

## Artículo científico

**Cytogenetics of *Lippia alba* (Mill.) Brown from Lules, Tucumán**

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**Abstract**

*Lippia alba* (Mill.) Brown is an aromatic, rhizomatous bush with thin branches and violet flowers. It is widely distributed in tropical and subtropical zones of South America. Its medicinal use is for haemorrhage and its leaves have digestive properties. There are some studies of meiosis in *Verbenaceae* family that report abnormal behaviour of chromosomes. For *L. alba* it was reported a chromosomal number of  $2n = 30$ . The aim of this work is to analyze the chromosomal number and behaviour in meiosis of *L. alba* from Lules, Tucumán, Argentina. It was made a squash of anthers and root tips with 2% haematoxylin and 1% ferric citrate. The determined chromosomal number was  $2n = 30$  with formation of bivalents, laggard chromosomes in telophase I and micronuclei. Taking into account these results, a percentage of inviable pollen grains, and a low production of viable seeds could be obtained. This species resorts to multiplication by means of both seeds and rhizomes, as a strategy of the species perpetuation. The presence of rhizomes could be an adaptation to the low viability of seeds. This topic will be explored in next studies.

**Key words:** mitosis, behavior chromosome, meiosis, micronuclei.

**Resumen**

*Lippia alba* (Mill.) Brown es un arbusto aromático, rizomatoso de ramas delgadas y flores violáceas. Está ampliamente distribuida en Sudamérica tropical y subtropical. Su uso medicinal es para tratamiento de hemorragias y las hojas poseen propiedades digestivas. Hay algunos estudios de meiosis en *Verbenaceae* que han reportado comportamiento anormal de cromosomas. Para *L. alba* se ha informado un número cromosómico  $2n = 30$ . El objetivo de este trabajo es analizar el número cromosómico y el comportamiento de la meiosis de *L. alba* de Lules, provincia de Tucumán, Argentina. Se realizó un aplastado de anteras y raíces con hematoxilina 2% y citrato férrico 1%. Se determinó un número cromosómico  $2n = 30$  con formación de bivalentes y se observaron cromosomas rezagados en telofase I y micronúcleos. Teniendo en cuenta estos resultados puede haber un porcentaje de granos de polen no viables, y una baja producción de semillas viables. Esta especie recurre a la multiplicación a través de semillas y además de rizomas, como una estrategia de perpetuación de la especie. La presencia de rizomas podría ser una adaptación a la baja viabilidad de las semillas. Esto será profundizado en estudios posteriores.

**Palabras clave:** mitosis, comportamiento cromosómico, meiosis, micronúcleo.

**Introduction**

The Verbenaceae family, Verbenoideae subfamily, comprises about 36 genus and more than 1035 species widely distributed in tropical and temperate regions (Judd *et al.*, 1999). Some genera of this family are appreciated by their medicinal properties, being *Lippia* one of the most important.

The genus *Lippia* comprises about 160 species mainly distributed in Brazil, Mexico, Central America, Africa, Paraguay and Argentina (Troncoso, 1974). *Lippia alba* (Mill.) is an

aromatic bush, with rhizomes, thin branches and violet flowers, distributed in tropical and subtropical South America regions, from Mexico to Argentina and Uruguay. The roots are used to heal hemorrhages and the leaves have digestive properties (Cabrera, 1993). In Tucumán province, Argentina, it grows at the side of roads and fields without cropping. It is used in popular medicine for digestive problem treatments, mainly by inhabitants in rural areas.

Despite the information regarding its medicinal properties, endemism, indiscriminate extraction and taxonomic problems to determine species, there are few cytogenetic studies of *Lippia* genus (Bose and Choudhury, 1960; Coleman, 1982; Filippa, 1984; Kumar and Dutt, 1989; Pastoriza, 2001). Darlington and Wile, (1955) reported for *Lippia* a basic number  $x = 8$  and  $x = 9$ , without

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mentioning the species studied in this paper. Bose and Choudhury (1960) determined the chromosomal number  $2n = 30$  for *L. alba*.

The meiotic studies have helped to confirm the relationship between polyploidy and meiosis, highly irregular in a lot of species (Nirmala and Kaul, 1993; Miller and Venable, 2000 and Stace, 2000 in Dias Brandao *et al.*, 2005). There are some studies of meiosis in Verbenaceae that reported abnormal behavior of chromosomes. In Tucumán, Andrada *et al.* (1998) observed abnormalities in *Lippia fissionalyx* Tronc. with univalent, multivalent and high abnormal pollen frequency (82,4 %). Pastoriza (2001) determined a  $2n = 30$  for *L. integrifolia* and a  $2n = 60$  for *L. turbinata* and *L. fissionalyx*. The cytogenetic analysis has been widely recognized like an auxiliary tool, because the chromosomal number is not influenced with environmental factors and constituted an important trait to characterize species and to establish evolutive tendencies (Stebbins, 1971).

In this work, the chromosomal number and meiosis behavior of *L. alba* from Lules (Tucumán, Argentina) are analyzed for the first time, contributing to the knowledge of local populations.

### Material and method

**Plant material:** the plants of *Lippia alba* used in this study were taken from a local population in the locality of Lules, Tucumán ( $26^{\circ} 57' 47''$  S,  $65^{\circ} 15' 09''$  O). Plants were taken at random and stakes were cut and rooted in water. Buds were taken directly of plants at random.

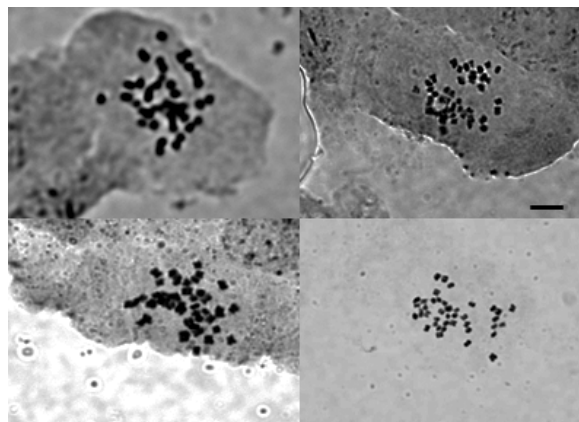
**Slide preparation:** for meiosis studies, immature buds were fixed in a Newcomer solution (isopropyl alcohol:glacial acetic acid:petroleum ether:acetone: dioxane; 6:3:1:1:1). Anthers of buds were squashed with 2 % haematoxylin as colorant and 1 % ferric citrate as mordant with the objective of observing mother pollen cells. For mitosis, stakes were put to take roots and 2 cm roots were pretreated with paradichlorobenzene for 2 h 20 min, then they were fixed in absolute ethylic alcohol:glacial acetic acid (3:1). The roots were hydrolyzed in hydrochloric acid 1N for 4 m at  $58^{\circ}\text{C}$  using the same meiosis coloration technique. The observations were made with a microscope ZEISS Primo Star, at 400 x and photographed with immersion at 1000 x.

**Viability assessment:** the viability of pollen grain was determined with cotton blue technique, according to D'Ambrogio de Argueso (1986). With this technique viability can be determined through the good formation of pollen grain

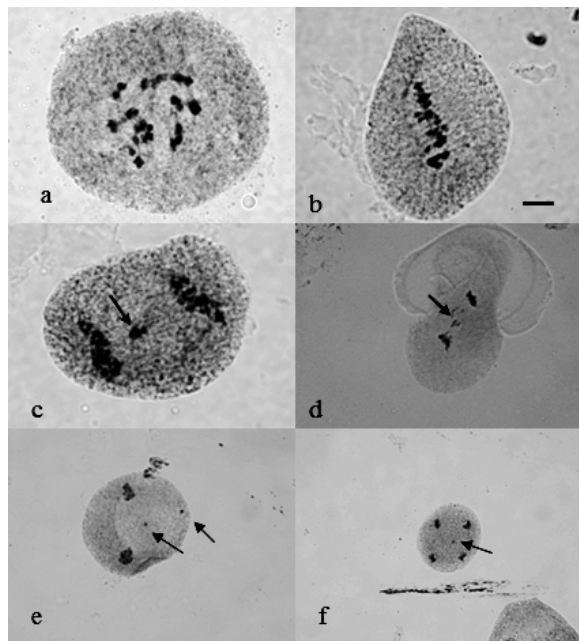
revealed by the blue color of well formed cells, counting viable and non viable pollen. Tree slides were made from random plants and in each one, 30 fields were observed. The results are expressed as percentage of viable grains.

### Results and Discussion

The chromosomal number determined was  $2n=30$  (Figure 1), which is coincident with the reported by Bose and Choudhury (1960) and Dias Brandao *et al.* (2007). In meiosis (Figure 2), laggards chromosomes in telophase I (Figure 2 c, d) and micronuclei in metaphase I were observed (Figure 2 e and f).



**Fig. 1.** *Lippia alba*. Chromosomes in metaphase of mitosis. Bar: 4  $\mu\text{m}$ .



**Fig. 2.** *Lippia alba*. Meiosis. (a) diakinesis, (b) metaphase I, (c) y (d) anaphase I with laggards (arrow), (e) metaphase I with micronuclei, (f) telophase II with micronuclei. Bar: 4  $\mu\text{m}$ .

Sanders (2001) support the hypothesis that  $x = 5$  is the ancestral basic number, and then *L. alba* would be an hexaploid. Other studies of the genus

*Lippia* have described chromosomal numbers of gametes corresponding to 12, 15, 16 and 18 (Bose and Choudhury, 1969; Coleman, 1982; Filippa, 1984; Andrada *et al.*, 1998; Pastoriza, 2001), however, a definitive basic number could not be established yet.

The meiosis analysis shows 15 bivalents and in some cells laggards have been observed. In studies made by Dias Brandao *et al.* (2007) in populations of *L. alba* of Brazil, they did not observe abnormalities related to chromosomes segregation. However, other investigations showed the existence of abnormal meiosis in *Lippia* (Pastoriza, 2001; Viccini *et al.*, 2006), being coincident with those observed in this work.

In the first division of meiosis, the univalents sometimes move to the poles at random without dividing, while in other cases, they divide equationally. Univalents that fail to move to either pole remain as laggards at the equatorial plate. At telophase I, those chromosomes that reach the poles organize dyad nuclei, while laggards often form micronuclei. According to Singh (2003) the second meiotic division is essentially normal and irregularities are restricted to the first meiotic division. Nevertheless, in this study it was observed micronuclei in metaphase II and telophase II. Based on the observations of meiosis and the origin of micronuclei proposed by Singh (2003), the laggards chromosomes in the population of *L. alba* studied, would take to the formation of unbalanced and inviable microspores.

In regard to pollen viability, 796 grains were counted: 578 viable and 218 non viable. The viability of this population was 72,6 %. It is known that pollen viability is related to normality of microsporogenesis; thus, regardless the abnormalities observed in meiosis the percentage of viable pollen can be considered high. Muñoz *et al.* (2006) reported a percentage of viability of 11 % to 18 % in three locations of Colombia, considering it low despite the high regularity observed in meiosis. This low viability may be due to the action of some genes that are mainly expressed in post-meiosis, degenerating the genetic material (Singh, 2003). Taking into account the results of pollen viability, those non viable must be due to the irregularities observed in meiosis.

Dias Brandao *et al.* (2005) suggest that the natural selection may have removed the problems caused by the ploidyization and produced a normal chromosomal behavior. The regular meiotic behavior seen in *L. alba* indicates a polyploid state sustained by diploid-like behavior

at meiosis, although a possible diploid origin cannot be excluded (Dias Brandao *et al.* 2005). Our results are opposite with the observations of Dias Brandao *et al.* (2005); they determined an irregular behavior in meiosis, with laggards which may have consequences in the formation of inviable pollen. This coincides with results found in pollen viability. The multiplication of *L. alba* is through seeds and rhizomes, as a strategy of the species perpetuation, and perhaps the presence of rhizomes could be an evolutionary consequence of the low viability of seeds.

The results reported here, provide important information regarding *L. alba* in the province of Tucumán, However, further studies are necessary to confirm the definitive basic chromosome number of the species and the consequences of meiotic behavior in possible domestication practices.

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