

Vegetative Propagation of *Balanites aegyptiaca* (L.) Del. by Air Layering under Sahelian Climate in Niger

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Authors' contributions

This work was carried out in collaboration among all authors. Author MKAH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript.

Authors HR and LA supervised the work and corrected the manuscript. Authors BMM and IBY participated in the correction of the manuscript. Author AM contributed in the scientific orientation of the work and the final revision of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Balanites aegyptiaca (L.) Del. is a fruit species from the Sahelian and Sudano-sahelian zones, highly appreciated by the population. It provides a source of income for the rural population and its various parts are used for human and animal food, handicrafts and traditional medicine. However, the lack of data on propagation techniques of the species is a significant limitation to its domestication. This study aims to evaluate the vegetative propagation capacity of *Balanites aegyptiaca* by air layering. The trials were conducted in the Arboretum of Sahara Sahel Foods in Zinder, Niger. The layers were placed on orthotropic branches. The variables selected were the diameter class (1-2 cm, 2-3 cm and 3-4 cm) and the substrates (sand+manure, sand+sawdust and sand). A total of 116 layers were made. The results show that 93.1% of the layers have taken root two months after realization. The diameter classes and the three substrates have no significant influence on the number of roots. In contrast, root length varied significantly with substrates ($P =$

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0.000) and branch diameter classes ($P = 0.031$). As for the diameter of the newly formed roots, a significant difference was observed between the diameter classes and the substrates have no significant effect on this parameter. One month after weaning of layers, an average survival rate of 68.5% was obtained. The seedlings of the diameter class 2-3 cm have the highest survival rate (83.1%). These results suggest that it is possible to produce *Balanites aegyptiaca* plants by air layering, and could contribute to the development of strategies adapted to its domestication.

Keywords: *Balanites aegyptiaca*; air layering; seedling production; domestication; Sahel; Niger.

1. INTRODUCTION

In the Sahel, woody species, and especially fruit trees, present a certain socio-economic interest for local populations. They provide income to households, contribute to food security and improve the health and nutritional status of these populations [1,2,3,4]. In recent years, the population explosion has led to overexploitation of multipurpose trees with a direct impact on the status of their natural stands and household incomes [5,6]. Many woody stands are thus in a regressive dynamic, characterized by rarefaction or the absence of young individuals [7,8]. It is therefore essential to maintain and reverse this regressive trend by reforestation using multipurpose woody or fruit trees.

B. aegyptiaca is a multipurpose species in Niger. It is exploited for its fruits, the wood for the manufacture of agricultural tools and for various medicinal products. Fruits are eaten raw and their almonds are transformed into oil and soap [9,10]. The young leaves and flowers are consumed by the populations, especially during the food lean season [11]. In Niger, Many households live through the exploitation, processing and marketing of fruits of this species.

Faced with these multiple uses, and in a context of degradation of ecosystems and climate change, it is important to conserve the genetic and economic potential of the species through research programs on propagation modes, and to consider its domestication.

Sexual reproduction of woody species remains the favored method of propagation for the conservation of the environment and the richness of its biodiversity [12]. However, for some species, the lack of seed or low germination capacity reduces the production seedling by the sexual way [13]. In addition, the ability of seedlings to move from the young to the adult stage is hampered by several factors including the tooth of animals, bush fires and especially

the long dry season [14,15]. Vegetative propagation can be a solution to the problems of natural regeneration [16]. It allows the conservation of a genetic patrimony and the capture of an interesting genotype by cloning agronomic interesting subjects. Air layering is one of the techniques used in vegetative propagation. It is carried out directly on the tree, on selected portions of stems or small branches. The time of root formation varies according to the layered species [17]. In Niger, in the field of vegetative propagation, work has been successfully conducted on species of high socio-economic interest [18,19]. But, scientific data on vegetative propagation of *B. aegyptiaca* are almost non-existent. As such, this work aims to evaluate the vegetative propagation capacity of *B. aegyptiaca* by air layering.

2. MATERIALS AND METHODS

2.1 Study Site

The trials were conducted in the Arboretum of Sahara Sahel Foods in Zinder, south-central Niger (13°08'00" North and 08°58'36" East). According to the phytogeographical subdivision of Niger proposed by Saadou [20], this site is located in the central South-Saharan sector (Compartment B2). The climate is marked by a long dry season (October to May) and a short rainy season (June to September) (Fig. 1). Average annual rainfall varies from 400 to 500 mm. Mean annual temperatures range from 25 to 30°C.

2.2 Setting up the Layers and Data Collection

The layers were placed from 6 to 8 July 2018 during the rainy season. The parts concerned by the layering on the branches were previously cleared leaves and thorns. The operation consisted of removing a complete ring of bark on the branches over a length of 5 cm (Fig. 1a). The stripped part was wrapped with a transparent

and sufficiently thick plastic sleeve containing the substrate. The two ends are then tightly sealed with adhesive tape (Fig. 2b). The treatments consisted of placing the layers on the median part of the orthotropic branches. The variables tested are: diameter classes of branches (1-2 cm, 2-3 cm and 3-4 cm) and substrates (sand+manure, sand+sawdust and sand). The numbers of layers placed per diameter class and per substrate are shown in Table 1.

The number of layered branches per tree of *B. aegyptiaca* varies from 3 to 6. This number depends on the availability and accessibility of orthotropic branches on the tree. A total of 26 trees of *B. aegyptiaca* were used for this operation. Each layer placed was numbered, and the nature of the substrate and the diameter class of the branch were also recorded. A quantity of water of 20 ml was injected into the layers with a syringe each time the state of

humidity of the substrate is low. After the injection, the holes caused by the introduction of the needle are immediately closed with the adhesive tape. Other care has been provided to the layers, in particular the replacement of the adhesive tape which yielded following the sunshine, the rain and the wind. The observations made every 48 h consisted of: the appearance and development of the roots in the substrate through the plastic, the possible signs of drying of the layers on the tree, the drying out and the appearance of buds on the layered branches.

Two (2) months after application, the rate of rooted layers, non-rooted layers and dead or rotten layers per treatment was recorded. On the roots formed, the following parameters were noted: the number, the length measured with a ruler and the diameter measured with an electronic caliper. These measurements were made on half of the rooted layers.

Table 1. Number of layers placed per diameter classes and substrates

Diameter classes (cm)	Substrates			Total
	Sand+manure	Sand+sawdust	Sand	
1-2	13	15	12	40
2-3	11	12	13	36
3-4	13	13	14	40
Total	37	40	39	116

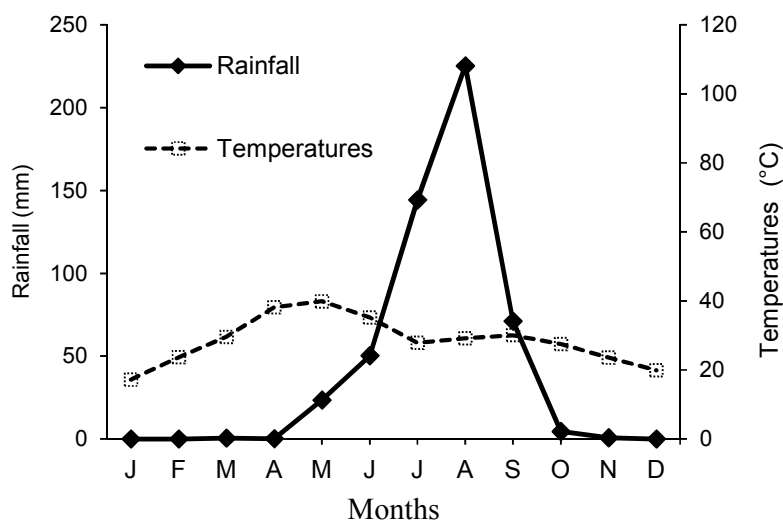


Fig. 1. Ombrothermic diagram of the Zinder meteorological station (Monthly averages from 1981 to 2017)

2.3 Weaning of Rooted Layers

The weaning was carried out on the second half of rooted layers. For this purpose, planting holes were dug. No organic amendments were made in the holes, considering the richness of the soil in well decomposed organic matter. The layers were separated from the mother plants by cutting the lower part with a horticultural saw (Fig. 3) and transplanted directly into the prepared holes, after having carefully removed the adhesive tape and the plastic. The layers had leaves at transplantation. The plants were watered in the absence of rainfall to ensure permanent soil moisture. Every day, the numbers of live and dead plants were recorded for one month. From this observation, the survival rate (SR) was calculated using the following formula:

$$SR = \frac{NLP}{NTP} \times 100 \quad (1)$$

With NAP being the number of live plants, and NTP the number of transplanted plants.

2.4 Statistical Analysis

An analysis of variance (ANOVA) was performed to determine the types of substrates and diameter classes that affected the number, length, and diameter of newly formed roots. To check if the success rate depends on layered diameter classes, an X^2 test was used. These data were analyzed using the Minitab 17 software.

3. RESULTS

3.1 State of the Layered Branches and the Rooting Capacity of the Layers

Two weeks after the realization of the layers, 56.3% of the layered branches all treatments (substrates and classes of diameter classes)

combined have leaf budding at the lower part of the incised area. No signs of drying were observed on the layered branches during the entire time of the experiment. At harvest, 107 layers rooted out of 116 realized, representing an overall success rate of 93.1% (Table 2). All diameter classes and substrates tested showed good rooting ability of layers, with success rates ranging from 83.3 to 100%. The X^2 independence test performed on the contingency table showed that the success of the layers is independent of the diameter classes ($X^2 = 0.71$, $P = 0.949$). Among the non-rooted layers, 3 have root callus and the other 5 have been found intact and without any sign of rotting.

3.2 Effects of Substrates and Diameter on Newly Formed Roots

The roots produced by the layers with the three substrates are vigorous and well branched (Fig. 4). Indeed, the number of roots was not significantly influenced by the substrates ($P = 0.998$) (Table 3). On the other hand, the average number of roots of the diameter class 3-4 cm differs from the diameter class 1-2 cm by a significantly high value ($P = 0.012$). ANOVA showed that the number of roots of the diameter class 3-4 cm and that of the diameter class 2-3 cm are statistically the same. Root length averages are significantly different between substrates ($P = 0.000$). In fact, the sand+manure substrate was well distinguished from other substrates with long roots (Table 4). Analysis of variance also showed that branch diameter classes have significant effect on root length ($P = 0.031$). The diameter class 2-3 cm seems to have long roots (13 ± 2.4 cm) compared to the other two diameter classes. As regards the diameter of root, the substrates tested did not have a significant effect on this parameter ($P = 0.332$) (Table 5). In contrast, the comparison of mean root diameter values between branch diameter classes revealed a significant difference ($P = 0.004$).

Table 2. Rooting rate (%) of layers per diameter classes and substrates (Mean \pm standard deviation)

Diameter classes (cm)	Substrates			MD
	Sand+manure	Sand+sawdust	Sand	
1-2	84.6	93.3	83.3	87.0 \pm 4.4
2-3	100	91.6	92.3	94.6 \pm 4.6
3-4	92.3	100	100	97.4 \pm 4.4
MS	92.3 \pm 7.7	95 \pm 4.4	91.8 \pm 8.3	93.1

MS: Averages of the three substrates; MD: Averages of the three diameter classes

Table 3. Average number of roots per diameter classes and substrates (Mean \pm standard deviation)

Diameter classes (cm)	Substrates			MD
	Sand+manure	Sand+sawdust	Sand	
1-2	27.1 \pm 13.5	35.7 \pm 9.6	38.6 \pm 19.4	33.8 \pm 15.2a
2-3	49.6 \pm 20.6	44.1 \pm 17.8	35.4 \pm 10.3	43 \pm 16.3ab
3-4	47.8 \pm 7.6	52.2 \pm 10.1	51.4 \pm 15.8	50.4 \pm 1.1b
MS	41.5 \pm 1.5a	44 \pm 15a	41.8 \pm 15.9a	-

For means per substrates (MS) and means per diameter classes (MD), the values followed by the same letter are not different at the 5% threshold

Table 4. Average values (cm) of root length per diameter classes and substrates (Mean \pm standard deviation)

Diameter classes (cm)	Substrates			MD
	Sand+manure	Sand+sawdust	Sand	
1-2	13.9 \pm 3.4	11.7 \pm 2.0	12.3 \pm 2.3	12.6 \pm 2.9a
2-3	13.6 \pm 2.3	13.1 \pm 2.7	12.2 \pm 1.9	13 \pm 2.4b
3-4	12.4 \pm 2.6	12.1 \pm 2.2	12.7 \pm 1.6	12.4 \pm 2.4a
MS	13.3 \pm 2.9a	12.4 \pm 2.5b	12.4 \pm 2.0b	-

For means per substrates (MS) and means per diameter classes (MD), the values followed by the same letter are not different at the 5% threshold

3.3 Survival Rates of *B. aegyptiaca* Plants from Layering after Weaning

Apparent mortality was observed 4 days after weaning in diameter class 2-3 cm plants, which reduces the survival rate of this diameter class to 83.3% and the overall rate at 95.1%. This is followed by the plants of diameter class 3-4 cm, whose mortality begins 6 days after transplantation. The disappearance seedlings of the 1-2 cm diameter class begin to be observed only from the 13th day, almost half of the follow-

up time (Fig. 5). At the end, the overall survival rate is 68.5%, plants in the class 2-3 cm in diameter have the highest survival rate (83.1%), followed by the class 1-2 cm (79.2%) and the class 3-4 cm (43.2%). Signs of successful weaning were observed on some plants in the first week, including the appearance of new leaves (Fig. 6a). It should also be noted that some plants presented a transient stress, characterized by the loss of the initial leaves and the beginning of desiccation of the twigs, before recovery a few days later (Fig. 6b).

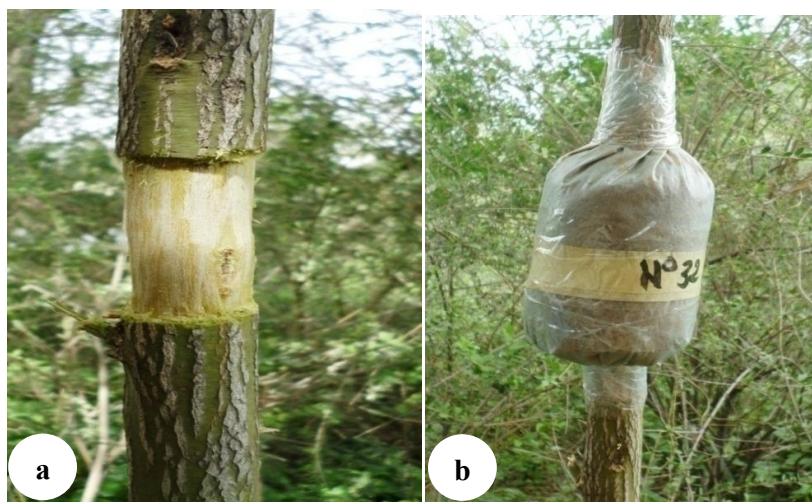


Fig. 2. Establishment of aerial layer *B. aegyptiaca*: (a) Annular incision of the branch; (b) substrate wrapped in the sleeve

Table 5. Average values (mm) of root diameter per diameter classes and substrates (Mean ± standard deviation)

Diameter classes (cm)	Substrates			MD
	Sand+manure	Sand+sawdust	Sand	
1-2	1.6±0.5	2.0±2.5	1.6±0.6	1.7±1.4a
2-3	2±0.6	1.9±0.4	2.0±0.5	1.9±0.5b
3-4	2.1±0.7	52±0.6	1.9±0.5	2±0.6b
MS	1.9±0.6a	1.9±1.8a	1.8±0.5a	-

For means per substrates (MS) and means per diameter classes (MD), the values followed by the same letter are not different at the 5% threshold



Fig. 3. *B. aegyptiaca* layers harvested for weaning



Fig. 4. Roots formed with three substrates: (a) Sand + manure; (b) Sand + sawdust; (c) Sand

4. DISCUSSION

The appearance of new leaf buds in the lower part of the layered branches is simply explained by the presence of well-differentiated nodes on these parts. The incision of the bark disturbs the circulation of the elaborated sap; its accumulation in the upper part of the incised zone could stimulate the formation of the new

leaves, especially when the setting up of the layers was carried out in of rainy season. This season corresponds to the period of strong photosynthetic activities resulting in an important vegetative development. All non-rooted branches were alive at harvest, which means that they are likely to take root later. This suggests that the rooting of *B. aegyptiaca* layers may extend beyond the time considered in this study.

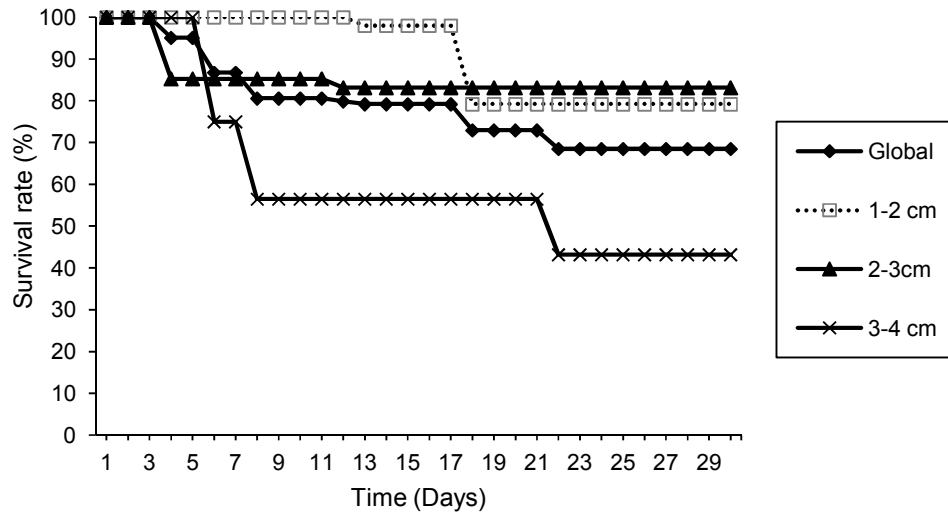


Fig. 5. Evolution of survival rate of plants by diameter classes as a function of time

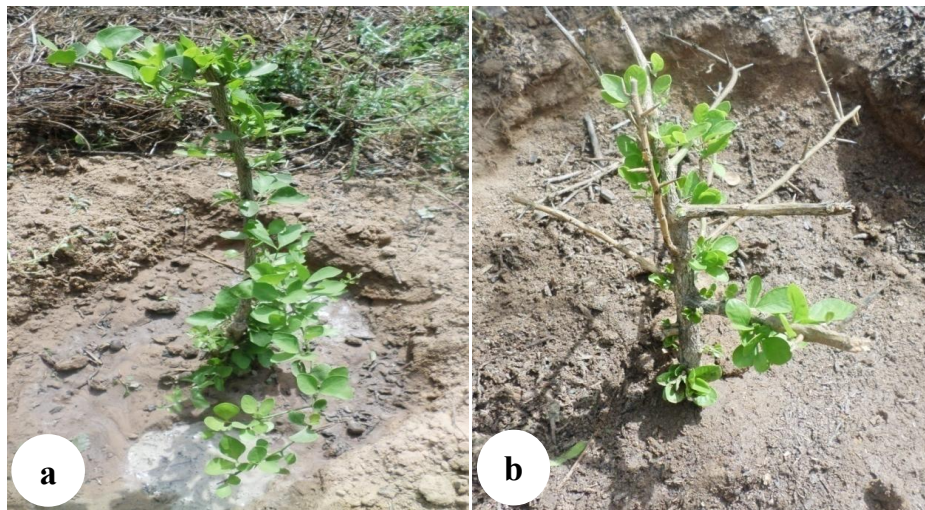


Fig. 6. *B. aegyptiaca* plants from aerial layering after transplantation: (a) Well acclimated plant with new leaves; (b) Plant having a transient stress

The rooting rate of the layers obtained in this study is similar to that obtained by Tchiagam et al. [21] on the same species. This rate could be related, on the one hand, to the humidity of the climate during the experimentation and also to the orthotropic orientation of the layered branches. These layers placed on the orthotropic branches could benefit from a supply of rainwater that runs along the branches passing under the tape. In their study, Elomo et al. [22] obtained a higher number of roots in orthotropic oriented layers compared to plagiotropic and oblique

orientations on *Dacryodes edulis* (G. Don) H.J. Lam. Mbète et al. [23] also observed rapid root emission on orthotropic branch in the kola tree (*Cola nitida*). Bonnèhin [24], in turn, asserts that in vegetative propagation, the plagiotropic or orthotropic orientation, as well as the origin of the axes used, affects the rooting capacity of the layers.

The diameter classes of the layered branches are relatively small in terms of their size; this could also be the basis of the high rate of rooting.

According to Meunier et al. [12], air layering is more likely to be successful when performed on young branches. In contrast, other studies [25, 26,27,28] have shown more significant results on large branches. This could be explained by the fact that for some species, young twigs have a small thickness of bark, cannot accumulate a significant amount of nutritive substances favorable to cellular differentiation towards the rhizogenesis [27,29].

Zida [30] obtained a success rate of 65 and 71.6% respectively in the median and basal part of the *B. aegyptiaca* stem. These rates are low compared to those observed in this study. This difference is largely due to the installation period of the device; because this author installed his trial of layering in February which corresponds to the dry season in Burkina Faso. Several studies [8,17,31] attribute the success of air layers to the trial period, to the age of the plant and to the test environment that would have a significant effect on rhizogenesis. Harivel et al. [29] estimate that the dry season would delay or even inhibit the development of layers, especially in the Sahelian zone where plants return to abscission to limit the loss of water.

The success rate of the layers observed in the present study is also higher than those obtained in several studies on other ligneous species, in particular on *Pterocarpus erinaceus* Poir. [27, 32], *Prosopis africana* (Guill & Perrot) Taub. [18, 33], *Detarium microcarpum* Guill. & Perr. [33], *Coula edulis* Baill. [16] and *Psidium guajava* L. [34]. This is explained by the fact that the rooting potential of the layers depends first of all on the genetic potential of the species or variety, with very marked differences between genera, species and even individuals [35,36]. In addition to the intrinsic variations of species, environmental, climatic and experimental conditions could explain these differences. The present experiment has shown that the three substrates tested have no influence on the rooting capacity of the layers and on the quality of roots, particularly on the number and the diameter. These results suggest that *B. aegyptiaca* layers can be rooted with several substrates, provided that watering is regularly ensured. In addition, these results testify to the ecological plasticity of the species, particularly to soils, as several authors have pointed out [9,37].

The assessment of the rate of rooting of layers should not be the only criterion to be taken into account, to qualify a species suitable or refractory to air layering. It is also necessary to

take into consideration the survival rate of the layers after weaning. The survival rate obtained in this study is 68.5% overall, with variations depending on plant diameter. This survival rate may not vary significantly even if the follow-up time is lengthened, because at the end of the observation all the living plants presented the sign of a good weaning, in particular the formation of the new leaves. The low survival rate observed for plants of diameter 3-4 cm could be related to the imbalance between the volumes of the aerial part, which is very important in relation to the volume of the part of the roots. In addition, transplantation conditions may not be appropriate because in some work [38, 39] weaning of aerial layering plants was initially done in pots prior to transplanting them to a real environment.

5. CONCLUSION

This study has shown the possibility to multiply *B. aegyptiaca* by air layering in two months, especially those with characteristics considered interesting by the population. The rooting of the layers is also possible on branches 1 to 4 cm in diameter and with substrates easy to find by farmers. In addition, the study has also shown that the propagation of *B. aegyptiaca* by air layering does not require hormones rhizogenesis, sphagnum or moss, inaccessible to farmers because of their high prices and their absence in local markets. The evaluation of the survival rate after transplantation of rooted layers showed that more than half of the plants survived after a month of monitoring. These prospects would allow *B. aegyptiaca* to be added to the list of sahelian woody species that are easily propagated by air layering and at a lower cost.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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