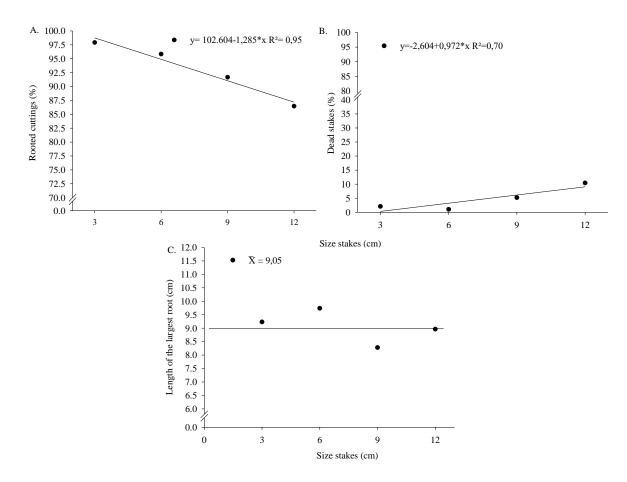
The proximity of the cuttings apical region has a lower degree of lignification and a higher concentration of rooting promoters due to the proximity to the auxin synthesis sites, which is associated with less differentiation of the tissues. This eases the return of the cells to the meristematic condition, which promotes the formation of lateral and adventitious roots (Taiz & Zeiger, 2004; Fachinello, et al., 1995; Kerbauy, 2013).

On the other hand, lower branch positions tend to be more lignified and less favorable to radial differentiation (Signor, et al., 2007). In addition to this characteristic, larger cuttings

have greater vegetative areas exposed to the environment, with greater susceptibility to dehydration (Lima, et al., 2006). These factors justify the mortality rate of the cuttings (Figure 1 B), since the results are proportional to the increase in size.

The times of year when the marjoram stems developed vegetatively (autumn and spring) and were established for seedling production significantly influenced the length of the largest root. As shown in Table 1, autumn cuttings, established for rooting and seedling formation during the beginning of the winter season, developed roots that were 2 cm longer than the spring cuttings, whose seedling formation occurred in the summer season. In turn, the length of the largest root in the marjoram seedlings did not have a significant effect on the size of the apical cuttings (Figure 1 C).

Figure 1 - Percentage of rooting (A), mortality (B), and length of the largest root (C) of marjoram seedlings (*Origanum majorana* L.) as a function of the size of apical cuttings.



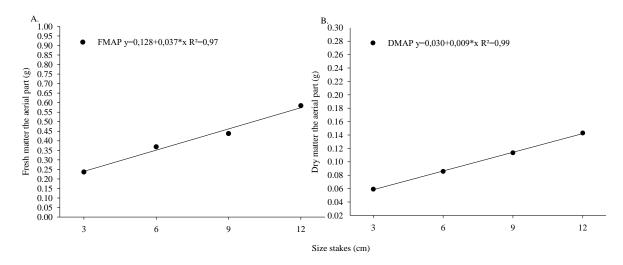
Source: Authors.

The collection season and the size of the cuttings significantly influenced the accumulation of fresh and dry mass of the aerial part of the marjoram seedlings, with no

significant interaction between the factors. Spring cuttings showed higher accumulation of fresh and dry shoot weight in comparison to autumn cuttings, with an average difference of 18.2% and 17.4%, respectively (Table 1). For the size of cuttings, the weights of fresh and dry mass of the aerial part were adjusted to the linear model for regression, with the highest values obtained for cuttings of larger size (Figure 2).

When testing different sizes of oregano cuttings, Signor, et al. (2007) found the same trend. This response is attributed to the fact that larger cuttings contributed to a larger amount of reserves, allowing for greater development during the evaluated period, in addition to the initial mass of cuttings being accounted for in the final average. They also have a greater number of buds, which allow (depending on the season) the emission of axillary shoots during the initial formation period of the seedlings, consequently increasing, in this sense, the formation of plant biomass.

Figure 2 – Fresh matter of the aerial part (FMAP) (A) and dry matter of the aerial part (DMAP) (B) of marjoram seedlings (*Origanum majorana* L.) plant propagation with different sizes of apical cuttings.



Source: Authors.

The height of the seedlings and the additional growth of the apical cuttings of marjoram responded significantly to both the time of collection and the different sizes, with significant interactions between the factors (Table 2).

Table 2 – Interaction between collection season and size of cuttings of marjoram (*Origanum majorana* L.) for seedling height and additional growth.

	Collection season		Collection season		
Cuttings size	Autumn	Spring	Autumn	Spring	
-	Seedling height (cm)		Additional growth (%)		
3 cm	3.9 dB*	9.8 cA	31 aB	226 aA	
6 cm	6.7 cB	12.5 bA	11 aB	109 bA	
9 cm	10.1 bB	14.3 aA	12 aB	59 cA	
12 cm	13.9 aA	14.6 aA	16 aA	22 dA	
Means	8.6 B	12.8 A	17.5 B	104 A	

* means followed by distinct letters, lower case in the column and upper case in the row, differ from each other by the Tukey test with a 5% probability of error ($p \le 0.05$). Source: Authors.

The interaction between the collection season and the size of cuttings indicates compared to the autumn cuttings, the spring cuttings which are 3 cm, 6 cm, and 9 cm in length demonstrated higher additional growth and height of the seedlings, since both variables differed significantly in case of spring cuttings from the autumn cuttings of the same size (Table 2). On the other hand, 12 cm cuttings showed a similar trend for the height of the seedlings, regardless of the collection season, with lower growth rates in comparison to the other sizes.

Increases in the growth rate, similar to that observed in the spring cuttings of the present study, were observed in the propagation of oregano, where the additional growth of the species was 312.6%, 109.6%, and 59% on 3, 6, and 9 cm long cuttings, respectively (Signor, et al., 2007).

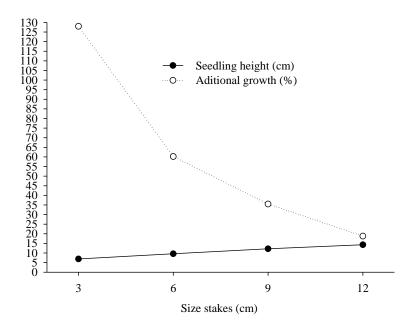
The spring cuttings showed an average growth increase of 104% compared to their initial size, yielding an average height of 12.8 cm in marjoram seedlings after 40 days. This differed statistically from autumn cuttings with an increase of only 17.5% in the height of the seedlings formed (Table 2), maintaining their respective initial heights.

In general, cuttings collected during the autumn and winter period tend to be more lignified, while spring and summer cuttings have a more herbaceous consistency (Fachinello, et al., 1995) which can be associated with full growth metabolic activity of the aerial part of

the plant that occurs mainly during spring (Andriolo, et al., 2006). It can, therefore, be inferred that cuttings at this time of the year present greater vegetative development.

The averages for seedling height and additional growth as a function of the tested pile sizes are shown in Figure 3. It was verified that the average additional growth of the cuttings was inversely proportional to the size initially tested, in which cuttings of 3, 6, 9, and 12 cm showed an average additional growth of 128%, 60%, 35%, and 19% in size at the end of the seedling formation period.

Figure 3 – Average seedling height (cm) and additional growth (%) in marjoram seedlings (*Origanum majorana* L.) as a function of different cuttings sizes in vegetative propagation.



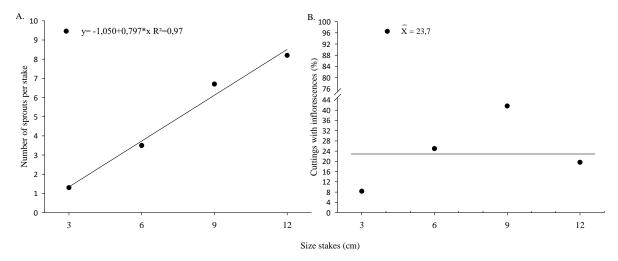
Source: Authors.

The superior growth rate of the 3 cm cuttings, which managed to compensate for their small initial size and helped the cutting reach heights similar to the other tested sizes, was also observed by Signor, et al. (2007), who attributed such a result to the proximity to the apical region of the plant. In this sense, it is possible that the greater auxin content in the apical region of the plant favors and results in the greater growth rate of the 3 cm cutting in comparison to the others, considering this hormone acts in the process of apical dominance (Taiz & Zeiger, 2004).

The total number of shoots were evaluated on the autumn, considering their development occurred during the seedling formation (40 days). The number of shoots per

cutting was adjusted to the growing linear model (Figure 4 A) according to the increase in cutting size, comparable to the results of Biase & Costa (2003) when analyzing different cuttings sizes of *Lippia alba* and that of Carvalho Junior, et al. (2009) with *Lippia sinoides* cuttings. This can probably be attributed to the greater number of axillary buds and the greater quantity of reserves present in piles of greater length. By contrast, Signor, et al. (2007) found that the size of cuttings in the propagation of oregano did not influence the number of shoots, with an average of 13.2 shoots per cutting.

Figure 4 – Total number of sprouts in autumn cuttings (A) and percentage of inflorescences in spring cuttings (B) in the vegetative propagation of marjoram (*Origanum majorana* L.).



Source: Authors.

The inflorescence emission in the rooting of cuttings for the formation of marjoram seedlings was observed only for spring cuttings (Figure 4 B). The results obtained did not adjust significantly to the regression models tested, with an average of 23.7% of cuttings with floral emission. One of the advantages of vegetative propagation, via cutting, is the reduction of the juvenile period; that is, the time until the plants start the reproductive phase (flowering and fruiting), provided adult matrix cuttings are used (Betanin, 2008). Thus, the spring cuttings collected at the end of the season were ripe and prone to flowering during this period.

Research related to the propagation of medicinal and aromatic species in different climatic conditions is essential to identify the time of collection and the size of the plant material, as they directly influence the quality and development potential of the seedlings. These studies would allow to reduce the time between planting and the beginning of the

harvests, providing incentives and improvements in the productive sector of medicinal and condiment species.

4. Conclusions

Under the conditions in which the study was developed, *Origanum majorana* L., has high natural rooting capacity for apical cuttings developed vegetatively in autumn and spring.

Apical cuttings of 3 to 12 cm can be used successfully for the vegetative propagation of the species; however, 3 and 6 cm cuttings are more efficient in rooting for initial seedling formation.

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