Meiotic behavior and pollen viability of *Aloysia gratissima* and *Aloysia triphylla* (Verbenaceae)

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Abstract

The use of medicinal plants for the treatment of diseases that attack human beings has been a practice for centuries and it is seen as one of the main therapeutic resource in many communities and ethnical groups, especially in developing countries. In Brazil, the economic potential of the germoplasm of medicinal plants is a wealth to be used and preserved. Native and exotic species are used medicinally in Brazil. Among them, *Aloysia gratissima* (Gill et Hook) Tronc. (native) and *Aloysia triphylla* (L'Her.) Britton (exotic) both from Verbenaceae Family are highlighted. In this study the meiotic behavior and pollen viability in populations of these species of the genus *Aloysia* from Rio Grande do Sul State were analyzed. Inflorescences of three populations of *A. gratissima* and one of *A. triphylla* were collected in their native local, fixed in absolute ethanol glacial acetic acid (3:1) for 24 hours at room temperature, and after that maintained in ethanol 70% until use. The chromosomal meiotic behavior and pollen viability analysis was performed with anthers by using the squashing technique, and staining in orcein acetic 2%. Our results show that occurred a good meiotic stability and a high viability of the pollen grains in the studied populations of *A. gratissima* and *A. triphylla.* And more, that both species present the gametic number n=18, allowing us to suggest the somatic number of 2n=36 chromosomes.

Index terms: medicinal plant, pollen, meiosis, meiotic index, chromosome number.

Resumo

O uso de plantas medicinais para o tratamento de doenças que acometem o ser humano tem sido uma prática secular e, em muitas comunidades e grupos étnicos, é um dos únicos recursos terapêuticos, especial-

mente em países em desenvolvimento. No Brasil, o potencial econômico de germoplasma de plantas medicinais é uma riqueza a ser utilizada e preservada. Espécies nativas e exóticas são usadas medicinalmente no Brasil. Dentre essas, destacam-se *Aloysia gratissima* (Gill et Hook) Tronc. (nativa) and *Aloysia triphylla* (L'Her.) Britton (exótica), ambas da família Verbenaceae. Nesse estudo, foram analisados o comportamento meiótico e viabilidade do pólen em populações dessas espécies do gênero *Aloysia* do estado do Rio Grande do Sul. Inflorescências de três populações de *A. gratissima* e uma de *A. triphylla* foram coletadas em seus locais de origem, fixadas em etanol absoluto e ácido acético glacial (3:1) por 24 horas em temperatura ambiente e depois mantidas em etanol 70% até o uso. A análise do comportamento meiótico e da viabilidade polínica foi feita pela técnica de esmagamento das anteras e coloração com orceína acética 2%. Duas populações de *A.gratissima* e a de *A. triphylla* apresentaram boa estabilidade meiótica e alta viabilidade polínica. Ambas as espécies mostraram número gamético n=18, o que sugere um número cromossômico somático de 2n=36 cromossomos.
Termos para indexação: planta medicinal, grãos de pólen, meiose, índice meiótico, número cromossômico

Introduction

The use of medicinal plants for the treatment of diseases is a non-controlled and a widely diffused practice in Brazil. Most of the species have been utilized in an exploratory way, and with a growing number of people and the occupation of native areas, an increase in the destructive pressure on this flora has been occurring (Rosa & Ferreira, 2001). Brito & Brito (1993) presented several studies carried out with species from the native flora highlighting their potential uses. They have also suggested the need of further and deeper studies on the Brazilian tropical flora. *Aloysia gratissima* and *Aloysia triphylla* are among the medicinal species of great interest.

Aloysia gratissima as a synonym of *Lippia sellowii* and *Aloysia sellowii* is a bush which reaches 1.5-2.5m height, with sub-coriaceous foliar blade, foliar oblanceolate, ovate or obovate with acute apices. The secondary venation is dorsally prominent, which is a common characteristic (Botta, 1979) for this species, which is commonly known as "erva-santa", "alfazema-do-brasil", or "erva-de-nossa-senhora". It can be used as antitumoral, antigripal, antirheumatic, balsamic, and digestive (Castro & Chemale, 1995). Souza & Wiest (2007) reinforced the antibacterial activity of *Aloysia gratissima.*

Aloysia triphylla as synonym of *Lippia citriodora* and *Aloysia citriodora* is a branchy shrub, which reaches 2-3 m height, erect, with a

citral aroma. It is native from South America, probably from Chile, and exotic in Brazil. This species is commonly known as "erva-cidreira", "cidró", "cidró-pessegueiro", "cidrão", "erva-Luisa", and "cidrozinho" (Lorenzi, 2002). It is an astringent and aromatic herb, rich in volatile oil, which reacts as mild sedative reducing fevers, and relieving spasms especially from digestive system (Bown, 1995).

According to Simões et al. (1989), *Aloysia triphylla* has leaves that can be used internally as digestive, stimulant, tonic, antispasmodic, carminative, and sedative. However, its long-term use may cause disturbs in the digestive system and sleep. It is important to emphasize that firstly it is necessary to carry out the taxonomic identification and characterization study of germoplasm species, mainly cytogenetic evaluation for further genetic breeding program (Fachinetto et al., 2008).

The meiosis is an event of high evolutionary stability which results in the reduction of the number of chromosomes. The usual and accurate way of the meiosis assures the gametic viability. The chromosome analysis during the meiotic cell division shows the fertility level, and thus make possible the estimative of the pollen grain viability (Fachinetto & Tedesco, 2009).

The aim of this study was to carry out the cytogenetic characterization (meiotic behavior and pollen viability) of the *Aloysia gratissima* and *Aloysia triphylla* populations from Rio Grande do Sul State, Brazil.

Material and methods

The young inflorescences were collected from three natural populations of *A. gratissima*, and one exotic population of *A. triphylla* in Rio Grande do Sul State, Brazil. From each population, a voucher specimen was deposited in the Herbarium SMDM (Santa Maria - Department of Biology) at the Universidade Federal de Santa Maria (UFSM), Rio Grande do Sul (RS), as shown in Table 1. The plants were taxonomically identified by Prof. Dr. Thais do Canto-Dorow.

The young plant inflorescences were collected to carry out the meiotic analysis and pollen viability estimative of the four populations (Table 1 and 2)

After collected, the inflorescences were fixed in ethanol-acetic acid 3:1 for 24 hours at room temperature, then transferred to ethanol 70%, maintained at a temperature of 4°C until the preparation of the slides. The slides were prepared by squashing and staining the anthers with acetic orcein 2% (Guerra & Souza, 2002). Five slides were observed per population that presented meiosis phases with good chromosomal visualization in optic microscopes and the best cells were photographed.

Table 1. Populations of *Aloysia gratissima* and *Aloysia triphylla* analyzed.

Species	Populations collected at RS		Registry number
	Population Nº	Locality	
A. gratissima	Population 1	Boca do Monte	9922
	Population 2	Santa Maria	9920
	Populatino 3	São Pedro	9921
A. triphylla		Santa Maria	9918

Diakinesis and/or metaphase I where considered normal when all the chromosomes are in bivalent associations. In anaphase/telophase I and II, chromosomal segregation was observed considering abnormal the cells with irregular chromosomal disjunction, retarded chromosomes, and bridges. The number of tetrads (quartets) was registered, and meiotic index (MI) calculated according to Love (1949): MI = nº normal quartets/ nº observed quartets x 100. The normal quartets were represented by four cells, and all the others which differ from this number were considered abnormal, like triads, dyads and polyads. Three hundred tetrads of each population of the *Aloysia* populations studied were observed.

For pollen viability estimative, the same method used for meiosis analysis was applied, with delimitation, at random, of six visual fields on each slide. Five hundred pollen grains were counted per studied population. Through the staining method, used by Tedesco et al. (2002), the estimative of the amount of viable pollen (stained) and unviable pollen (not stained) was possible, since the fertility of the pollen is determined by the pollen tube growth.

Results and discussion

The results obtained by the analysis of microsporogenesis of *Aloysia gratissima* and *Aloysia triphylla* are presented in Table 2.

The analysis of the chromosomes in diakinesis and metaphase I, show that n=18 (Figures 1A, 1B and 1C) is the gametic number for *A. gratissima* and *A. triphylla*.

The analysis of the meiotic behavior was considered regular during meiosis I and II for the populations studied of *A. gratissima* (Table 2, Figures 1A, 1B and 1C). However, some irregularities such as the presence of triads, irregular disjunction of the chromosomes in anaphase II (Figures 2A, 2B), and telophase II with 3 and 5 poles (Figure 2C) were observed.

Table 2. Microsporogenis analysis of *Aloysia gratissima* and *Aloysia triphylla*.

Species		Meiosis I		Meiosis II		Meiotic Index (%)	Pollen Viability (%)
		Diakinesis/ Metaphase I	AnaphaseI/ Telophase I	Metaphase II	Anaphase II/ Telophaase II		
A. gratissima	Population 1	111	50	15	1	-	-
	Population 2	163	75	6	15[1]	96,1	99,1
	Population 3	170	91	27	44[2]	93,3	97,9
A. triphylla		193	109[3]	145	128[4]	87,1	98,6

[1] 1 cell with irregular anaphase II; 3 cells with spindle tripolar in telophase II
[2] 2 cell with spindle tripolar in telophase II; 1 polyad
[3] 1 cell anaphase with 1 univalent chromosome and 1 cell with laggard chromosome in telophase I
[4] 9 cells with spindle tripolar in telophase II.

The population of *A. triphylla* presented irregular meiosis with a higher meiotic instability though. Some irregularities, such as laggard chromosomes and the presence of triads and dyads (Figure 3B) were also observed in this population.

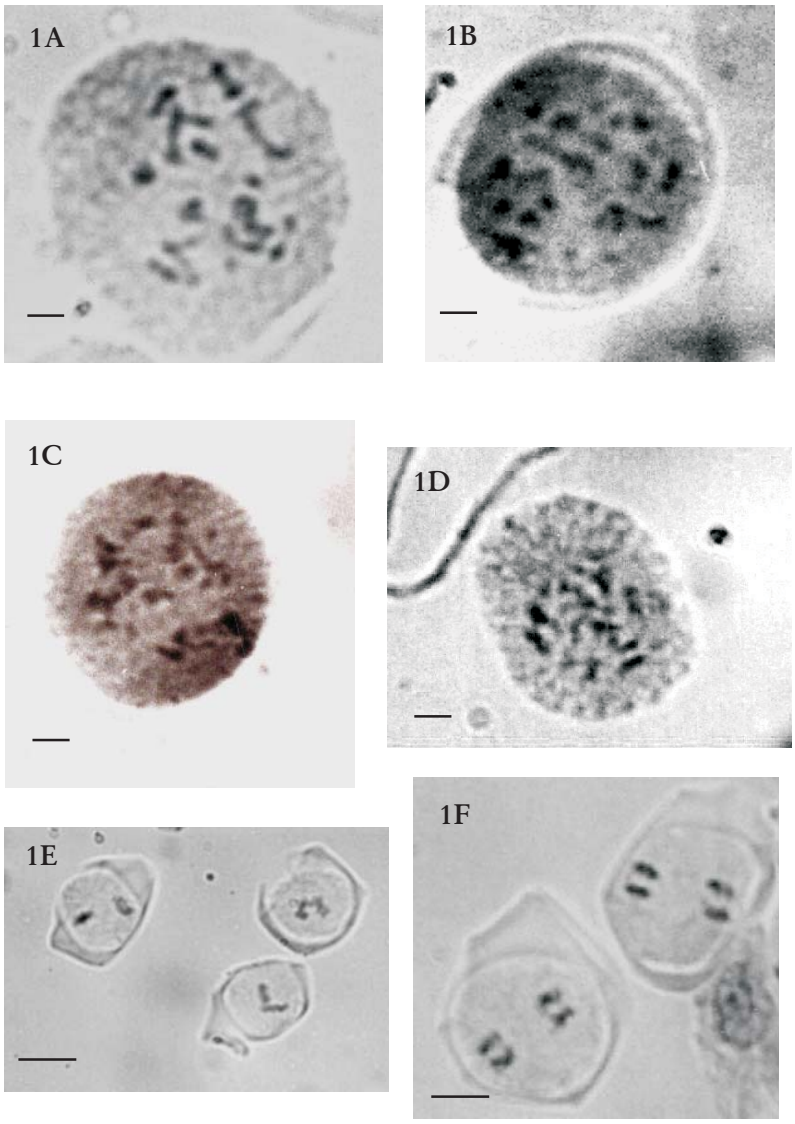

Figure 1. Meiotic chromosomes from populations of *Aloysia gratissima* (A, B and C) and *Aloysia triphylla* (D, E and F). A – population 2, diakinesis regular (18 II); B – population 3, metaphase I regular; C – population 3, diakinesis regular (18II) 3. D – *Aloysia triphylla*, diakinesis regular (18II); E – metaphase II; F – anaphase II. Scale= 2µm

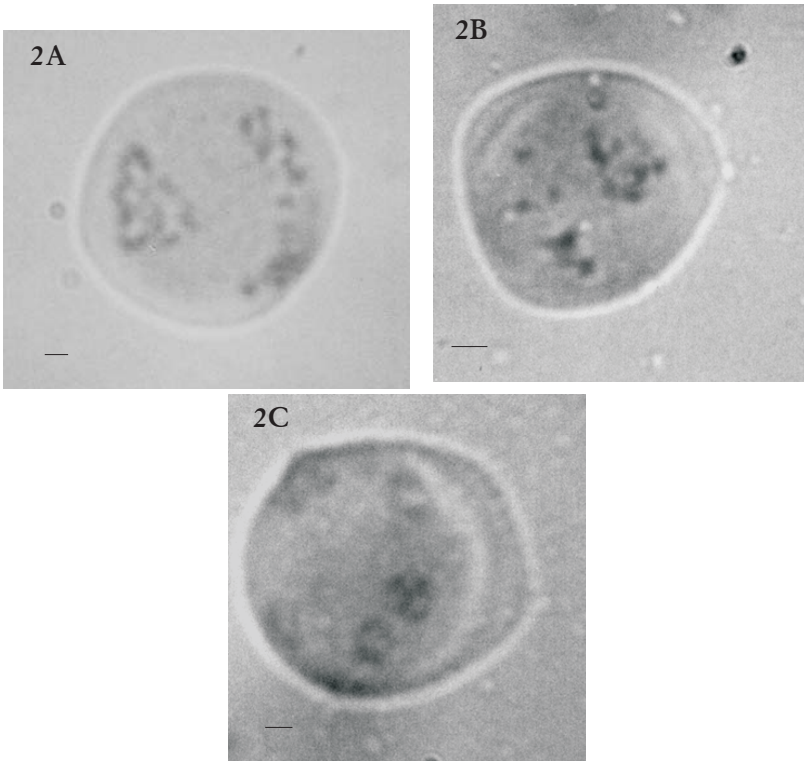

Figure 2. Meiotic chromosomes from populations of *Aloysia gratissima* (A, B and C). A – population 2, anaphase II with chromosome irregularity (non disjunction); B – population 3, anaphase II with irregularity; C – population 3, Telophase II with multipolar spindle. Scale= 2μm.

The meiotic index presented a variation among populations from 87.1 to 96.1% (Table 2), being that abnormal and normal tetrads were found (Figure 3).

Despite the irregularities during the meiosis, high pollinic viability was observed (Table 2) from 97.9 to 99.1%. Unviable pollen grains were found in all populations, though.

Aloysia gratissima presented regular meiosis, however, some irregularities were found in the anaphase and telophase II. (Figs. 2A, 2B, and 2C). These irregularities refer to chromosomal non disjunction during these phases, what consequently leads to the formation of unviable gametes. High viability of pollen grains was observed. However, this high pollinic viability carried out through the staining method does not assure high fertility, serving just as an indicative. This way, we can suggest that the

meiotic irregularities observed turn into problems of fertility, despite the pollen grains present good staining capacity and seem viable

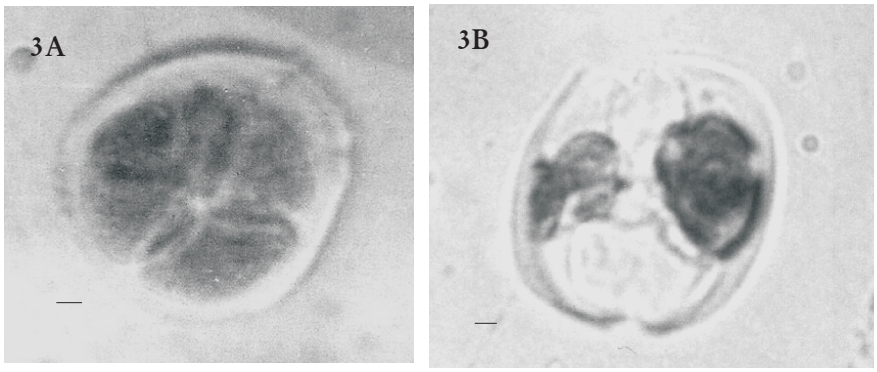

Figure 3. Estimate of the IM of *Aloysia gratissima* (A, B). A – population 3, tetrad. B – population 2, dyad. Scale= 2μm.

Chromosomal irregularities are reportedly common in *Aloysia*, *Lippia*, and *Lantana* (Siedo, 2006). Specific abnormalities observed in *Aloysia gratissima* include precocious chromosome disjunction contributing to irregular chromosomal segregation (Pagliarini, 2000). This results in the formations of univalents, multiple diakinetic chromosomal associations, abnormal segregation during meiosis II, multiple spindle formation, and multipolar spindle formation (Corazza Nunes et al., 1993).

The first chromosome number reported for *Aloysia* was a mitotic count of 2n=36 by Doulat (1943) for *A. citrodora*. Andrada et al. (1998) reported mitotic counts of 2n=54 for *A. gratissima*, 2n=36 for *A. polystachya* and 2n=60 for *Lippia fissicalyx*. All of them presented small chromosomes, which makes the caryological study more difficult. The chromosome number found showed that *A. polystachia* and *L. fissicalyx* are tetraploids, and *A. gratissima* is hexaploid. Considering the results obtained in our studies (Table 2), it is possible to assure that these species which were evaluated and showed n=18 are diploids.

Darlington & Wyle (1955), established the basic number for *Aloysia* x=9, and for *Lippia* (in discussion) x=8 or 9, but they do not mention the chromosome number of these species. On the other hand, Filippa (1984) found out that the gametic number for *Lippia turbinata* is n=15.

Brandão et al. (2003) observed the normal behavior of the chromosomes with bivalent formations of 15, 18 and 22 during diplotene

and diakinesis for *Lippia alba*, *Aloysia virgata* and *Lantana camara*, respectively. Cytogenetic analysis of *A. virgata*, performed Brandão (2009), revealed 2n=36 small metacentric chromosomes. According to this author, *A. virgata* showed regular meiotic behavior with a high percentage of seed germination (about 80%), unlike *Aloysia* species.

The occurrence of polyploid series seems to be one of the most important and known phenomena in the evolution of the species. The apparent disadvantage of the polyploidy makes us discuss how the polyploid plants managed to establish and distribute geographically. It is known that tetraploids get adjusted to more severe environment conditions. This fact indicates that their selective value is a compensatory factor to the excess of genes, as well as to their deficiencies in reproduction, which many times resort to apomitic mechanisms to get their multiplication. Thus, this mechanism represents that many polyploid species may sacrifice their evolution due to their adjustment (Andrada et al., 1998).

Conclusions

Our results show that occurred a good meiotic stability and a high viability of the pollen grains in the studied populations of *A. gratissima* and *A. triphylla.* And more, that both species present the gametic number n=18, allowing us to suggest the somatic number of 2n=36 chromosomes.

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