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STATUS OF VEGETATIVE PROPAGATION OF BAOBAB: A REVIEW

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ABSTRACT

The African baobab (*Adansonia digitata* L.) is a large tree of great socio-economic and cultural importance in Africa, with almost all the parts of the species used for various purposes. A major concern about baobab fruit pulp production is the long time it takes for first fruiting (about 15 years). Vegetative propagation offers several advantages with regard to consumers' preferences and precociousness of fructification. The objective of this study was to synthesise existent knowledge related to vegetative propagation methods of baobab and examine future prospects for improving the species propagation. This will ultimately contribute to better integrate baobab-based agroforestry systems into the diversification and poverty alleviation programmes. It is clear that cutting, grafting and *in vitro* multiplication are the vegetative propagation methods already tested on baobab. The success of grafting methods ranges from 10 to 89%, depending on the technique used. The Murashige and Skoog environment, supplemented with or without growth regulator hormones is by far the best condition for the *in vitro* reactivity of baobab explants, regardless of their types. With regards to cuttings, the average success rates stand around 30% when Indole-3-butyric acid (IBA) hormone is used. Other approaches such as marcotting techniques are yet to be tested and data on fruit production using these techniques are still needed in order to determine the best promising method for rapid and efficient vegetative propagation of baobab.

Key Words: African baobab, cuttings, grafting, marcotting

RESUME

Adansonia digitata L. est un grand arbre d'importance socio-économique et culturelle en Afrique. Toutes les parties de l'arbre sont utilisées à diverses fins. L'une des principales préoccupations concernant la pulpe de baobab est le temps nécessaire à la première fructification de l'arbre (environ 15 ans). La multiplication végétative présente plusieurs avantages en ce qui concerne les préférences des consommateurs et permet la précocité de fructification. Ce travail a synthétisé les connaissances actuelles sur les méthodes de propagation végétative du baobab et a discuté des perspectives futures pour l'intégration des systèmes agroforestiers à base de baobab dans les programmes de diversification agricole et de réduction de la pauvreté. Les résultats ont montré que le bouturage, le greffage et la multiplication *in vitro* sont les méthodes de multiplication végétative déjà testées sur le baobab à ce jour. Le succès du greffage varie de 10 à 89% selon la technique de greffage utilisée. L'environnement Murashige and Skoog complété avec ou sans phytohormones de croissance est à l'heure actuelle la meilleure condition pour la réactivité *in vitro* des explants de baobab, quels que soient leurs types. En ce qui concerne le bouturage, le taux de réussite moyen s'élevait à 30% avec utilisation de l'hormone Acide Indole-3-butyrique (IBA). D'autres approches telles que la technique du marcottage aérien devront être testées. Aussi, les données sur la production de fruits issus de ces techniques sont nécessaires. Ces informations permettront de déterminer la meilleure méthode à recommander pour une propagation végétative rapide et efficace du baobab.

Mots Clés : Baobab, bouturage, greffage, marcottage

INTRODUCTION

The African baobab (*Adansonia digitata* L.) is characteristic of the Sahelian zones and belongs to the Malvaceae family. It is the only species among the eight of the genus Adansonia occurring in West Africa (Wickens and Lowé, 2008). The tree yields hanging flowers and produces capsules that contain numerous seeds, surrounded by a floury pulp. The leaves are rich in vitamin C, pro-vitamin A, mineral salt, and iron; and are used as vegetables (Chadare *et al.*, 2009; Kamatou *et al.*, 2011). Leaves and bark are used to treat several diseases such as malaria, tuberculosis, microbial infections, diarrhoea and anaemia (Van Wyk and Gericke, 2000; Brendler *et al.,* 2003; Tapsoba and Deschamps, 2006; Wickens and Lowe, 2008; De Caluwe *et al.,* 2009). Pulp of the fruit is used in folk medicine as a febrifuge (Sidibe and Williams, 2002; Bosch *et al.,* 2004).

Recently, baobab has been referred to as a "super fruit" based on its nutritional profile (e.g. vitamin, fatty acid, and mineral). Its fruit pulp has a very high vitamin C content (up to 500 mg 100 g dw^{-1}, approximately ten times more than that of orange, and three times that of chocolate milk); hence a high anti-oxidant property. For instance, consumption of 40 g of baobab pulp provides 100% of the recommended daily intake of vitamin C in pregnant women (19 - 30 years) (Chadare *et al.*, 2009). Due to this exceptional nutritional value, baobab has been acknowledged as a novel food by the European Union in 2008 (regulation EC N°258/97 of the European Parliament); and also accepted as food ingredient in the USA (Addy 2009).

When propagated from the seed, the first flowers of the baobab appear 8-23 years later (Diop *et al.*, 2005), and the young trees are often not identical to the parental ones due to cross-pollination (Assogbadjo *et al.*, 2006). This makes vegetative propagation the option of choice, if attributes of mother trees are to be conserved.

Vegetative propagation is the type of propagation that uses fragments of the vegetative system to propagate the plant. It can be divided into two groups: macro-propagation and micro-propagation. The different variants of macro-propagation most commonly known are cuttings, layering and grafting (ICRAF, 2011). Micro-propagation mostly deals with *in vitro* culture techniques because they are so far the best for micropropagation; and the techniques mostly result in true to type plants. However, the high cost of the needed settings and the technologies make it inaccessible to farmers compared to macro-propagation techniques (Singh 2015).

The main advantage of vegetative propagation is to avoid a great deal of heterogeneity, which often results from seed propagation (Sidibé and Williams, 2002). Other advantages of vegetative propagation over seed-based propagation include earlier fruiting of trees; the integral transfer of hereditary characters of the mother tree to progenies; the multiplication of species whose seeds are not available; and the good timing of the production because it no longer depends on the fruiting seasons (Assogbadjo *et al.*, 2009). However, the risk of intensification of diseases is great because the chances of creating new varieties are reduced and the techniques may be more expensive and may require greater expertise (ICRAF, 2011).

Vegetative propagation also offers the advantage to fix desirable traits, especially about consumer's preferences (Tchoundjeu *et al.*, 2006). For example, grafted plants have similar characteristic traits to the parental plants and can shorten period for fruit production. These grafted plants begin to flower in less than 5 years after grafting, compared to 8-23 years for the case of seed propagation (ICUC, 2002; Sidibe and Williams, 2002). In fact, grafting is a method of vegetative propagation that allows the production of individuals of the same genetic constitution as the ortet, and hence facilitates the multiplication of desirable genotypes (Sanou *et al*., 2004).

This vegetative propagation method can be a powerful tool for reproducing the desirable phenotypic traits of baobab (Simons and Leakey, 2004; Akinnifesi *et al.*, 2008). Studies have addressed vegetative propagation methods in baobab; however, no synthesis of these studies have been performed so far. Moreover, this is needed to guide further research aimed at improving the propagation of the species. The objective of this study was, therefore, to assess the status of the available information on different methods of vegetative propagation of baobab tree and provide future prospects for the improvement of the species propagation.

METHODOLOGY

A systematic literature review was conducted in order to gather the available information on the subject. To do so, the following research questions were addressed: (i) what studies have been done so far on vegetative propagation of baobab tree? (ii) what were the main findings and which aspects remained to be addressed?

To answer these questions, documents that focused on the vegetative propagation of baobab were searched for (both in French and English) in, among other online resources, Google scholar™, Academia, ResearchGate and African Journals Online. Because there appeared no previous reviews on this subject, this review was not restricted to a specific time period. Rather, all documents that fell within the scope of this review were considered. The key words used for document search included, though not restricted to cuttings, grafting, layering, multiplication, *in vitro* propagation, and micro-propagation in combination with African baobab, baobab or *Adansonia digitata*.

Selection study. Search results were carefully screened based on our inclusion criteria, which was all articles focusing on vegetative propagation of baobab tree, regardless the publication year. Figure 1 provides a flowchart for inclusion and exclusion criteria of studies that were considered in the review. From an initial 54 documents found, only 6 articles actually focused on vegetative propagation of baobab tree (Assogbadjo *et al.*, 2009; N'doyé *et al.*, 2012; Rolli *et al.,* 2014; Anjarwalla *et al.*, 2016; Mukhtar *et al.*, 2016; Jenya *et al.*, 2018).

The above-mentioned articles were published in the period between 2009 and

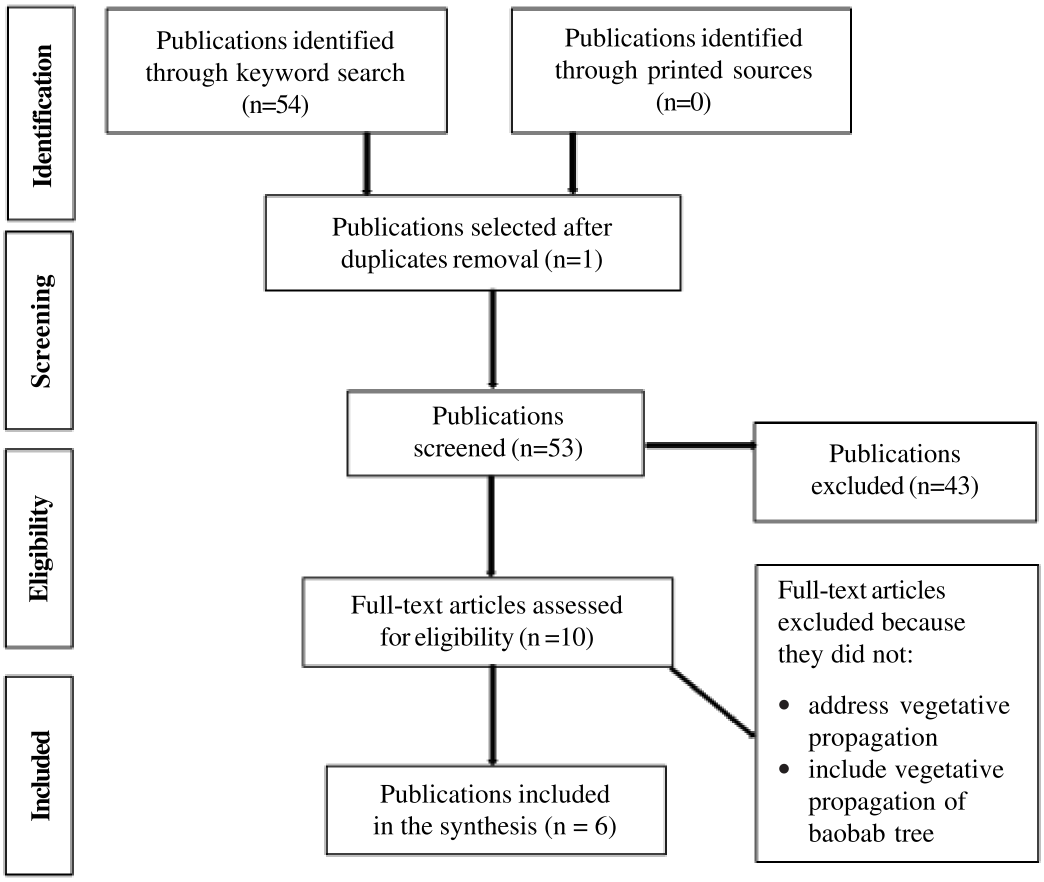

Figure 1. Flowchart of search literature on vegetative propagation of baobab tree.

2018. These six articles were selected through the following steps: (i) the relevance of document which was checked based on its title; (ii) screening of the title and abstract of all articles identified; (iii) downloading and reading of the full text of these article as they matched step two criteria; and (iv) inclusion of only publications that focused on vegetative propagation of baobab tree. Among the six articles that met our inclusion criteria, three focused on macro-propagation and three on micropropagation. These articles were thoroughly, analysed read and summarised.

FINDINGS

Macropropagation. Macropropagation is an *in situ* asexual method for multiplying planting materials which provides simple and relatively rapid techniques for vegetative propagation (Baiyeri and Aba, 2007). It includes several techniques such as grafting, cuttings and air or terrestrial layering. Macropropagation is useful for reducing the very long reproduction time, typical of certain trees like baobab. Furthermore, the method is affordable and can be easily implemented by farmers. Only cuttings and grafting are the techniques that have been tested on baobab (Assogbadjo *et al.,* 2009; Anjarwalla *et al.*, 2016; Jenya *et al.,* 2018).

There is no evidence of a study so far on baobab involving layering technique in baobab propagation. The application of marcotting has been successful in the improvement of some trees and shrubs which have multiple desirable traits without genetic segregation (Thirunanvoukkarasu *et al*, 2004).

Bridgemohan *et al.* (2016) tested air layering and reported a successful rate of rooting for marcotting breadfruit (91%). According to Bridgemohan *et al.* (2016), marcotting ensured more rooting success, including clones which do not root easily. Additionally, it is simple to perform and allows for larger plants which readily mature, to be produced in a faster manner. Information on such methods applied to baobab tree will contribute to a better knowledge of effective vegetation propagation method of baobab.

A major weakness with the macropropagation methods is the rebellious character of some trees, thus depending on hormonal growth use or squarely *in vitro* methods. Use of Indole-3-butyric acid (IBA) hormone in the macropropagation of baobab is required because cuttings treated with IBA produced more precursors during the rooting period (Ludwig-Müller, 2000). However, there are several other growth regulating hormones (e.g. BAP: 6-benzyl aminopurine, and IAA: Indole-3- Butyric Acid) (Asghari *et al.*, 2012) usually used to induce early rooting in plants. Asghari *et al.* (2012) assessed the effect of explants source on Murashige and Skoog medium, supplemented with different concentrations of BAP and the effects of different levels of BAP and IAA individually or in combination on direct regeneration of basil plants (*Ocimum basilicum* L.). The authors found that maximum regeneration percent (96.67+/-0.33) and average number of shoot (5.6+/-1.15) were observed on the medium containing 11 muM BAP + 0 muM IAA; while increasing BAP concentration led to decreased rooting. It is suggested that growth hormones should be concentrated in the buds in order to promote differentiation of vascular elements in the graft tissues (Hartmann *et al*., 2002). Future studies should be extended to these hormones and their comparative cost and accessibility by local communities assessed.

Cuttings. A cutting is a piece of the stem or root of the source plant that is used in horticulture for vegetative propagation. It regenerates a whole new copy of the source plant (McKey *et al.*, 2010). For the baobab tree, cuttings of about 10 cm have been used (Assogbadjo *et al.*, 2009). A more detailed treatise on baobab tree propagation by cuttings is available in Assogbadjo *et al.* (2009).

The success rate for baobab propagation by the cuttings method is only close to 2%. However, when supplemented with IBA hormone, the success rate jumps to about 30% (Assogbadjo *et al.,* 2009). This attests to the effectiveness of IBA hormones to improve the success rate for this particular technique with baobab tree. This method cannot then be easily applied in order to propagate baobab trees by local communities, unless there is an easy supply of this hormone in rural areas. In addition, the success rate with IBA hormone is still dismal (30 %), making it somewhat not effective for a massive propagation of baobab. Further investigations are needed to enhance our knowledge on this technique, particularly in relation to baobab. Combining IBA with nutrient solutions such as N-$NO3^-$, N-$NH4^+$ at different concentrations for fertigation of the cuttings may improve the success rate of cuttings in baobab tree. In fact, Filho *et al.* (2017) reported up to 97% of mini-cutting success rate for *Khaya anthotheca* when solutions with 100 and 50% of these nutrients concentration were combined with 2 g L^{-1} IBA.

Grafting. Grafting is a method of vegetative propagation through which individuals of the same genetic constitution as the ortet are produced. The method facilitates the multiplication of desirable genotypes (Sanou *et al*., 2004). For baobab tree, grafts of about 10 cm long and 1 cm in diameter have been taken from mature tree; and a rootstock from a young baobab tree are often considered for this purpose (Assogbadjo *et al.*, 2009). Grafting can be with a side veneer or top cleft. A side veneer is done by creating two opposite wounds (small and large) on the graft and inserting it into the rootstock, taking care to arrange the large wound against the sapwood and the small against the bark while a top cleft is done by inserting scion into the slot made in the rootstock (Agbohessou *et al.*, 2018a). A detailed procedure for grafting using the side veneer and top cleft technique has been described in Agbohessou *et al.* (2018a,b) and Anjarwalla *et al.* (2016).

The choice of between grafts and rootstocks is based on desired characters of mother trees from where grafts are collected, and vigour of rootstocks (Sanou *et al.*, 2004). Grafting has the characteristics of adding the quantitative traits of the strains used, thus shortening the reproduction period, and being realised at any time of the year (McKey *et al.*, 2010). Anjarwalla *et al.* (2016) tested side veneer grafting and top cleft grafting techniques on baobab using grafts of about 20 cm size; and observed that compared to 1-year-old rootstocks, 2-year-old rootstocks (young trees) performed better, 55 and 71% for top cleft grafting and side veneer grafting method, respectively. Jenya *et al.* (2018) obtained 66.6 and 63.3% survival rates for top cleft and side veneer grafting, respectively; using rootstocks of one year old. In addition, the average shoots length with the top cleft technique is higher compared to the average shoot length with the side veneer grafting method (Anjarwalla *et al.,* 2016).

Success in grafting is affected by the time of the year during which it is performed, owing to the rainy season (Jenya *et al.,* 2018). For instance, Jenya *et al.* (2018) in Malawi obtained one response in October and yet the value changed in November of the same year. The authors have found October as the better time compared to November for grafting baobab; however, they gave no explanation on this effect. This might be linked to environmental conditions (e.g. temperature, relative humidity, etc.) which might vary between these two months. Such an effect has been reported in jackfruit (Selvi *et al.*, 2008). It is therefore, imperative that proper timing is established to maximise success in baobab grafting efforts. No study has so far assessed the best time of the year for baobab grafting, although it has been advised that grafts should be harvested and grafted at different times over the year when they are at different physiological and at growth stages, while considering the variation in climatic conditions (Anjarwalla *et al.,* 2016). In addition, the study by Jenya *et al.* (2018) revealed significant differences between grafting methods in two different months. Shoot growth was higher for side veneer than for top cleft, implying that baobab is easily amenable to grafting when done at the right time, which is October month (Jenya *et al.*, 2018).

Overall, among the macropropagation techniques, grafting was the most tested, though additional insights are still needed on this technique. For instance, its success is affected by the seasons of the year, which vary across climatic regions in Africa. Extending the testing of this technique across several climatic regions will substantially advance our knowledge on the value of this technique. Also, monitoring of grafts until fructification has not been alluded to in the literature, yet this is required in order to better master aspects related to the technique's effectiveness for achieving the overarching goal of early mass and quality fruiting.

Micro-propagation: *In vitro* procedures. Micropropagation implies the culture of tissues and organs in vessels with defined culture medium and under controlled environmental conditions (Singh, 2015). N'Doyé *et al.* (2012) studied the optimal conditions for *in vitro* propagation of the baobab tree, to achieve massive production of plants for their subsequent introduction into the planting areas. The reactivity rate was 100% for apex, cotyledonary and axillary nodes cultured in various media. The number of newly formed shoots varied according to the type of explant, but also the nature and concentration of the hormone. Similar patterns were observed for the average shoot length and the mean number of newly formed nodes. Murashige and Skoog (MS) environment, supplemented with growth enhancer phytohormones (IBA, BAP or NAA), is efficient for the *in vitro* reactivity of baobab explants regardless of where the explants were collected from. Baobab explants react spontaneously in MS culture media containing or lacking growth regulators (N'Doyé *et al.*, 2012). The number of newly formed shoots depends not only on the type of explants (cotyledonary nodes, axillary nodes or terminal apex), but also on which phytohormone (IBA, BAP or NAA) is used and at which concentration; the latter varying depending on the phytohormone (N'Doyé *et al.*, 2012).

The average shoot length and the number of newly formed nodes also depends on the type of explants, nature and concentration of hormones (N'Doyé *et al.*, 2012). Growth regulators affect the formation of baobab roots in the *in vitro* culture (Mroginski *et al.*, 1981). For instance, N'Doyé *et al.* (2012) observed at thirty days after planting that the best medium for the growth of *A. digitata* explants was the medium MS + BAP 0.5 mg L^{-1}. Comparing the growth rate of explants grown on MS media containing different cytokinins like BAP and kinetin has allowed to demonstrate that the BAP is more effective than kinetin for the formation of new shoots, irrespective of the type of explant considered (N'Doyé *et al.*, 2012). Therefore, in the absence of polyphenolic interactions, which could interact on morphogenetic capacities of baobab explants due to the power of activated charcoal adsorbent on baobab explants, the BAP is more organogenic at the same concentration compared to Kinetin.

Regarding *in vitro* propagation, Rolli *et al.* (2014) tested clonal propagation of explants from *in vivo*-grown seedlings, using two-nodes segment. *In vitro* shoot multiplication was achieved through enhanced axillary bud proliferation of sterilised two-node segments. MS environment supplemented with 10 mM of zeatin riboside (ZR) alone or with combinations of 1.0 or 10.0 mM ZR and 10.0 mM IBA on shoots development, had the best results. In the presence of these proliferative conditions, the highest percentage of regenerating explants was obtained, callus formation was limited to the basal part of the two-nodes segment, and completely absent in the regenerated shoots (Rolli *et al.*, 2014). In addition, the shoots obtained developed well, both in terms of mean number of nodes and shoot length.

Use of IBA at 10μM is the best option for having high rate of rooting (Rolli *et al.*, 2014). The best rates of survival were 77.77, 72.72 and 57.14 % for plants formed from the apex, plants stemming, respectively, from the axillary nodes and for those formed from cotyledons. Using two-node segments from *in vivo*-germinated seedlings represents a good starting material for *A. digitata* in vitro culture because plant tissues in vegetative phase were resilient to sterilisation with sodium hypochlorite.

KNOWLEDGE GAPS AND FUTURE PROSPECTS

There is paucity of information on vegetative propagation of baobab tree using the different procedures available. This is probably due to its socio-economic value being virtually restricted to local use in major growing areas like west Africa. The very few publications on vegetative propagation available, shows the novelty of this topic and the need for research in this and related issues, which can speed the valorisation and integration of the species in formal programmes of agricultural diversification. Furthermore, there is no information available on the propagation of the species by marcotting which is also an innovative method of vegetative propagation. The performance of this method can be compared with that of other methods to provide additional insights into the species vegetative propagation. Regarding micropropagation, the optimum rooting conditions (temperature, humidity and light) and the time required for rooting still need to be investigated. Further works on assessing the rootability of adult material of *Adansonia digitata* using each method is also recommended. One crucial point is also how the existing techniques have been made accessible to local people who mostly depend on the species.

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