Conservation of Medicinal and Aromatic Plants in Brazil

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THE BRAZILIAN VEGETATION

Approximately two thirds of the biological diversity of the world is found in tropical zones, mainly in developing countries. Brazil is considered the country with the greatest biodiversity on the planet, with nearly 55,000 native species distributed over six major biomes (Fig 1): Amazon (30,000); Cerrado (10,000); Caatinga (4,000); Atlantic rainforest (10,000), Pantanal (10,000) and the subtropical forest (3,000).

The Brazilian Amazon Forest (tropical rainforest) covers nearly 40% of all national territory, with about 20% legally preserved. This ecosystem is rather fragile, and its productivity and stability depend on the recycling of nutrients, whose efficiency is directly related to the biological diversity and the structural complexity of the forest (Anon. 1995). Giacometti (1990) estimated that there are about 800 plant species of economic or social value in the Amazon. Of these, 190 are fruit-bearing plants, 20 are oil plants, and there are hundreds of medicinal plants (Berg 1982).

The "Cerrado" is the second largest ecological dominion of Brazil, where a continuous herbaceous stratum is joined to an arboreal stratum, with variable density of woody species. The cerrados cover a surface area of approximately 25% of Brazilian territory and around 220 species from cerrado are reported as used in the traditional medicine (Vieira and Martins 1998).

The "Caatinga" extends over areas of the states of the Brazilian Northeast and is characterized by the xerophitic vegetation typical of a semi-arid climate. The soils that are fertile, due to the nature of their original materials and the low level of rainfall, experience minor runoff (Anon. 1995). Various fruit species and medicinal plants have their centers of genetic diversity in this region, and the use of local folk medicines is common. Several important aromatic species are reported for this region (Craveiro et al. 1994), such as *Lippia* spp. and *Vanillosmopsis arborea*.

The Atlantic Forest extends over nearly the whole Brazilian coastline, and is one of the most endangered ecosystems of the world, with less than 10% of the original vegetation remaining. The climate is predomi-

nantly hot and tropical, and precipitation ranges from 1,000 to 1,750 mm. The land is composed of hills and coastal plains, accompanied by a mountain range (Anon. 1995). Several important medicinal species are found in this region, such as *Mikania glomerata*, *Bauhinia forficata*, *Psychotria ipecacuanha*, and *Ocotea odorifera*.

The territory of the Meridional Forests and Grasslands includes the mesophytic tropical forests, the subtropical forests, and the meridional grasslands of the states of southern Brazil. The climate is tropical and subtropical, humid, with some areas of temperate climate. The naturally fertile soils, associated with the mild climate, allowed a rapid colonization during the last century, mainly by European and, more recently, by Japanese immigrants (Anon. 1995). Several medicinal plants, such as chamomile (*Matricaria recutita*), calendula (*Calendula officinalis*), lemon balm (*Melissa officinalis*), rosemary (*Rosmarinus officinalis*), basil (*Ocimum basilicum*), and oregano (*Origanum vulgare*), were introduced and adapted by immigrants.



Fig. 1. Major biomes of Brazil, source: Embrapa, Cenargen.

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The Pantanal is a geologically lowered area filled with sediments which have settled in the basin of the Paraguay River. Pantanal flora is formed by species from both Cerrado and Amazon vegetation. More than 200 species useful for human and animal consumption as well as for industrial use have been recorded in this region (Anon. 1995).

GERMPLASM CONSERVATION

In the last decade, serious efforts to collect and preserve the genetic variability of medicinal plants have been initiated in Brazil. The National Center for Genetic Resources and Biotechnology—Cenargen, in collaboration with other research centers of Embrapa (Brazilian Agricultural Research Corporation), and several universities, has a program to establish germplasm banks for medicinal and aromatic species (Table 1).

The first step is to establish criteria to define a species priority, based on economic and social importance, markets, and potential genetic erosion. Vieira and Skorupa (1993) proposed the following criteria to define priority, as follows: (1) species with proven medicinal value including those containing known active substance(s) or precursor(s) used in the chemical–pharmaceutical industry with proven pharmacological action, or at least demonstrating pre-clinical and toxicological results; (2) species with ethnopharmacological information widely used in traditional medicine; and which are threatened or vulnerable to extinction; (3) species with chemotaxonomical affinity to botanical groups which produce specific natural products.

Conservation of threatened germplasm includes seed banks, field preservation, tissue culture, and cryopreservation. Seed storage is considered the ideal method; seeds considered orthodox can be dried and are able to be preserved at sub-zero temperatures (-20°C), while recalcitrant seeds, including most tropical species, lose their seed viability when subjected to the same conditions. Maintenance of the germplasm in field collections is costly, requires large areas, and can be affected by adverse environmental conditions. Tissue culture or cryopreservation techniques can be also considered in some cases.

The next step is to decide which germplasm conservation method will be applied: ex situ or in situ. In an ex situ procedure, the germplasm is collected from fields, markets, small farms, and other sites, in form of seeds, cuttings, underground systems, and sprouts. The collected samples should represent the original population with passport data and herbarium vouchers. In a long term, mutation can take place over the years in a cold chamber or in vitro conservation. In contrast, in situ conservation maintains population in its preserved natural area, allowing the evolutionary process to continue, although genetic reserves are subject to anthropogenic action and environmental effects. Most in situ conservation has focused in forest species, with some medicinal species included, such as *Pilocarpus microphyllus* and *Aniba roseodora*. The establishment of genetic reserves in Brazil has relied on National Parks and conservation areas established by the environmental protection agency of Brazil, Ibama.

There are now five forest genetic reserves in Brazil: one in the Amazon Tropical Rainforest, state of Para; one in the Caatinga, state of Minas Gerais; two in the Cerrado in the Federal District, and one in the Meridional Forest (Subtropical) in the state of Santa Catarina. Four other genetic reserves are being created; two in the Atlantic Forest in the states of Rio de Janeiro and Espirito Santo, one in the Caatinga in the state of Piaui, and another in the Tropical Humid Forest in transition with Cerrado in the state of Minas Gerais (Anon. 1995). These reserves aim to conserve the most endangered species and those of greatest economic interest, including medicinal and aromatic plants.

The Brazilian program on medicinal germplasm conservation has three foci: (1) ethnobotanical studies; (2) germplasm collection and characterization; and (3) in situ conservation. Ethnobotanic and phytogeographic studies on the medicinal flora of Cerrado have been able to identify and collect genetic material for conservation. About 110 species used in traditional medicine were reported in the Cerrado region (Vieira et al. 1998). Bibliography review and a herbaria search were carried out allowing an estimation of the medicinal potential of each species studied, their geographic distribution, and period of fruit maturation.

In 1994, a cooperative project between the Brasília Botanical Garden and Embrapa/Cenargen was established. An in vivo collection of medicinal plants from Cerrado, now contains 161 accessions (Dias et al. 1995). The collection has facilited phytochemical and pharmacological studies of this plant materials, and an anti-inflamatory agent has been identified on *Lychnophora salicifolia* (Miguel et al. 1997).

PRIORITY SPECIES

A few germplasm collections of medicinal and aromatic plants have been established in Brazil (Table 2). The following species, listed alphabetically, have been recognized as priority for germplasm conservation.

Maytenus ilicifolia Martius ex Reiss., Celastraceae (Espinheira Santa)

Espinheira santa is a small shrub evergreen tree reaching up to 5 m height. It is native to many parts of southern Brazil, mainly in Paraná and Santa Catarina states.

Leaves of *Maytenus* species are used in the popular medicine of Brazil for their reported antiacid and antiulcerogenic activity. The effects of a boiling water extract of equal parts of *M. aquifolium* and *M. ilicifolia* leaves have been tested in rats and mice. Attempts to detect general depressant, hypnotic, anticonvulsant, and analgesic effects were reported by Oliveira et al. (1991). The potent antiulcerogenic effect of espinheira santa

Table 1. List of institutions, germplasm collections of medicinal and aromatic plants, accessions, curator, and contact.

Institution	Major germplasm collections	No. accessions	Curator	Contact	
Agronomic Institute of Paraná	Pfaffia	Unknown	P. Guilherme	www.pr.gov.br/iapar	
Brasília Botanical Garden	Medicinal plants from cerrado	165	A. Lucia	Jardim Botanico de Brasilia, Lago Sul, Brasilia, DF	
Embrapa–Genetic Resources and Biotechnology	Phyllanthus, Pilocarpus, Stevia, Solanum, plants from cerrado	335	T. Dias R. Vieira	www.cenargen.embrapa.br	
Embrapa-Ocidental Amazon	Croton cajucara, general collection of medicinal and aromatic plants	Unknown	A. Franco	www.embrapa.br/cpaa, Rodovia Am - 010, km 24, CP 319, 69048-660, Manaus	
Embrapa–Oriental Amazon	Psychotria, Pilocarpus	109	I. Rodrigues	Trav. Dr. Enéas Pinheiro s/n, Marco, CP 48 66095-100, Belém, PA	
Maranhão State Univ.	Pilocarpus	27	G. Silva	Campus Universitário Paulo VI, CP 09, São Luis-MA	
São Paulo State Univ., Botucatu	Lippia, Ocimum	Unknown	L. Ming	Unesp, Faculdade de Ciencias Agrarias, Departamento de Agronomia, Botucatu, SP	
Univ. of Brasilia	Pfaffia, Mentha spp., Labiatae, Phitolacca dodecandra	Unknown	J. Kleber	Universidade de Brasília, Departamento de Agronomia, CP 04364, 70000, Brasília, DF	
Univ. of Campinas, Cpqba	Maytenus, Artemisia, Phyllanthus, Pfaffia, Cordia, Stevia	330	P. Melillo	Unicamp, Cpqba, C.P. 6171, Campinas, SP	
Univ. of Ceará	Aromatic plants: Lippia, Croton, Cymbopogon	224	F. Mattos	Lab. de Produtos Naturais/UFC, Campus do Pici, 60021-970, Fortaleza,CE	
Univ. of North Fluminense	Psychotria	10	E. Martins	Universidade Estadual do Norte Fluminense, Lab. de Melhoramento Genetico Vegetal, Av. Alberto Lamego, 2000, Horto, 28015-620, Campos dos Goytacazes, RJ	
Univ. of Paraná	Maytenus	78	M. Scheffer	Universidade Federal do Parana, Escola de Florestas, Departamento de Silvicultura e Manejo, Rua Bom Jesus, 650, Juveve, 80035-010, Curitiba, PR	

Table 2. List of medicinal and aromatic species with high priority for germplasm collection and conservation in Brazil.

Species	Common name	Habit	Active substance/ pharmacological action	Region	Conservation form
Achyrocline satureioides L.	Macela	Herb	Hypotensive, spasmolytic	Cerrado	Field collection
Ageratum conyzoides L.	Mentrasto	Herb	Anti-inflammatory	Ruderal	Field collection
Aniba roseodora Ducke	Pau rosa	Tree	Linalool	Amazon forest	In situ
Astronium urundeuva (Fr. All.) Engl.	Aroeira	Tree	Anti-inflammatory, anti-ulceric	Cerrado chamber	In situ, cold
Baccharis trimera DC.	Carqueja	Herb	Hepatic disturbs	Ruderal	Field collection
Bauhinia forficata L.	Pata de Vaca	Tree	Diabetes	Atlantic forest	Cold chamber
Caryocar brasiliensis Camb.	Pequi	Tree	Anti-inflamatory	Cerrado	In situ
Copaifera langsdorffi Desf.	Copaiba	Tree	Oil, anti-inflamatory	Cerrado	In situ, cold chamber
Croton cajucara Benth.	Sacaca	Herb	Linalool	Amazon	Field collection
Croton zehntneri Pax et Hoff.	Cunha	Shrub	Anetol, eugenol	Caatinga	Field collection
Datura insignis B. Rodr.	Toe	Shrub	Escopolamina	Amazon forest	Cold chamber
Dimorphandra mollis Benth.	Faveiro	Tree	Rutin, anti-hemorragic	Cerrado	Cold chamber
Echinodorus macrophyllus (Kunth.) Mich	Chapeu de Couro	Herb	Diuretic	Cerrado	Field collection, cold chamber
Jatropha elliptica (Pohl) Baill.	Batat de Tiu	Shrub	Jatrophone	Cerrado	In situ, field collection
<i>Lippia</i> spp.	Alecrim pimenta	Shrub	Source of volatile oils, anti-microbial	Caatinga	Field collection
Lychnophora ericoides Mart.; L. salicifolia Mart.	Arnica do Cerrado	Shrub	Volatile oils	Cerrado	Field collection, in situ
Mandevilla vellutina Mart.		Shrub	Anti-inflamatory, bradykynin antagonist	Cerrado	In situ, field collection
Maytenus ilicifolia Mart. ex. Reiss; M. aquifolium Mart.	Espinheira Santa	Tree	Anti-ulceric	Meridional forest	Cold chamber, in situ
Mikania glomerata Spreng.	Guaco	Herb	Bronchitis, coughs	Atlantic forest	Field collection
Ocotea odorifera (Vell.) Rohwer	Canela Sassafraz		Safrol, metileugenol	Atlantic forest	In situ
Operculina macrocarpa (L.) Farwel	Batata de Purga	Herb	Purgative	Caatinga	Cold chamber
Piper hispidinervum DC.	Pimenta longa	Herb	Safrol	Amazon	Cold chamber, field collection
Pfaffia paniculata (Martius) Kuntze	Ginseng brasileiro	Herb	Antitumor compounds	Margins of Parana river	Cold chamber, field collection
Phyllanthus niruri L.	Quebra pedra	Herb	Hepatitis B, renal calculus	Ruderal	Cold chamber
Pilocarpus microphyllus Stapf.	Jaborandi	Shrub	Pilocarpine	Amazon forest	Cold chamber, in situ
Psychotria ipecacuanha (Brot.) Stokes	Ipecac	Herb	Emetin, cefaline	Amazon and Atlantic forest	Cold chamber, in situ
Pterodon emarginatus Vogel	Sucupira	Tree	Analgesic, antinoceptive, cercaricide		In situ, cold chamber
Solanum mauritianum Scopoli	Cuvitinga	Shrub	Solasodine	Ruderal, southeast and southern Brazil	Cold chamber
Stryphnodendron adstringens (Mart.) Coville	Barbatimao	Tree	Tannin, anti-inflamatory		In situ, cold chamber
Tabebuia avellanedae (Lor.) ex. Griseb.	Ipe roxo	Tree	Lapachol	Cerrado	In situ
Vanillosmopsis arborea (Aguiar) Ducke	Candeia	Shrub	Bisabolol	Caatinga	In situ, field collection

leaves was demonstrated effective compared to two leading anti-ulcer drugs, Ranitidine and Cimetidine (Souza-Formigoni et al., 1991). Toxicological studies demonstrated the plant's safety.

Seeds of *Maytenus ilicifolia* can be classified as orthodox and stored at -20° C in long-term cold chambers (Eira et al. 1995). The Forestry Department of the University of Paraná began a project in 1995 to study the genetic variability of natural populations of *Maytenus ilicifolia* and 78 accession were collected in the states of Parana, Santa Catarina, and Rio Grande do Sul. Field collections are maintained at the university campus (Scheffer et al. 1998). Although cultivation of *M. ilicifolia* is the object of several studies in Brazil, a research focus on in situ conservation and sustainable systems of harvesting are required.

Pfaffia paniculata Martius, Amaranthaceae (Brazilian Ginseng)

Pfaffia is a large, shrubby ground vine, which has a deep root system. *Pfaffia* is well known in Central and South America with over 50 species growing in the warmer tropical regions of the area and has been exploited for more than 15 years. The species grow in the borders of Paraná river, but predatory collection has greatly reduced the natural populations.

In Brazil, *Pfaffia* is known as *para tudo*, which means "for all things" and also as Brazilian ginseng, since it is widely used like American and Asian ginseng (*Panax* spp.). The active substances are found in the roots.

This action is attributed to the anabolic agent, beta-ecdysterone as well as three novel ecdysteroid glycosides which are found in high amounts in *Pfaffia*. This species is such a rich source of beta-ecdysterone. The extraction methods employed to obtain it from this root is protected by a Japanese patent (Nishimoto et al. 1988).

The root of *Pfaffia* contains about 11% saponins. These saponins include a group of novel chemicals called pfaffosides as well as pfaffic acids, glycosides, and nortriperpenes. These saponins have clinically demonstrated the ability to inhibit cultured tumor cell melanomas and help to regulate blood sugar levels (Takumoto et al. 1983; Nishimoto 1984). The pfaffosides and pfaffic acid derivatives in *Pfaffia* have been patented as antitumor compounds in two Japanese patents (Japanese Patent 84184198, Oct. 19, 1984 by Rohto Pharmaceutical Co., Ltd.).

Few accessions of *Pfaffia* are available in any of the present field collections. This species requires an immediate recollection to preserve the plant. Due to its economic importance a germplasm collection and characterization of its chemical constituents, is fully warranted.

Phyllanthus niruri L., Euphorbiaceae (Quebra Pedra)

Quebra pedra is a small erect annual herb growing up to 30 to 40 cm. height. Although several species are recognized by this common name, *P. niruri* and *P. sellovianus* are the most scientifically studied. The antispasmodic activity of alkaloids in *Phyllanthus sellovianus* explained the popular use of the plant for kidney and bladder stones. The alkaloid extract demonstrated smooth muscle relaxation specific to the urinary and biliary tract which facilitates the expulsion of kidney or bladder calculi (Calixto 1984; Santos 1994, 1995)

Quebra pedra has gained world-wide attention due to its effects against Hepatitis B (Thyagarajan 1982; Mehrotra 1990; Yeh, et al. 1993; Wang 1995). Recent research on quebra pedra reveals that its antiviral activity extends to the human immunodeficiency virus (HIV). The HIV-1 reverse transcriptase inhibition properties of *P. niruri* can be obtained with a simple water extract of the plant (Qian-Cutrone 1996). There have been no side effects or toxicity reported in any of the clinical studies or in its many years of reported use in herbal medicine.

Several species, called quebra pedra, contain the same or similar active compounds. A germplasm collection to study the genetic and chemical variation, as well as the seed physiology of this species is necessary and warranted.

Pilocarpus microphyllus Stapf., Rutaceae (Jaborandi)

Jaborandi is an indigenous name (*ia-mbor-end*) of this species. *Pilocarpus microphyllus* contain the highest pilocarpine content in the leaves. The plant is an understory species, 6 to 8 m in height, of the pre-Amazonian rain forest in the states of Pará, Maranhão, and Piauí.

Pilocarpine is an imidazolic alkaloid that stimulates the secretions of the respiratory tract, the salivary, lachrymal, gastric and other glands, weakens the heart action, accelerates the pulse rate, increases intestinal peristalsis and promotes uterine contractions (Morton 1977). In the treatment of glaucoma, the alkaloid pilocarpine acts directly on cholinergic receptor sites, thus mimicing the action of acetylcholine. Intraocular pressure is thereby reduced, and despite its short-term action, pilocarpine is the standard drug used for initial and maintenance therapy in certain types of primary glaucoma (Lewis and Elvin-Lewis 1977). Recently, the US Food and Drug Administration approved pilocarpine for use to treat post-irradiation xerostomia (dry mouth) in patients with head and neck cancer (Pinheiro 1997).

The exploration of this product, due to its high economic value, has led to great scientific interest in research and development effects for domestication and conservation. Pinheiro (1997) reports that the price of jaborandi leaves has reached US\$4.00/kg. The wild harvest or collection of leaves from wild *P. microphyllus* has been carrying out to such an extent that it has significantly reduced the natural populations, and this species is included in the official list of endangered plants from Brazilian flora (Anon. 1992).

In 1991, the Cenargen initiated a project for recollecting and conservation of the genetic variability of *Pilocarpus microphyllus* and related species. From 1991 to 1993, two collection expeditions were undertaken, covering the states of Pará and Maranhão. A total of 27 accessions were collected in form of seeds and seedlings (Vieira, 1993). A germplasm bank of Jaborandi was established at Maranhão State University, São Luis, and at Embrapa—Ocidental Amazon, Belém, Pará State.

Studies on the methodology of *P. microphyllus* conservation led to the conclusion that seeds of this species are considered orthodox. Seeds can be dried down to 6–8% moisture content and be conserved for a long period at –18°C and 5% relative humidity (Eira et al. 1993). A seed sample of all collected accessions is being maintained at Embrapa, Cenargen.

Native populations of *P. microphyllus* have suffered from anthropogenic activity, with plants of shorter size than normal due to intensive harvesting of leaves. It will be challenge to stimulate the management and cultivation of this species in its native habitat. Although seeds can be preserved for long periods, in situ conservation must be initiated and natural reserves established. This species can be only found in indigenous areas, and some private lands.

Psychotria ipecacuanha (Brot.) Stokes, Rubiaceae (Ipecac)

Psychotria ipecacuanha (Brot.) Stokes [= Cephaelis ipecacuanha (Brot.) A.Rich.] is a shrub, whose medicinal value relates to the production of emetine in the roots. Ipecac is found in the humid forests of Central America, Colombia, southern part of the Amazon Forest in the States of Rondônia, Mato Grosso, and Atlantic forest, in the States of Bahia, Espírito Santo, Minas Gerais, and Rio de Janeiro (Skorupa and Assis 1998).

Ipecac as a powerful emetic, is used in gastrointestinal diseases, diarrhea, and intermitent fevers. It is employed as an expectorant, in bronchitis, broncopneumonia, asthma and mumps, and also as a vasoconstrictor. In 1959, dihydroxi-emetine, an emetine analogue, was presented as an amoebicide due to its reduced toxic effect on cardiac muscle (Lewis and Elvin-Lewis 1977).

The global production of ipecac averages 100 t a year, originated mainly from Nicarágua, Brazil and India (Husain 1991). Considering the economic and medicinal values of ipecac, the deforestation of the areas of occurrence and the extrativist nature of its production, in 1988, Cenargen has began a program for the recollecting and conservation of the genetic variability of this species. From 1988 to 1991, five collecting expeditions were undertaken, covering the States of Rondonia, Mato Grosso, Pernambuco, Bahia, Espirito Santo, Rio de Janeiro, and Minas Gerais, and a total of 86 accessions were collected (Skorupa and Assis 1998) and now maintained in field germplasm banks at Embrapa—Ocidental Amazon, Belém, Para, and at Florestas Rio doce, Linhares, Espirito Santo. Recently, other germplasm collections was established at the University of North Fluminense, which contains 10 accessions originated from the Atlantic Forest area (states of Rio de Janeiro and São Paulo).

Solanum mauritianum Scop., Solanaceae (Cuvitinga)

The steroidal alkaloids of the Solanaceae are compounds of considerable pharmaceutical interest as starting materials for the synthesis of steroid compounds such as anticontraceptive steroids and corticosteroids. The

world demand for steroid precursors continues to increase while some of the traditional sources of steroidal raw material, such as yams (*Dioscorea* spp.) of Mexico and Central America, are becoming rapidly depleted (Roddick 1986). Solasodine is a chemical analog of diosgenin, and may be a substitute for this drug.

There are around 1,100 species of *Solanum* in South America, and *S. mauritianum* is among the species with the highest solasodine content (Vieira and Carvalho 1993). *Solanum mauritianum* is a subtropical shrub which grow all over southern Brazil. The solasodine content of *S. mauritianum* was evaluated in green fruits of natural populations growing on two different soils. High contents of solasodine were found in both population of *S. mauritianum* (from 2% to 3.5% of total dry weight) (Vieira 1989). Germplasm collections are needed to continue the study of genetic and environmental variation of solasodine, and to provide foundation study for future development programs.

Exotic Species

Although the major focus of germplasm conservation is on native species, several exotic, introduced and adapted species have been widely used and cultivated in Brazil. Many of them, such as lemongrass [Cymbopogon citratus (D.C.) Stapf.] and aloe (Aloe spp.), are cultivated in backyard gardens. Others, such as picão-preto (Bidens pilosum L.), mastruço (Chenopodium ambrosioides L.), and mentrasto (Ageratum conyzoides L.), whose adaptation through the years, has allowed a spontaneous wide distribution throughout the country, have had their use well disseminated (Dias 1995). In southern Brazil, due to favorable cultural and environmental conditions, several exotic species are cultivated in large areas. These include chamomile (Matricaria recutita), calendula (Calendula officinalis), rosemary (Rosmarinus officinalis), Duboisia sp., and Japanese mint (Mentha arvensis), all of which are deserving of collection and preservation due to the use of their natural products and the agricultural-based industries that produce these crops. The germplasm collection of exotic species also needs to be expanded to provide genetic resource for species adapted in Brazil. Although Brazil is not their genetic center of origin, different chemotypes have been naturalized (Mattos, pers. commun. 1994) and need to be conserved. One example is Coleus barbatus, which was introduced from Africa, and is clonally propagated in Brazil. However, several volatile oils chemotypes are found in Brazil for this species, probably due to different introductions from Africa in the past.

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