Journal of Applied Biosciences 172: 17894 – 17904
ISSN 1997-5902

Propagation by stem cutting of *Euphorbia balsamifera (Aiton), a* galactogenic plant in Benin

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Submitted on 22rd March 2022. Published online at www.m.elewa.org/journals/ on 30th April 2022 **https://doi.org/10.35759/JABs.172.4**

ABSTRACT

Objectives: Cutting is a method of asexual vegetative plant propagation that allows new plants from a plant cuttings cut from the mother plant. The present study aims to test this plant reproduction method using cuttings of the stem of *Euphorbia balsamifera* for the domestication of the species as it induces milk production in cows.

Methodology and results: The experiment was carried out on the experimental site of the botanical garden of University of Abomey-Calavi in southern Benin. The *Euphorbia balsamifera*'s stem of 15 and 25 cm of length were planted in two different substrates: soil alone and mixture of soil + compost for rooting. The mixture of soil + compost was used in a ratio of 3/4 of soil and 1/4 of compost. The experimental design used was a split plot with four replications. The main factor was the stem length and sub factors were type of substrate. The results revealed that the substrate and stem cuttings length of plant material induced significant ($p < 0.01$ to $p < 0.001$ respectively) effect on the proportion (85%) of stem cuttings that were recovered. The stem cuttings with the length 25 cm planted in the soil alone reduced the duration of stem cuttings recovered (7.00±0.00 days).

Conclusion and application of findings: Stem cuttings (length 25 cm) transplanted in soil alone was the efficient way of regeneration of *Euphorbia balsamifera*. This finding is suggested to the agro-pastoralists to enhance milk production of cows. This study is the first step in the process of domestication of the species. However, propagation by stem of this galactogenic plant species could also be tested during different seasons of the year in order to determine the best plantation period in the field in a context of *in situ* and *ex situ* conservation.

Keywords: Budburst, cuttings, domestication, lactogenic plant, biodiversity conservation.

INTRODUCTION

In Africa, it is widely known that spontaneous plant species plays a very crucial role in the socio-economic balance of local populations and enable them to meet their basic needs. For several ethnic groups of the continent, natural resources contribute, among other things, to poverty alleviation, food security and health care (Loubelo, 2012). These natural resources provide many products including food, fodder, medicines, timber, and firewood. (Loubelo, 2012). Among these resources, certain species are known to stimulate milk production of cows, improve animal growth and maintain the animal in good health during dry periods (Hosseinzadeh *et al*., 2013; Silué *et al*., 2014; Badgujar and Bandivdekar, 2015; Sahoo *et al*., 2016). For example, plants with galactogenic components are widely used by herders to stimulate dairy cows to increase their milk production (Hêdji *et al.*, 2014; Imorou *et al.*, 2020). The anthropic pressure exerted on species in general and lactogenic species in particular which would lead to extinction if adequate conservation measures were not taken. Therefore, it is necessary to set up domestication program for certain plant species. However, in-depth knowledge on the reproduction and production of these specific planting materials are lacking. Many wild plant species are characterized by sexual and vegetative reproduction systems. The present study focuses on *Euphorbia balsamifera* (Aiton) used by local herders in Benin to stimulate milk production of cows. This species is found in the northern Benin and distributed from Senegal to Chad, and grow on sandy or rocky soils (Arbonnier, 2000). The genus Euphorbia is the largest of the Euphorbiaceae family consisting of about 20,000 well described species (Jassbi, 2006) and the plants of this family are well known for the chemical diversity of their isoprinoid constituents (Shi *et al*., 2008). It was reported that *Euphorbia* species contain triterpene alcohol in their latex as chemotaxonomic markers (Giner and Schroeder, 2015). In addition, the genus contains cerebrosides, phlorethophenones, glycerols, sesquiterpe noids, steroids, and flavonoids (Shi *et al.*, 2008). Some species of the genus *Euphorbia* are used in the folkloric medicine to cure skin disorders, gonorrhoea, migraines and wart (Ertas *et al*., 2015). The latex of this plant is effective in stimulating milk production in nursing and its leaves are lactogenic for cattle (Arbonnier, 2000). Camels and goats graze the leaves. Sheep prefer to eat the leaves when they have fallen in the ground (Arbonnier, 2002). Latex treats tooth decay and is used for external treatment of snake bites, and insect stings. It is also used for its magic properties (Arbonnier, 2002). *Euphorbia balsamifera* contains many natural compounds, some of which are of therapeutic importance or of commercial use (Giner and Schroeder, 2015). The latex usually protects forages from animal grazing because of its toxic nature (Al-Sultan and Hussein, 2006).

Considering the diversity and the importance of *Euphorbia balsamifera* (Figure 1), it is valuable to integrate the species in the cropping systems. There is a knowledge gap on the duplication technique of the plant. *Euphorbia balsamifera* is cultivated in the world as a cover plant used for field delimitation in Mali and Niger (Burkill, 1994; Koudoussou, 2020). The general objective of the present study was to evaluate the propagation ability of the stem cuttings of *Euphorbia balsamifera* for its conservation and valorisation perspective. Specifically, the study aims to: i) test the effect of stem cuttings lengths on the duration of the plant recovery, ii) determine the effect of substrate types on plant cuttings recovered development.

Figure 1: *Euphorbia balsamifera*

MATERIALS AND METHODS

Study area: The study was carried out in the botanical garden of the University of Abomey-Calavi (UAC) from January 16 to May 16, 2020. It is located in the district of Abomey-Calavi (06°25.092'N; 002°20.607'E and at 24 m above the sea level). The study area is located in the sub-equatorial climatic zone of Benin characterized by a bimodal rainfall pattern: April to June and September to November (Ahoyo, 2014). This rainfall regime is currently highly variable with climate change. The soil type belongs to ferric ferrasol, developed on sandy clay sediments.

Experimental design: A split plot was the experimental design used with stem cuttings length as the main factor and substrates as sub factors with four replications. Stem cuttings lengths studied were 15 cm and 25 cm. Types of substrate used were soil alone and soil + compost. Four treatments were used (T1: 15 cm stem length transplanted in the soil alone; T2: 25 cm stem cuttings length transplanted in the soil alone; T3: 15 cm stem cuttings lengths transplanted in the mixture of soil + compost and T4: 25 cm stem cuttings length transplanted in the mixture of soil + compost) (Figure 1). This leads to 20 pots per replication corresponding to a total of 80 pots (20 pots x 4 replications). The mixture of soil + compost was used in a ratio of 3/4 of soil and 1/4 of compost. The compost was purchased at the laboratory of genetic and horticulture's experimental farm at the Faculty of Agronomic Sciences of the University of Abomey-Calavi. It was made from cow dung, poultry droppings, peanut tops, water hyacinth, pineapple residues, weeds and grass clippings. The physical and chemical characteristics of the two substrates used are presented in Table 1. The soil has an acidic pH (6.47) and contains 82.5% sand. As for the compost, it has a basic pH (7.92). The planting materials used had average diameter of 1.7 cm. Planting materials were harvested the day before transplanting in January 15, 2020. Before filling the bags, soil was firstly sieved with a 2 mm mesh sieve to remove coarse particles and elements that may disturb rooting. After filling the bags, they were placed under trees' shade at the botanical garden. The pots were moistened with 20 l of water before transplanting cuttings. After transplanting, the plants were watered with 20 l of water every morning for the first 15 days. The plants were maintained in the pots over a period of 4 months from January 16 to May 16, 2020. Polyethylene bags of 20 x 10 x 8 cm^3 were used for the pot experiment, which represent the experimental unit. The plant materials were treated one week after transplanting with Sunpyrifos 48% EC insecticide against termites.

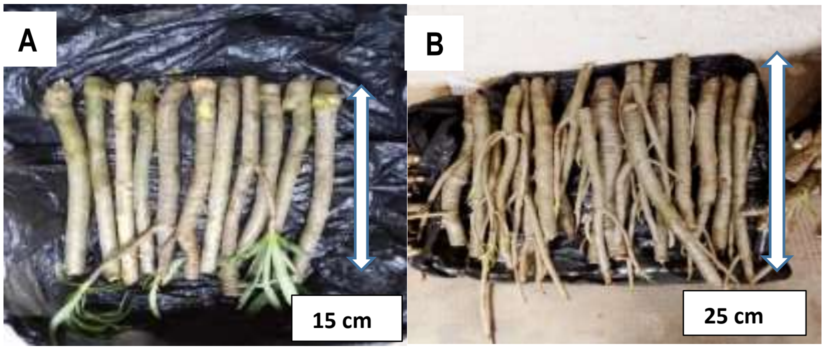

Figure 2: *Euphorbia balsamifera* cuttings 15 cm (A) length and 25 cm (B) length.

Table 1: The physical and chemical characteristics of the substrates used (soil and compost) during the trial

Characteristics	Units	Soil	Compost
pH (H_2O)		6.47	7.92
pH(KCl)		5.92	7.19
Total N	(g kg^{-1})	0.4	2.3
Available P	(mg kg^{-1})	14.30	-
CEC	(cmol kg^{-1})	7.5	-
Exchange K	(cmol kg^{-1})	1.26	-
Clay	(%)	5.2	-
Silt	(%)	12	-
Sand	(%)	82.6	-
Total P	(g kg^{-1})	-	4.8
Total K	(g kg^{-1})	-	2.81
Total Ca	(g kg^{-1})	-	1.16
Total Mg	(g kg^{-1})	-	1.99

Data collection: The dates of observed budburst were recorded daily from January 23 to 16 May 2020. The budburst was the stage of vegetation recovery and then follows the growth of the newly formed buds. Regarding the cuttings, parameters collected were number of recovered plant per treatment (cuttings forming new leaves). Data collected allowed to determine the duration of stem cuttings recovered of the plant material (time elapsed between the planting and the appearance of the first leaves). From the number of stem cuttings recovered, we computed the proportion of stem cuttings recovered.

Statistical analyses: The Statistical Analysis System version 9.4 (SAS v. 9.4) software was used for the statistical analyses. They consisted mainly of two-ways analysis of variance followed by the Student Newman-Keuls test for the separation of means at the 5% threshold. The purpose of this analysis was to compare the combined effect of stem cuttings length and substrate type on the measured parameters. In case of significant interaction, the analysis is continued at the level of one factor (depending on the type of substrate and the stem cuttings length. To obtain normal distributions (analysis of variance hypothesis) requirement, the proportion of cuttings recovered were transformed with $2\arcsin\sqrt{x/n}$ (Dagnelie, 1998).

RESULTS

Effect of stem cuttings lengths and substrate on the proportion of stem cuttings recovered

Table 2 presents the results of the analysis of variance considering the stem cuttings lengths and types of substrate used. The substrates and stem cuttings lengths of plant material had highly significant ($p < 0.01$ and $p < 0.001$ respectively) effect on the proportion of stem cuttings recovered. Interaction length and substrate had also a significant ($p < 0.05$) effect on plant cuttings recovery. Figure 3 showed the effect of the stem cuttings lengths and types of substrate on the proportion and duration of the stem cuttings recovered. The results showed that the stem cuttings length 15 and 25 cm transplanted in the soil alone recorded the highest proportion of stem cuttings recovered (60± 8.16 and 85.00 ± 9.57 % respectively) comparatively to those transplanted in the mixture of soil + compost. However, we noticed a significant difference between stem length 15 cm for both substrates. The highest proportion (85.00 ± 9.57%) was recorded with stem length 25 cm transplanted in soil alone and the lowest proportion (30.00 ± 5.77%) was obtained with stem length 15 cm transplanted in the mixture of soil + compost. It is also noted that the stem length 25 cm transplanted in the soil alone improved significantly the proportion of cuttings recovered.

Table 2: Result of the analysis of variance (F values) of the proportion of stem cuttings recovered considering length of stem cuttings and types of substrate

Source of variation	DF	Value of Fisher	
		Proportion of stem cuttings recovered	
Length	1	61.36***	
Substrate	1	13.36**	
Replication	3	11.18**	
Length* substrate	1	6.82*	

* P < 0.05; ** p < 0.01; *** p < 0.001

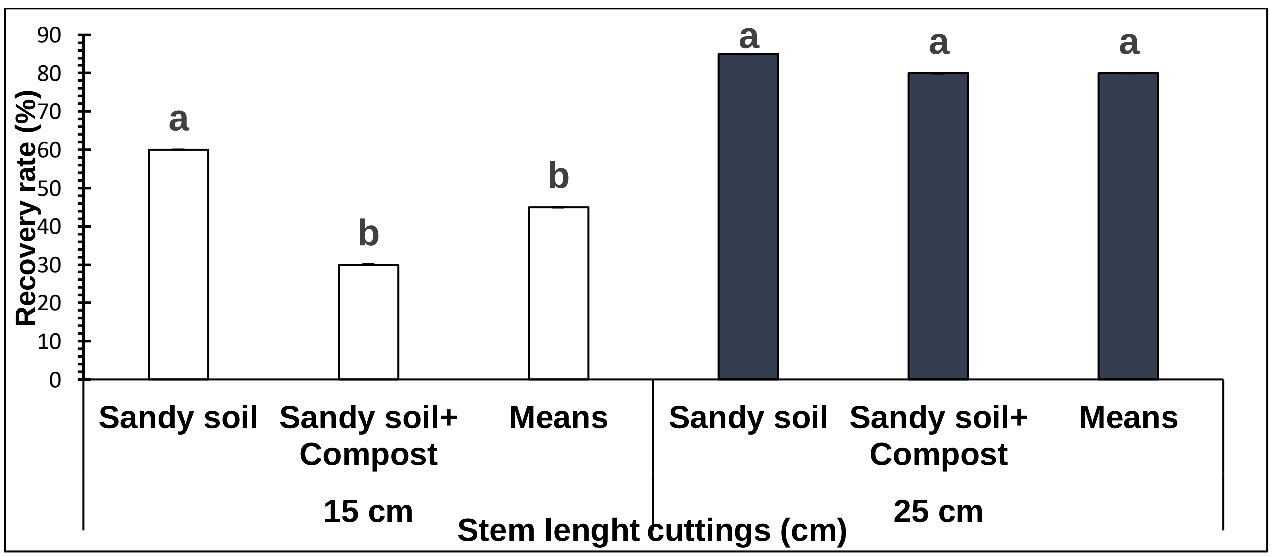

Figure 3: Effect of stem cuttings lengths and types of substrate on proportion (%) of stem cuttings recovered

The bars on the histograms show standard errors. The affected histograms of the same alphabetical letters are not significantly different (p >0.05) according to the Student Newman-Keuls test.

Effect of stem cuttings lengths and types of substrate on the duration (days) of recovered: Table 3 presents the results of the analysis of variance considering the stem cuttings lengths and substrates. The types of substrate and length of planting material had no significant ($p > 0.05$) effect on the duration of stem cuttings recovered. The interaction of planting material length and type of substrate had also no significant effect ($p > 0.05$) on the duration of the recovery. Figure 4 indicates the effect of the stem cuttings lengths of planting material and types of substrate on the duration of stem cuttings recovery. The results showed that the stem cuttings length 25 cm reduced the duration of the stem cuttings recovery (7.00±0.00 and 8.75±1.18 days) in the soil alone and the mixture soil + compost respectively compared with the 15 cm length. The shortest recovery duration (7.00±0.00 days) was obtained with stem cuttings length 25 cm in soil alone. In general, the stem cuttings length of 25 cm transplanted in the soil alone has reduced the duration of the stem cuttings recovered.

Table 3: Result of the analysis of variance (F values) of the proportion and the duration (days) of the cuttings recovered considering length of stem cuttings and types of substrate

Source of variation	Degree of Freedom	Value of Fisher	
			The duration of stem cuttings recovered
Length	1		2.77 ns
Substrate	1		0.74 ns
Replication	3		1.19 ns
Length* substrate	1		0.00 ns

ns: no significant

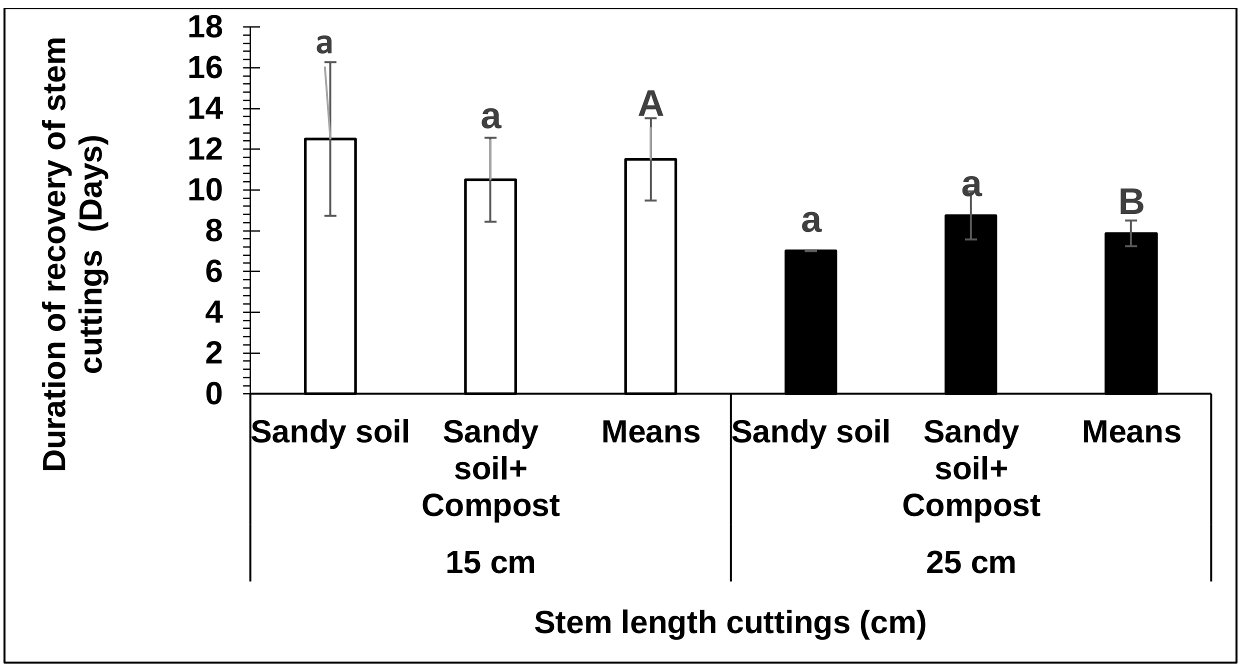

Figure 4: Effect of stem length and types of substrate on the duration (days) of stem cuttings recovered

Bars on the histograms indicate standard errors. The affected histograms of the same alphabetical letters are not significantly different ($p > 0.05$) according to the Student Newman-Keuls test.

DISCUSSION

Seven days after transplanting, some stem cuttings had their first budburst indicating their recovery. These first buds are the signs marking the process of growth and later development. The stem length of 25 cm showed the best proportion of cuttings recovered. The budburst marking the recovery process is quickly noticed on the apical stems, which are mostly smaller than the other stems. This more rapid recovery of these types of stem cuttings could be explained by the juvenility of the apical part of these stems compared to the basal part. The apical part of the plant, which is chronologically the oldest, is the least mature part in terms of ontogeny (Wiesman and Jaenicke, 2003). The highest percentage of rooting, observed in the apical stem cuttings, can be related to a low level of phenolic compounds at these locations and the fact that hormones (mainly auxins) and cofactors that act in rooting are in high concentrations at the apex of branches (Hartmann *et al*., 2010; Pigatto *et al*., 2018). Pigatto *et al.* (2018) added that apical cuttings have higher rates of auxin synthesis and may be less tissue differentiated, but they are more sensitive to dehydration. Similarly, Sanogo *et al.* (2008) found in their study on the possibilities of *in situ* cuttings of *Lawsonia inermis* L. (henné) of different ages, 99% recovery proportion for young cuttings and 71% for adult cuttings. The difference in the recovery proportion in this study was due to the age of the cuttings (6 months, and one year) which induces a significant effect on the proportion of the stem cuttings. According to various authors (Hounmey *et al.,* 2007; Maile and Nieuwenhuis, 2010; Sanoussi *et al.*, 2012), the recovery capacity of cuttings might have linked to the following internal factors: the presence of lignin, carbohydrate content of plants, growth hormones and nutrient contents. Hannah and Jan (2003) reported that the presence of undifferentiated meristematic cells, capable of rapid multiplication, forms the necessary tissues which, in turn, form organs such as buds and rootlets. Like many species, several environmental factors can influence the proportion of recovery of cuttings because according to Dembélé *et al.* (2012), the relative humidity of the ambient air and the substrate, the ambient air temperature and the light in some cases are all factors that influence the rooting of the cuttings. The success proportions of stem cuttings are often variable. The work of Harrouni (2002) showed that the cutting season also has an effect on rooting with an increase in the rooting proportion and a reduction in rot. In Mali, for example, the success proportion for the cuttings of *Euphorbia balsamifera* reached around 70% when the period suitable for cutting and its establishment takes place at the beginning of May until the beginning of July that is the end of the dry season and the start of the rainy season (IER, 1994). In the context of our work on the species, the recovery proportion was higher than that obtained in Mali due to the environmental conditions, which were probably more favourable than those of Mali were because the trial was implemented in the south of Benin in sub-equatorial climate condition. Season when the cuttings are taken is one of the major factors for rooting success of cuttings and plant species respond differently to this. This might be very important to some plant species, whereas in others it might not make any difference in root development (Hartmann and Kester, 1983; Klein, Cohen, Hebbe, 2000). The recovery of cuttings of stems of species presents significant variability. Several studies reported variable proportions of recovery of cuttings. Houehounha *et al*. (2009) reported a low recovery proportion of stem cuttings on *Daniellia oliveri* of 38.8%. Sanoussi *et al.* (2012) reported 46.62% of the recovery proportion of stem cuttings from *Vitex doniana*. Khativaran *et al*. (2009) obtained 68% of the proportion of stems cuttings recovered from *Jatropha curcas* L. Dan Guimbo *et al.* (2017) obtained 91.67% of the proportion of recovery in rainy period of *Leptadenia hastata* (Pers. communication). These differences could be probably due to several factors such as species, age, diameter, length of cuttings, period of sampling, origin (samples from young or old mother plants), types of cuttings (basal, intermediate or terminal cuttings) and environmental conditions. In the present study, the stem cuttings length of 25 cm transplanted in soil alone had reduced the number of days of recovery. This confirms on the one hand the performance of the 25 cm stem cuttings length and on the other hand the physico-chemical characteristics of the soil and its high water retention capacity and permeability. The characteristics of the substrates have also shown that the pH (water) of the soil is close to neutral (6.47) while that of the compost was alkaline (7.92). Most of the nursery plants grow well in soils with a pH between 5.0 and 7.2 (MAAO and MAR, 2014). The reduction of the duration recovery days could be explained by soil texture which was mainly sandy (82.6%) compared to the other elements (clay and silt respectively 5.2 and 12%). The sandy texture is the sign of a well-aerated, well-drained and permeable soil. According to Schimitz *et al*. (2002), sand is a material capable of ensuring higher water availability. Several research works have shown that the particle size of the substrate has an influence on plant growth (M'Sadak *et al.,* 2014). The choice of a substrate for plant production should take into account a number of physical factors such as texture and density that interfere with aeration, water retention capacity and substrate aggregation (Gimenes *et al.*, 2015; Hartmann *et al*., 2011). The significant effect of the culture medium on different root characteristics such as number and length of root (Severino *et al.,* 2011), and number of primary roots and amount of root dry matter (Caspa *et al.,* 2009; Aghdaei *et al.*, 2019) has been reported in different studies. Likewise, according to Arbonnier (2000), *Euphoria balsamifera* used to grow on soils or rocky places. Based on the results obtained in the present work, it is showed that the supply of suitable substrate (soil) and length of stem cutting can create favourable conditions for rooting stem cuttings for *Euphorbia balsamifera.* This shows an important result for the domestication of the plant for cow milk production improvement by the herders.

CONCLUSION

This study showed that the best proportion of stem cuttings recovered is obtained from stems length of 25 cm transplanted in soil alone. Stems length of 25 cm planted in soil alone had a reduced the duration before stem cuttings were recovered. With its capacity to induce more stem cuttings recovered and reduce the duration of recovery, sandy soil alone can be recommended as the best substrate. Managing the propagation method is the first step in the domestication process of lactogenic plant species and an important tool for the conservation to enrich biodiversity. The nursery and materials used in this study were appropriate, cheap and accessible to any agro-pastoralists aiming to improve cow milk production. Therefore, additional investigation (period of cutting) may be required to optimize the percentage of stem cutting. Future studies would take account the season of cuttings collection and age of cuttings for rooting success.

CONFLICT OF INTEREST

There are no conflicts of interest.

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