

Contribution to the Improvement of Vegetative Propagation by Greenhouse Cuttings of Odorous Verbena (*Aloysia citrodora*)

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Abstract. Currently several species are threatened with extinction due to climatic factors, population pressure and the strong global demand for a continuous and uniform supply of fragrant, aromatic and medicinal plants. The multiplication and domestication of these species remains the only way to save them from extinction, our study is within the framework of propagation by cuttings of odorous verbena (*Aloysia citrodora*). In fact, some parameters influencing the success of cuttings have been studied, namely the nature of the substrate, the effect of certain rooting products and the position of the cutting in relation to the mother plant (basal or apical), the tests were carried out in a tunnel greenhouse at the Ibn Tofail University, Faculty of Sciences Kenitra, Morocco. Out of 144 trials, the success rate of cuttings according to the type of substrate was 91.66%, 75% and 83.33% respectively for substrates S1 (soil only), S2 (1/2 sand and 1/2 compost) and S3 (1/3 soil; 1/3 compost and 1/3 peat). For the three products, we didn't make combinations, but we followed the effect of each product alone. For the treatment effect, 77%, 0% and 69% respectively were obtained for Product 1 (auxin), Product 2 (based on mineral matter and amino acids) and Product 3 (seaweed extract). With regard to the position of the cutting, high percentages were obtained for cuttings from the basal position.

Keywords: *Aloysia citrodora*, apical, basal, cutting, domestication, rooting

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1. Introduction

The odorous verbena (*Aloysia citrodora*), (*Aloysia triphylla*), or (*Lippia citriodora*) belong to the family Verbenaceae, class Magnoliopsida and order Lamiales. The fragrant verbena is a perennial shrub that grows quickly and can reach in the best conditions 2 to 3 meters high. Its stems, straight branched into narrow and thin branches, bear whole and lanceolate leaves, a little rough, deciduous in winter (Figure 1), It originates from Chile and was introduced in Europe around 1784 [1]. It is traditionally used as an infusion mainly for its sedative and muscle relaxant properties, mainly in newborns [2].

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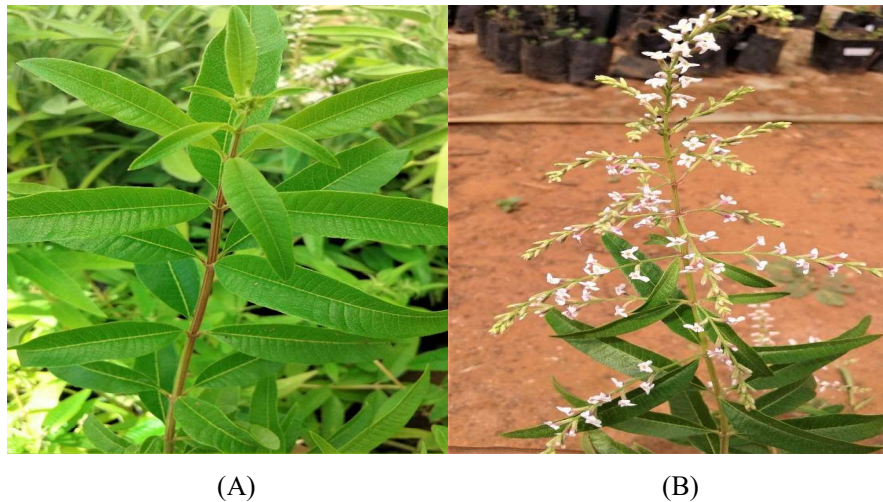


Figure 1. Odorous verbena (*Aloysia citrodora*) with the leaves (A) and with the flowers (B)

Reproductive systems are an important part of the adaptive strategy of plant species. They are subject to intraspecific variations that are under genetic control. The domestication of plant species is an evolutionary process that is driven by the constraint of man, who applies cultivation techniques designed to increase productivity. During this domestication, vegetative propagation has taken an important place in different species, either by using vegetative propagation existing in the spontaneous form, or by inducing vegetative propagation techniques. Biologically, one of the most striking features of the domestication of vegetatively propagated species is the frequent loss or diminution of sexuality [3]. Cutting is a fast and very advantageous way to supply the plants necessary for reforestation of very intensive types [4]. This technique consists in reproducing plants genetically conforming to the mother plant by making use of the parts of the plant (stem, root, leaf) and putting them in suitable conditions to produce roots and consequently a whole plant [5]. It is a method of multiplication that allows to obtain a large number of plants quickly and cheaply.

Many studies have focused on the effect of substrate on the rooting of cuttings in woody species as it remains a key factor in the success of the cutting. The most commonly used substrates are peat, sand, vermiculite, perlite, Styrofoam and various mixtures of these media [6]-[8]. Usually a mixture of an organic component (the peat for example) and a mineral component (for example: perlite, vermiculite, coarse sand) meets these conditions. Generally, the volume of air should not be below 15% of substrate volume to ensure aeration of the substrate and the presence of oxygen [9]. Growth regulators are also among the factors influencing the success of cuttings. Auxin, for example, is a plant hormone that plays an essential role in several plant development processes [10]. It acts in a synergistic or antagonistic manner with other hormones to trigger a series of processes involved in adventitious root formation [11], [12].

2. Materials and Methods

2.1. Plant material

The tests were carried out in a tunnel greenhouse at the Ibn Tofail University, Faculty of Sciences Kenitra; Morocco (2018). The objective of our work is to improve the success rate of cuttings of aromatic and medicinal plants, including odorous verbena (*Aloysia citrodora*) (Figure 1), while favouring the rooting conditions. For this purpose we studied some parameters, namely the effect of the substrate, the effect of some rooting products and the position of the cutting in relation to the mother plant (basal or apical). 8-12 cm long cuttings were used for both apical and basal cuttings.

2.2. Preparation of Substrates and Rooting Products

To study the effect of the substrate, three types of compositions were chosen :

- Substrate 1: Composed only of soil (Maâmora Forest soil, Kenitra);
- Substrate 2: Composed of 50% sand and 50% compost;
- Substrate 3: Mixture of three compounds: 1/3 of soil, 1/3 of commercial peat, and 1/3 of compost.

The sand used is that of the Maâmora forest, the compost is recovered from the 'Saknia' urban forest in Kenitra. A step of preparation of the cuttings was made before the culture, this step consists in defoliation of the cuttings, by decreasing the number of leaves and the leaf surface, in order to minimize evaporation and to avoid the drying of the cuttings. Also the apex of each cuttings is cut to cancel the apical dominance and stimulate the development of axillary buds and the rooting process. In the second step, the cuttings were soaked in 3 products (P1, P2, P3) which differ in chemical composition and active ingredient (Figure 2). The preparation of dipping solution was carried out according to the instructions for each product:

- Product 1: auxin
- Product 2: mineral matter
- Product 3: seaweed extract based on mineral matter and amino acids



Figure 2. Soaking the Cuttings in the Rooting Products after Preparation of the Solutions in the Laboratory (Photo in the Greenhouse)

To realize our tests we adopted an experimental device (Table 1), which consists of 24 pots for each type of cuttings (apical and basal) or 48 pots for each substrate (S1, S2, S3) or a final number of 144 tests.

Table 1. Experimental Device Adopted

<i>Aloysia citrodora</i>	Substrate	P1	Ap
			Ba
		P2	Ap
			Ba
		P3	Ap
			Ba
		Control	Ap
			Ba

(Ap = apical / Ba = basal / P = Product)

Data processing was carried out using IBM SPSS Statistics 21 and Microsoft EXCEL 2016.

3. Results and Discussion

3.1. Percentage of Successful Cuttings

The success rate of fragrant verbena cuttings varies according to the type of treatment, the type of substrate and also the position of the cuttings in the mother plant. According to Figure 3, there are very important variations in the percentage of success of odorous verbena (*Aloysia citrodora*) cuttings in the three substrates in relation to the position of the cuttings and the rooting products.

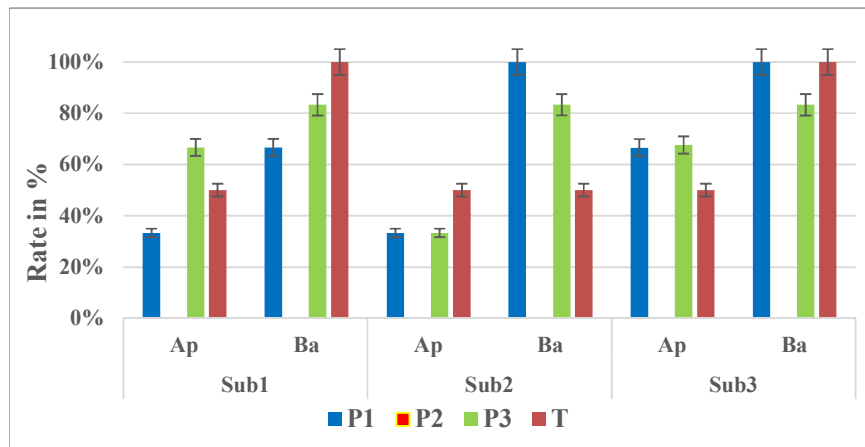


Figure 3. Success Rate (in %) of Odorous Verbena (*Aloysia citrodora*) Cuttings in the Three Substrates that Underwent the Treatments (Sub = substrate / Ap = apical cutting / Ba = basal cutting / P = product / T = control)

In Substrate 1 (soil only) the success rate for apical cuttings ranged from 33.33% to 67%, while for basal cuttings the success rate ranged from 67% to 100%. For substrate 2 (1/2 sand + 1/2 compost) and substrate 3 the ANOVA test also showed that there is no significant difference between the two types of cuttings. Concerning the applied treatments it was found that the three

products had variable effects; product 2 had a lethal effect on the cuttings of odorous verbena (0% survival rate) in the three substrates, while products 1 and 3 had a positive effect especially on the basal cuttings.

The ANOVA test showed that there is a very significant difference ($P < 0.05$) between the means of three treatments (Table 2 and Table 3).

Table 2. Statistical Analysis by the One-Factor ANOVA Test on the Success Rate of Odorous Verbena Cuttings

Source of variation	Sum of squares	Degree of freedom	Mean of squares	F	Probability	Critical value for F
Between Groups	2.06	3	0.69	14.22	0.00	3.10
Within the groups	0.97	20	0.05			
Total	3.03	23				

Our results showed that product 1 (auxin) was more favorable than the other products, while product 2 caused mortality of all cuttings. The results obtained for odorous verbena show that basal cuttings are more effective than the apical part and substrate 3 (Soil + Peat + Compost) are more favourable for rooting and regeneration of odorous verbena by stem cuttings. In addition, product 1 (auxin) is the best of the three products tested.

Table 3. One-Factor ANOVA Test on the Success Rate of Odorous Verbena (*Aloysia citrodora*) Cuttings in the Three Substrates that Underwent the Treatments

Variables	P	Signification
Substrate 1 Apical Basal	0.21	NS
Substrate 2 Apical Basal	0.38	NS
Substrate 3 Apical Basal	0.31	NS
Sub1+Sub 2+Sub 3	0.002	S

NS = not significant / S = significant / P = probability

3.2. Study of the Aerial Part and Root System Development

3.2.1. Effect of treatment and substrate on root system development

The results obtained in our study showed a great diversity in the morphology of the root system. as shown in the photos in Figure 4, there is a clear difference between the treated cuttings and the untreated controls.



A: Product 1

B: Product 3

C: Control

Figure 4. Morphology of the Root System of Odorous Verbena (*Aloysia citrodora*) after 6 Weeks of Cultivation

It was found that product 1 (Auxin) gave better results both in terms of root quality and quantity, while for product 3 (Seaweed extract) the root system is less developed.



Figure 5. The Cutting of Odorous Verbena (*Aloysia citrodora*) at t0

For the effect of the substrate, it was found that the substrate 3 composed of soil, peat and compost; gave a good rooting (Figure 6).



Sub 1

Sub 2

Sub 3

Figure 6. Morphology of the Control Cuttings of Odorous Verbena (*Aloysia citrodora*) in the Three Substrates after 5 Weeks of Culture (Sub = substrate)

3.2.2. Effect of the substrate on the length of the aerial part

The curves in Figure 7 show that substrates have a variable effect on the stem length of odorous verbena (*Aloysia citrodora*). After two weeks (S2) of culture, the stem length was found to be stable (cuttings kept their initial length) (Figure 5). The stem length varied between 3 and 4 cm in the three substrates. From the third week (S3), significant variations in stem length were obtained; in substrate 1 (soil only) the length increased from 4cm (week 3) to 15.3cm at week 7.

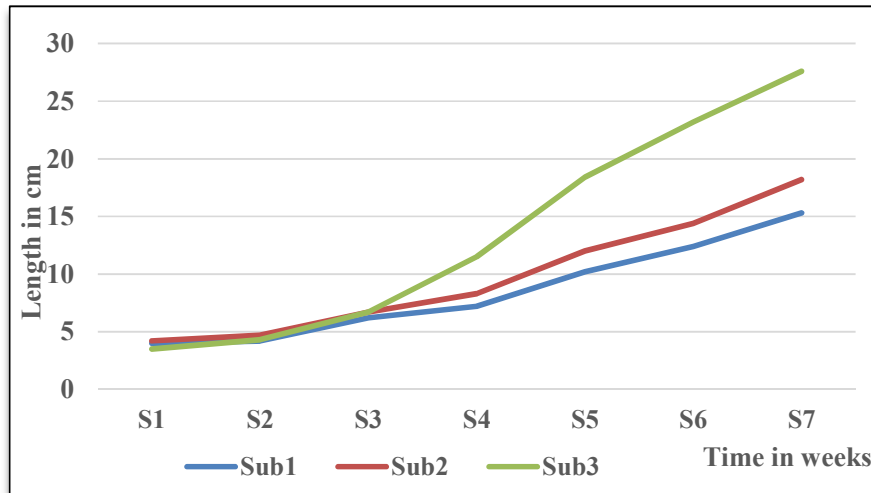


Figure 7. Effect of Substrate on the Evolution of the Stem Length of Odorous Verbena (*Aloysia citrodora*) (Sub=substrate)

For substrate 2 (Sand + Compost) it increased from 4.2 cm (week 3) to 18.2 cm at week 7. It is in substrate 3 that a considerable increase in stem length was obtained, on average from 3.5 cm (week 3) to 27.6 cm in week 7 (Figure 8).

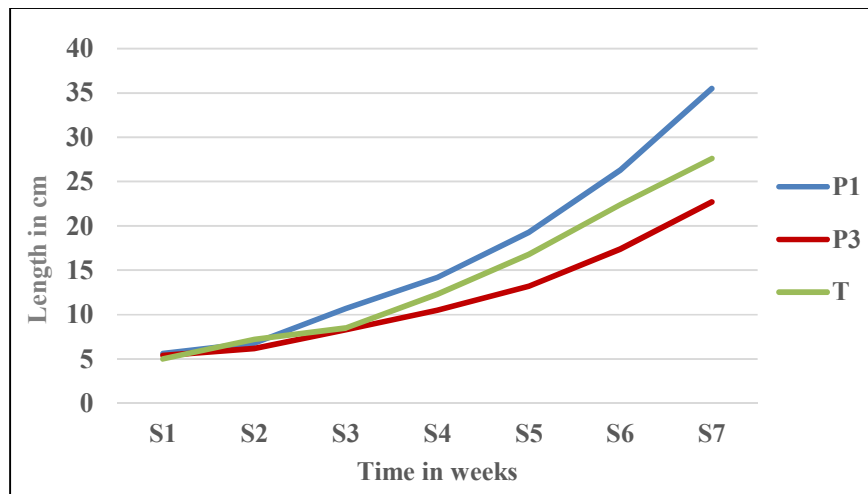


Figure 8. Effect of the Rooting Treatment on the Stem Length of Odorous Verbena (*Aloysia citrodora*) (P=product / T=control)

Statistical analysis by the one-factor ANOVA test showed that there was no significant difference between the means of the three substrates used ($P>0.05$) (Table 4).

Table 4. One-Factor ANOVA Statistical Test on the Effect of Three Substrates on the Length of the Aerial Part of Odorous Verbena (*Aloysia citrodora*)

Source of variation	Sum of squares	Degree of freedom	Mean of squares	F	Probability	Critical value for F
Between Groups	204.96	5	40.99	1.28	0.29	2.48
Within the groups	1151.59	36	31.99			
Total	1356.56	41				

3.2.3. Rooting treatment effect on the stem length of odorous verbena

Figure 8 shows that the effect of treatment on the evolution of the stem length of odorous verbena is different according to the treatment and the type of cutting. This is also confirmed by the ANOVA statistical test which shows a significant difference ($P<0.05$) (Table 5). The growth of the aerial part started after three weeks of cultivation;

- After 3 weeks of culture a stem length of 10.7cm, 8.3cm and 8.5cm was obtained for P1, P3 and control cuttings respectively (Figure 7).
- At week 7, the stem length reaches 35.5 cm; 27.6cm and 27.6cm for P1, P3 and controls respectively.

Table 5. Statistical ANOVA Test for the Treatment Effect on the Stem Length of Odorous Verbena (*Aloysia citrodora*)

Source of variation	Sum of squares	Degree of freedom	Mean of squares	F	Probability	Critical value for F
Between Groups	626.77	3	208.92	3.64	0.03	3.01
Within the groups	1377.10	24	57.38			
Total	2003.87	27				

The technique of cuttings has many advantages, which can be summarized by the faithful reproduction of the characteristics of the mother plant in thousands of individuals, all homogeneous in growth [13], [14]. In addition, it produces faster growing plants than those obtained by sowing. Although this technique has many advantages, it presents some difficulties related to the rooting of cuttings, the choice of substrate, and the maintenance of adequate temperature and relative humidity. During this study, greenhouse cultivation promoted good growth and viability of cuttings at the budding and rooting stage. This could be explained by the microclimate inside the greenhouse characterized by a high relative humidity close to 92% and an average temperature of $30\pm 2^\circ\text{C}$. These conditions are sufficiently close to the natural

conditions of the plant. [15] Showed that high light intensities accelerated bud break (a decrease in the time between cutting and bud break of the cutting) and slightly increased the percentage of buds broken.

The results obtained in this study showed the existence of a remarkable effect of chemical treatment especially the product 1 (Auxin), several authors confirm this. It was reported that the application of exogenous auxins to the roots increased up to six times the root regeneration of oak seedlings, also [16] stated that β -Indolbutyric Acid (BIA) had a favourable effect on rooting in *Hinpahae rhamnoid* softwood cuttings taken from the base of the shoot, but had no marked effect on the apical cuttings. In the case of the control cuttings, a success percentage ranging from 50% to 100% was obtained. While in the work of [17], he found a rooting rate of 17% for control cuttings. [18] Also reported a low rooting rate of *Anogeissus leiocarpus* cuttings treated with various concentrations of BIA. [19] and [20] observed no rooting on control *Anogeissus pendula* cuttings. For the position of cuttings in relation to the mother plant, better rooting percentages were found in basal cuttings, which is also confirmed in the work of [21]-[23] who observed that cuttings in the basal position rooted better than those in the median or terminal position. In the same direction [24] also showed that the position of cuttings on the mother tree has a significant effect on the rooting rate and root elongation. They found that cuttings taken from basal positions have a high ability of root elongation, as well as cuttings taken from shoots developed at the base of the trees have a greater ability to produce roots compared to other positions.

The success of a cuttings process depends on several important factors, among which we distinguish the growing medium. The rooting of the cuttings is linked to the quality of the substrate. Substrates favorable for good rooting are light, porous, have a good water retention capacity. Thus, they allow a good circulation of water and oxygen. This is the case of substrates composed of a mixture of black soil/wood sawdust and black soil/sand [25].

It is therefore for these properties that the substrate consisting of a mixture of soil/peat/compost was chosen in this study, and which gave high success rates. According to [26], the quality of the substrate is a very important parameter for the success of the rooting process of the cuttings. In addition, the requirements of the species with respect to the different substrates depend on their hydromorphic or xeromorphic character. Thus, it would seem that there is a relationship between the water content of the substrate and that of the cuttings. For example, it is known that different substrates affect the water supply of cuttings [27] and have an effect on photosynthesis and stomatal conductance of cuttings [28].

4. Conclusion and Recommendation

4.1. Conclusion

Vegetative propagation of odorous verbena by cuttings has not only improved the cutting process in the tunnel greenhouse, but has also saved money and time. The success rate of the cuttings varies according to the species; some species have a high survival rate while others have a low survival rate. The present work allowed us to find out the effect of some parameters on the survival power and rooting success of odorous verbena, namely the type of cuttings, the type of substrate, and the effect of rooting products. In our study, the vegetative multiplication by cuttings of the odorous verbena, not only allowed us to improve this multiplication process, but also saved money and time. Thus, the recorded results allow us to conclude that the semi-linear cuttings (basal part), substratum 3 (soil+compost+peat) and product 1 (Auxin) gave high percentages of success and a very important power of survival.

4.2. Recommendation

In the context of the development of aromatic and medicinal plants, it is essential to preserve them from disappearing due to several factors of degradation, such as population pressure, climate change, overexploitation, overgrazing and the strong world demand for a continuous and uniform supply of perfume, aromatic and medicinal plants, etc.

At the national level, the production and exploitation of these plants remains generally spontaneous, for that, our study proposes a method of vegetative propagation by cuttings in greenhouses, which deserves to be applied in the field to meet the needs of field crops and to go from spontaneous plants to the field crop stage.

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