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Methods for Overcoming Seed Dormancy and Vegetative Propagation of Marula – a review

Métodos para superar a dormência das sementes e a propagação vegetativa de Marula – revisão

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A B S T R A C T

The production of marula in Africa holds significant social, economic, and medicinal importance. The semi-arid region of Minas Gerais, Brazil, has great potential for marula cultivation, as the soil and climatic conditions resemble those of its native habitat. Therefore, studies related to the propagation of this species in the semi-arid conditions are essential for advancements in various areas related to marula production. Marula seeds exhibit an as yet undefined dormancy, and methods for overcoming this dormancy have not been well-established. Additionally, a protocol for vegetative propagation has not been established. Consequently, there are obstacles to overcome and enable the commercial production of marula seedlings. Furthermore, there is limited research on this crop in Brazil, despite the potential to establish marula cultivation in the semi-arid region of the country, providing another food option for the Brazilian population. In addition to its medicinal properties and the potential for job creation and income generation in dryland areas, the cultivation play a role in poverty reduction and strengthening agriculture in the semi-arid region.

Keywords: medicinal plant, dormancy, *Sclerocarya birrea*

R E S U M O

A produção de marula na África tem uma importância social, econômica e medicinal significativa. A região semiárida de Minas Gerais, Brasil, apresenta grande potencial para o cultivo de marula, pois as condições edafoclimáticas se assemelham às de seu habitat nativo. Portanto, estudos relacionados à propagação desta espécie no semiárido são essenciais para avanços em diversas áreas relacionadas à produção de marula. As sementes de marula apresentam uma dormência ainda indefinida e os métodos para superar esta dormência não estão bem estabelecidos. Além disso, não foi estabelecido um protocolo para propagação vegetativa. Consequentemente, existem obstáculos a serem superados e viabilizar a produção comercial de mudas de marula. Constatou-se que há poucas pesquisas sobre esta cultura no Brasil, apesar do potencial para estabelecer o cultivo de marula na região semiárida do país, proporcionando mais uma opção alimentar para a população brasileira. Além das suas propriedades medicinais e do potencial de criação de emprego e geração de rendimentos em zonas áridas, o cultivo desempenha um papel na redução da pobreza e no fortalecimento da agricultura na região semiárida.

Palavras-chave: planta medicinal, dormência, *Sclerocarya birrea*

INTRODUCTION

Marula (*Sclerocarya birrea* (A. Rich.) Hochst.) belongs to the Anacardiaceae family and is native to the savannah biomes of South Africa and the eastern African region. It is one of the most highly valued native trees in southern Africa due to its significant medicinal and nutritional properties. The fruit pulp is commercially used in the production of an alcoholic beverage known as Amarula®. The liquor is produced by fermenting its juice.

As the marula fruit pulp is juicy, sweet-tart, and highly fermentable, there is no need for fungi or bacteria to convert the pulp into alcohol. Therefore, most marula fruits are destined for beverage production. The propagation of fruit species like marula can be done through sexual and asexual means, both of which are of great importance for species perpetuation and maintenance (Fachinello *et al*., 2005).

When propagation is carried out sexually, plants exhibit inconveniences such as genetic segregation, a long juvenile period, and slower growth (Villalobos, 2001), as marula seeds have dormancy, making seedling emergence difficult (Maruzane *et al*., 2002). According to Coates (1977), marula is a dioecious plant, with male and female flowers on separate plants, and fruiting requires cross-pollination between these plants.

In asexual propagation, the resulting plants maintain the basic characteristics of the mother plant, with greater uniformity and vigor in production, reducing the juvenile period, accelerating flowering and production. It is commonly used for species that have difficulty with root formation (Tosta *et al*., 2012).

This study provides an overview of marula cultivation to provide theoretical support for future projects and scientific productions, as well as to share the research foundation with key scientific findings.

METHODOLOGY

This review is intended to present the current state of knowledge and priorities for future research concerning dormancy and vegetative propagation of marula assessment methods. The state-of-the-art review may offer new perspectives on an issue or highlight an area needing further research. The performance of a State-of-the-Art review follows the next steps:

Anacardiaceae Family

The *Anacardiaceae* Lindl. family is represented by approximately 80 genera and 600 species (Barroso *et al*., 2002), with a predominantly pantropical distribution and some species in temperate regions (Cronquist, 1981). This family is characterized by the presence of resin ducts or latex-secretory channels. These plants also commonly contain tannin compounds and calcium oxalate crystals in parenchymal tissue, as well as silica grains in some cells of the xylem tissue (Cronquist, 1981). In the Americas, there are approximately 32 native genera, with 77% of the species endemic to the American continent, and only the genera *Antrocaryon, Campnosperma*, *Cotinus*, *Pistacia*, *Rhus*, *Spondias*, and *Toxicodendron* having representatives on other continents as well (Terrazas, 1999).

In Brazil, 14 genera with 57 *Anacardiaceae* species are cataloged, with 14 of them being restricted to the country (Silva-Luz and Pirani, 2010). According to Souza and Lorenzi (2005), several species in the *Anacardiaceae* family have economic importance for providing edible fruits, useful woods, or ornamental species. Cashew (*Anacardium occidentale* L.) yields cashew nuts from the fruit, and the thickened floral pedicel (hypocarp or accessory fruit) is sold in its natural state. Other commercially and regionally important fruits include mango (*Mangifera indica* L.), caja (*Spondias* spp.), umbu (*Spondias tuberosa* Arruda), and seriguela (*Spondia purpuria* L.).

Schinus terebinthifolius Raddi, *Schinus molle* L., and *Rhus succedanea* L. are examples of plants used for street and square ornamentation. Among the species with good-quality wood are Gonçalo-Alves (*Astronium fraxinifolium* Schott ex Spreng.), guarita (*Astronium graveolens* Jacq.), aroeira (*Myracrodruo murundeuva* Allemão), white-aroeira (*Lithream olleoides* (Vell.) Engl.), and braúna (*Schinopsis brasiliensis* Engl.). *Spondias tuberosa* Arruda, popularly known as umbuzeiro or ambuzeiro, among others, is native to the Brazilian semiarid region (Lorenzi, 1992) and is a species of great socio-economic importance within the *Anacardiaceae* family. These species, in addition to providing tasty and nutritious fruits and xilopods rich in water (Mendes, 1990), it represents a significant source of income through extractivism (Araújo and Neto, 2002).

Some *Anacardiaceae* species are used in Brazilian folk medicine for their potential antifungal activity (Fenner *et al*., 2006). Approximately 32 genera in the family contain known species that can cause dermatitis (Mitchell, 1990). According to Midgley *et al.* (2010), marula, is a fruit-bearing species used by both humans and wild animals, typical of a healthy recruitment tree for the population.

Origin and Botanical Classification

Marula, or maruleira, is native to the semiarid savannahs of South Africa (Muok *et al.,* 2007). According to Coates (1997), it is a deciduous, dioecious tree, although there are reports of monoecious trees. It reaches average heights between 7 and 17 meters, with gray, fissured bark, a sturdy appearance, and pale foliage. It produces pale yellow fruits, approximately 3-4 cm in diameter, with succulent pulp, and when mature, they weigh about 15-25g, according to Shackleton *et al.* (2003).

According to Van de Venter *et al.* (2008), the stem bark of the marula tree is rough, with a spotted appearance due to the contrast between gray and pale brown. The leaves are divided into 10 or more pairs of leaflets, each about 60 mm long, with dark green color and sharp tips. The flowers are grouped in small clusters, and being a dioecious species, the sexes are separated on different plants. The flowers are small, with red sepals and yellow petals. Marula seeds do not easily germinate because they exhibit dormancy after fruit harvesting, and when they fall from the canopy, they can remain inactive or dormant in the soil for more than six months (Shone, 1979).

Marula thrives in a wide range of soils but is best adapted to well-drained soils. Planting altitudes can vary from sea level to 1800 m, and it can grow in areas with an annual precipitation range of 200-1500 mm (Wynberg *et al*., 2002).

Socioeconomic Importance

The marula tree is one of the most valued native trees in Africa (Von Teichman and Robbertse, 1986) for various reasons, including its numerous medicinal uses (Eloff, 2001). According to Jaenicke and Thiong'o (2000) and Mojeremane and Tshwenyane (2004), the importance of marula is supported by its different characteristics, such as high levels of vitamin C, proteins, and the palatable taste of its fruits. The vitamin C content of the fruit juice is about four to five times higher than that found in orange juice (Jaenicke and Thiong'o, 2000; Mojeremane and Tshwenyane, 2004). As the industrial demand for its products is increasing, there is a growing concern for sustainable production (Nwonwu, 2006).

Marula has a wide range of uses, including food for humans and animals, as well as medicinal uses in various regions of Africa (Hilman *et al.,* 2008; Ojewole *et al*., 2009; Gouwakinnou *et al*., 2011). Marula has been identified as one of the five fruit species that should be integrated into the domestication of agricultural systems in Africa to meet the nutritional and income security needs of the local population (Jama *et al.,* 2008). In addition to its medicinal uses, marula can be consumed fresh or used to make jams and liqueurs. Its nuts can be consumed as a snack or used for oil extraction for domestic or cosmetic purposes (Shackleton *et al*., 2002; Bationo *et al*., 2008; Gouwakinnou *et al*., 2011).

According to Wollenberg and Ingles (1998) and Koziell and Saunders (2001), a wide range of people worldwide have made prolonged use of a variety of natural resources, including non-timber forest products (NTFPs), to meet their subsistence requirements. Palmer and Pitman (1974) assert that marula stands out in the African landscape, both now and throughout the continent's history, as a significant tree in every sense, in terms of its stature, range, and value to people. Still a wild resource, the explosive surge of interest in its commercial potential in recent years has become a high priority for domestication.

Marula also has considerable social and cultural importance through its byproducts, promoting local development and generating jobs (Junod, 1938; Shone, 1979; Shackleton *et al*., 2002). Due to its widespread occurrence and utilization in Africa, marula has been identified as a key species in the development of rural enterprises that use the fruit, beer, nuts, or oil, and therefore, as a species of localized domestication.

Thus, interest in this species has been renewed after the development of a liquor that has gained wide acceptance in the market. This has promoted the development of some regions in South Africa, particularly market-oriented initiatives to benefit the poorest rural communities, including initiatives in Botswana, Malawi, and Namibia (Taylor and Moss, 1983; Pretorius *et al*., 1985; Holtzhausen *et al.,* 1990; Nerd and Mizrahi, 1993; Leakey and Simons, 1998; Leakey, 1999; Leakey and Tomich, 1999).

Nutritional Composition and Medicinal Use

According to Watt and Breyer-Brandwijk (1962), the fruit of the marula tree (marula) is rich in ascorbic acid, and the fruit juice contains sesquiterpene hydrocarbons (including caryophyllene, α-humulene, and copaene). The seeds contain a high amount of oil and protein, while the bark has tannin contents ranging from 3.5% to 20.5%, 10.7% tanning material, and traces of alkaloids. It contains two to three edible kernels, which contain 53.0%, 28.0%, and 8.0% oil, protein, and carbohydrates, respectively. The pulp contains citric and malic acids, ascorbic acid, sugar, and the gum or resin of the tree is rich in tannin.

According to Galvez *et al.* (1992), dried marula seeds and nuts are widely consumed by local populations in Africa, especially those living in rural areas. In some African countries, marula stem bark, roots, and leaves are used for a variety of human diseases, including malaria and fevers, diarrhea and dysentery, stomach ailments, headaches, eye pain, toothaches, backaches and body pains, infertility, schistosomiasis, constipation, abdominal cramps, toothache, swollen or infected gums, coughs, hypertension, arthritis, proctitis, epilepsy, diabetes mellitus, wounds, boils, carbuncles, abscesses, and various other unspecified gastrointestinal problems.

In East Africa, marula roots are an ingredient in an alcoholic medicine ingested to treat an internal illness known as "kati" (Watt, 1962). In addition to its use for treating inflammatory diseases in folk medicine (Fotio *et al*., 2009), marula also has antidiabetic effects, and its hypoglycemic activity and ethanolic extract of the bark can be used as a complementary remedy for type II diabetes (Gondwe *et al*., 2008).

Propagation Methods: Vegetative and Seed

Propagation refers to a set of practices aimed at perpetuating species through sexual and asexual methods. Its goal is to increase the number of individuals and ensure the maintenance of desirable agronomic characteristics of cultivars (Fachinello *et al*., 2005).

When propagation is done through seeds, it increases the variability of resulting progenies, which is important for genetic improvement but undesirable in the cultivation of most tropical fruits. In such cases, genetic segregation, a long juvenile phase, and slow growth are likely to occur when compared to asexual propagation (Muok *et al*., 2007; Villalobos, 2001).

Propagation of marula by seeds, despite having germination difficulties due to dormancy that can last for more than six months, produces desirable seedlings (Shone, 1979). Marula seeds germinate easily when the connection between the operculum and the rest of the endocarp is weakened. However, if this does not happen, the seeds do not germinate due to the interruption of water passage and gas exchange with the environment (Msanga, 1998). Due to this fact, marula seeds require pre-germination treatment to overcome dormancy.

Seeds of the caffra subspecies (*Sclerocarya birrea* (A.Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro) have been described as orthodox and capable of long-term storage (Msanga, 1998). Mbuya *et al.* (1994) reported that the viability of caffra subspecies seeds in Tanzania lasts for three months at room temperature, and the viability of stored seeds is estimated to be up to four years.

In commercial seedling production, vegetative propagation is sometimes more important than sexual propagation (Fachinello *et al*., 2005).

Vegetative propagation allows the obtaining of plants identical to the mother plant, reduces juvenility, increases uniformity and vigor in production, and can be an important tool for propagating species with difficult rooting (Tosta *et al*., 2012).

Vegetative propagation through cutting is one of the most important cloning methods used in horticulture because it allows the fixation of selected genotypes, population uniformity, ease of propagation, anticipation of the flowering period, reduction of the juvenile stage, and greater control over development stages (Franco *et al*., 2007).

The vegetative propagation of marula is important for two main reasons: obtaining more female plants than male ones, which in turn serve as pollinators since the plant is dioecious, and the uniformity and early development of plants, as well as higher-quality fruits (Mbuya *et al*., 1994). Maundu *et al*. (1999) observed better fruit production in grafted marula plants of three caffra subspecies. Grafting is successful when done immediately after breaking dormancy, around September in southern Africa, using scion material from the tips of branches.

Dormancy and Seed Quality

According to Carvalho and Nakagawa (2012), dormancy is the phenomenon by which seeds of a particular species, even if viable and under all the necessary environmental conditions, fail to germinate. This is reiterated by Popinigis (1977), who refers to seeds as dormant when they do not germinate, despite being placed under favorable environmental conditions for germination. Bewley and Black (1994) state that dormancy is the state in which seeds capable of germination temporarily suspend the development process until all external conditions ordinarily required for their growth are met. Two universally known types of dormancy exist: natural or primary dormancy and induced or secondary dormancy. Primary dormancy always occurs, although its intensity varies from year to year and from location to location. It is, therefore, a species characteristic (Carvalho and Nakagawa, 2012).

Immediately after harvest, seeds do not germinate (Popinigis, 1977). In some cases, primary dormancy can be overcome simply by storing the dried seeds for some time. Secondary dormancy is a type that does not always occur. When it does, it is induced by a special environmental condition, usually occurring when seeds are provided with all the favorable conditions for germination, except one (Popinigis, 1977; Carvalho and Nakagawa, 2012).

Water impermeability of the seed coat is common in seeds of the *Fabaceae, Cannaceae, Chenopodiaceae, Convallariaceae, Poaceae, Malvaceae, Solanaceae, Anacardiaceae,* and *Rhamnaceae* families, with legume seeds accounting for approximately 85% of the species examined. Such seeds are referred to as impermeable or hard (Rolston, 1978). Therefore, the impermeability of the seed coat to water entry is likely determined by the deposition of substances such as suberin, lignin, cutin, and mucilage on the seed coat, pericarp, or nuclear membrane, with this being the most common dormancy mechanism among legume species (Mayer and Poljakoff-Mayber, 1978; Bewley and Black, 1994).

One of the factors that hinder large-scale marula propagation is the dormancy of its seeds, which leads to slow and uneven emergence (Maruzane *et al*., 2002). The marula seed exhibits dormancy due to its rigid, woody, and lignified endocarp (Msanga, 1998). This dormancy can hinder marula seed germination for more than six months (Shone, 1979). Seed dormancy, caused by factors inherent to the seed coat, can be interrupted by scarification, a term that applies to any process that weakens or breaks the seed coat to allow imbibition and subsequent germination (Nassif and Perez, 1977).

The breaking of the seed coat through scarification, in addition to increasing water permeability, can lead to increased sensitivity to light and temperature, increased gas permeability, removal of inhibitors and promoters, and potential tissue damage, thus significantly affecting seed metabolism (Mundim and Salomão, 1999). Methods such as immersion in solvents (hot water, alcohol, acetone, and others), rapid cooling, exposure to high temperatures, increased oxygen tension, shocks, and impacts against hard surfaces can overcome dormancy caused by impermeability and mechanical restrictions of the seed coat (Popinigis, 1977).

Seed quality is highest at the physiological maturity stage. After this point, degenerative changes begin to occur, making the determination of maturity and the ideal harvest time crucial for producing high-quality seeds (Carvalho and Nakagawa, 2012). Conserving the physiological quality of seeds during storage is also essential in seed production and supply programs. In storage, conditions of high temperature and humidity contribute to seed deterioration, primarily due to lipid peroxidation (Vieira and Carvalho, 1994). High relative humidity of the air promotes the resumption of embryo metabolic activities, while elevated temperatures lead to increased respiratory activity and depletion of stored reserves. Moreover, such conditions can facilitate the action of fungi and insects, reducing seed quality (Carvalho and Nakagawa, 2012).

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