Economic importance, conservation and *in vitro* propagation of *Vanilla* spp. Mill.: a literature review

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Abstract. The genus *Vanilla* Mill, which belongs to the Orchidaceae family, is known for having species that are sources of aromatic compounds with high commercial value, used mainly in the food, pharmaceutical and cosmetics industries. The importance of discovering the pollinating agents and how vanilla is pollinated is of major commercial interest, given that vanillin is extracted from the dried vanilla pods. Due to the extreme extraction of native plant species and the destruction of natural habitats, there is an urgent need to apply methods that favor the sustainable use of biodiversity. In addition to extractivism, vanilla is susceptible to fungal and viral diseases, which result in severe damage to its cultivation. Orchids have a difficult time germinating seeds, which are considered slow, in addition to the low survival rate of seedlings of most species in the wild. The growing demand for cultivation and the complex activity of producing vanilla for oil extraction exposes the need to establish technologies for propagation, conservation and improvement of species of the genus. Considering these problems, biotechnological techniques, such as tissue culture, allow for the rapid and massive propagation of stable and disease-free genotypes of the most diverse plant species for various purposes, and these plants can be used commercially and in germplasm conservation and/or seedling propagation.

Keywords. *in vitro* cultivation, organogenesis, embryogenesis, *in vitro* germination.

1. Introduction

Vanilla Mill., a genus of the Orchidaceae family, is an epiphytic liana with adventitious roots opposite the leaves that arise from the nodes. The leaves are fleshy and linear, with a generally terrestrial stem. There are 119 species accepted worldwide, 20 of which are endemic to Brazil. They occur in the Amazon, Caatinga, Cerrado and Atlantic Forest biomes [1] [2].

Vanilla is susceptible to fungal and viral diseases, which result in major harvest losses, usually caused by the fungus *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, which is responsible for severe damage to vanilla cultivation [3] [4].

Biotechnology is defined as a multidisciplinary activity that uses biological agents to produce products that solve problems [5]. Biotechnological techniques, such as tissue culture, allow the rapid and massive propagation of stable and disease-free genotypes of the most diverse plant species for various commercial, research or conservation purposes [6].

Plant growth regulators are widely used in tissue culture to increase efficiency in seedling production, such as inducing cell division and differentiation, stimulating root formation and plant elongation [7].

2. Cultivation of vanilla species and economic importance

Some orchids have a commercial interest that goes beyond ornamentation, and this is the case with some species of the *Vanilla* genus [8]. In the *Vanilla* Mill. genus, some species are sources of the world-renowned organic compound vanillin. One of these species of economic importance is *Vanilla pompona* Schiede, and especially *Vanilla planifolia* Jacks. ex Andrew [9] [10] [11].

Some fruits of this taxon are natural sources of vanillin, the main constituent of vanilla absolute oil, with a history of use in different cuisines, as well as medicinal and industrial applications [12] [13] [14].

The first data on vanilla dates back to 1427-1440 AD, when the Aztecs conquered the Totonaca Empire and were introduced to the spice through the local people, who offered vanilla to the conquerors of the Empire. The Aztecs used vanilla as a flavor and aroma ingredient for chocolate, a drink intended only for noble families. The Spanish took vanilla to Europe in 1519, when Hernán Cortés sent Francisco Montejo y Portocarrero to Spain with the profits of the expedition, along with other new products. In 1793, vanilla was taken to the botanical gardens of Paris and then to England. In 1822, vanilla plants from France were sent to Reunion Island, from where the orchid was propagated to the countries of the Indian Ocean. In 1850, more plants were taken to Madagascar, where the cultivation and harvesting of the fruit became an important source of income for the region [10].

Widely used in industry as a flavoring for various products, on a small scale, the fruits are also used for medicinal purposes, as well as being an aphrodisiac, antiseptic and digestive plant [15] [16]. The absolute oil of vanilla is also cited by traditional populations as being effective in soothing anger through inhalation, which brings calm and tranquillity to the person, and is considered the oil of inner satisfaction [17].

Demand for this spice is growing by an average of around 10% a year. In addition, it differs from other orchid species in that it is also sold for medicinal purposes by traditional populations, who use the plant's leaves and flowers [18]. Due to the difficulty in producing and extracting the absolute oil, vanilla is one of the most expensive spices on the market, second only to saffron [19] [20].

[21] identified Madagascar, Indonesia, China, Tonga and Mexico as the main vanilla producing countries. In 2000, vanilla production in Madagascar employed 20,000 growers and 5,000 producers. A significant portion of the more than 70% of the working population in rural areas is involved in vanilla production. In 2005, the value of pure natural vanillin extract was worth US$4,000.00/kg. Cyclone Enawo hit the island of Madagascar in 2017 and devastated much of the annual plantation; since then there have been difficulties in production and export, as it takes around 3 to 4 years for the plant to reach maturity [22].

The municipality of Mexico City is the largest vanilla marketing center, with an estimated 4,000 families - the majority of whom are indigenous, although six private companies and four farmers' cooperatives should be highlighted - dedicated to growing the plant, which is marketed exclusively as green pods. Annual production varies from 80 to 200 tons of green vanilla, depending on climatic conditions [23]. Fruit development can be affected by droughts and high temperatures, as well as the risk of contamination by the fungus *F. oxysporum*, the main pest that attacks the crop, causing significant damage [14].

The pods of *V. phaeantha* appear to be an alternative source of vanilla flavor, since they have been shown to express some of the most important enzymes in the biosynthesis of vanilla flavor compounds [24]. Another main indicator of this species' potential as an alternative source of natural vanilla flavor is the production of vanillin itself, which was also successfully identified in the study. This same species has been assessed as 'threatened' in relation to conservation status', one of the factors for this assessment can be defined on account of the difficulty of seed germination, which is considered slow, in addition to the low survival rate of seedlings of most orchid species in the wild [25] [26].

The expansion of the study of the conservation of the genetic basis of cultivars between cultivated species and related wild species is extremely important, guaranteeing the recognition of the potential of new cultivable species among all the diversity of wild species, known or not, that can be exploited for domestication and inclusion in human food [27].

3. Diseases and pests that affect the *Vanilla* genus

The temperature and humidity conditions in which vanillas grow can favor the development of fungi. Fungal and viral diseases result in major harvest losses. Root and stem rot is caused by the fungus *Fusarium oxysporum* Schltdl. which is mainly responsible for severe damage to the vanilla crop, preventing the supply of water and food to the aerial parts of the plant [14] [4].

In the study carried out by [28], the vanilla plantation showed necrosis and dark brown to black coloration on the leaves, stems and fruit, and the fungus Scytalidium lignicola Pesante was identified. The disease known as "YELLOWING OF YOUNG FRUIT", caused by *Fusarium incarnatum-equiseti* and *Colletotrichum* sp., causes young fruit to yellow and fall or even rot. Plants attacked by the Vanilla Necrosis Potyvirus show chlorophyll-deficient and necrotic leaves, resulting in defoliation and plant death. [14].

The presence of larvae from the genus Montella was described by [29], in the species Vanilla planifolia Jacks. ex Andrew, which caused serious damage to the plant, such as galleries in the stem, yellowing at the site of predation and absence of the apical bud. [30] observed that beetles of the genus Montella sp. feed on parts of the flower of the orchid species Dichaea pendula [31]. Because of the attack, the same genus of beetle is identified in the plantations as the main cause of the reduction in production.

Two species of caterpillar have been identified in V. planifolia plantations during periods of higher rainfall (December to March), Hyphilaria thasus Stoll and Spodoptera spp. which feed on adult leaves and young shoots, causing severe defoliation throughout the vanilla's development cycle. The Spodoptera attack is characterized as the most serious, causing monetary losses in agriculture. The Conchaspis angraeci Cockerell mealybug has been identified as a vanilla pest due to its chewing-sucking mouthparts. The insect crawls on the leaves and stems of plants, injecting toxins, and chlorotic spots appear followed by necrosis [32] [33].

Taking care to ensure that this genus is free from attacks by any type of pest or disease is of the utmost importance, since the main focus of marketing is on quality broad beans, from which the organic compound known as vanillin is obtained, which is used in the pharmaceutical, food and cosmetic fields [10] [34].

Due to the economic importance of orchids, it is extremely important to use means that mitigate their scarcity and threaten the conservation of these species. The high price of vanillin from the natural extract of vanilla has stimulated research into its production and biotechnological propagation by cell culture of the plant [35].

[36] refer to tissue culture as a method used to recover plants free of viruses and other disease-causing agents. Tissue culture can be used to multiply species that are difficult to propagate, such as some species native to the Cerrado [37].

4. Conservation methods and *in vitro* propagation of vanilla specimens

4.1 *In vitro* germination

Orchid germination is naturally low (1 to 10%), but the use of *in vitro* cultivation can increase the percentage by up to 98%, depending on the species [38].

For the *in vitro* establishment of *V. planifolia*, [38] used seeds from the still closed fruit, inoculated in semi-solid culture medium at room temperature with a 16-hour photoperiod in the light, obtaining the emission of protocorms (orchid germination) in four months (Figure 1). After three months, the shoots formed were multiplied by the axillary bud.

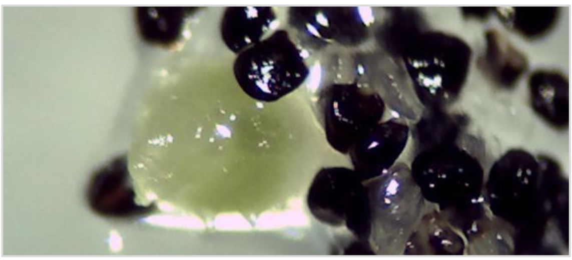

Figure 1: Vanilla seed emitting the protocorm (greenish circular sphere).

(Source: authorial, 2023)

There are hypotheses that the size of the fruit interferes with the germination of the seeds, as predicted by [39], who obtained germination (90%) of *V. planifolia* after two months of cultivation of fruits with a size of 9 cm. However, the cultivation was carried out in the absence of light.

Some authors suggest dormancy in *V. planifolia* seeds and using chemical agents to manage the seeds, as in the case of [40] who obtained 64% germination in four months with scarification using sulphuric acid for 60 seconds.

Seed propagation has become an efficient method for producing seedlings that are more likely to be pathogen-free due to their variability and continuous adaptation to the environment [41].

4.2 Plant tissue culture

The use of biotechnology and tissue culture in orchid propagation and conservation has resulted in plants with better phytosanitary quality and genetic vigor [35].

Plant tissue culture is a set of techniques that allows large-scale propagation from a small part of the plant. This technique is indicated when there is a need to propagate and conserve overexploited species [38].

According to [42], the main advantages of tissue culture have been the high rates of plant multiplication, long-term storage of germplasm and continuous propagation throughout the year, which can be achieved through different morphogenetic routes, the two basic levels being: Organogenesis and embryogenesis.

4.3 Somatic Organogenesis

Organogenesis is an in vitro multiplication process by which cells and tissues are induced to undergo changes (processes of differentiation and dedifferentiation), which lead to the production of an aerial part or root structure, whose vascular system is often linked to the tissues of the initial explant (Figure 2). These stem axes are induced to root in vitro, resulting in complete seedlings [43] [44] [45].

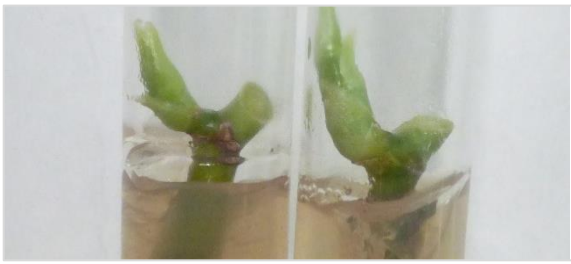

Figure 2: Shoot emission in the process of somatic organogenesis induction using the vanilla axillary bud.

(Source: authorial, 2023)

The *in vitro* formation of vanilla shoots (*V. planifolia*) through bud induction takes an average of nine to twelve days. The use of Benzyl Amino Purine (BAP) speeds up the formation process. After fourteen days of inoculation, bud formation reached 67-100% [46].

There are variables for the formation of a more robust shoot, differing according to the type of growth regulator and concentration used. The use of BAP generates a greater number of well-developed V. planifolia shoots, as well as a larger average size [47].

For clonal micropropagation purposes, the formation of callus is undesirable, since the chromosomal constitution of this material is generally unstable and can give rise to genetic or epigenetic variants, through a process called somaclonal variation [48].

4.4 Somatic Embryogenesis

Somatic embryogenesis has been an alternative technique with potential application in the clonal propagation of plants on a large scale [40] and consists of forming embryos using the principle of plant cell totipotency [49]. Embryogenesis begins with the production of cells with embryogenic competence, the creation of the somatic embryo, maturation, conversion and regeneration of the plant [50].

One of the techniques used in the embryogenesis process is the Thin Cell Layer (TCL), in which the plant tissue is transversely sectioned (2 mm) and induced in an *in vitro* medium [52] (Figure 3). In the propagation of *V. planifolia*, the use of TCL is favorable in conjunction with the use of BAP (1 mg L^{-1}) or TDZ (3 mg L^{-1}), promoting embryos in four weeks [52].

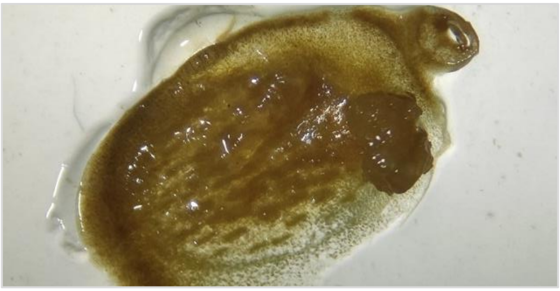

Figure 3: Beginning of embryo development in the axillary bud explant, using the TCL technique.

(Source: authorial, 2023)

Not all plant tissues produce embryos, which is why it is important to carry out inductions in order to find the one with the best competence. The use of antibiotics can interfere with embryo development [53].

Endogenous levels of auxin and cytokinin are fundamental in regulating somatic embryogenesis, with auxin being the main hormone capable of controlling cell division and growth. Low levels can inhibit the embryogenic capacity of the explant [54].

5. Conclusion

According to the facts covered in this bibliographical research, it is concluded that it is of paramount importance to propose strategies for the propagation, conservation and proper management of vanilla species, considering the current forest fragmentation and exacerbated extractivism, becoming a concern.

The studies found on digital platforms on *in vitro* propagation methods are still scarce for the various vanilla species, concentrating on the one in constant use, *Vanilla planifolia*. The concentration of studies on a single species reduces the possibility of recognizing the potential of other species that occur naturally in forests, as well as being a risk of extinction for species that have not yet been recognized and registered.

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