

UNIVERSIDADE FEDERAL DE JUIZ DE FORA
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
IMUNOLOGIA E DIP/ GENÉTICA E BIOTECNOLOGIA

Victória Rabelo Campos

**REPRODUCTIVE STRATEGIES AND CYTOTYPES FORMATION IN
Lippia alba (MILL.) N.E. BROWN (VERBENACEAE) POLYPLOID
COMPLEX**

Tese de doutorado

Juiz de Fora

2022

VICTÓRIA RABELO CAMPOS

**REPRODUCTIVE STRATEGIES AND CYTOTYPES FORMATION IN
Lippia alba (MILL.) N.E. BROWN (VERBENACEAE) POLYPLOID
COMPLEX**

Tese de Doutorado do Curso de Pós-
Graduação em Ciências Biológicas: Área:
Genética e Biotecnologia, para obtenção do
Título de Doutor em Ciências Biológicas:
Área: Genética e Biotecnologia

Orientador: Lyderson Facio Viccini

Juiz de Fora

2022

Ficha catalográfica elaborada através do programa de geração automática da Biblioteca Universitária da UFJF, com os dados fornecidos pelo(a) autor(a)

Campos, Victória Rabelo.

REPRODUCTIVE STRATEGIES AND CYTOTYPES
FORMATION IN *Lippia alba* (MILL.) N.E. BROWN (VERBENACEAE)
POLYPLOID COMPLEX / Victória Rabelo Campos. -- 2022.
193 f. : il.

Orientador: Lyderson Facio Viccini

Tese (doutorado) - Universidade Federal de Juiz de Fora, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Ciências Biológicas: Imunologia e Genética, 2022.

1. Reproductive biology. 2. Microsatellite. 3. Flow cytometry. 4. Polyploid complex. I. Viccini, Lyderson Facio, orient. II. Título.

VICTÓRIA RABELO CAMPOS

**"REPRODUCTIVE STRATEGIES AND CYTOTYPES FORMATION IN *Lippia alba* (MILL.)
N.E.BROWN (VERBENACEAE) POLYPLOYD COMPLEX"**

Tese de doutorado
apresentada ao
Programa de Pós-
Graduação em
Ciências Biológicas -
Imunologia e
DIP/Genética e
Biotecnologia
da Universidade
Federal de Juiz de
Fora como requisito
parcial à obtenção do
título de doutora em
Ciências Biológicas.
Área de
concentração: Genética
e Biotecnologia

Aprovada em 20 de maio de 2022.

BANCA EXAMINADORA

Prof. Dr. Lyderson Facio Viccini

Orientador

Universidade Federal de Juiz de Fora

Prof. Dr. Wagner Campos Otoni

Universidade Federal de Viçosa

Dr.^a Ana Luisa Sousa Azevedo

Embrapa

Dr.^a Juliana Mainenti Leal Lopes
Universidade de Trás-os-Montes e Alto Douro
Portugal

Prof.Dr. Saulo Marçal de Sousa
Universidade Federal de Juiz de Fora

Juiz de Fora, 02/05/2022.



Documento assinado eletronicamente por **Lyderson Facio Viccini, Professor(a)**, em 21/05/2022, às 15:16, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do [Decreto nº 10.543, de 13 de novembro de 2020](#).



Documento assinado eletronicamente por **Saulo Marçal de Sousa, Professor(a)**, em 22/05/2022, às 16:03, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do [Decreto nº 10.543, de 13 de novembro de 2020](#).



Documento assinado eletronicamente por **Wagner Campos Otoni, Usuário Externo**, em 25/05/2022, às 14:46, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do [Decreto nº 10.543, de 13 de novembro de 2020](#).



Documento assinado eletronicamente por **Ana Luiza Souza Azevedo, Usuário Externo**, em 25/05/2022, às 14:52, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do [Decreto nº 10.543, de 13 de novembro de 2020](#).



Documento assinado eletronicamente por **Juliana Mainenti Leal Lopes, Usuário Externo**, em 25/05/2022, às 20:31, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do [Decreto nº 10.543, de 13 de novembro de 2020](#).



A autenticidade deste documento pode ser conferida no Portal do SEI-Ufjf (www2.ufjf.br/SEI) através do ícone Conferência de Documentos, informando o código verificador **0760727** e o código CRC **6C4F4C18**.

À minha amada mãe Luzia,
Minhas amigas e irmãs Luiza e Giovanna,
Amado pai Luiz,
Amado companheiro e amigo Robson,
DEDICO

AGRADECIMENTOS

Agradeço primeiramente a Deus pela força e coragem em toda minha trajetória acadêmica e pela possibilidade de estudar o universo maravilhoso das plantas;

À Universidade Federal de Juiz de Fora e ao Programa de Pós-graduação em Ciências Biológicas pela oportunidade de execução do trabalho proposto;

Às agências de fomento CAPES, CNPq e FAPEMIG, pelo apoio financeiro e pela concessão da bolsa durante a execução do projeto, tanto no Brasil quanto no Programa de Doutorado Sanduíche realizado na Universidade de Saskatchewan, Canadá.

Ao meu orientador Doutor Lyderson Facio Viccini, pela confiança em me proporcionar este grande desafio de trabalhar com reprodução, genética e evolução; e por me fazer entender que a beleza da ciência está mais nas perguntas do que nas próprias respostas.

Ao professor Doutor Saulo Marçal de Sousa por acompanhar meus passos desde minha entrada no laboratório e mostrar o que é amar a Ciência, sendo exemplo de profissional e ser humano;

Ao professor Doutor José Marcello Salabert de Campos, pelos ensinamentos, conversas e grande apoio desde minha entrada no laboratório;

Aos demais professores Doutor Marcelo de Oliveira Santos, Doutora Michelle Munk e Doutor Carlos Maranduba, pelas ajudas e conselhos do dia a dia;

Aos professores da botânica Doutora Fátima Salimena, Doutor Luiz Menini, Doutor Paulo Peixoto e Doutora Nádia Somavilla por compartilharem seus conhecimentos e pela ajuda sempre que preciso;

Ao professor Doutor Wagner Campos Otoni pela disponibilidade e grande ajuda em parte dos experimentos e na partilha de conhecimentos;

A professora Doutora Denise Maria Trombert de Oliveira por toda colaboração e compartilhamentos de conhecimentos, me mostrando cada vez mais o quão incrível é o universo das plantas;

Ao professor Doutor Richard Grazul pela parceria no estudo dos óleos essenciais de *Lippia alba*;

À professora Doutora Sueli Maria Gomes, pela prontidão em nos auxiliar e pelos conhecimentos compartilhados;

Ao professor Doutor Timothy Sharbel por aceitar fazer parte deste trabalho e me receber tão prontamente em seu laboratório da Universidade de Saskatchewan. Ao pesquisador Rick Goertzen por todo auxílio na coleta de dados de citometria de sementes e a todos

os membros do GIFS (*Global Institute for Food Security*) pela receptividade e compartilhamento de ensinamentos e experiências.

Aos pesquisadores da Embrapa Gado de Leite Ana Luisa de Sousa Azevedo e Marco Antônio Machado, pela disponibilidade e auxílio na coleta de dados moleculares e no compartilhamento de conhecimentos;

Aos pesquisadores Lorena Vieira e Victor Bonifacio pela grande ajuda e disponibilidade na coleta de dados de anatomia;

Ao Doutor Elyabe Monteiro por toda o auxílio não só na coleta de dados de citometria, mas também em toda a minha trajetória na pós graduação, sendo exemplo de dedicação e conhecimento;

À Doutora Juliana Mainenti, que me acompanha desde o mestrado e que me ensina com muito carinho e persistência cada vez mais sobre o universo molecular;

À Doutora Aryane Reis pela querida parceria de 11 anos de laboratório e pela partilha de experiências de ciência e de vida e por dividir comigo perguntas e mais perguntas sobre *Lippia alba*.

À Thalita Bordignon pela grande ajuda e dedicação na coleta de dados e pelo companheirismo no estudo da reprodução da espécie;

À Ana Luiza Franco e Flávia Rangel de Sousa pela grande amizade e oportunidade de caminharmos juntas; mostrando-me sempre que, apesar dos obstáculos, nunca podemos desistir;

Aos queridos amigos de laboratório: Juliana Siqueira, Marizia Trevizani, Rafael Valotti, Moisés Gusmão e Silvioney, pelo companheirismo, ajuda e partilha de conhecimentos de ciência e de vida;

Às amigas de graduação Paola Oliveira e Ludymila Cruz pela trajetória compartilhada com muito carinho e amizade;

Às secretárias da pós-graduação Graça e Fernanda pelo auxílio quando necessário;

Aos amigos de infância que mesmo de longe torcem sempre por mim;

À Silvia Lessa que me recebeu com tanto carinho em um país com costumes e idioma diferentes, assim como todos os amigos que fiz no doutorado sanduíche e que levarei para vida, em especial Luciana, Lucas, Márcia e Diana;

À minha mãe Luzia, exemplo para mim de ser humano e de vida. Ao meu amado pai Luiz, que mesmo distante sempre me apoiou na busca de minha felicidade; às minhas amadas irmãs Luiza e Giovanna pela admiração e amor ao longo de toda uma vida;

Ao Robson, pela admiração, companheirismo e amor que trouxe para meu trabalho e minha vida;

À grande amiga Rita Mota;

A todos os meus familiares que sempre fizeram parte da minha trajetória,

Aos que partiram e deixaram saudades eternas;

Aos mestres da minha caminhada!

RESUMO

Lippia alba (Verbenaceae), de ampla ocorrência no Brasil e originária da América do Sul, é conhecida por vários nomes populares como “erva cidreira”, “falsa melissa”, “chá de tabuleiro”, “erva cidreira do campo”, “salva do Brasil”, “salva-limão” e “erva cidreira brava”. Possuindo importância medicinal pela riqueza de seu óleo essencial, diversas atividades biológicas são atribuídas à espécie. Formada por um complexo poliploide com cinco diferentes números cromossômicos: $2n=30$, $2n=38$, $2n=45$, $2n=60$ e $2n=90$, poucas informações sobre o sistema reprodutivo estão disponíveis. Nenhum estudo sistemático sobre este tema foi relatado até o momento. O presente trabalho teve como objetivo investigar aspectos da biologia reprodutiva do complexo poliploide *Lippia alba*, a fim de se obter informações sobre a origem dos diferentes citótipos da espécie. Analisou-se de forma comparativa entre os citótipos (intra e inter ploidia) a produção de sementes sob polinizações aberta e controlada e a morfometria e germinação das mesmas. Estimativas de quantidade de DNA permitiram identificar o nível de ploidia da progênie provinda dos diferentes citótipos. Análises cromossômicas foram realizadas em progênies oriundas de cruzamentos naturais e artificiais. Padrões moleculares de marcadores SSR foram analisados, comparando-se o perfil alélico das plantas-mães e seus descendentes. Para observar a produção de gametas não reduzidos, foi realizada uma análise do tamanho dos grãos de pólen em diploides, possivelmente fonte dos demais citótipos. Análises histológicas do microgametofito e megagametofito de diferentes ploidias também foi realizada. Como resultado, foi possível observar que os diploides apresentam maior produção de sementes sob polinização aberta, enquanto os poliploides apresentam maior produção quando as inflorescências foram isoladas. Além disso, foi possível observar diferenças entre os níveis de ploidia ao se analisar a morfometria e a germinação das sementes. Comparativamente sob polinização aberta, os diploides apresentaram maior sucesso reprodutivo, porém, sob polinização controlada, os poliploides se mostraram mais eficientes. A análise da citometria de fluxo revelou a presença de autofecundação como um dos mecanismos reprodutivos na espécie, apesar de ser preferencialmente alógama. Além disso, foram observados cruzamentos entre diferentes níveis de ploidia, mas com uma possível reprodução preferencial entre os diploides. Marcadores microsatélites, por sua vez, confirmaram tanto o sucesso do cruzamento artificial quanto

a presença de descendentes provindos de autofecundação. Também foi possível observar a presença de pólenes não reduzidos em praticamente todos os diploides da espécie. Já a análise da formação do microgametófito e macrogametófito indicaram uma maior dificuldade dos tetraploides em produzir gametas viáveis. Reunidos, os dados sugerem uma complexidade ainda maior envolvida na formação do complexo poliploide *Lippia alba*. Em conjunto com dados até agora obtidos, contribuiu-se para se compreender o processo evolutivo em *Lippia alba* assim como demonstrar processos envolvidos na origem da biodiversidade tropical. Paralelamente os dados podem ser também utilizados para se estabelecer estratégias para o melhoramento genético da espécie.

Palavras-chave: Biologia reprodutiva. Microsatélite. Citometria de fluxo. Complexo poliploide.

ABSTRACT

Lippia alba (Verbenaceae), a South American species of wide occurrence in Brazil, is known by several popular names such as “erva cidreira”, “falsa melissa”, “chá de tabuleiro”, “erva cidreira do campo”, “salva do Brasil”, “salva-limão” and “erva cidreira brava”. With medicinal importance due to the richness of the essential oil, several biological activities are attributed to the species. Being a polyploid complex with five different chromosome numbers: $2n=30$, $2n=38$, $2n=45$, $2n=60$ and $2n=90$, few data about the reproductive system is available and no systematic study on this topic has been reported so far. The present work aimed to investigate aspects of the reproductive biology of *Lippia alba* polyploid complex contributing on the understanding of the origin of the different cytotypes. The production of seeds under open and controlled pollination as well as the morphometry and germination of seeds were analyzed in a comparative way between the cytotypes (intra and inter ploidal levels). DNA amount estimation made possible to identify the ploidal level of the offspring from the different cytotypes. Chromosomal analyses were performed on progenies from natural and artificial crosses. Molecular patterns of SSR markers were analyzed, comparing the allelic profile of mother plants and their offspring. To observe the production of unreduced gametes, an analysis of the size of pollen grains in diploids was performed. Histological analyzes of microgametophyte and megagametophyte from different ploidal levels were also performed. As a result, it was possible to observe that diploids have higher seed production under open pollination, while polyploids have higher production when inflorescences were isolated. In addition, it was possible to observe differences between the ploidal levels when analyzing the morphometry and germination of the seeds. Comparatively, under open pollination, diploids showed greater reproductive success, however, under controlled pollination, polyploids were more efficient. Flow cytometry analysis revealed the presence of self-fertilization as one of the reproductive mechanisms in the species, despite being preferentially allogamous. In addition, crosses were observed among different ploidal levels, but with possible preferential reproduction among diploids. Microsatellite markers, in turn, confirmed both the success of the artificial crossing and the presence of offspring from self-fertilization. It was also possible to observe the presence of unreduced pollens in practically all diploids of the species. The analysis of the formation of microgametophyte and macrogametophyte indicated a greater difficulty of tetraploids in producing viable gametes.

Taken together, the data suggest an even greater complexity involved in the formation of the *Lippia alba* polyploid complex. Together with the data obtained so far, this work has contributed on the understanding of the evolutionary process in *Lippia alba* as well as demonstrating processes involved in the origin of tropical biodiversity. At the same time, the data can also be used to establish strategies for the genetic improvement of the species.

Key words: Reproductive biology. Microsatellite. Flow cytometry. Polyploid complex.

LISTA DE FIGURAS

Figure 1	Figure 1- A: Inflorescence detail of <i>Lippia alba</i> species cultivated at the Experimental Plant Station at UFJF. B: Internal morphology of <i>L. alba</i> flower. C: <i>L. alba</i> individual.....35
Figure 2	<i>Lippia alba</i> inflorescences representing the isolation method of flowers at the Experimental Station of Plant Cultivation and Maintenance at the Universidade Federal de Juiz de Fora, Minas Gerais.....40
Figure 3	Representative diagram of the samples analyzed with the SSR markers. The red color represents diploid ($2n=30$) and the green color represents triploid ($2n=45$). The numbers 1, 2 and 3 respectively comprise the diploid mother plant (BGEN-77) of the artificial cross, the offspring of the artificial cross and the triploid paternal provider (BGEN-59) of the artificial cross. The number 4 is a triploid (BGEN-59) offspring from controlled pollination. The number 5 is a triploid (BGEN-27) while its offspring from controlled pollination comprises the numbers 6, 7 and 8. The number 9 is a triploid (BGEN-10) while its offspring from controlled pollination comprises the numbers 10, 11 and 12.....49
Figure 4	Average of seeds per inflorescence from controlled (blue bars) and open pollination (orange bars) of diploid accessions of <i>Lippia alba</i> . The difference between the number of seeds produced from both conditions for each accession were all significative by the chi-square test ($p<0.05$) except for accession BGEN-02 (ns=non-significant).....54
Figure 5	Average of seeds produced by triploid and tetraploid accessions of <i>Lippia alba</i> under controlled (blue bars) and open (orange bars) pollination. The asterisk indicates a significant difference between the means by the chi-square test ($p<0.05$).....55
Figure 6	Average of seed production in <i>Lippia alba</i> for diploids (red bars) and polyploids (blue bars) under open (OP) and controlled (CP) pollination. Values are presented as mean and standard error of the mean of seeds

produced per reproductive unit. Different letters represent statistical differences at $p < 0.05$ according to Dunn's test.....56

Figure 7 Index of self-incompatibility at different ploidal levels of *Lippia alba*: diploids, triploids and tetraploids. A: Index average in the ploidal levels. B: distribution diagram of all individuals index analyzed.....57

Figure 8 Germination index from open pollination of diploid (red bars), triploids (green bars) and tetraploids (blue bars) accessions of *Lippia alba*...58

Figure 9 Germination rate of seeds produced from individuals under controlled pollinated in *Lippia alba*. Red bars: diploids; green bars: triploids; blue bar: tetraploid. The numbers above the bars represent the total amount of seeds analyzed.....59

Figure 10 Length (A) and width (B) average of seeds from diploid and polyploid accessions of *Lippia alba* under open pollination.....60

Figure 11 Length (A) and width (B) of seeds from diploid and polyploid individuals of *Lippia alba* under controlled pollination.....61

Figure 12 Seed length and width measurements in *Lippia alba* for diploids (red bars) and polyploids (blue bars) under open (A) and controlled (B) pollination. Values are presented as mean and standard error of the mean of seed length and width. Different letters represent statistical differences at $p < 0.05$ according to Dunn's test.....62

Figure 13 Seed length and width measurements in *Lippia alba* for diploids (red bars) and polyploids (blue bars) under open (A) and controlled (B) pollination. Values are presented as mean and standard error of the mean of seed length and width. Different letters represent statistical differences at $p < 0.05$ according to Dunn's test.....63

Figure 14 Average seed weight of diploid, triploid and tetraploid individuals. Presence of significant difference between diploids and triploids ($p < 0.05$) according to Dunn's test.....63

Figure 15 Principal component analysis (PCA) of five seed characteristics analyzed from 34 samples of *Lippia alba*. The colors represent different ploidal levels: diploid in red circle, triploid in green, tetraploid in blue..64

Figure 16	Fitness values (W) and selection coefficient (s) considering the average of seed production in diploids (red), triploids (green) and tetraploids (blue) of <i>Lippia alba</i> under open pollination (A) and controlled pollination (B).....	65
Figure 17	Fitness values (W) and selection coefficient (s) considering seed length (A), width (B), weight (C) and germination (D) in diploids (red), triploids (green) and tetraploids (blue) of <i>Lippia alba</i> under open pollination..	67
Figure 18	Fitness values (W) and selection coefficient (s) considering the average of seed production in diploids (red), triploids (green) and tetraploids (blue) of <i>Lippia alba</i> under open pollination (A, B and C) and controlled pollination (D, E and F).....	68
Figure 19	Fitness values (W) and selection coefficient (s) from seed length and width of diploids (red), triploids (green) and tetraploids (blue) under open pollination.....	69
Figure 20	Fitness values (W) and selection coefficient (s) of seed weight and germination index in diploids (red), triploids (green) and tetraploids (blue) of <i>Lippia alba</i> under open pollination.....	70
Figure 21	Fitness (W) value of average of seed production in <i>Lippia alba</i> for diploids (red bars) and polyploids (blue bars) under open pollination (O.P.) and controlled pollination (C.P.). Values are presented as mean and standard error of the mean. Different letters represent statistical differences at $p < 0.05$	71
Figure 22	Fitness (W) value of seed length (A), seed width (B), germination index (C) and seed weight (D) in <i>Lippia alba</i> for diploids (red bars) and polyploids (blue bars) under open pollination. Values are presented as mean and standard error of the mean. Different letters represent statistical differences at $p < 0.05$	72
Figure 23	Representative histogram of DNA amount estimation of seeds from diploid accessions of <i>Lippia alba</i> under from open pollination. A: diploid from sexual reproduction. B: aneuploid. C: triploid from sexual	

	reproduction. P1 and P2 represents respectively the embryo and endosperm informations.....75
Figure 24	Representative histogram of DNA amount estimation of seeds from triploid accessions of <i>Lippia alba</i> under from open pollination. A: diploid of sexual reproduction. B: aneuploid. C: triploid of sexual reproduction. P1 and P2 represents respectively the embryo and endosperm informations.....76
Figure 25	Representative histogram of DNA amount estimation of seeds from tetraploid accessions of <i>Lippia alba</i> under from open pollination. A: triploid from sexual reproduction. B: aneuploid. C: tetraploid with only the embryo peak. P1 and P2 represents respectively the embryo and endosperm informations.....77
Figure 26	Representative histogram of DNA amount estimation of seeds from diploid accessions of <i>Lippia alba</i> under controlled pollination showing ratio 2:3 (embryo:endosperm) of sexual reproduction. P1 and P2 represents respectively the embryo and endosperm informations....78
Figure 27	Representative histogram of DNA amount estimation of seeds from triploid accessions of <i>Lippia alba</i> under controlled pollination showing ratio 3:4 (embryo:endosperm)of sexual reproduction. P1 and P2 represents respectively the embryo and endosperm informations....78
Figure 28	Offspring ploidal level from diploid, triploid and tetraploid accessions of <i>Lippia alba</i> under open pollination.....79
Figure 29	Offspring ploidal level from triploid accessions of <i>Lippia alba</i> under controlled pollination.....80
Figure 30	Karyogram and DNA content of the offspring individuals in <i>Lippia alba</i> . A: offspring with $2n=35$ from the artificial crossing between the diploid BGEN-77 and the triploid BGEN-59; B: offspring with $2n= 30$ from open pollination of the diploid BGEN-77; C: offspring with $2n=30$ from open pollination of the tetraploid BGEN-64; D: offspring with $2n=48$ from controlled pollination of the triploid BGEN-59. The asterisk indicates the chromosomes with extra copies. Bar= $5\mu\text{m}$83

Figure 31	Ideograms of the offspring individuals in <i>Lippia alba</i> . A: offspring with 2n=35 from the artificial crossing between the diploid BGEN-77 and the triploid BGEN-59; B: offspring with 2n= 30 from open pollination of the diploid BGEN-77; C: offspring with 2n=30 from open pollination of the tetraploid BGEN-64; D: offspring with 2n=48 from controlled pollination of the triploid BGEN-59.....84
Figure 32	Terpenes found in the analyzed offspring of <i>Lippia alba</i> . A: 2n=35 from artificial crossing; 2n=30 of a diploid from open pollination and 2n=30 of a tetraploid from open pollination. B: 2n=48 of a triploid from controlled pollination.....88
Figure 33	Chromatograms from offspring individuals. A: Artificial crossing (2n=35) with geranial as the majority component. B: Offspring (2n=30) from diploid of open pollination with geranial as major component. C: Offspring (2n=30) from tetraploid of open pollination with geranial as major component. D: Offspring (2n=48) from triploid of controlled pollination with linalool as major component.....89
Figure 34	Representative scheme of the samples analyzed by the SSR technique. (A) The numbers 1, 2 and 3, respectively, comprise the diploid mother plant (BGEN-77) of the artificial cross; the offspring 2n=35 of the artificial cross and the triploid paternal genitor (BGEN-59) of the artificial cross; (B) The number 4 is the 2n=48 offspring from controlled pollination of the sample 3 (BGEN-59); (C)The number 5 is a triploid (BGEN-27) while its offspring from controlled pollination comprises the numbers 6, 7 and 8; (D) The number 9 is a triploid (BGEN-10) while its offspring from controlled pollination comprises the numbers 10, 11 and 12.....90
Figure 35	Representative scheme of the allele pattern in 14 primers in the different analyzed individuals of <i>Lippia alba</i> . The numbers 1 to 12 represent the samples and different colors were chosen for each allele.....91,92

Figure 36	Representative electropherograms of primer LAC4 of three individuals of <i>Lippia alba</i> . The sequence of samples corresponds to the diploid mother plant BGEN-77, the offspring with $2n=35$ (from artificial crossing) and the male-parent triploid BGEN-59.....93
Figure 37	Representative electropherograms of primer LA08 of three individuals of <i>Lippia alba</i> . The sequence of samples corresponds to the triploid mother plant BGEN-27 (sample 5) and two offspring (sample 6 and 7) from controlled pollination.....95
Figure 38	Representative electropherograms of primer p229 of three individuals of <i>Lippia alba</i> . The sequence of samples corresponds to the triploid mother plant BGEN-10 (sample 9) and two offspring (samples 10 and 12) from controlled pollination.....96
Figure 39	Representative electropherograms of primer LA08 of two individuals of <i>Lippia alba</i> . The sequence of samples corresponds to the triploid mother plant BGEN-59 (sample 3) and its offspring (sample 4) from controlled pollination.....97
Figure 40	Representative scatter plots of pollen grains showing wide variations in two diploid individuals of <i>Lippia alba</i> (A: BGEN-58; B: BGEN-89) and less variations (C: BGEN-21; D: Planta F).....100
Figure 41	Pollen grains from different diploid individuals of <i>Lippia alba</i> . A and B: individuals with greater variation. C and D: individuals with lesser variation. Red arrows highlight bigger pollens and green arrows highlight smaller pollens. Bar=50 μ m.....101
Figure 42	Mature anthers of <i>Lippia alba</i> in transversal sections stained with Toluidine Blue. A-E . Diploid individuals. A . General view of anther in dehiscence, showing the two theca. B . Detail of a theca; note the density of most pollen grains. C-E . Pollen grain details; in D-E , typical pollen grains in different section planes, with the ellipsoid generative cell, long and tapered, sectioned transversally (D) and longitudinally (E). F-J . triploid individuals. F . General view of anther in dehiscence, showing the two theca with several dense and typical shaped grains

and others more hyaline, with an atypical contour that indicates their inviability. **G.** Detail of a theca; note pollen grains presented in monads or tetrads, all of which are not viable in this field. **H-J.** Pollen details. **H.** Pollen grains with typical characteristics. **I.** Grain with slightly lobed contour and protoplast more hyaline than typical grains. **J.** Grains with typical contour, but the protoplast is more hyaline than that seen in H and the generative cell does not have the characteristic contour, but rather curved ends. **K-N.** tetraploid individuals. **K.** General view of the anther at the beginning of the dehiscence, showing the two theca with several hyaline grains and with an atypical contour, aspects that indicate their inviability; observe that the anther is clearly smaller than those from diploid and triploid individuals. **L.** Detail of a theca; to notice nonviable pollen grains, with variable contours. **M-N.** Details of pollen that has some obvious content. **M.** Bicellular pollen grain, with very vacuolated vegetative cell and atypical generative cell, with curved ends. **N.** Grain with lobed contour, protoplast more hyaline than typical grains and only the vegetative cell formed. (asterisk: non-viable pollen, with irregular and atypical wall contour, without evident content; arrowhead: pollen grains emitting pollen tube; arrow: generative cell; vc: vegetative cell).....103

Figure 43

Lippia alba flower ovules in anthesis, seen in longitudinal sections stained with Toluidine Blue. **A-F** Diploid individuals. **A.** General view of the unitegumented ovule, with phenolic compounds deposited in the chalazal cells (characterizing hypostasis) and in the inner epidermis of the integument (endothelium), which delimits the megagametophyte. **B-F.** Ovules details showing different views of the megagametophyte. **G-I.** Triploid individuals; note the expansion of phenolic deposits, both tegumentary and bordering the megagametophyte (the latter in detail in I). **J-L.** Tetraploid individuals; note the immature-looking megagametophyte, accompanied by the more evident nucellus. **K-L.** Details of megagametophyte in which only the central cell is

recognized. (asterisk: hypostasis; arrowhead: deposition of phenolic compounds in the tegument; double arrowhead: deposition of phenolic compounds in the megagametophyte; arrow: nucellar residues; an: antipode; cc: central cell; ec: oosphere; en: endothelium; in: tegument; mi: micropyle; sy: synergid).....106

Figure 44 Representative scheme proposed by Reis et al (2014) for the origin of the different cytotypes in *L. alba*. Each cytotype is depicted by a different color. Continuous arrows represent unreduced gametes; dashed arrows represent reduced gametes; dotted arrows represent chromosome duplication (2x).....121

Figure 45 Representative scheme regarding the origin of *Lippia alba* cytotypes. Each cytotype is depicted by a different color. Continuous arrows represent unreduced gametes; dashed arrows represent reduced gametes; dotted arrows represent chromosome duplication (adaptated from Reis et al., 2014).....124

LISTA DE TABELAS

Table 1	Number of seeds of <i>Lippia alba</i> from controlled pollination analyzed for germination index.....	43
Table 2	Number of seeds of <i>Lippia alba</i> from controlled pollination analyzed for morphometry analysis.....	43
Table 3	Number of open pollination offspring from different ploidal level of <i>Lippia alba</i> analyzed by flow cytometry.....	46,47
Table 4	Number of open pollination offspring from triploids of <i>Lippia alba</i> analyzed by flow cytometry.....	47
Table 5	Data about of 16 microsatellite primers used to perform a genealogy investigation in twelve accessions of <i>Lippia alba</i> accessions.....	52,53
Table 6	Mean and standard deviation of length (mm) and width (mm) of seeds from diploid and polyploid accessions of <i>Lippia alba</i> under controlled and open pollination seeds.....	63
Table 7	Quantitative data from seed flow cytometry in the different ploidal level under open and controlled pollination.....	75
Table 8	Qualitative data from seed flow cytometry in the different ploidal level under open and controlled pollination.....	75
Table 9	Ranges of DNA amount corresponding to ploidal levels in the offspring of <i>Lippia alba</i>	80
Table 10	Morphometric data of the offspring with 2n=35 from the artificial crossing between the diploid BGEN-77 and the triploid BGEN-59.....	86
Table 11	Morphometric data of the offspring with 2n=30 from open pollination of the diploid BGEN-77.....	86
Table 12	Morphometric data of the offspring with 2n=30 from open pollination of the tetraploid BGEN-64.....	87
Table 13	Morphometric data of the offspring with 2n=48 from controlled pollination of the triploid BGEN-59.....	87

Table 14	Karyotypic asymmetry, chromosome number and DNA content of <i>Lippia alba</i> offspring.....88
Table 15	Offspring samples and their respective primers with a pattern of bands different from its mother plant.....95
Table 16	Pollen measurement data from 29 diploid individuals of <i>L.alba</i>100
Table 17	Number of measurements present in the quartiles calculated for the averages of pollen dimensions of the diploid accession BGEN-38 of <i>Lippia alba</i>100
APPENDIX A	Identification, ploidal level, DNA content, registration number and origin of different individuals of <i>Lippia alba</i>131,132
APPENDIX B	Fitness (W) and coefficient of selection (s) regarding average of seed production under open pollination in different ploidal levels of <i>Lippia alba</i>133,134
APPENDIX C	Fitness (W) and coefficient of selection (s) regarding average of seed production under controlled pollination in different ploidal levels of <i>Lippia alba</i>135,136
APPENDIX D	Fitness (W) and coefficient of selection (s) regarding seed length under open pollination in different ploidal levels of <i>Lippia alba</i>137,138
APPENDIX E	Fitness (W) and coefficient of selection (s) regarding seed width under open pollination in different ploidal levels of <i>Lippia alba</i>139,140
APPENDIX F	Fitness (W) and coefficient of selection (s) regarding seed weight under open pollination in different ploidal levels of <i>Lippia alba</i>141,142
APPENDIX G	Fitness (W) and coefficient of selection (s) regarding seed germination under open pollination in different ploidal levels of <i>Lippia alba</i>143,144

APPENDIX H.1	Fitness (W) and selection coefficient (s) regarding average of seed production within diploids under open pollination in <i>Lippia alba</i>	145
APPENDIX H.2	Fitness (W) and selection coefficient (s) regarding average of seed production within triploids under open pollination in <i>Lippia alba</i>	146
APPENDIX H.3	Fitness (W) and selection coefficient (s) regarding average of seed production within tetraploids under open pollination in <i>Lippia alba</i>	146
APPENDIX I.1	Fitness (W) and selection coefficient (s) regarding average of seed production within diploids under controlled pollination in <i>Lippia alba</i>	147
APPENDIX I.2	Fitness (W) and selection coefficient (s) regarding average of seed production within triploids under controlled pollination in <i>Lippia alba</i>	148
APPENDIX I.3	Fitness (W) and selection coefficient (s) regarding average of seed production within tetraploids under controlled pollination in <i>Lippia alba</i>	148
APPENDIX J.1	Fitness (W) and selection coefficient (s) regarding seed length within diploids under open pollination in <i>Lippia alba</i>	149
APPENDIX J.2	Fitness (W) and selection coefficient (s) regarding seed length within triploids under open pollination in <i>Lippia alba</i>	150
APPENDIX J.3	Fitness (W) and selection coefficient (s) regarding seed length within tetraploids under open pollination in <i>Lippia alba</i>	150
APPENDIX K.1	Fitness (W) and selection coefficient (s) regarding seed width within diploids under open pollination in <i>Lippia alba</i>	151
APPENDIX K.2	Fitness (W) and selection coefficient (s) regarding seed width within triploids under open pollination in <i>Lippia alba</i>	152
APPENDIX K.3	Fitness (W) and selection coefficient (s) regarding seed width within tetraploids under open pollination in <i>Lippia alba</i>	152

APPENDIX L.1	Fitness (W) and selection coefficient (s) regarding seed weight within diploids under open pollination in <i>Lippia alba</i>153
APPENDIX L.2	Fitness (W) and selection coefficient (s) regarding seed weight within triploids under open pollination in <i>Lippia alba</i>154
APPENDIX L.3	Fitness (W) and selection coefficient (s) regarding seed weight within tetraploids under open pollination in <i>Lippia alba</i>154
APPENDIX M.1	Fitness (W) and selection coefficient (s) regarding seed germination index within diploids under open pollination in <i>Lippia alba</i>155
APPENDIX M.2	Fitness (W) and selection coefficient (s) regarding seed germination index within triploids under open pollination in <i>Lippia alba</i>156
APPENDIX M.3	Fitness (W) and selection coefficient (s) regarding seed germination index within tetraploids under open pollination in <i>Lippia alba</i>156
APPENDIX N.1	DNA amount of diploids offsprings in <i>Lippia alba</i> under open pollination.....157,158,159,160,161,162,163,164,165,166
APPENDIX N.2	DNA amount of triploids offsprings in <i>Lippia alba</i> under open pollination.....167,168,169
APPENDIX N.3	DNA amount of tetraploids offsprings in <i>Lippia alba</i> under open pollination.....170,171
APPENDIX O	DNA amount of triploids offsprings in <i>Lippia alba</i> under controlled pollination.....172

LISTA DE ABREVIATURAS, SIGLAS E ACRÔNIMOS

BGEN	Banco do Germoplasma de Espécies Nativas
µL	Microlitro
°C	graus Celsius
CESJ	Herbário Leopoldo Krieger
CTAB	Cetyltrimethyl ammonium bromide
DAPI	4',6-diamidino-2-phenylindole
DNA	“deoxyribonuclei acid” – ácido desoxirribonucleico
dNTP	deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
EEP-UFJF	Estação Experimental de Plantas da Universidade Federal de Juiz de Fora
eV	electron volt
KHz	kilohertz
m	meter
mg	milligram
mL	milliliter
µL	microliter
mm	millimeter
PVP	Polyvinylpyrrolidone
PCR	“Polymerase Chain Reaction” – Reação em Cadeia de Polimerase
rpm	Rotation per minute
UFJF	Universidade Federal de Juiz de Fora

SUMÁRIO

1- INTRODUCTION	26
1.1- Angiosperm reproduction	26
1.2- Sexual vs asexual reproduction	26
1.3- Apomixis	28
1.4- Polyploidy in plants	31
1.5- Polyploidy and reproduction	33
1.6- <i>Lippia alba</i> polyploid complex	36
2- OBJECTIVE	40
3- MATERIAL AND METHODS	41
3.1- Plant material	41
3.2- Seed production	41
3.3- Seed germination, morphometry and weight.....	42
3.4- Fitness	44
3.5- Seed flow cytometry	45
3.6- Offspring ploidal level analysis	46
3.7- Offspring: case studies	47
3.7.1- Offspring from natural reproduction	48
3.7.2- Offspring from an artificial crossing	49
3.8- Microsatellite markers	50
3.9- Pollen analysis	54
3.10- Comparative anatomy of <i>Lippia alba</i> anthers and ovules	54
4- RESULTS	55
4.1- Seed production, germination and morphometry	55
4.1.1 - Seed production	55
4.1.2 – Germination rate	59
4.1.3. Seed morphometry	61
4.2- Fitness (W)	66
4.3- Seed flow cytometry and offspring ploidal level	74
4.4- Offspring: case studies	82
4.4.1 Cytogenetic analysis and DNA content estimation	82
4.4.2 Essential oil constituents	89
4.5- Microsatellite analysis	91
4.6- Pollen analysis	99

4.7- Comparative anatomy of <i>Lippia alba</i> anthers and ovules	103
4.7.1- Pollen grains and microgametophytes	103
4.7.2- Ovules and megagametophytes	105
5- DISCUSSION	107
5.1- Diploids and polyploids of <i>Lippia alba</i> show different behavior regarding seed production and germination	108
5.2- Selfing as an alternative way of reproduction in <i>Lippia alba</i>	110
5.3- Fitness and adaptability under different scenarios.....	113
5.4- Artificial crossing and the role of triploids and aneuploids in the formation of the <i>L.alba</i> polyploid complex	115
5.5- Crosses between cytotypes and the emergence of different chromosomal numbers.....	118
5.6- The role of unreduced pollens in the formation of the <i>L. alba</i> polyploid complex	124
5.7- The importance of asexual reproduction in <i>Lippia alba</i>	127
5.8- Final considerations	128
6- CONCLUSION	130
7- APPENDIX.....	131
8- REFERENCES	173

1- INTRODUCTION

1.1- Angiosperm reproduction

With over 350,000 species (THE PLANT LIST, 2022), angiosperms are the largest and most diverse group of plants in existence. Among the major factors for the success of these individuals are the presence of double fertilization, the development of closed carpels for greater seed protection and the presence of the fruit (coming from the mature ovary wall). In addition, it is believed that early angiosperms experienced a wide variety of pollinating agents, being pollinated by water, wind or animals. However, it was the association with animals that provided its greatest diversification throughout its evolutionary history (ARMBRUSTER, 2014; SOLTIS et al., 2019).

The reproductive system of angiosperms can generally be classified, according to the mode of reproduction, in sexual and asexual forms. Several species of plants may present exclusively one mode of reproduction (sexual or asexual). In the case of sexual reproduction, the mating system can be autogamous (ie. self-fertilizing plants), allogamous (plants that cross-fertilize, are usually self-incompatible) or mixed (plants that self-fertilize and cross-fertilize) (FRYXEL, 1957; HARDER and BARRET, 2006). Typically, there is a reproduction mode and a predominant crossover system and sporadic events of the other (BODANESE-ZANETTINI and CAVALLI, 2003).

1.2- Sexual vs asexual reproduction

Sexual reproduction in plants includes mechanisms that somehow lead to gamete fusion and consequently fertilization (CARDOSO et al., 2018). Since they cannot directly choose reproductive partners, the result of sexual reproduction in plants depends on the pollen that reaches the stigma. The sexual reproduction system results in increased gene recombination and the possibility of a competitively

superior progeny, as well as a reduction in homozygosity (WANG and COPENHAVER, 2018).

In higher plants, reproductive success is associated with the evolution of diversified and versatile strategies. The formation of the male (pollen) and female (embryo sac) gametophytes, which harbour the male (sperm) and female (egg and central cell) gametes, occur inside specialized reproductive tissues of the flower (anther and ovule). The multicellular gametophytes formation, after the meiosis of spore mother cells, produces reduced gametes that harbour half the chromosome number of the maternal sporophyte (haploid in case of diploid plants). During sexual reproduction, the embryo and endosperm are originated from the double fertilization: the fusion of sperm cells with both the egg and the central cell, respectively. The embryo constitutes the next sporophytic generation, while the endosperm works as a nourishing tissue for the embryo (SCHMIDT et al., 2015).

Asexual forms, in turn, comprise all mechanisms that give rise to clones (genetically identical to the mother plant). The two existing types are the vegetative propagation, which is the formation of a new individual by sprouting or branching from roots, stems, leaves and inflorescences, not involving seeds (SILVERTOWN, 2008; VAN DRUNEN and HUSBAND, 2018), and apomitic reproduction, which is the asexual formation through seeds, with the development of one or more embryos from egg tissues and omission of meiosis and double fertilization (BICKNELL and KOLTUNOW, 2004; CARDOSO et al., 2018). Its advantage over vegetative propagation lies in the fact that the apomixis dispersion units allow a larger area of occupation. It is also important to emphasize that in the apomitic process, individuals originate from a single cell (unfertilized egg cell or somatic cell) whereas in vegetative propagation development occurs from multicellular tissues (MOGIE, 1992).

The fact is that although in theory a clone is genetically uniform, 100% clone individuals are rare in nature (AVISE, 2008). The mechanisms linked to DNA repair in gene replication are efficient, but not perfect, and it is possible to have some mutations that will be retained during the development of the individual. Importantly, some factors such as gene conversion (process that occurs without having had a

reciprocal recombination event), mitotic recombination, and epigenetic events (changes in phenotype that are not caused by changes in the DNA sequence) can influence genotypic development and plasticity resulting from differential gene regulation, creating genetically different clones (VERHOEVEN and PREITE, 2014; DOUHOVNIKOFF and DODD, 2015).

It is known that the adaptation of species to environmental variations is closely related to their reproductive mode (SCHIMIDT et al., 2015). In nature, sexual and asexual reproductions have distinct consequences on natural populations. While sexual reproduction gives rise to genetically distinct individuals, asexual reproduction in turn produces individuals identical to the parental plant. Combined, both types of reproduction (sexual and asexual), it is possible to rapidly expand and at the same time maintain genetic variability, allowing the formation of strains that can diversify by speciation (HÖRANDL and HOJSGAARD, 2012; VISGER et al., 2016). These processes often occur accompanied by events of entire genome duplication, such as polyploidy (SOLTIS et al., 2015). Thus, to understand the genetic structure of a population, it is essential to understand these mechanisms, which are crucial factors for the dispersal and success of Angiosperms (MANGLA et al., 2015).

An important tool used to analyze the reproductive mode (sexual vs. apomixis) performed by an individual and also used in the present work is the “flow cytometric seed screening” (MATZK et al., 2000). Based on the same principles as for the analysis of DNA ploidal levels, this approach consists of establishing the ratio between the relative DNA contents of the embryo and the endosperm in the seed. In an general way, seeds from most flowering plants formed via sexual pathway are expected to display an embryo-endosperm ratio of 2C:3C resulting from double fertilization. Any deviation from this ratio is assumed to have arisen from a variety of apomictic pathways (HOJSGAARD and HÖRANDL, 2019; PELLICER et al., 2021).

1.3- Apomixis

Apomixis is a mechanism of seed formation without fertilization, being observed in at least 78 of 460 angiosperm families and seems to have arisen

independently multiple times (BARCACCIA et al., 2020). Despite its range, the occurrence of apomixis is clearly concentrated in certain groups, such as Asteraceae, Melastomataceae, Orchidaceae, Poaceae and Rosaceae (DOS SANTOS et al., 2012; HOJSGAARD et al., 2014; CARDOSO et al., 2018; LEÓN-MARTÍNEZ and VIELLE-CALZADA, 2019). Confronting the previous presume that this mode of reproduction would be an “evolutionary dead end” (STEBBINS, 1950) apomixis has been correlated with high taxonomic diversity within some clades. Keeping up the genetic structure and hence fixing superior genotypes after reproduction, the apomixis process combines the advantages of propagation through seed with those of propagation by clone. Genetic studies reveal that, though complicated, the trait is controlled by a few genes (BARCACCIA and ALBERTINI, 2013). The benefits of apomixis are estimated to surpass those of the green revolution in terms of crop improvement (HOJSGAARD and HÖRANDL, 2019). The reproduction through apomixis can be a feasible strategy for preserving hybrid seed qualities for multiple generations (FIAZ et al., 2020).

Apomict plants have evolved some mechanisms that circumvent sexual pathways as: functional female gametophytes without meiosis (apomeiosis); development of embryos without fertilization (parthenogenesis), and formation of a functional endosperm. There are two main developmental pathways in which unreduced gametophytes can develop: (1) two unreduced MCs (mother-cells) are formed via restitutional meiosis or via mitotic division (diplospory), found on *Tripsacum*, *Eragrostis*, and *Taraxacum*; (2) a somatic, unreduced cell of the nucellus develops into an embryo sac (apospory). In aposporous apomicts, the megaspore cell in the sexual ovule starts to develop but stops at some stage. One or more somatic cells in the ovule and their nuclei start to develop and they generally resemble megaspore mother cells. At a stage before the mature embryo sac formation, the megaspore or young embryo is aborted and replaced by developing aposporous sacs. Apospory is by far the most common mechanism in higher plants and has been reported in *Beta*, *Brachiaria*, *Cenchrus*, *Chloris*, *Eriochloa*, *Heteropogon*, *Hieracium*, *Hyparrhenia*, *Hypericum*, *Panicum*, *Paspalum*, *Pennisetum*, *Ranunculus*, *Sorghum*, *Themeda* and *Urochloa* (BARCACCIA and

ALBERTINI, 2013). Despite the strict requirement of a gamete fusion for the initiation of seed development in nature, apomictic plants can produce seeds through a single fertilization of the polar nuclei (pseudogamy) or without fertilization (autonomously) (HOJSGAARD and HÖRANDL, 2019).

Due to their great plasticity linked to polyploid lineages, apomitic species tend to colonize new habitats promoting greater geographic expansions (HORANDL and PAUN, 2007; DOS SANTOS et al., 2012; HOJSGAARD et al., 2014). The phenomenon called “geographical parthenogenesis” refers to the fact that apomitic individuals have a wider distribution in regions of higher altitude than those of sexual reproduction, and may also be found in large numbers in previously glacial regions (BIERZYCHUDEK, 1985; KEARNEY, 2005; HÖRANDL, 2006; HÖRANDL et al., 2008). Combined both types of reproduction (sexual and asexual), rapid expansion and at the same time maintaining genetic variability are possible and may lead to the formation of geographically isolated sexual lineages that diversify by ecological to allopatric speciation (HÖRANDL and HOJSGAARD, 2012).

Despite polyploidization and hybridization process being recognized as important preconditions for emergence of apomixis (DE STORME and GEELEN, 2013), there is no evidence suggesting that the presence of both mechanisms is sufficient to generate reproductive innovations leading to apomeiosis or autonomous embryogenesis from a previously established and strictly sexual developmental pathway (LEÓN-MARTÍNEZ and VIELLE-CALZADA, 2019).

The ecological and evolutionary success of apomitic plants is linked to the presence of uniparental reproduction, lower sexual cost, maintenance of adapted genotypes and also greater dispersal ability (HORANDL, 2006; NAKAMURA et al., 2021). Due to the possibility of finding apomitic and sexual reproduction in the same individual or even the same egg, asexual reproduction is believed to be nothing more than a deregulation in the process of gametogenesis during sexual reproduction (KOLTUNOW and GROSSNIKLAUS, 2003; FEI et al., 2019).

The study of apomixis has been increasing not only for evolutionary research but also in plant breeding and agriculture (DALL'AGNOL and SCHIFINO-WHITTMANN, 2005). The greatest benefit of an “apomitic technology” for agriculture

include the hybrid vigor fixation in cultivated plants (SPILLANE et al., 2004; FIAZ et al., 2020).

1.4- Polyploidy in plants

Polyploidization (multiplication of the complete set of chromosomes) is considered one of the main mechanisms of plant speciation and evolution (HUSBAND et al., 2013; LANDIS et al., 2018). It is now known that all existing seed plants have suffered one or more old polyploidization events (JIAO et al., 2011; SOLTIS et al., 2014). The underlying processes by which polyploids deal with reproductive disadvantages in the early stages after their formation are still poorly understood.

Different mechanisms have been proposed to explain how polyploids arise in nature. Two major pathways are known to lead to the appearance of polyploidy in plants: somatic duplication and formation of unreduced reproductive cells. Somatic duplication is associated with mitotic events such as endomitosis or endoduplication, which can occur in both zygotic cells and apical meristematic tissues, giving rise to myxoploids or even completely polyploid organisms. Despite being constantly used to obtain artificial polyploids, somatic duplication is supposed to play a minor role in the origin of natural polyploid organisms (RAMSEY and SCHEMSKE, 1998; YASMIN et al., 2017).

Production and fusion of unreduced reproductive cells has been pointed out as the most predominant pathway leading to plant polyploidy (KREINER et al., 2017). The ability to produce unreduced gametes is an inherited trait evidenced in many plant species. In addition to genetic control, environmental factors such as temperature, herbivory, injury, water deficit and nutrient scarcity influence the production of unreduced gametes (RAMSEY and SCHEMSKE, 1998; LEI et al., 2020). Once formed, the unreduced gamete can fuse with another unreduced gamete (bilateral polyploidization) or with a reduced gamete (unilateral polyploidization) (SATTLER et al., 2016).

Recent studies have shown that polyploid genomes can be highly dynamic and undergo structural and functional changes (eg, diploidization). These findings

renewed interest in examining genome multiplication as an evolutionary process (DODSWORTH et al., 2016; JOACHIMIAK et al., 2018).

In the long run, duplicate genetic material can experience multiple destinations. Many duplicate genes are lost or silenced over time, but some are retained and develop new functions (neofunctionalization) or more specific functions (subfunctionalization) (LYNCH, 2004; DOYLE et al., 2008; SOLTIS et al., 2015; MANDÁKOVÁ and LYSÁK, 2018). These processes are capable of producing remarkable biological novelties (LEVIN, 1983; FLAGEL and WENDEL, 2009; SOLTIS et al., 2014; SOLTIS and SOLTIS, 2016), such as the enzymatic pathways that produce glucosinolate compounds in Brassicales (EDGER et al. 2015, VAN DEN BERGH et al., 2016). A comprehensive understanding of the causes and consequences of polyploidy is vital to understanding the evolution of these organisms given the near ubiquity of old or recent polyploidy among some of the species-rich plant clades (SOLTIS et al., 2009; TANK et al., 2015 SPOELHOF et al., 2017).

The polyploids are classified into two main categories: so-called autopolyploids are traditionally considered to originate from a single species by duplication of structurally similar homologous genomes (AAAA) and it may involve either a single individual or a cross between two individuals of the same species that represent genetically distinct lineages, while allopolyploids arise via interspecific hybridization and subsequent duplication of non-homologous (ie homoeologous) genomes (AABB) (SPOELHOF et al., 2017; MASON and WENDEL, 2020; BOROWSKA-ZUCHOWSKA et al., 2022). Most species, especially those with tropical distribution, remain poorly studied regarding genome size and polyploidy (HUSBAND et al., 2013; ZHANG et al., 2019). Some researchers have attempted to determine the approximate prevalence of autopolyploidy and allopolyploidy by analyzing unreduced gamete formation or available data on chromosome counts, flow cytometry, and phylogenetic reconstruction; (RAMSEY and SCHEMSKE, 1998; BARKER et al., 2015), and eventually estimated that autopolyploids and allopolyploids occur at similar rates. Unfortunately, these methods are not always consistent in their results (DOYLE and SHERMAN-BROYLES, 2017), so the true

prevalence of both processes will likely remain a point of debate until taxonomic and geographic sampling of genome size has vastly improved. However, it is quite clear that no form of polyploidy is rare and that both forms have widespread and important evolutionary impacts (SOLTIS & SOLTIS, 1999; COMAI, 2005; PARISOD et al., 2010b; SPOELHOF et al., 2017).

1.5- Polyploidy and reproduction

Two main aspects regarding an evolutionary association between reproductive modes and polyploidy have been studied for decades (e.g. STEBBINS, 1950): the rate and occurrence of asexual vs. sexual reproduction, and the transitions in mating systems (i.e. self-compatibility or sexual dimorphism). The association between sex dimorphism (e.g. dioecy) and polyploidy can be explained by direct or indirect effects of one process on the other (ASHMAN et al., 2013), since evolutionary transitions usually occur simultaneously, and different factors can affect these transitions in different clades (GLICK et al., 2016). Despite some exceptions (e.g. in the *Fragaria* genus; LISTON et al., 2014), polyploidy is usually correlated with self-compatibility (BARRINGER, 2007; ALIX et al., 2008; HUSBAND et al., 2008; ROBERTSON et al., 2011). Thus, the evolutionary link between mating system and ploidy has been fairly well documented, but the association of polyploidy with asexual reproduction (beyond the odd ploidies such as 3x or 5x), and particularly clonal reproduction (i.e. vegetative growth in plants), has been less studied, particularly in wild species (HERBEN et al., 2017).

Polyploidization plays a significant role enhancing adaptability to extreme habitats. Through the introduction of epigenetic changes, variation in gene expression and also shifting heterozygosity levels and allelic combinations, the genome duplication provides genetic redundancy, creates genomic and phenotypic novelty and influences the ecophysiological and genetic diversity of individuals (OSBORN et al., 2003; CHEN, 2010; FINIGAN et al., 2012; KARUNARATHNE et al., 2018).

Natural intraspecific reproductive variation associated with polyploidy is widely known to affect plant physiology, ecology and dispersal abilities. Studies reveal that the community ecology can also be significantly affected by this intraspecific variation (BOLNICK et al., 2011; ARAÚJO and COSTA-PEREIRA, 2013), modifying population density, community structure, niche breadth, interaction among phenotypes, ecological opportunities and adaptive ecoevolutionary dynamics (BOLNICK et al., 2011; WELLBORN and LANGERHANS, 2015). In order to understand local and regional population dynamics, studies of traits such as cytotype diversity, dispersal ability, phenology, reproductive modes are essential (e.g. CASTRO et al., 2012; SONNLEITNER et al., 2016; VISGER et al., 2016), as they provide insights into evolutionary pathways driving species coexistence, ecological opportunity and adaptive diversification (ARRIGO and BARKER, 2012; WELLBORN et al., 2015; KARUNARATHNE et al., 2018).

According to Herben et al. (2017) there is an indicator of correlated evolution between polyploidy and vegetative growth, mostly mediated by increased distance of spread. An experimental 'garden' data nicely confirmed that while polyploids rely more on vegetative spread, the diploids rely more on seed reproduction. They also show that vegetative reproduction may often evolve before polyploidization and could then enhance the rates of polyploid speciation.

Despite frequent affirmation of a relation between clonality and polyploidy in the literature (STEBBINS, 1971; OTTO and WHITTON, 2000; WEISS-SCHNEEWEISS et al., 2013; DUCHOSLAV and STANKOVA, 2015) this relationship has received comparatively little attention. One hypothesis regarding a direct evolutionary association between clonality and polyploidy is the ability to clonal reproduction predispose yield successful polyploids. It is also expected that clonal reproduction decreases the negative effects of MCE (minority cytotype exclusion) on new polyploids throughout its establishment since the sexual reproduction is temporarily hampered. (HUSBAND et al., 2008; HUSBAND et al., 2013) Furthermore, clonal offspring itself also contributes to polyploid fitness in the absence of functional sexual reproduction (STEBBINS, 1971; RAMSEY and SCHEMSKE, 2002; HUSBAND et al., 2008). Old studies emphasize the hypothesis

that the presence of clonal reproduction is influenced by polyploidy and the origin of a perennial life history and the ability to reproduce clonally could be direct consequences of a whole-genome duplication event (WGD)(MUENTZING, 1936; GUSTAFSSON, 1948). Recent studies in turn, suggest the process of polyploidy as a consequence of clonality (HERBEN et al., 2017).

Regarding the apomixis process in natural systems, the effects of polyploidy for its functionality are not yet clear. Delgado et al. (2014) observed in polyploidized *Paspalum rufum*, a positive effect of autopolyploidization on establishing higher frequencies of apomictic seed formation. Ozias-akins and Van dijk (2007) also detected allele dosage effects of apospory or diplospory-specific genomic regions in polyploids on frequencies of apospory/diplospory. These dosage effects including may further enhance development of unreduced embryo sac formation compared to meiotic reduced ones (SHARBEL et al., 2010; HOJSGAARD et al., 2013). In 1984, Nogler suggested that the apospory-controlling factors would have lethal effects in haploid gametes, hence requiring diploid gametes for inheritance. However, Barke et. al (2018), found in diploid *R. auricomus* hybrids the inheritance of apospory by haploid gametes.

On the establishment of apomictic individuals, the polyploidization process might have several indirect positive effects. An important example is the creation of an immediate reproductive barrier against the diploid parental and progenitor population. The breakdown of genetic self-incompatibility (SI) is also needed to establish self-fertility of pseudogamous apomicts. Furthermore, the establishment of an apomictic cytotype could be indirectly helped by polyploidy by changing the overall physiological features and adaptive potentials of the plants (HOJSGAARD and HÖRANDL, 2019).

1.6- *Lippia alba* polyploid complex

Lippia alba (Verbenaceae) (Fig.1), native from Brazil, is known by several popular names such as “erva cidreira”, “falsa melissa”, “chá de tabuleiro”, “erva cidreira do campo”, “salva do Brasil”, “salva-limão” and “erva cidreira brava” (PIERRE, 2004). Its distribution varies from tropical regions to temperate regions, and can be found on the banks of rivers, lakes and dams where the soils are sandier (CORREA et al., 1994; STEFANINI et al., 2001). Possessing medicinal importance due to the presence of different chemical components, several biological activities are attributed to the species. Among them analgesic, antipyretic, antioxidant, bactericide, acaricide, fungicide, insecticide, anticonvulsant, antiinflammatory, antiparasitic, antispasmodic, sedative and also in its use as a repellent (PEIXOTO et al., 2015; SOUSA et al., 2015; SOARES et al., 2016; DE FREITAS SOUZA et al., 2018; NUNES et al., 2018; AZIZ et al., 2019; COSTA et al., 2020). Its hydroethanolic extract is also linked to the treatment of migraine in women (CARMONA et al., 2013).



Figure 1- A: Inflorescence detail of *Lippia alba* cultivated at the Experimental Plant Station at UFJF. B: Internal morphology of *L. alba* flower. C: *L. alba* individual.

Individuals of the species have a perennial life cycle and shrubby habit with a height of about two meters, with many lateral branches and rapid growth (SALIMENA, 2000). The flowers are violet, pink or white, with insects as the main pollinator (VENÂNCIO et al., 2016). Its phyllotaxis is opposite dystic presenting simple leaves of whole limb with arrangement of one pair per node, being classified as acute oblong serrated (SCHOCKEN, 2007). Additionally, individuals of the species have a wide phenotypic plasticity, having significant morphological variation (TORRES and LOPEZ, 2007), as well as differences in leaf blade size and density and type of trichomes present in the blade. Vale's study (2010) reports a wide variation in the size and habit of different accessions of the species maintained under the same environmental conditions. Techniques such as fluorescent in situ hybridization (FISH), silver nitrate banding and impregnation helped in the analysis of diploid ($2n = 30$) and tetraploid ($2n = 60$) individuals of the species (BRANDÃO et al., 2007; SOUSA et al., 2009; PIERRE, 2004; 2008; REIS et al., 2014). These works demonstrate that in addition to phenotypic plasticity, the species has broad genomic plasticity.

Despite several studies on the pharmacological properties and chemical constitution of *L. alba*, little is known about the biology of the species, noting that the main work was done by our research group of the Laboratory of Genetics and Biotechnology of the Universidade Federal de Juiz de Fora. Initially the species was described by Bose and Choudhy (1960) and Brandão (2007) as possibly diploid with $2n = 30$ chromosomes. Pierre et al. (2011) studied the cariology of three *L. alba* chemotypes (citral, carvone and linalol), and observed differences in chromosome number and morphology. The citral chemotype presented $2n = 30$ chromosomes while the carvone chemotype $2n = 60$, whereas in the linalol chemotype there was a large numerical variation from $2n = 12$ to 60 chromosomes, being a mixoploid chemotype. Variations in DNA amounts estimated by flow cytometry were also observed for these chemotypes (CAMPOS, 2011), and variations compatible with the hypothesis of natural autopolyploidy were detected.

On the other hand, it was observed different chemotypes in five *L. alba* accessions with $2n = 30$ (SOUSA et al., 2009). This fact points to the complexity

within the species and the need for broader investigations involving a larger number of accessions. Additionally, three other new chromosomal numbers ($2n=38$, $2n=45$ and $2n=90$) were found for the species, showing that the variation is even greater than that previously described (REIS et al., 2014).

Viccini et al. (2014) reported variation in secondary metabolite production between different *L.alba* cytotypes. Diploids and tetraploids presented citral as the main component, and linalool as the main component in triploids, suggesting a possible correlation between the chemicals and the amount of DNA. Another work by Gomes et al (2019) also described a seasonal variation of metabolites of polar extracts of carvone and linalool chemotypes, identified by GC-MS analyses of the essential oils.

In an even more recent study by our research group (REIS, 2021), it was possible to observe by mapping satellite probes specific to *L. alba* that for one of them there was stability in the size and position of their sites, and the number of signals remained constant and proportional to the ploidy levels in the different accessions. Although small differences in the distribution profile of this repetition are observed, the uniformity of the results reinforces the hypothesis of autopolyploidy in the formation of the *L.alba* complex.

Regarding medicinal, pharmacological, and economic applications as well as addressing intrinsic questions involved in the polyploidization process in tropical plants, Julião et. al (2020) established a polyploid induction protocol for the species using colchicine where tetraploids and triploids individuals were produced. Lopes et. al (2020) on the other hand, reveals linkages among the ploidal levels using microsatellite and DNA sequences of ITS and *trnL-F*.

Despite the great interest of study in the species, little information about the reproductive system is available. No systematic studies on this topic have been reported so far. There is a dearth of data not only in gender but in the family in general. Recently, the work of Venâncio et al. (2016), about its pollination and flowering reported the presence of open flowers throughout the year, being visited by different types of insects, including the main one, the bee.

Regarding some reproductive aspects of *L. alba*, preliminary data from our research group (CAMPOS et al., in preparation) showed an intriguing behavior regarding seed production between different cytotypes and even within the same ploidal level. Most diploids do not seem to produce seeds when their inflorescences are isolated, revealing a preference of the species for allogamy. However, for some diploids it was possible to collect seeds even when the inflorescences were isolated. On the other hand, most polyploids (triploids and tetraploids) produced seeds when their inflorescences were isolated or not, a result that reflects possible seed formation by self-fertilization or asexual reproduction.

In general, being a plant of medicinal interest and a source of compounds also used in industry and considering the vast chemical plasticity associated with variation in ploidy levels as well, understanding the mode of reproduction becomes essential to understand the base/structure genetics of the species and thereby enable new approaches to the use and genetic improvement of this important Brazilian genetic resource.

2- OBJECTIVE

The present study has as a general objective the investigation of reproductive biology of *Lippia alba* polyploid complex.

More specifically, the objectives include:

1. Analyze seed production under different conditions (open and controlled pollination);
2. Analyze the size and germination rate of seeds, under open and controlled pollination conditions;
3. Analyze, by seed flow cytometry, the type of reproduction performed by the different ploidal level;
4. Identify the offspring ploidal level from diploids, triploids and tetraploids;
5. Analyze chromosome traits in individuals from known parents;
6. Perform a possible artificial crossing and characterize the progeny;
7. Analyze, by microsatellite markers, the profile of the parental individual and its offspring from controlled pollination;
8. Analyze the production of unreduced male gametes in diploid individuals;
9. Analyze the anatomy of *Lippia alba* anthers and ovules between the different cytotypes under different conditions.

3- MATERIAL AND METHODS

3.1- Plant material

Accessions of *Lippia alba* representing different chromosome numbers maintained at the Experimental Plant Station of the Universidade Federal de Juiz de Fora (EEP-UFJF) were used in the study (Appendix A). Voucher copies were deposited at the Leopoldo Krieger Herbarium (CESJ) according to usual techniques. The varied number of samples used in the different approaches is linked to the availability of the material during the study.

3.2- Seed production

As it is a perennial species, the individuals studied showed flowering throughout the year. Flower buds from 47 accessions of different ploidal levels of *Lippia alba* (32 diploids; 6 triploids and 9 tetraploids) were isolated from March 2015 to January 2022. After the flowering period, the number of seeds from each isolated and open pollinated inflorescence was quantified (Figure 2). After collecting and quantifying the seeds, the average number of seeds per inflorescence was compared using the chi-square test at 5%.



Figure 2- *Lippia alba* inflorescences representing the isolation method of flowers at the Experimental Station of Plant Cultivation and Maintenance at the Universidade Federal de Juiz de Fora, Minas Gerais.

The difference between the means of seed production between diploids and polyploids under both conditions (open and controlled pollination) was analyzed by the Kruskal-Wallis test followed by the Dunn's Test for multiple comparisons on PAST v. 2.17c software (HAMMER et al. 2001).

Additionally, the index of self-incompatibility (ISI) was calculated for each diploid, triploid and tetraploid plant using the following expression:

$$ISI = 1 - (SP_{S-P} / SP_{OP})$$

Where SP = seed production, S-P = self-pollination, and OP = outcross pollination. (LLOYD, 1965; SIOPA et al., 2020).

ISI can range from 0–1, where fully self-compatible plants score 0 and complete self-incompatible plants score 1.

3.3- Seed germination, morphometry and weight

For germination index, 20 seeds from 29 diploids, 6 triploids and 5 tetraploids from open pollination were collected while those from controlled pollination relied on the quantity provided by individuals under this condition. In the table 1, it is possible to observe the number of seeds from controlled pollination that were placed to germinate from each accession. The seeds were placed to germinate in a moistened filter paper in petri dish (9 cm in diameter) and subjected to 6 hours at 25°C, followed by 4 hours at 40°C, 4 hours at 25°C and 10 hours at 18°C. This temperature regime was based on the environmental variation to which natural populations of the species are subjected.

For morphometry analysis, 20 seeds from the 32 diploids, 6 triploids and 6 tetraploids from open pollination were analyzed. Regarding the controlled pollination, the number of seeds analyzed is described in table 2. Their length and width were measured using a 6-steel caliper rule. The difference between diploid and polyploid under open and controlled pollination and also within diploids and polyploids in both conditions were compared by the Kruskal-Wallis on ranks test through PAST program. 2.17c (HAMMER et al., 2001).

Table 1- Number of seeds per accession of *Lippia alba* from controlled pollination germination analysis

Accession	Ploidal level	Nº of seeds
BGEN-12	2x	7
BGEN-43	2x	5
BGEN-48	2x	1
BGEN-58	2x	1
BGEN-67	2x	29
BGEN-73	2x	9
BGEN-96	2x	53
BGEN-01	3x	2
BGEN-10	3x	8
BGEN-27	3x	9
BGEN-49	3x	7
BGEN-59	3x	20
BGEN-03	4x	20
BGEN-45	4x	14
BGEN-64	4x	30
BGEN-86	4x	20
BGEN-87	4x	37

Table 2- Number of seeds per accession of *Lippia alba* from controlled pollination for morphometric analysis

Individual	Ploidal level	Nº of seeds
BGEN-12	2x	7
BGEN-13	2x	20
BGEN-43	2x	5
BGEN-46	2x	1
BGEN-48	2x	3
BGEN-49	2x	7
BGEN-58	2x	1
BGEN-59	2x	20
BGEN-62	2x	13
BGEN-71	2x	2
BGEN-73	2x	9
BGEN-81	2x	20
BGEN-89	2x	4
BGEN-96	2x	20
BGEN-45	4x	20
BGEN-64	4x	20

For weight analysis, the weight of 20 seeds of 29 diploids, 6 triploids and 6 tetraploids were compared by the Kruskal-Wallis on ranks test, followed by the Dunn's Test for multiple comparisons through PAST program 2.17c (HAMMER et al., 2001).

A Principal Component Analysis (PCA) was conducted for the following seed characteristics: average production, germination, length, width and weight. It was analyzed 25 diploids, 4 triploids and 5 tetraploids using the PAST program 2.17c (HAMMER et al., 2001).

3.4- Fitness

Attempting to establish a value corresponding to the "fitness" for each individual, the following seed characteristics were analyzed: average of production, germination, length, width and weight. The average of seed production was the only trait evaluated under both different conditions: open and controlled pollination. This is due to the fact that few seeds were produced under the controlled pollination condition.

For the average of seed production, 32 diploids, 6 triploids and 9 tetraploids were used. For germination: 29 diploids, 6 triploids and 5 tetraploids. For both length and width: 29 diploids, 6 triploids, 6 tetraploids. Lastly, for weight: 29 diploids, 6 triploids and 6 tetraploids.

To compare the performance of different individuals, the individual with the highest value was used as a reference $W=1$ (higher *fitness*). Based on the following formula,

$$W = 1 - s$$

where W is the aptitude and "s" the selection coefficient, the reference individual always had $s = 0$. The fitness of the other accessions was calculated in relation to the reference individual.

As an example, if the individual X had 100% (higher seed germination index) it was assigned as a reference individual with $W = 1$ and $s = 0$. If the second highest value was observed for the individual Y with 95% seed germination, the W value of this individual corresponds to 95 (germination index for individual Y)/ 100 (germination index for individual X). So, $W = 0.95$ and $s = 1 - 0.95 = 0.05$. The same procedure was done for all other individuals.

A comparison of the averages of *fitness* (W) value between diploids and polyploids and between open and controlled pollination was performed by the Kruskal-Wallis on ranks test, followed by the Dunn's Test for multiple comparisons through PAST program 2.17c (HAMMER et al., 2001).

3.5- Seed flow cytometry

For seed flow cytometry 18 diploids, 4 triploids and 6 tetraploids were analyzed.

Two protocols were used to obtain these data. One of them in a collaboration with Dr. Timothy Sharbel from the University of Saskatchewan, Canada, seeds were placed individually along with a bead in each well of a 96-well plate. Then, they were ground for 15 seconds and observed. Again, they were ground for another 15 seconds and centrifuged, 50 μ l of CyStain UV Precise P buffer was added and then the samples were ground again. A second centrifugation was performed and then another 70 μ l of buffer was added. Samples were stored at 4°C for 10 minutes. Subsequently, they were filtered and 120 μ l of DAPI dye was added.

On the second protocol held at the Universidade Federal de Juiz de Fora, individual seeds were ground in 300 μ l of WPB buffer (LOUREIRO et al., 2007) and filtered in a 30 μ m mesh. Samples were stored at 4°C for 10 minutes. Then 25 μ l of propidium iodide dye was added.

Both analyses were performed in a Cytoflex cytometer (Becton Dickinson) and showed similar results.

3.6- Offspring ploidal level analysis

To estimate the offspring amount of DNA, leaves from seedlings germinated in Petri dishes were individually collected, macerated in WBP buffer (LOUREIRO et al., 2007), filtered in a 30µm mesh and stained with propidium iodide. The analysis was performed using a Cytoflex flow cytometer (Beckman Coulter). From open pollination, it was analyzed 20 diploids, 5 triploids and 3 tetraploids. In table 3, it is possible to observe the number of samples from each accession.

Table 3- Number of open pollination offspring from different ploidal level of *Lippia alba* analyzed by flow cytometry

Accession	Ploidal level	Nº of samples
BGEN-02	2x	29
BGEN-09	2x	4
BGEN-12	2x	35
BGEN-13	2x	21
BGEN-21	2x	22
BGEN-38	2x	4
BGEN-40	2x	24
BGEN-48	2x	30
BGEN-56	2x	4
BGEN-58	2x	20
BGEN-67	2x	21
BGEN-68	2x	21
BGEN-72	2x	10
BGEN-73	2x	19
BGEN-75	2x	9
BGEN-77	2x	2
BGEN-80	2x	28
BGEN-81	2x	16
BGEN-96	2x	18
BGEN-98	2x	32
BGEN-23	3x	19
BGEN-27	3x	29
BGEN-59	3x	21
BGEN-60	3x	7

Table 3- Number of open pollination offspring from different ploidal level of *Lippia alba* analyzed by flow cytometry (cont.)

Accession	Ploidal level	Nº of samples
BGEN-109	3x	20
BGEN-07	4x	12
BGEN-64	4x	40
BGEN-86	4x	4

From the controlled pollinated individuals, it was possible to analyze the offspring of three triploids (Table 4), in according to the material availability.

Table 4- Number of controlled pollination offspring from triploids of *Lippia alba* analyzed by flow cytometry.

Individual	Ploidal level	Nº of samples
BGEN-10	3x	2
BGEN-27	3x	4
BGEN-59	3x	1

3.7- Offspring: case studies

Despite the large number of offspring analyzed with other approaches, such as flow cytometry, the vast majority of individuals showed lower capacity to growth and many of them died after 3 weeks. Other times, the germinated sample was fully used in flow cytometry analysis. Thus, four individuals showed ideal conditions to perform the cytogenetic and essential oil analysis. Two of them came from open pollination: one from a diploid (BGEN-77) and another from a tetraploid (BGEN-64). The third individual was an offspring from a triploid (BGEN-59) that were kept under a controlled pollination, i.e., it represents a case of selfing or apomixis. The fourth individual came from an artificial cross between a diploid (BGEN-77) and a triploid (BGEN-59).

All of them were cultivated in pots with soil and commercial substrate BioPlant (2:1) in the greenhouse of the Experimental Plant Station of the Universidade Federal de Juiz de Fora. After two years of growth, cuttings from each one was collected and immediately transferred for a container containing only water for later collection of meristems for cytogenetic analysis.

3.7.1- Offspring from natural reproduction

The offspring from natural reproduction comprises both from open pollination (one of a diploid and the other from a tetraploid) and also the one from a triploid of controlled pollination.

To estimate the amount of DNA of each individual, leaves were macerated in WBP buffer (LOUREIRO et al., 2007) along with an internal standard (*Pisum sativum*), filtered in a 30µm mesh and stained with propidium iodide. The analysis was performed using a Cytoflex model cytometer (Beckman Coulter).

For cytogenetic analysis, slides were prepared from young root tips pre-treated with sodium chloride (NaCl) at room temperature for 8 h and then fixed in 3:1 v/v ethanol–acetic acid for 24h at – 20 °C. The roots tips were submitted to enzymatic maceration in 4% (w/v) cellulase (Onozuka R-10, Serva) and 40% (v/v) pectinase (Sigma) for 3 h at 37 °C. Cytogenetic preparations were performed according to Carvalho and Saraiva (1993).

Chromosome length, short and long arms, ratio between chromosome arms, and the index of karyotype asymmetry for each of the accessions were determined according to Watanabe et al. (1999). The chromosomes were classified according to Levan et al. (1964).

For essential oil microextraction, approximately 100 mg of fresh leaves of the samples were transferred to 2 mL tubes. It was added approximately 1 mL of solution containing n-hexane with internal standard (linalool 0.2×10^{-3} mg/mL for diploid and tetraploid samples; citral 0.4×10^{-3} mg/mL for triploids). The samples were kept for 10 min in an ultrasound bath (ThorntonINPEC, Vinhedo, SP, Br) with a frequency of

70KHz. Then hexane extract was filtered through pipette containing cotton and anhydrous sodium (Shimadzu, Kyoto, JA) (SILVA et al., 2010. adapted).

Quantitative analysis of the major compound was performed in a chromatograph gas coupled to a GCMS-QP2010 Plus 40 eV mass spectrometer (Shimadzu, Kyoto, JA) and RTx-Wax-Restek column (Restek, Bellefonte, PA) of 30m x 0.25mm.

An aliquot (1 μ L) of the clear solution obtained containing the extracted oils was analyzed. The analysis started with the temperature at 100°C, maintained for 3 minutes, followed by an increase of 7°C for 1 minute, until 190°C and hold for 15 min. The injector was operated in 'split' mode (1:20), at 220°C. Helium was used as a carrier gas, with a flow of 19.6 mL/min⁻¹. The identification of the constituents was carried out according to the internal standardization method (LEGGIO et al., 2017).

3.7.2.- Offspring from an artificial crossing

For the artificial crossing, one diploid individual BGEN-77 was selected as the maternal genitor. The choice was based on seed production behavior, as this individual does not produce any seed when subjected to reproductive isolation. The paternal genitor was a triploid accession, BGEN-59. This choice was based on the availability of the material and the particular behavior of this triploid regarding seed germination (reaching 75%).

In the field, flower buds of individual BGEN-77 were isolated. After opening the flowers, pollen grains from the triploid BGEN-59 individual (paternal genitor) were artificially inserted through a syringe in different flowers and then the buds were isolated again. After the flowering time, the dried inflorescences were collected and as a result, one seed was produced.

This single seed was placed to germinate and after the individual had grown, the DNA amount estimation, cytogenetic analysis and essential oil microextraction (see item 3.7.1) were performed.

3.8- Microsatellite markers

Twelve individuals were selected for this analysis. The diagram below illustrates the analyzed individuals (Figure 3). The different colors represent different ploidal level (red=diploid, green=triploid, grey= possible aneuploid offspring) while the solid arrows represent the artificial crossing and the dotted arrow represents the origin of offspring from isolated inflorescence.

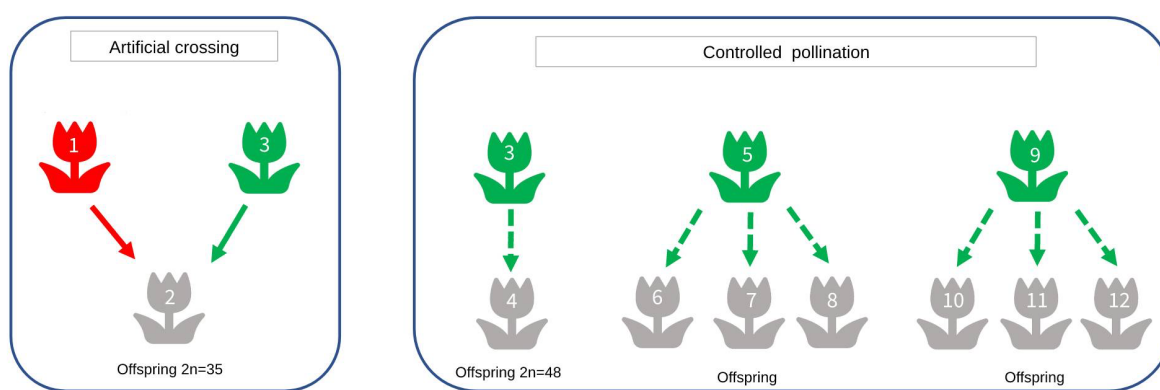


Figure 3- Representative diagram of the samples analyzed with the SSR markers. The red color represents diploid ($2n=30$) and the green color represents triploid ($2n=45$). The numbers 1, 2 and 3 respectively comprise the diploid mother plant (BGEN-77) of the artificial cross, the offspring of the artificial cross and the triploid paternal provider (BGEN-59) of the artificial cross. The number 4 is a triploid (BGEN-59) offspring from controlled pollination. The number 5 is a triploid (BGEN-27) while its offspring from controlled pollination comprises the numbers 6, 7 and 8. The number 9 is a triploid (BGEN-10) while its offspring from controlled pollination comprises the numbers 10, 11 and 12.

To compare the genetic profile and infer the reproductive mode, the DNA from the parents and their respective offspring leaves were macerated in liquid nitrogen for extraction (DOYLE., 1990), with modifications. Immediately after maceration, 800 μ L of CTAB buffer, pre-warmed to 65°C, containing 100 mM Tris-HCl (pH 8), 20 mM EDTA, 1.4M NaCl, 2% CTAB, 1% PVP and 0.002% 2-mercaptoethanol. The samples were incubated in a water bath at 65°C for 30 min and homogenized every 5 min. The proteins were removed in two steps, with the addition of 2/3 of the sample volume (approximately 400 μ L) of a chloroform:isoamyl alcohol (24:1) solution. The samples were then centrifuged for 10 min at 14000rpm, recovering the supernatant. The DNA was precipitated with 0.54% (v/v) ice-cold isopropanol and 0.08% (v/v)

ammonium acetate at -20°C for 30 min. The material was centrifuged at 14000 rpm for 10 min, discarding the supernatant and washing the precipitate in 700 µL of cold 70% ethanol and once in cold 95% ethanol. The pellet was dried overnight at room temperature and resuspended in 30 µL of autoclaved ultrapure water. The samples were incubated at 4°C during 24 hours for complete resuspension of the DNA and were stored at -20°C. The DNA concentration of the samples was estimated using a nanometric spectrometer (Nanodrop).

For DNA amplification, 14 primers were used (Table 5) (RAY et al., 2012; SANTOS et al., 2012; LOPES et al., 2020a e 2020b). The primers were selected based on polymorphism data previously obtained by Lopes et al (2020). For the polymerase chain reaction (PCR), the samples were diluted in ultra-pure autoclaved water to obtain the final concentration of 25ng/µl. After defining the annealing temperature of the primers and the PCR conditions, the technique was performed with the GoTaq® kit Flexi Promega, in a volume of 10 µl: 1X buffer, 0.5 µM primer, 1.5/2.5/3 mM MgCl₂ (varied between the primers), 0.3 mM dNTP, 1 unit of Taq DNA Polymerase and 30ng of genomic DNA. The reaction of PCR was performed in a Mastercycler gradient Eppendorf® thermocycler and optimized from the following conditions: 94°C (3 min), 35 cycles at 94°C(45s), annealing temperature 50 to 55°C for each primer (45s) with touch-down followed by 72°C (45s) and final extension for 20 min at 72°C. The fluorescent products were detected by capillary electrophoresis using a SeqStudio sequencer (Applied Biosystems). Fragment size was measured using Microsatellite Analysis Software (MSA), available on Thermo Fisher Cloud.

Table 5- Fourteen microsatellite primers in twelve accessions of *Lippia alba* accessions.

<i>Loci</i>	<i>Primers</i>	Primer sequences (5'–3')	Repeat motif	Size (bp)	Ta °C	Reference
p077	77F 77R	*AAGGTCACGTAAACATACCG AGGAGAAGCATGAGCATGTA	(AATAC) ₁₅	179–209	55	LOPES et al., 2020
p229	229F 229R	*GAGGAAGTTTCCTGTACCAGT GTGTACAGAGAGATGGCAAAG	(GCCCTT) ₄	147–169	50	LOPES et al., 2020
p249	249F 249R	*ACACCAGTTGATGAAGTCAGT TTGTTACACAGGACGATAAGC	(CAA) ₆	186–227	46	LOPES et al., 2020
p270	270F 270R	*GAAGTTGGAGATGTGTGTGTT ACTGAAACATGGAGGACCTAT	(GA) ₉	113–126	57	LOPES et al., 2020
p283	283F 283R	*CATTCTAGAGGTCCATTTCCCT CGGAGAAGGAGGTATATGTTT	(CAAT) ₄	189–190	55	LOPES et al., 2020
p294	294F 294R	*CACCAGTGCGACTACATAGAT CCTATGATTCTCGAACACTTC	(GACAC) ₅	110–194	55	LOPES et al., 2020
p370	370F 370R	*ATGTCACAGGGATAGGTTTG TATCTCTCGGTCATCTGAGAA	(GGAGG) ₅	173–279	55	LOPES et al., 2020

Table 5- Fourteen microsatellite primers in twelve accessions of *Lippia alba* accessions (cont.).

<i>Loci</i>	<i>Primers</i>	Primer sequences (5'–3')	Repeat motif	Size (bp)	Ta °C	Reference
p412	412F 412R	*CCTCTCTCGTTCTCACTGAC CAAGTTTTCTCACACTCGTTC	(GTC) ₇	122	56	LOPES et al., 2020
p432	432F 432R	*GTCTTCGTCGTCGTCATT CAATATAGTCGCCTGTGTCTC	(GGC) ₆	171–182	56	LOPES et al., 2020
p473	473F 473R	*GAAACAATATCTCTCGTGTGC GGTAGCTTCCGAGAAAACA	(TATC) ₂₃	107	59	LOPES et al., 2020
p484	484F 484R	*GCCTAGTCAATCTTGGACAAT TTGTCATCGAGACTTATCTCC	(TG) ₇	167–173	55	LOPES et al., 2020
LAC4	LAC4F LAC4R	*CCTACAGCCGAACGACCTAC TGCTTCCGCCTTTCTGTTAC	(AAC) ₅	263-276	54	RAY et al.,2012
LAC5	LAC5F LAC5R	*GCTTCGGACACGTATCCACT GGTTGAGGATTCGGTGAATG	(CCT) ₈	313-335	50	RAY et al.,2012
LA08	P08F P08R	*GCCTTGTGGTAAGATCCTGTTC GGTTTATGTCCAGATCAGCTCC	(TA)5C(GT) ₁₀	139-150	50	*

*Primers designed and tested using the Primer 3 online program (<http://bioinfo.ut.ee/primer3-0.4.0/>).

*Tail M13 (5'-TGTAACGACGGCCAGT-3').

Ta. annealing temperature.

3.9- Pollen analysis

Later stage flower buds from 29 diploid individuals were collected and fixed in Carnoy (3 alcohol:1 acetic acid). After 24 hours, the anthers were isolated from the bud and macerated with a needle to release the pollen grains. Afterwards, the material was stained with acetic orcein and covered with a cover slip. One thousand pollen grains from each individual were analyzed. In the analysis, the average of three measurements of each pollen was performed for the final average of each structure. The t test ($p < 0.05$) was performed to compare the mean of reduced (n) and non-reduced (2n) pollen.

3.10- Comparative anatomy of *Lippia alba* anthers and ovules

Flowers from open and controlled pollination of *Lippia alba* in anthesis, from diploid, triploid and tetraploid individuals, were fixed in the Karnovsky mixture (KARNOVSKY, 1965), and then passed under vacuum. After 24 hours, the fixed flowers were dehydrated in a gradual ethanol series, being stored in 50% ethanol until further processing. The structures of the anthers and ovaries were separated and then dehydration in ethyl series was completed. The inclusion of samples was performed in Leica 2-hydroxyethyl-methacrylate (PAIVA et al, 2011) and transversal and longitudinal series were sectioned with a thickness of 6µm using a Zeiss Hyrax M40 rotary microtome. To stain the sections, 0.05% toluidine blue in acetate buffer pH 4.7 (modified O'BRIEN et al. 1964) was used and the sections were mounted in synthetic resin (Entellan). The images were obtained using an Olympus CX41 microscope with an LC20 camera attached. The CorelDraw Graphics Suite program was used to edit the figures.

4- RESULTS

4.1- Seed production, germination and morphometry

4.1.1 - Seed production

Regarding diploid individuals, 32 individuals were investigated. It was observed that 9 accessions did not produce any seeds when their inflorescences were isolated: BGEN-11, BGEN-17, BGEN-20, BGEN-30, BGEN-40, BGEN-56, BGEN-74, BGEN-77 and BGEN-90. Another 19 accessions were able to produce seeds, most of them in small quantities: BGEN-02, BGEN-09, BGEN-12, BGEN-13, BGEN-21, BGEN-43, BGEN-46, BGEN-48, BGEN-57, BGEN-58, BGEN-65, BGEN-67, BGEN-71, BGEN-72, BGEN-73, BGEN-78, BGEN-88, BGEN-89 and BGEN-98 (Figure 4). Four of them were able to produce a greater average of seeds per inflorescence (from 2.45 to 5.95) when the inflorescences were isolated: BGEN-75, BGEN-80, BGEN-81 and BGEN-96. Only one individual (BGEN-02) showed a non-significant difference between the means of seeds produced from open and controlled pollination, probably due to the smaller sample size available.

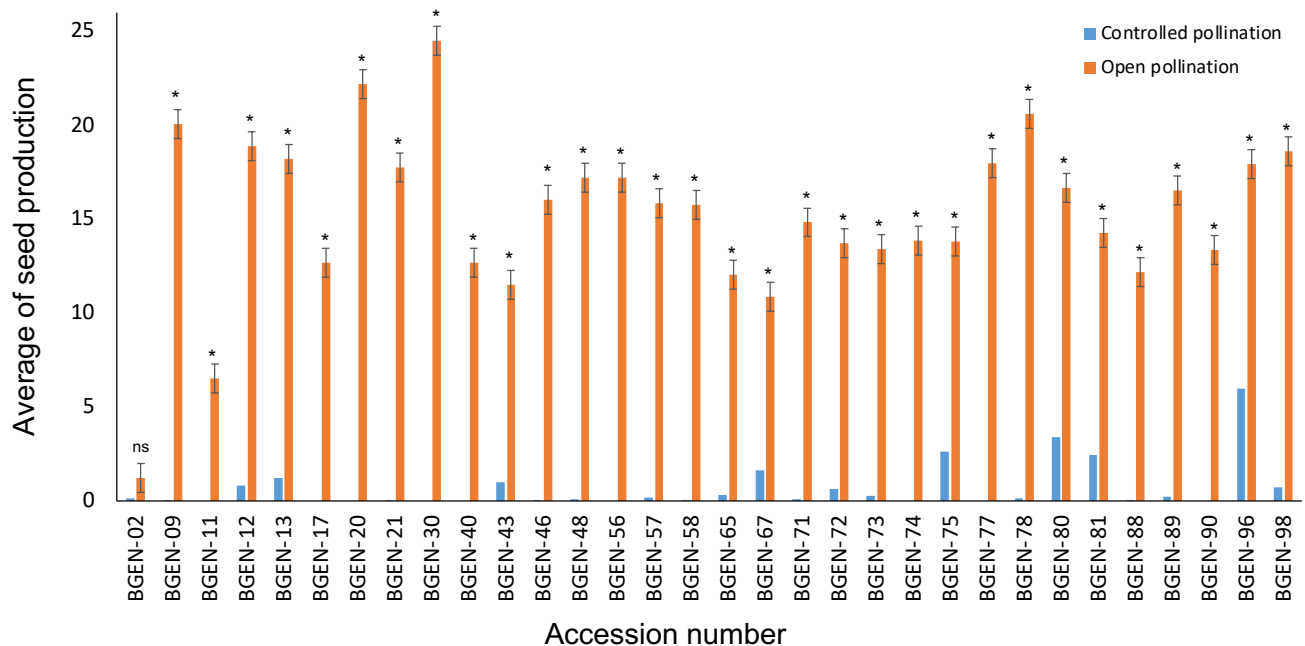


Figure 4- Average of seeds per inflorescence from controlled (blue bars) and open pollination (orange bars) of diploid accessions of *Lippia alba*. The difference between the number of seeds produced from both conditions for each accession were all significant by the chi-square test ($p < 0.05$) except for accession BGEN-02 (ns=non-significant).

On the other hand, when analyzing the seed production of polyploid individuals (triploids and tetraploids) (Figure 5), it was observed that all individuals produced seeds when the pollination was controlled (inflorescences were isolated). Unlike diploids, for practically all accessions there was no significant difference between the means of seeds from isolation and non-isolation, except for accession BGEN-27.

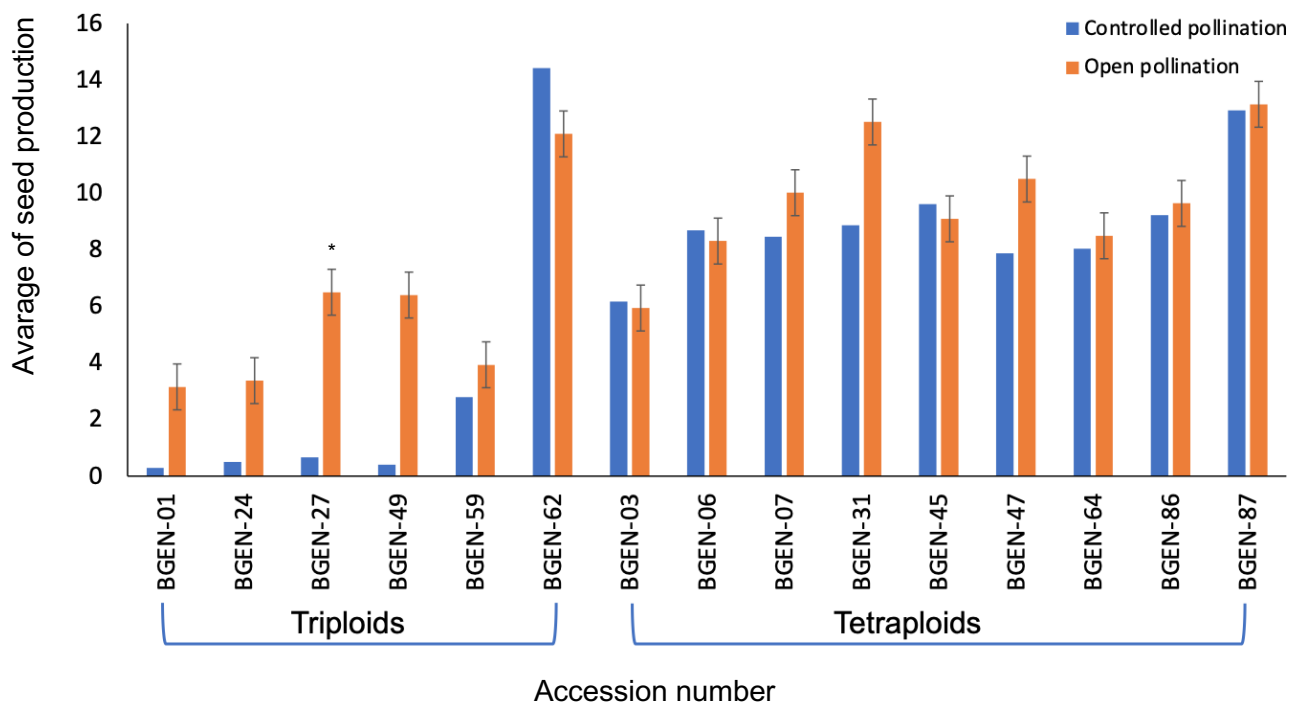


Figure 5- Average of seeds produced by triploid and tetraploid accessions of *Lippia alba* under controlled (blue bars) and open (orange bars) pollination. The asterisk indicates a significant difference between the means by the chi-square test ($p < 0.05$).

In general, it was possible to observe that diploids produced more seeds than polyploids when the accessions were kept under open pollination. On the other hand, under controlled pollination, diploids produced less seeds than polyploids. Analyzing only the diploids, it was possible to observe a higher seed production in open pollination condition, while in polyploids, no difference was observed between both conditions (Figure 6).

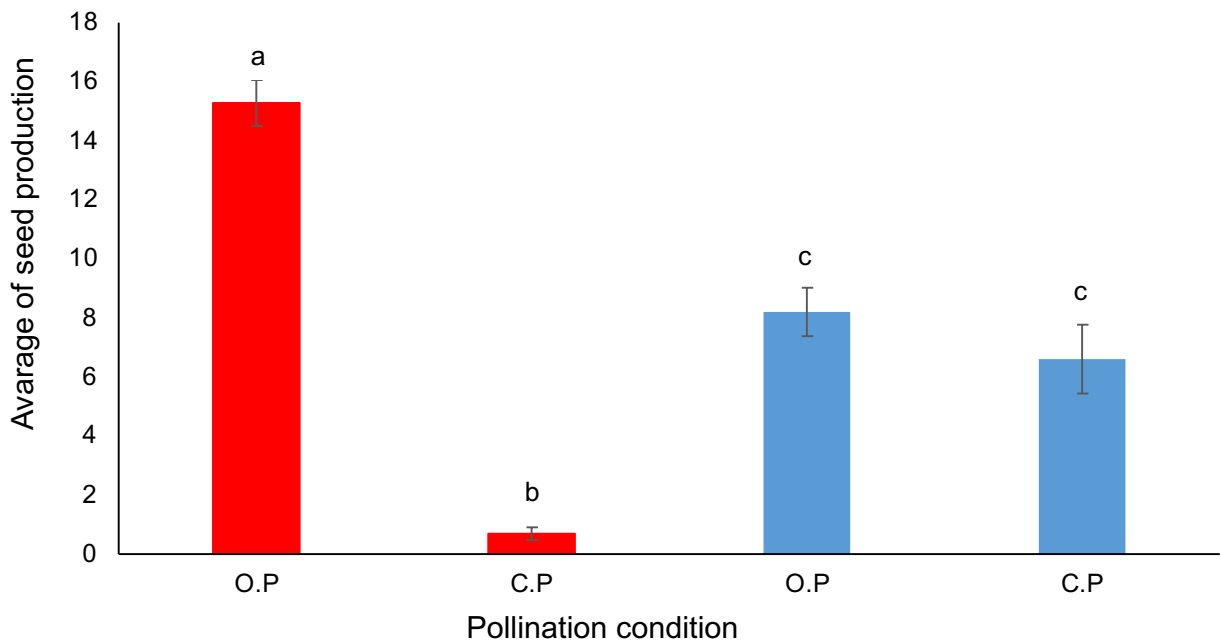


Figure 6- Average of seed production in *Lippia alba* for diploids (red bars) and polyploids (blue bars) under open (OP) and controlled (CP) pollination. Values are presented as mean and standard error of the mean of seeds produced per reproductive unit. Different letters represent statistical differences at $p < 0.05$ according to Dunn's test.

Analyzing the index of self-incompatibility (ISI), it was noticed a different behavior among diploids, triploids and tetraploids (Figure 7A). It seems that diploids are much more self-incompatible than polyploids, although some triploids also show high index of ISI. Triploids revealed a mix of self-incompatible resources taking that some individuals show low ISI. Tetraploids, on the other hand, are apparently more self-compatible. In general, different profiles of self-incompatibility was observed for diploids and polyploids revealing high variability for this character in *Lippia alba* accessions (Figure 7B).

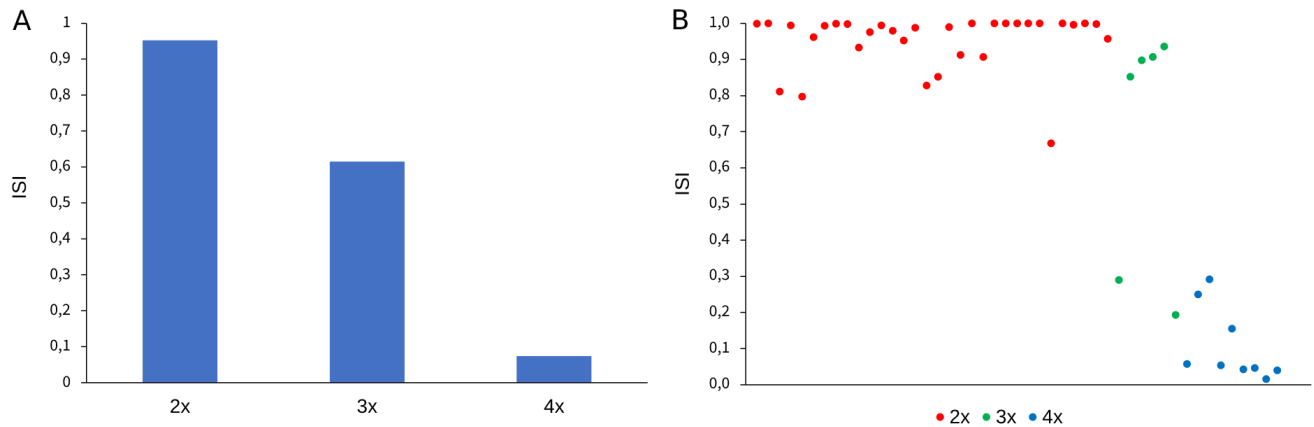


Figure 7- Index of self-incompatibility at different ploidal levels of *Lippia alba*: diploids, triploids and tetraploids. A: Index average in the ploidal levels. B: distribution diagram of all individuals index analyzed.

4.1.2 – Germination rate

Regarding the germination of seeds from open pollination, diploid individuals, represented by 29 individuals (red bars), had a high germination rate, some even reaching 100% (Figure 8). Polyploid individuals (green and blue bars) represented by 6 triploids (BGEN-01, BGEN-24, BGEN-27, BGEN-59, BGEN-60, BGEN-109; green bars) and 5 tetraploids (BGEN-06, BGEN-07, BGEN-64, BGEN-70, BGEN-86; blue bars), had a lower germination rate (ranging from 20% to 60%), except for BGEN-59, a triploid individual, that showed germination index of 75%, similar to some diploids.

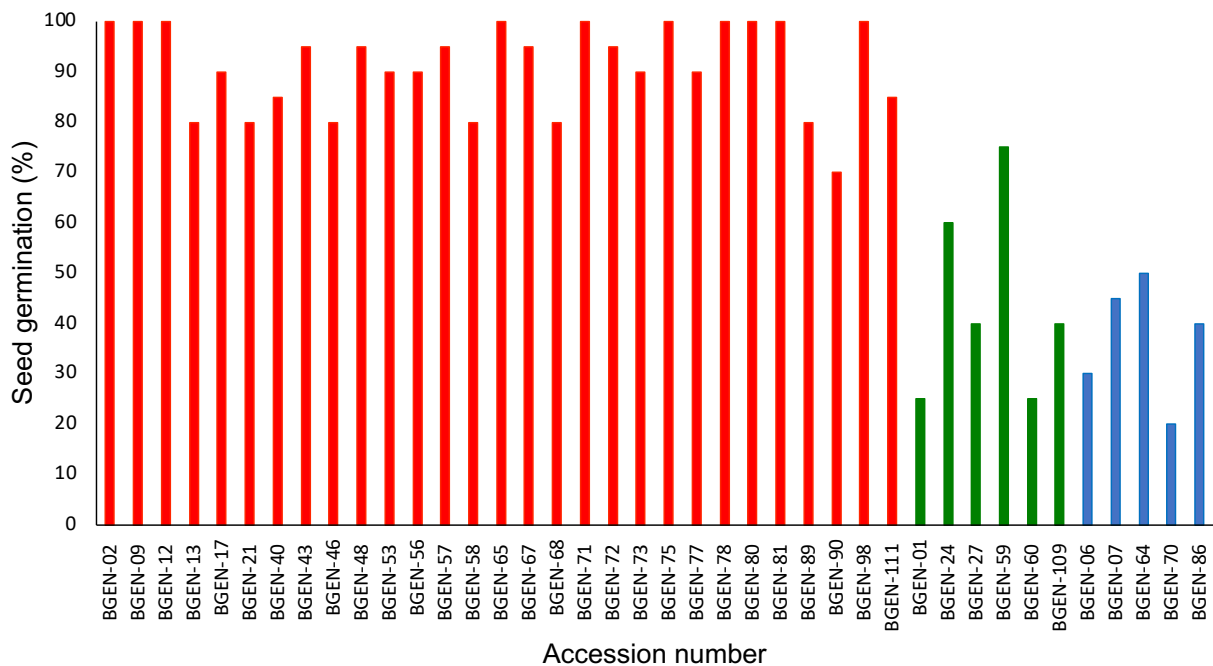


Figure 8- Germination index from open pollination of diploid (red bars), triploids (green bars) and tetraploids (blue bars) accessions of *Lippia alba*.

Regarding seed germination from individuals kept under controlled pollination, seven diploids, five triploids and five tetraploid individuals were analyzed. As a result, diploids varied from 0% to 100% of germination rate, triploids from 0% to 45% and tetraploids from 0% to 5% (Figure 9).

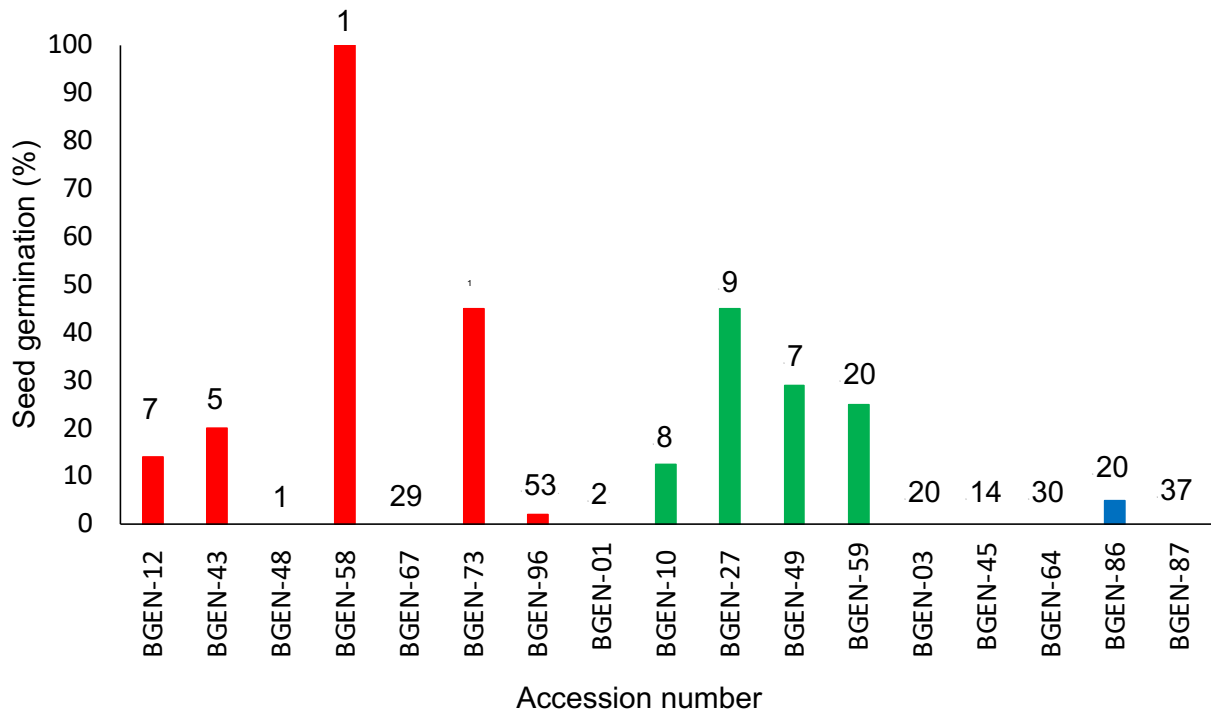


Figure 9- Germination rate of seeds produced from individuals under controlled pollinated in *Lippia alba*. Red bars: diploids; green bars: triploids; blue bar: tetraploid. The numbers above the bars represent the total amount of seeds analyzed.

4.1.3. Seed morphometry

Measurements of length and width of seeds from individuals with different ploidal levels under open pollination are represented in the box plot below (Figure 10). In open pollination condition there was no significant difference in length and width measurements between diploids and polyploids.

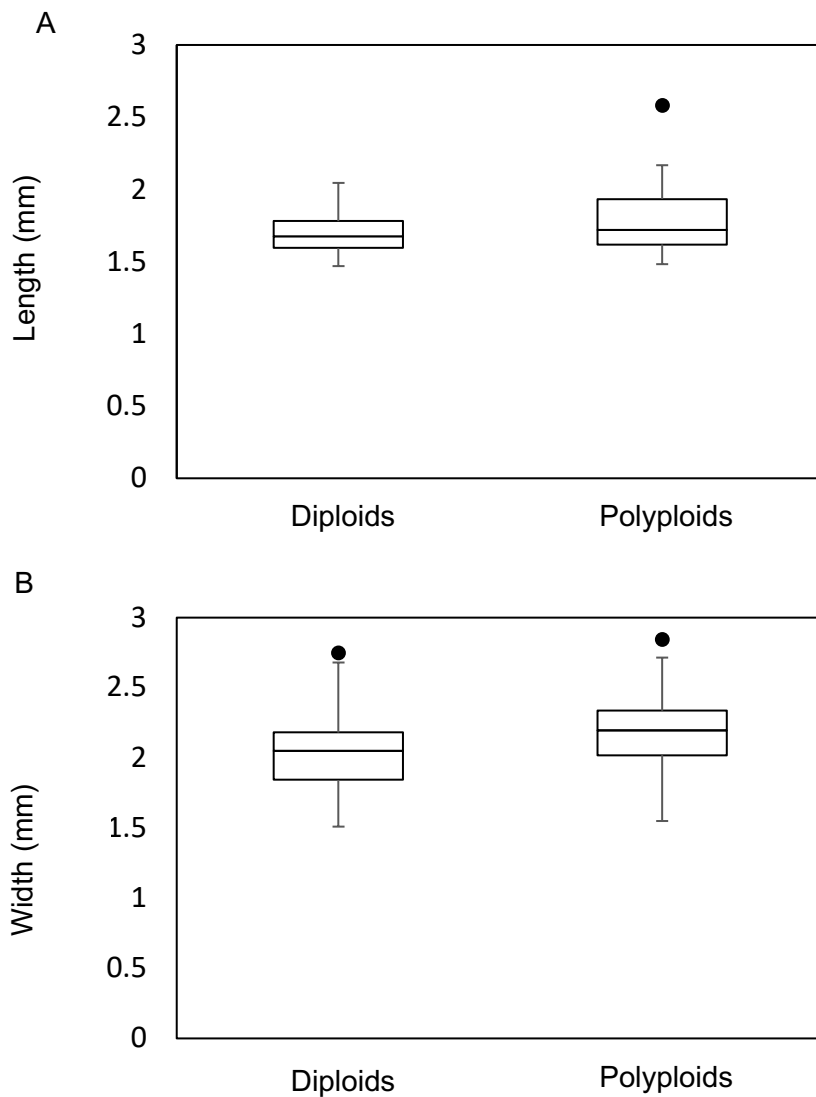


Figure 10- Length (A) and width (B) average of seeds from diploid and polyploid accessions of *Lippia alba* under open pollination.

Morphometric data of seeds from individuals kept under controlled pollination are represented in the box plot below (Figure 11). The mean and the standard deviation of seed length and width under different conditions are summarized in Table 6.

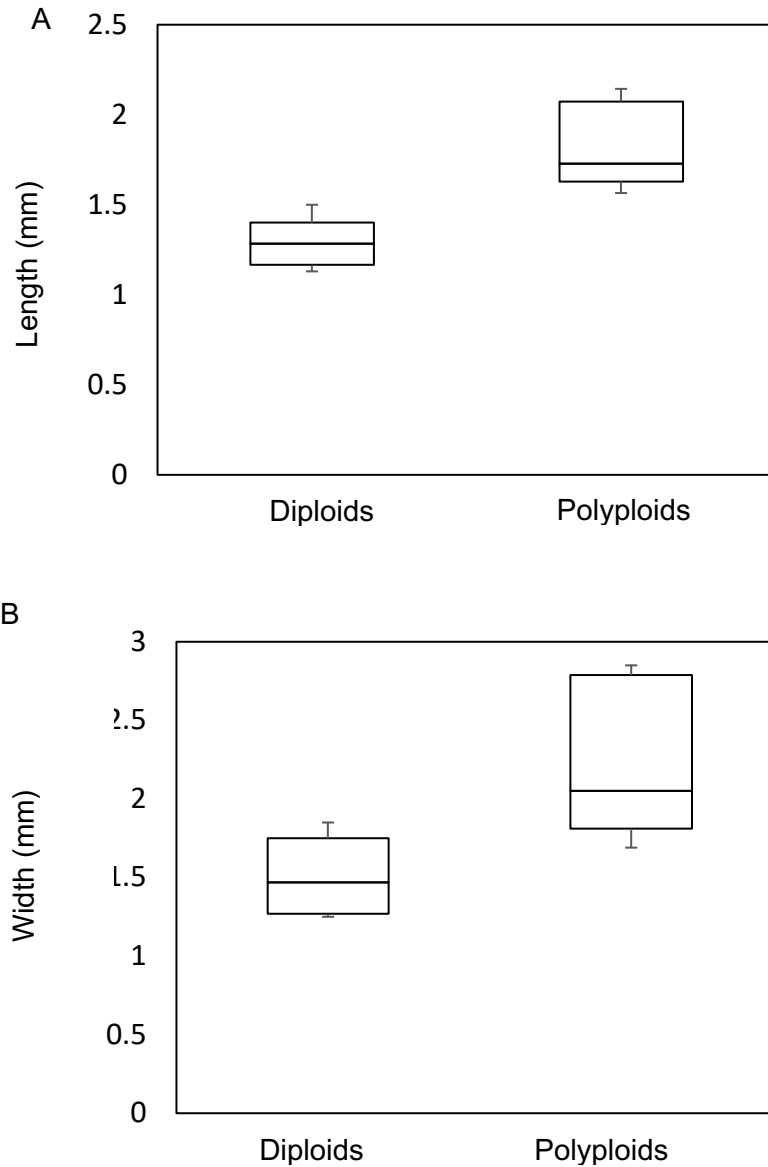


Figure 11- Length (A) and width (B) of seeds from diploid and polyploid individuals of *Lippia alba* under controlled pollination.

Table 6- Mean and standard deviation of length (mm) and width (mm) of seeds from diploid and polyploid accessions of *Lippia alba* under controlled and open pollination seeds.

	Controlled pollination		Open pollination	
	Lenght (mm)	Width (mm)	Lenght (mm)	Width (mm)
Diploid	1.29 ± 0.13	1.51 ± 0.24	1.70 ± 0.14	2.05 ± 0.28
Polyploid	1.82 ± 0.23	2.24 ± 0.48	1.80 ± 0.31	2.20 ± 0.36

In open pollination, no significant difference was found between diploids and polyploids (Figure 12A). Under controlled pollination in turn, there was a significant difference in length and width between diploids and polyploids (Figure 12B).

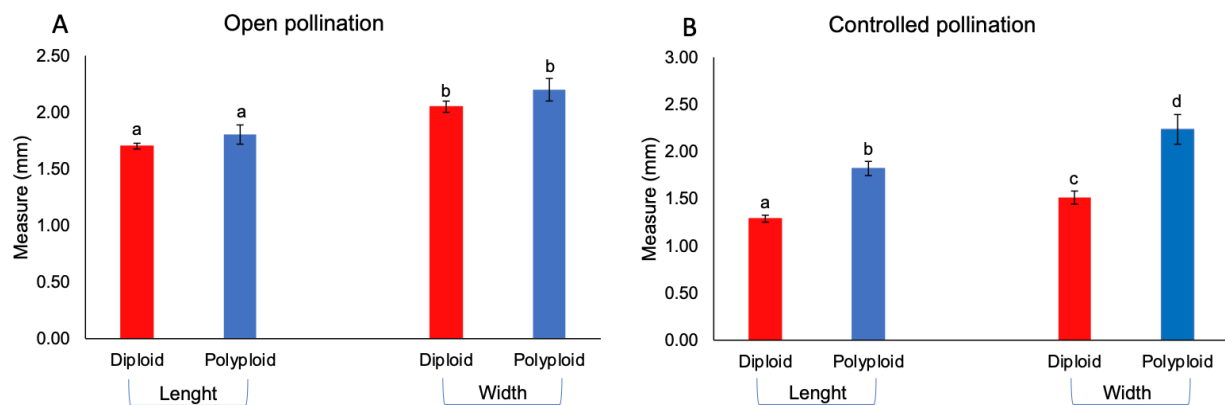


Figure 12- Seed length and width measurements in *Lippia alba* for diploids (red bars) and polyploids (blue bars) under open (A) and controlled (B) pollination. Values are presented as mean and standard error of the mean of seed lenght and width. Different letters represent statistical differences at $p < 0.05$ according to Dunn's test.

When comparing the measurements of length and width of diploids under different pollination conditions, it is observed that in open pollination the measurements are significantly higher than in controlled pollination. The polyploids, in turn, showed no significant difference in either the length or width of the seeds under different conditions (Figure 13).

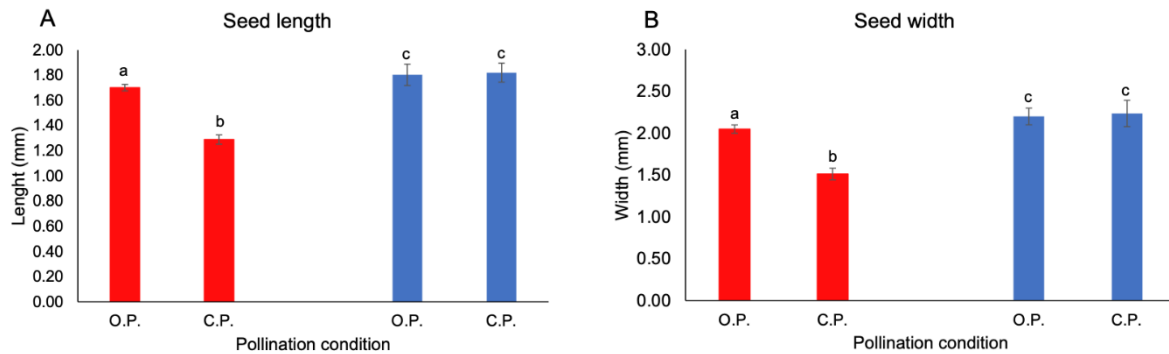


Figure 13- Seed length and width measurements in *Lippia alba* for diploids (red bars) and polyploids (blue bars) under open (A) and controlled (B) pollination. Values are presented as mean and standard error of the mean of seed length and width. Different letters represent statistical differences at $p < 0.05$ according to Dunn's test.

The analysis of seed weight revealed, in turn, that diploids had the lowest value, followed by tetraploids (Figure 14). Triploid individuals showed the highest weight. Statistical analysis revealed a significant difference only between diploids and triploids.

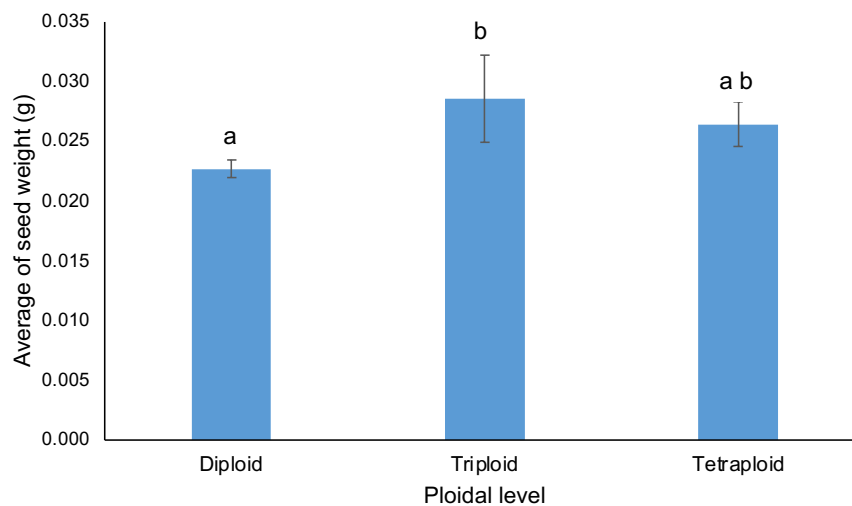


Figure 14- Average seed weight of diploid, triploid and tetraploid individuals. Presence of significant difference between diploids and triploids ($p < 0.05$) according to Dunn's test.

The principal component analysis (PCA) revealed a tendency to group ploidal levels based on the analyzed seed characteristics: production, germination, length, width and weight. Two main components (components 1 and 2) are the ones that influence the individual's distribution (Figure 15). The first one corresponded to approximately 99.78% of influence while the second component corresponded to 0.20%. Diploids were mostly distributed in the upper quadrants, while triploids were grouped exclusively in the third quadrant. Most tetraploids were concentrated in the lower quadrants, except for one individual.

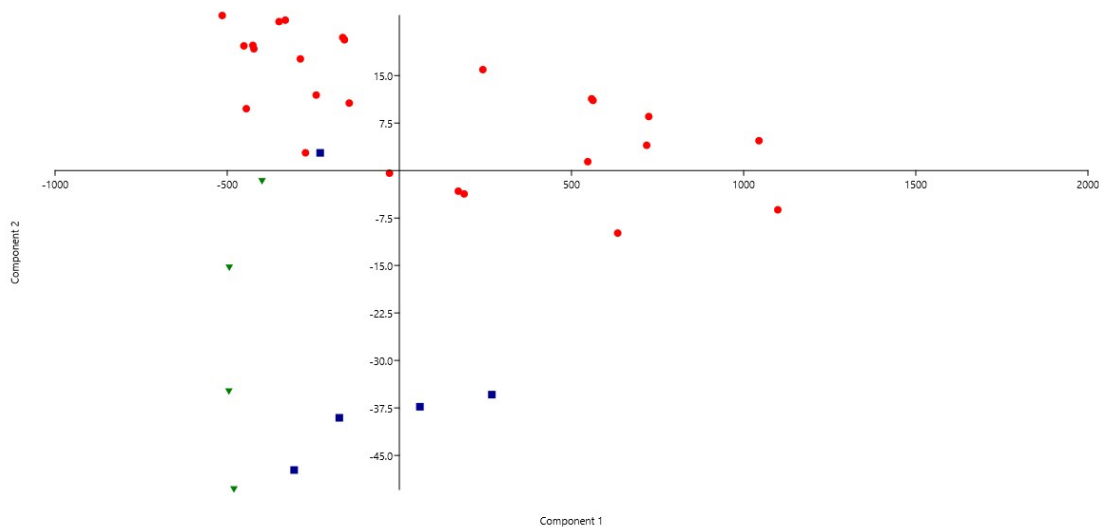


Figure 15- Principal component analysis (PCA) of five seed characteristics analyzed from 34 samples of *Lippia alba*. The colors represent different ploidal levels: diploid in red circle, triploid in green, tetraploid in blue.

4.2- Fitness (W)

In attempting to observe the performance of each individual seed traits (number, length, width, weight and germination) were comparatively evaluated.

Regarding the average of seed production, it was possible to observe that under open pollination condition, diploid individuals tend to have a better fitness value (W) and consequent lower selection coefficient (s) when compared to polyploid individuals. It is important to note that despite this tendency, both the highest and the lowest value of W were attributed to the diploids BGEN-30 and BGEN-02, respectively (Figure 16A) pointed out the fitness variability within diploids.

On the other hand, when analyzing the average of seed production under controlled pollination, the polyploids (mainly tetraploids) tend to present the highest fitness values (W). The highest value of W belongs to the triploid BGEN-62 and the lowest to the diploid BGEN-17 (Figure 16B).

In general, we can conclude that in the absence of cross fertilization, polyploid individuals tend to have a better adaptation. The appendix B and C present in detail the values of W (in descending order) and “ s ” (in ascending order) in the different individuals.

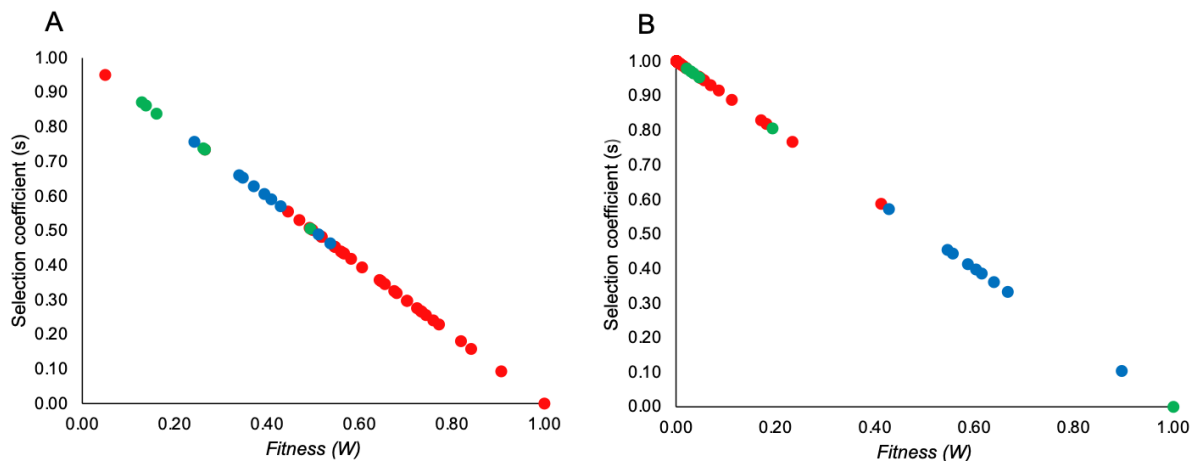


Figure 16- Fitness values (W) and selection coefficient (s) considering the average of seed production in diploids (red), triploids (green) and tetraploids (blue) of *Lippia alba* under open pollination (A) and controlled pollination (B).

As previously reported, length, width, weight and germination of seeds were also analyzed only under open pollination.

Regarding the seed length and width, we observed that triploids have the highest W value, followed by some diploids and tetraploids. The largest length and width value of W belongs to the triploid BGEN-59 while the smallest values belong to the diploids BGEN-90 and BGEN-67, respectively (Figure 17A and 17B).

The seed weight and germination in turn, revealed an interesting difference. While polyploids have the highest W values on the seed weight, they have the lowest W values on seed germination index. Diploids, however, have lower W values regarding the seed weight and the highest W regarding seed germination (Figure 17C and 17D). The triploids BGEN-27 and BGEN-60 correspond respectively to the highest and lowest value of W on seed weight while the diploid BGEN-75 and the triploid BGEN-01 correspond to the highest and lowest value of W on seed germination. The appendix D, E, F and G presents in detail the values of W (in descending order) and “s” (in ascending order) for different individuals.

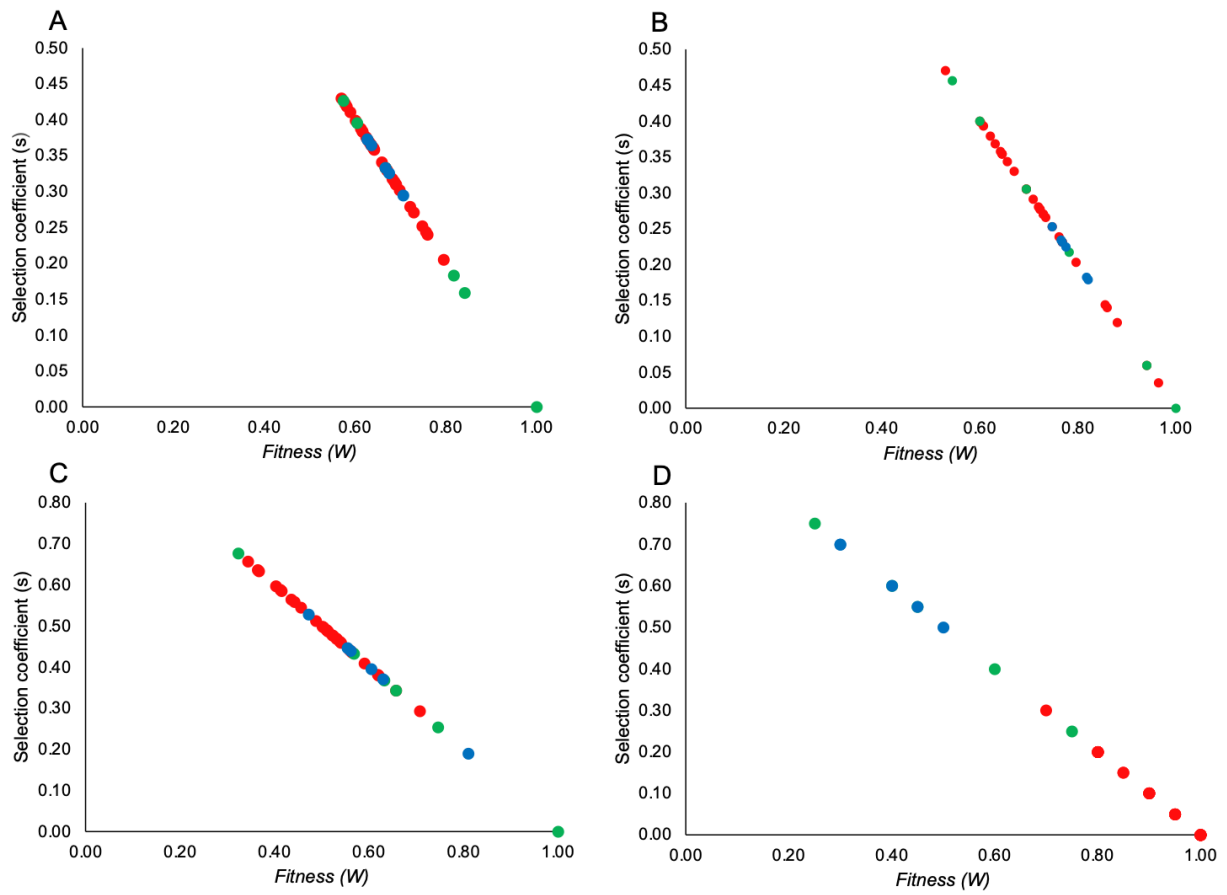


Figure 17- Fitness values (W) and selection coefficient (s) considering seed length (A), width (B), weight (C) and germination (D) in diploids (red), triploids (green) and tetraploids (blue) of *Lippia alba* under open pollination.

Analyzing the same variables within the same ploidal level, some interesting information was obtained. Regarding the average of seed production, diploids, as previously presented, have a higher fitness under open pollination when compared to the controlled pollination. The values of W ranged from 0.049 (BGEN-02) to 1 (BGEN-30) in open pollination while in controlled pollination the value of W ranged from 0 (BGEN-17) to 1 (BGEN-96) (Figure 18A and 18D).

The triploids in open pollination showed the values of W varying from 0.26 (BGEN-01) to 1 (BGEN-62) while under controlled pollination W values varied from 0.020 (BGEN-01) to 1 (BGEN-62) (Figure 18B and 18E).

The tetraploids in turn, showed a variation in the value of W from 0.45 (BGEN-03) to 1 (BGEN-87) in open pollination and from 0.47 (BGEN-87) to 1 (BGEN-03) in controlled pollination condition (Fig. 18C and 18F). The detailed values can be found in appendix H.1, H.2, H.3, I.1, I.2 and I.3.

It is important to note that tetraploids showed the smallest variation in the value of W in both situations (open and controlled pollination), followed by triploids and diploids.

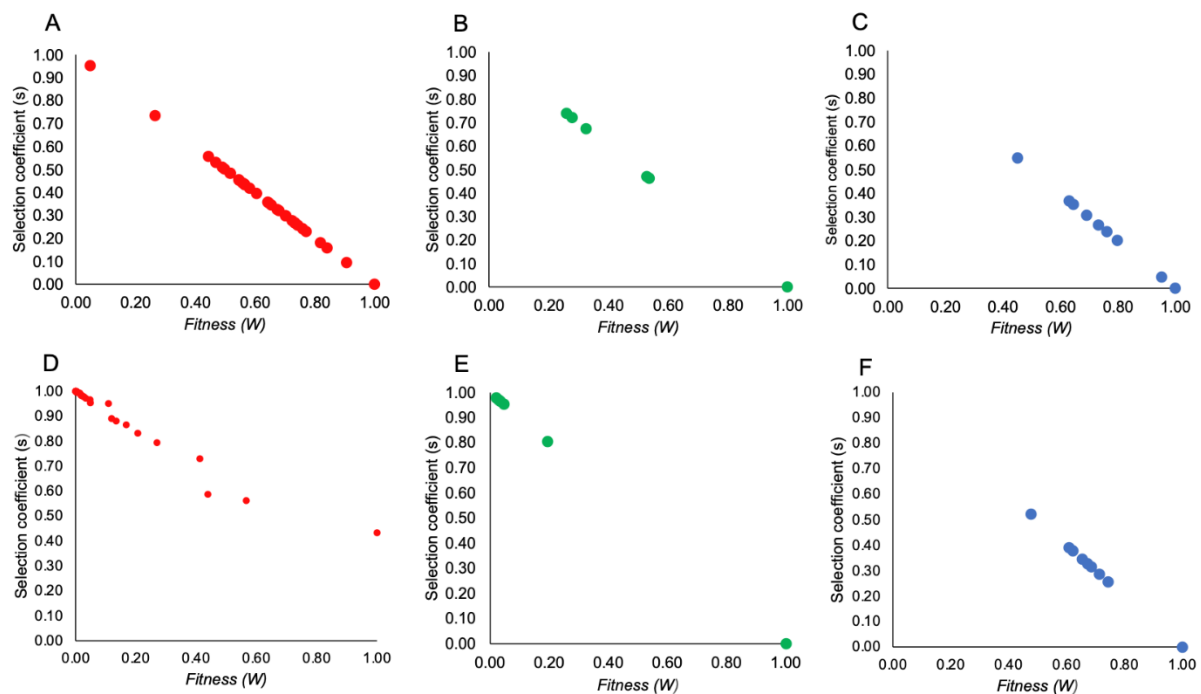


Figure 18- Fitness values (W) and selection coefficient (s) considering the average of seed production in diploids (red), triploids (green) and tetraploids (blue) of *Lippia alba* under open pollination (A, B and C) and controlled pollination (D, E and F).

Regarding the variable length and width of seeds, the highest and lowest values of W belonged to different individuals in the diploids. Taken the length of seeds, the values of W ranged from 0.71 (from BGEN-90) to 1 (from BGEN-65) while for seed width, the values of W ranged from 0.54 (from BGEN-67) to 1 (from BGEN-72) (Figure 19A and 19D).

The triploids, in turn, presented for both characteristics the accession BGEN-59 with the highest value of W and the BGEN-60 with the lowest value of W . For seed length, the value of W varied from 0.57 to 1 and for width, the value of W ranged from 0.54 to 1 (Figure 19B and 19E).

The tetraploids had a W value ranging from 0.88 (BGEN-70) to 1 (BGEN-86) in length and from 0.91 (BGEN-45) to 1 (BGEN-64) in width (Figure 19C and 19F). It is important to highlight that the tetraploids showed the smallest variation in W values, followed by diploids and triploids. The detailed values can be found in appendix J.1, J.2, J.3, K.1, K.2 and K.3.

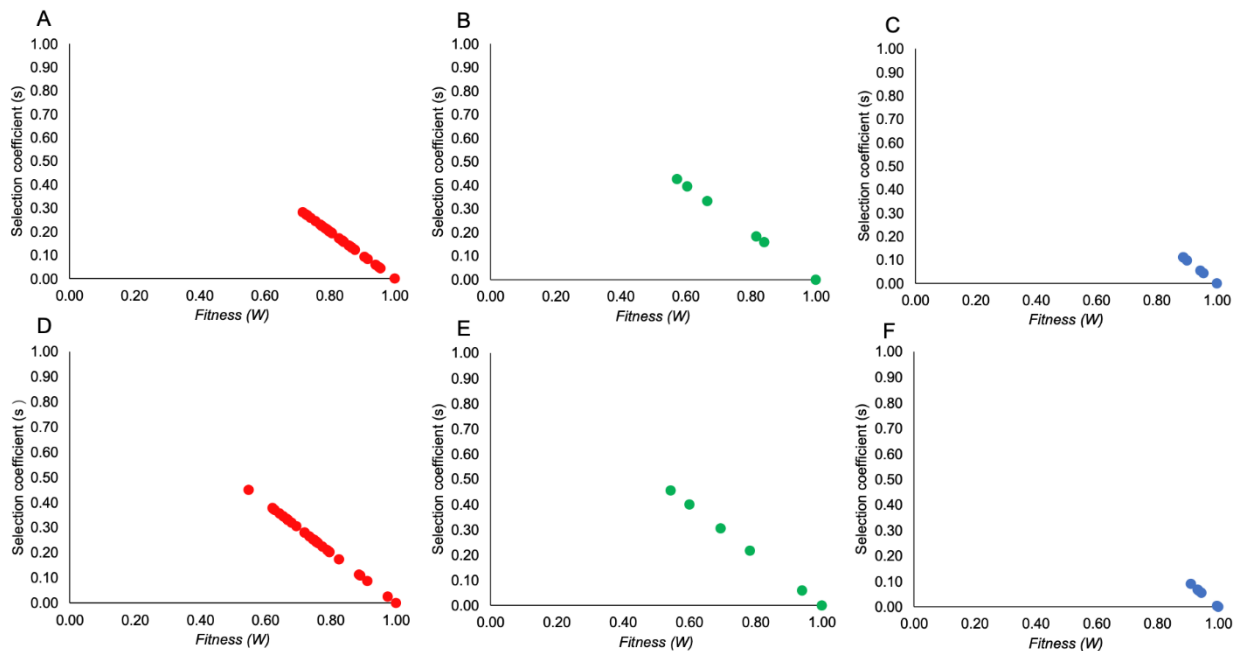


Figure 19- Fitness values (W) and selection coefficient (s) from seed length and width of diploids (red), triploids (green) and tetraploids (blue) under open pollination.

Regarding the characteristics of seed weight and germination, the diploid individuals showed a variation of the W value from 0.48 (BGEN-56) to 1 (BGEN-65) and from 0.7 (BGEN-90) to 1 (BGEN-75), respectively. Triploids, in turn, presented a W variation from 0.32 (BGEN-60) to 1 (BGEN-27) in weight and from 0.33 (BGEN-01) to 1 (BGEN-59) in germination index while the tetraploids had a W value ranging from 0.58 (BGEN-06) to 1 (BGEN-86) in weight and from 0.6 (BGEN-70) to 1 (BGEN-64) in germination index (Figure 20). The detailed values can be found in appendix L.1, L.2, L.3, M.1, M.2, and M.3.

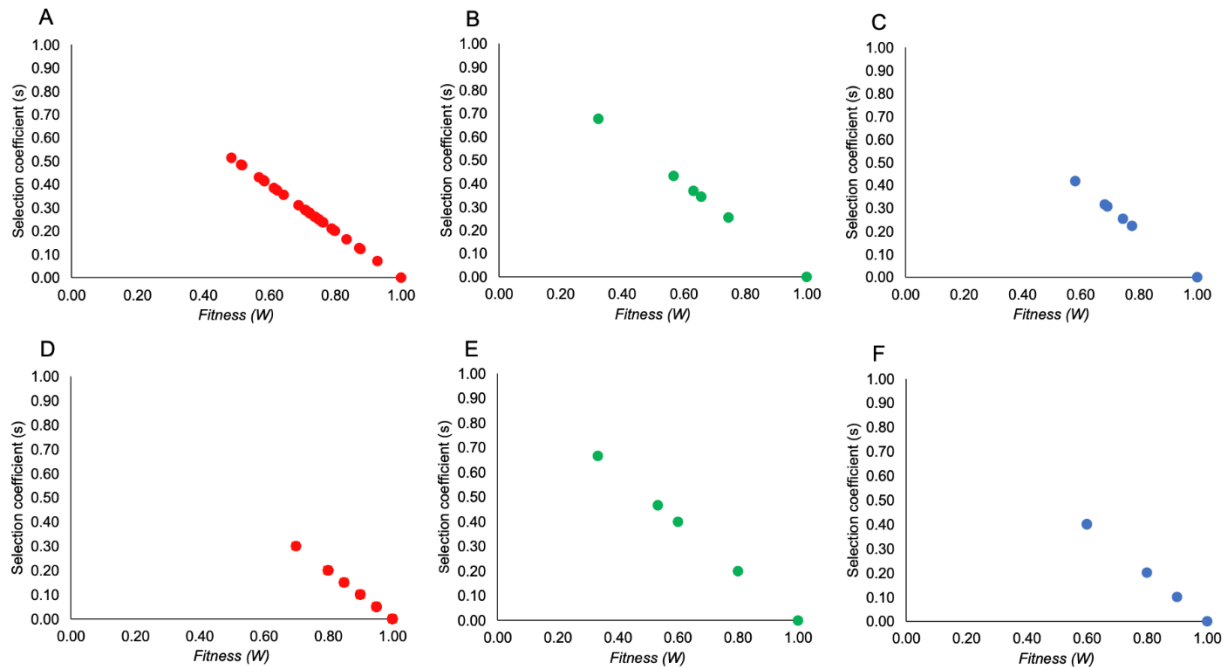


Figure 20- Fitness values (W) and selection coefficient (s) of seed weight and germination index in diploids (red), triploids (green) and tetraploids (blue) of *Lippia alba* under open pollination.

The statistical analysis revealed that for the open pollination condition, the *fitness* (*W*) value of the average of seed production differed significantly between diploids and polyploids, with diploids showing the highest mean production. Under the controlled pollination condition the opposite is observed, polyploids have a significantly higher average seed production than diploids. Interestingly, the polyploids under controlled conditions did not show a significant difference in the average of seed production when compared with the diploids and polyploids from open pollination (Figure 21).

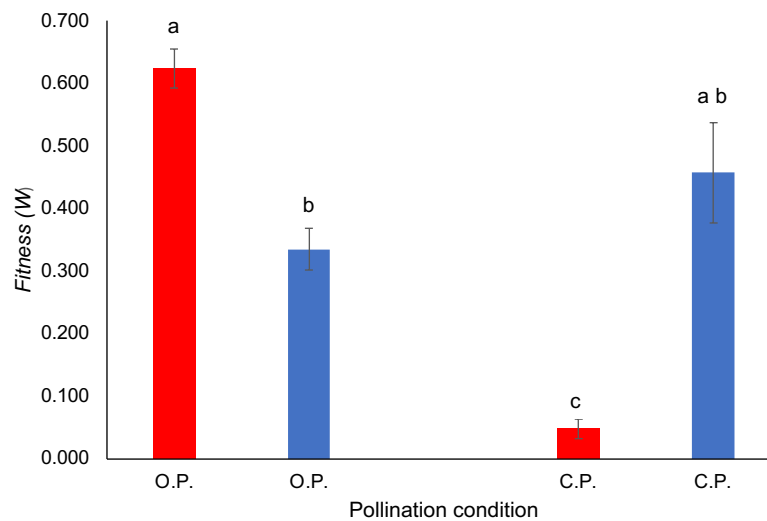


Figure 21- Fitness (*W*) value of average of seed production in *Lippia alba* for diploids (red bars) and polyploids (blue bars) under open pollination (O.P.) and controlled pollination (C.P.). Values are presented as mean and standard error of the mean. Different letters represent statistical differences at $p < 0.05$.

Regarding the length and width of the seeds, the *fitness(W)* value did not differ significantly between diploids and polyploids (Figure 22A and 22B) while the germination index and seed weight, had a significant difference between diploids and polyploids (Figure 22C and 22D). All these four traits were analyzed only under open pollination condition.

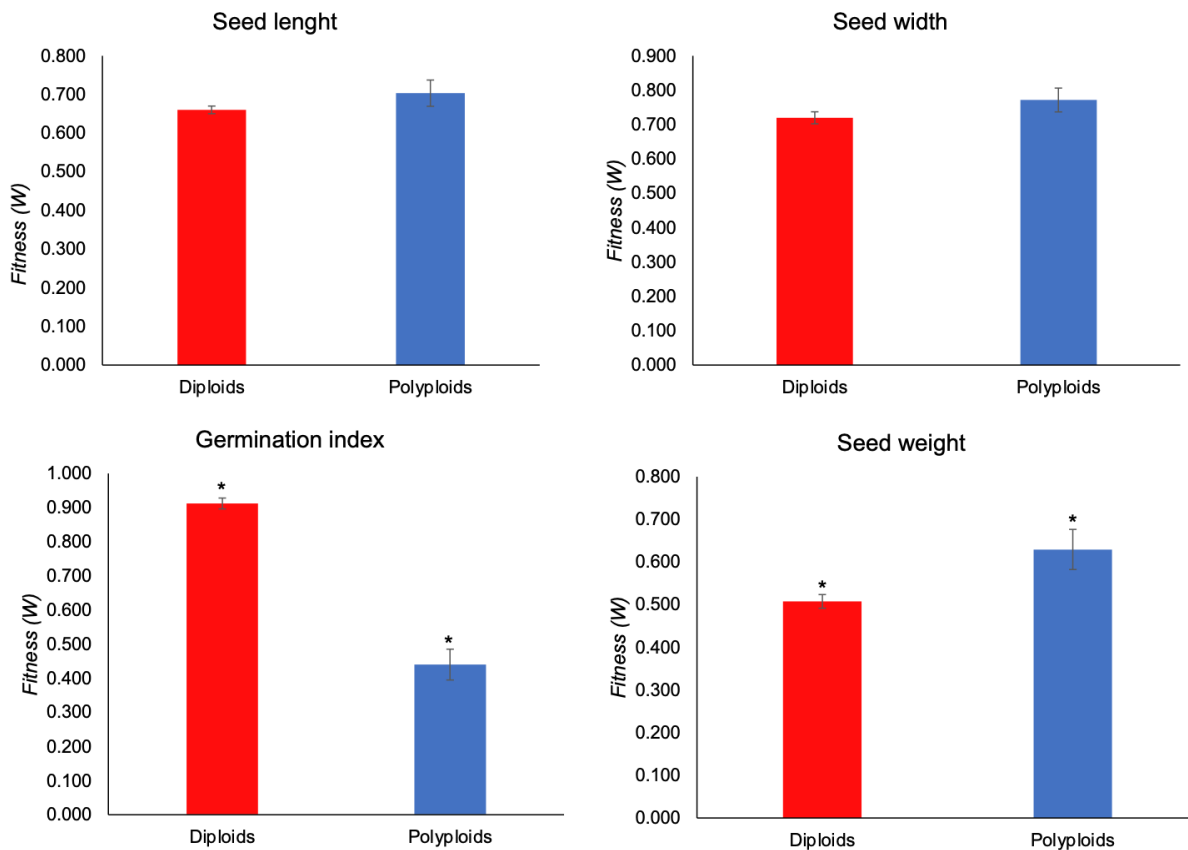


Figure 22- Fitness (W) value of seed length (A), seed width (B), germination index (C) and seed weight (D) in *Lippia alba* for diploids (red bars) and polyploids (blue bars) under open pollination. Values are presented as mean and standard error of the mean. Different letters represent statistical differences at $p < 0.05$.

4.3- Seed flow cytometry and offspring ploidal level

The seed flow cytometry provides important information about the reproduction way by analyzing the ratio between the amount of DNA in the embryo and the endosperm.

The table 7 shows the quantitative data of the analysis i.e., the number of samples analyzed in each ploidal level and under the two different conditions: open and controlled pollination. It was possible to observe for how many samples it was possible to identify the endosperm peak (P.E.- presence of endosperm); for how many it was not possible to identify the endosperm peak (A.E.- absence of endosperm) and for how many samples it was not possible to identify any peak, neither the embryo nor the endosperm (A- aborted).

Table 8, in turn, presents the qualitative data i.e., which embryo/endosperm ratio appear for each ploidal level under both conditions (open and controlled pollination).

Regarding the ratios of the embryo/endosperm peaks, the ratios 2:3, 3:4 and 3:5 correspond to the formation of seeds by sexual reproduction. For 2:X, 3:X and 4:X ratios, we observed only the embryo peak corresponding to diploid, triploid and tetraploid, respectively, being not possible to confirm the type of reproduction involved. Samples called aneuploids correspond to those with an intermediate amount of DNA (not corresponding to diploids, triploids or tetraploids), being not possible to determine the corresponding ratios.

Table 7- Quantitative data from seed flow cytometry in the different ploidal level under open and controlled pollination.

	Open pollination			Controlled pollination		
	2x	3x	4x	2x	3x	4x
P.E.	218	46	19	12	2	0
A.E.	247	74	140	36	0	17
A	84	30	163	34	8	65
Total	549	150	322	82	10	82

P.E.= presence of endosperm

A.E.= absence of endosperm

A= aborted

Table 8- Qualitative data from seed flow cytometry in the different ploidal level under open and controlled pollination.

Ratio (embryo:endosperm)	Open pollination (%)			Controlled pollination (%)		
	2x	3x	4x	2x	3x	4x
2:X	41.72	6.66	-	75	-	-
2:3	40.86	16.66	-	25	-	-
3:X	0.21	6.66	22.01	-	-	82.35
3:4	0.64	8.33	1.88	-	100	-
3:5	0.43	8.33	3.14	-	-	-
4:X	-	-	15.09	-	-	-
Aneuploid?	16.34	52.5	57.86	-	-	17.64

As observed in both tables, in cross-fertilization, diploid individuals had a greater number of histograms with the presence and/or absence of endosperm when compared to the number of aborted ones. These individuals produced mostly diploids (Figure 24A), followed by aneuploids (Figure 24B) and triploids (Figure 24C). When observing the ratios presented by these individuals, the most common was 2:3 and it was possible to observe the diploid peak of the embryo (2) and of the endosperm (3), indicating the sexual reproduction.

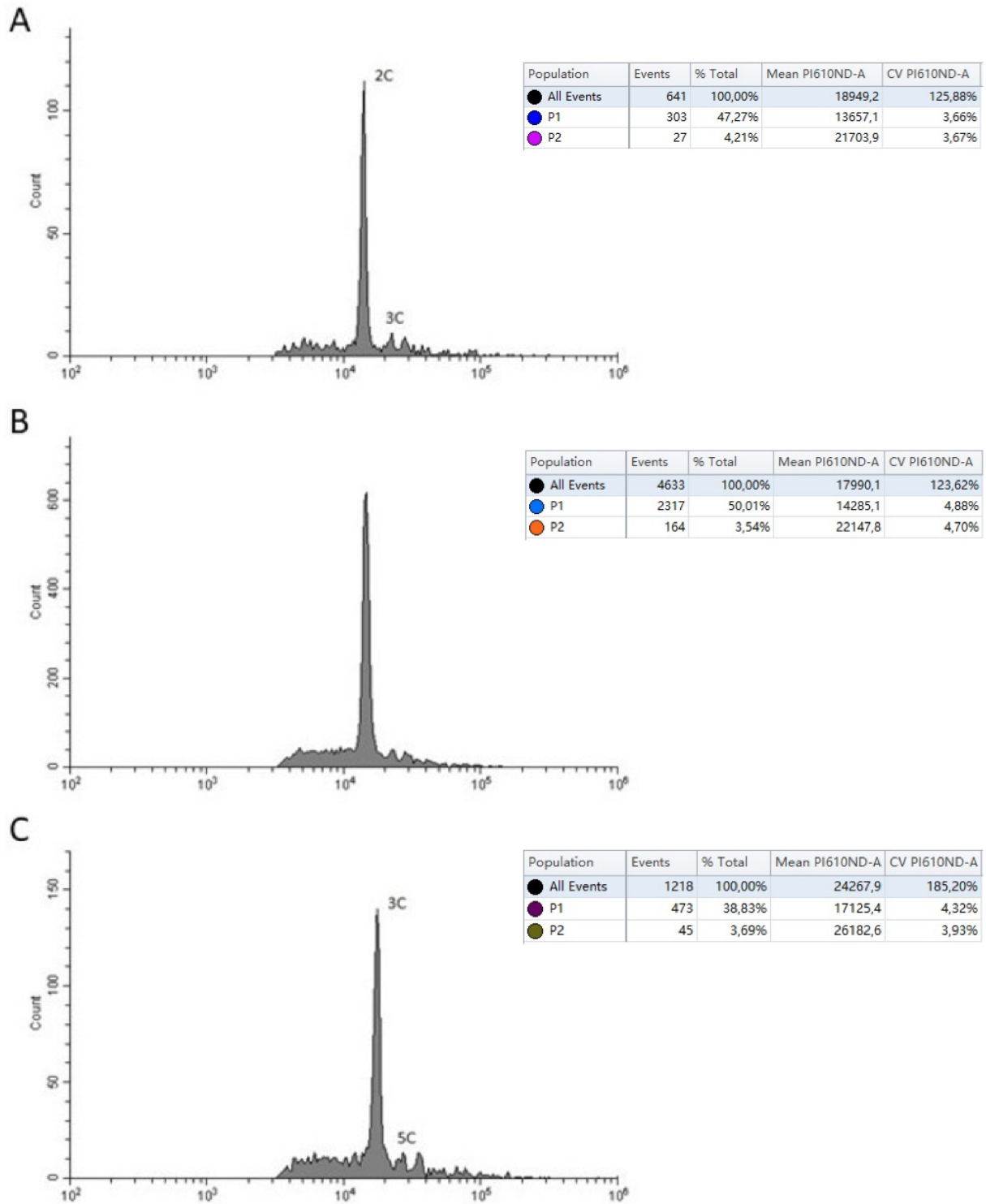


Figure 23- Representative histogram of DNA amount estimation of seeds from diploid accessions of *Lippia alba* under from open pollination. A: diploid from sexual reproduction. B: aneuploid. C: triploid from sexual reproduction. P1 and P2 represents respectively the embryo and endosperm informations.

The triploids in turn, showed a greater number of histograms with absence of the endosperm peak. The offspring presented diploids (Figure 24A), mostly aneuploids (Figure 24B) and triploids (Figure 24C). The most common embryo/endosperm ratios were 2:3 (Figure 24A), 3:4 (Figure 24C) and 3:5, indicating all of them sexual reproduction.

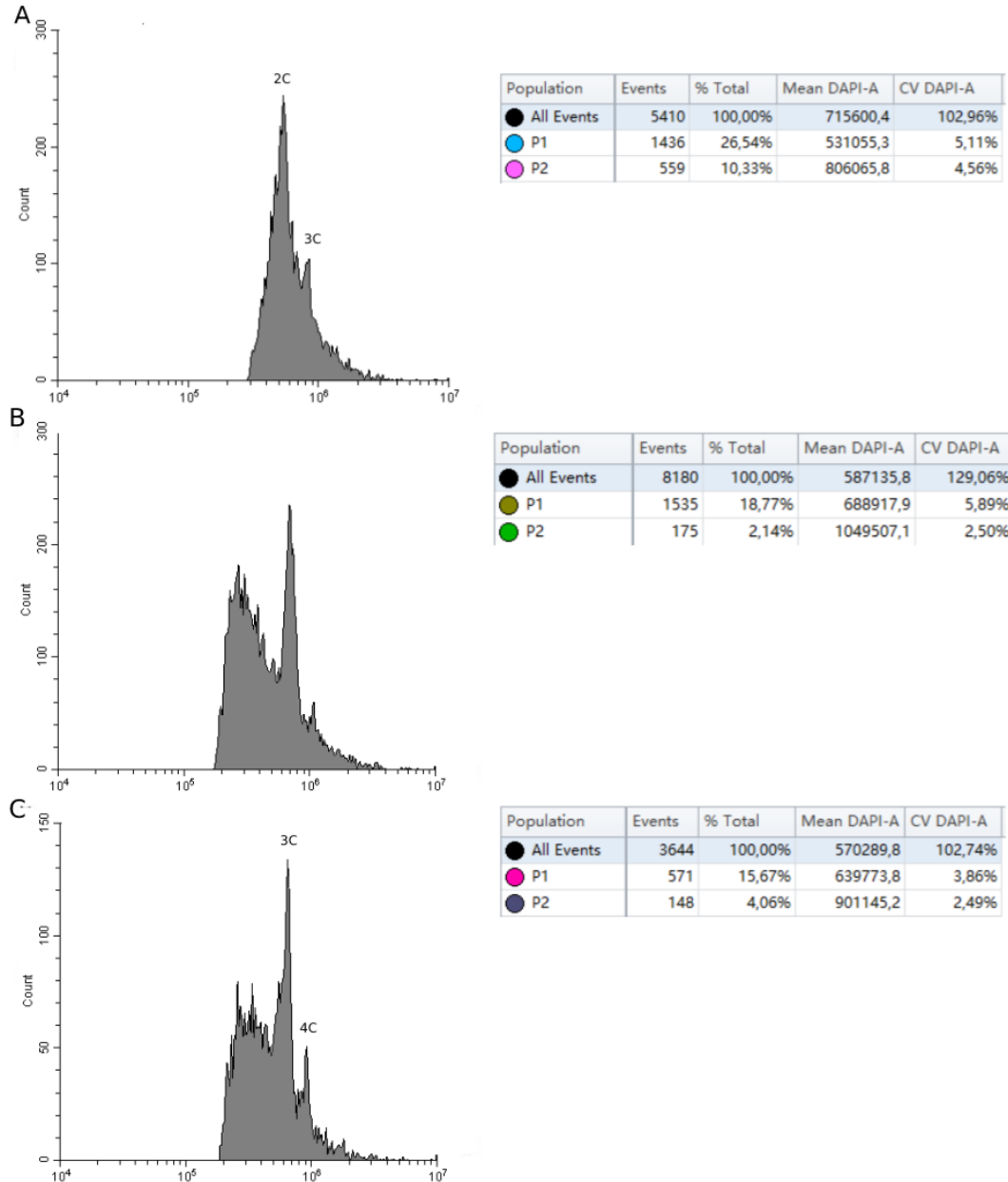


Figure 24- Representative histogram of DNA amount estimation of seeds from triploid accessions of *Lippia alba* under from open pollination. A: diploid of sexual reproduction. B: aneuploid. C: triploid of sexual reproduction. P1 and P2 represents respectively the embryo and endosperm informations

In tetraploid offspring, it was possible to observe triploids (Figure 25A), aneuploids in greater number (Figure 25B) and tetraploids (Figure 25C). The ratios 3:4 and 3:5 was detected, indicating also sexual reproduction.

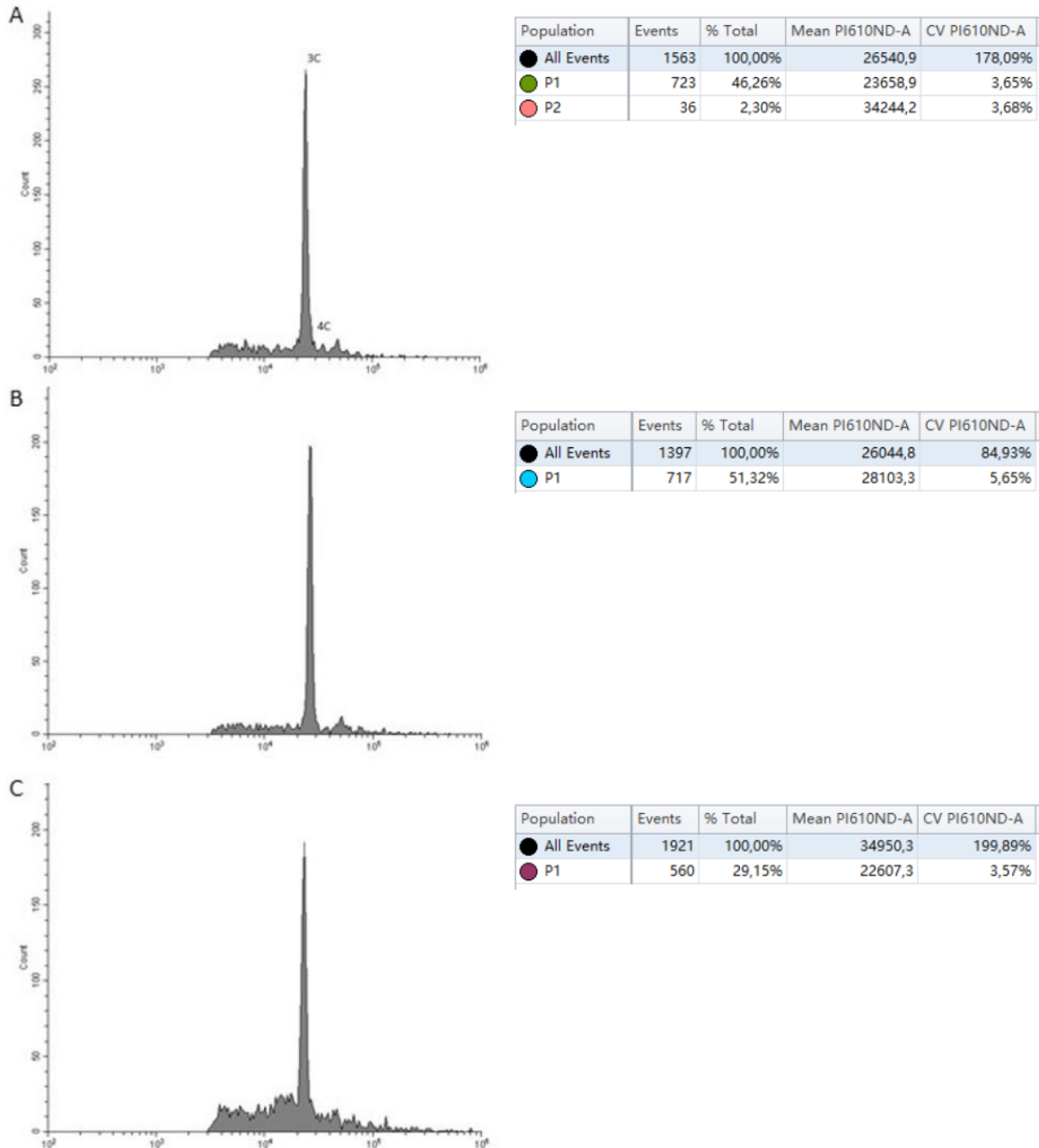


Figure 25- Representative histogram of DNA amount estimation of seeds from tetraploid accessions of *Lippia alba* under from open pollination. A: triploid from sexual reproduction. B: aneuploid. C: tetraploid with only the embryo peak. P1 and P2 represents respectively the embryo and endosperm informations.

Analyzing controlled pollination, the diploids showed only offspring of diploid embryo peak, some of them with ratio of 2:3, indicating a sexual reproduction and consequently, self-fertilization.

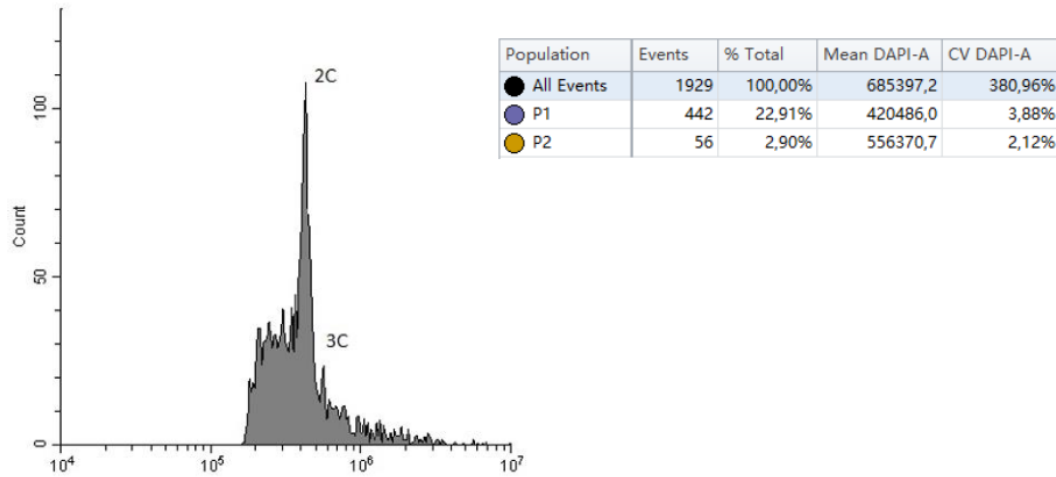


Figure 26- Representative histogram of DNA amount estimation of seeds from diploid accessions of *Lippia alba* under controlled pollination showing ratio 2:3 (embryo:endosperm) of sexual reproduction. P1 and P2 represents respectively the embryo and endosperm informations.

The triploids in turn, presented triploids in the offspring of controlled pollination with the ratio 3:4, also suggesting a sexual reproduction and consequently, self-fertilization (Figure 27). The tetraploid group, presented triploid and aneuploid offspring, being possible to infer, despite the absence of the endosperm, that the self-fertilization process is occurring.

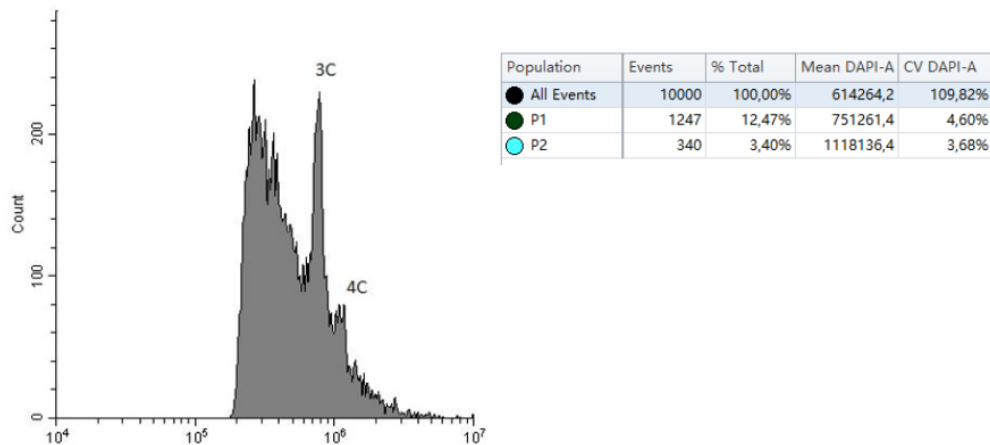


Figure 27- Representative histogram of DNA amount estimation of seeds from triploid accessions of *Lippia alba* under controlled pollination showing ratio 3:4 (embryo:endosperm) of sexual reproduction. P1 and P2 represents respectively the embryo and endosperm informations.

In addition to the material analyzed by seed cytometry, germinated seedlings (in a petri dish) were also used to investigate the ploidal level of the offspring.

The amount of DNA used to classify the ploidal level of the progeny is showed in table 9.

Table 9- Ranges of DNA amount corresponding to ploidal levels in the offspring of *Lippia alba*

DNA content (pg)	Ploidal level
up to 2.80	2x
2.81-3.78	Aneuploid
3.79-4.14	3x
4.15-4.55	Aneuploid
4.56-4.90	4x
4.91-6.89	Aneuploid
6.90	6x

As a result, the diploids individuals showed a greater number of diploid offspring (98.91%), followed by aneuploids (1.08%). The triploids in turn, showed mainly aneuploids (81.25%), followed by triploids (8.33%), diploids (9.37%) and tetraploids (1.04%). Lastly, the tetraploids showed a greater number of aneuploids (82.14%), followed by diploids (12.5%) and triploids (5.35%) (Figure 28) (Appendix N.1, N.2 and N.3).

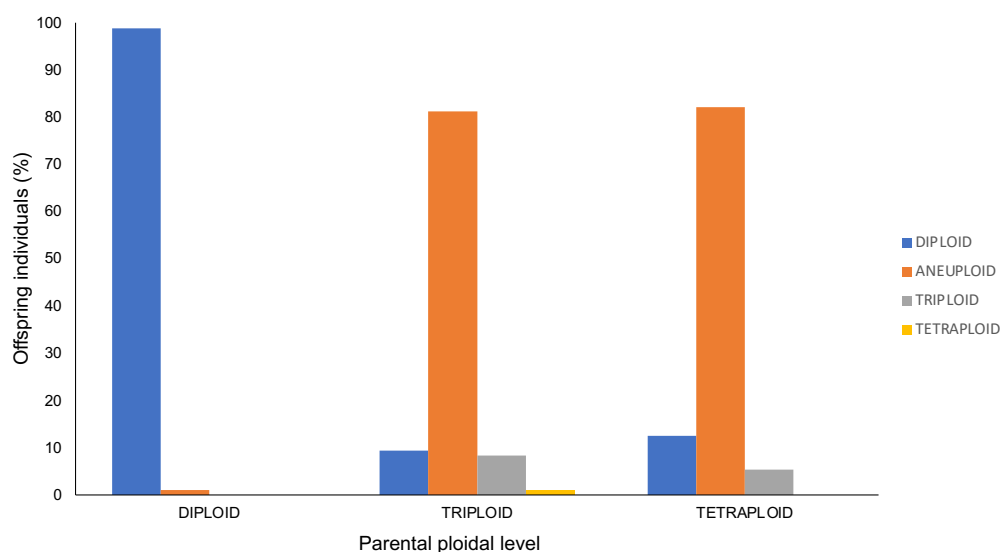


Figure 28- Offspring ploidal level from diploid, triploid and tetraploid accessions of *Lippia alba* under open pollination.

Considering individuals that were under controlled pollination, it was possible to observe that the most of the offspring were aneuploid. The estimation of the DNA amount of these individuals revealed that it is compatible with triploids and tetraploids (Figure 29) (Appendix O).

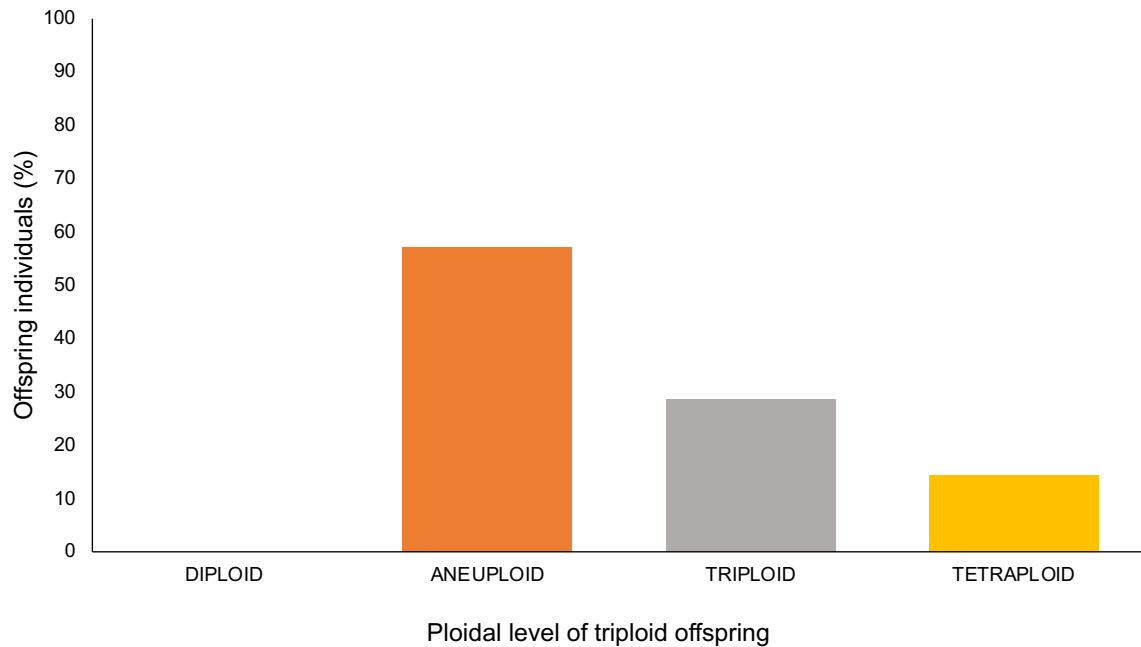


Figure 29- Offspring ploidal level from triploid accessions of *Lippia alba* under controlled pollination.

4.4- Offspring: case studies

4.4.1 Cytogenetic analysis and DNA content estimation

To understand possible karyotypic configuration and how the chromosome set is inherited, a preliminary cytogenetic survey was done in four individuals, here called, case studies. The first individual is the offspring from artificial crossing between the diploid mother plant (BGEN-77) and the triploid BGEN-59 (male parent). The second individual is an offspring from open pollination of the diploid BGEN-77. The third is an offspring from open pollination of the tetraploid BGEN-64. The fourth is an offspring from controlled pollination of the triploid BGEN-59.

The karyogram of the first individual obtained from the artificial crossing between a diploid and a triploid show $2n=35$ chromosomes which correspond to an aneuploid (Figure 30A). It was possible to observe the presence of 12 median chromosomes and three submedian chromosomes, presenting the karyotypic formula of $12m+3sm$. Details of these chromosomes are summarized on figure 31A. It was also possible to notice that the extra homologues have been grouped on the chromosomes I, II, VIII, IX and XIII (Figure 30A, table 10). Regarding its morphometric data (short arm - sa, the long arm - la, ratio la/sa and relative size), it was observed that the total chromosome size varied from $1.87\mu m$ to $2.98\mu m$. The short arm in turn, had a variation of $0.77\mu m$ to $1.23\mu m$ while the long arm had of $1.05\mu m$ to $1.83\mu m$ (Table 10).

The figure 30B, shows the offspring of the diploid BGEN-77 from open pollination with $2n=30$. Its ideogram (Figure 31B) showed a karyotypic formula of $6m+9sm$. The morphometric data showed a variation in chromosome size of $1.27\mu m$ to $2.85\mu m$. The short arm in turn, had a variation of $0.43\mu m$ to $1.24\mu m$ while the long arm varied from $0.77\mu m$ to $1.83\mu m$ (Table 11).

The offspring of the tetraploid BGEN-64 from open pollination also presented $2n=30$ (Figure 30C). Its ideogram (Figure 31C) shows a karyotypic formula of $12m+3sm$ and its morphometric data revealed a chromosome variation of $1.80\mu m$ to $2.80\mu m$. The variation in the size of the short arm and long arm was respectively, $0.71\mu m$ to $1.04\mu m$ and $0.99\mu m$ to $1.99\mu m$ (Table 12).

Lastly, the figure 30D shows the offspring of the triploid BGEN-59 from controlled pollination with $2n=48$. Its ideogram showed a karyotypic formula of $9m+6sm$ (Figure 31D). The extra homologues have been grouped on the chromosomes XIII, X and XI (Figure 30D, table 13). Its morphometric data showed a variation in chromosome size of $1.72\mu\text{m}$ to $4.11\mu\text{m}$. The short arm in turn, had a variation of $0.7\mu\text{m}$ to $1.63\mu\text{m}$ while the long arm had of $1.02\mu\text{m}$ to $2.73\mu\text{m}$ (Table 13).

Furthermore, it is possible to observe in table 14 the data of karyotypic asymmetry and amount of DNA of the four individuals analyzed.

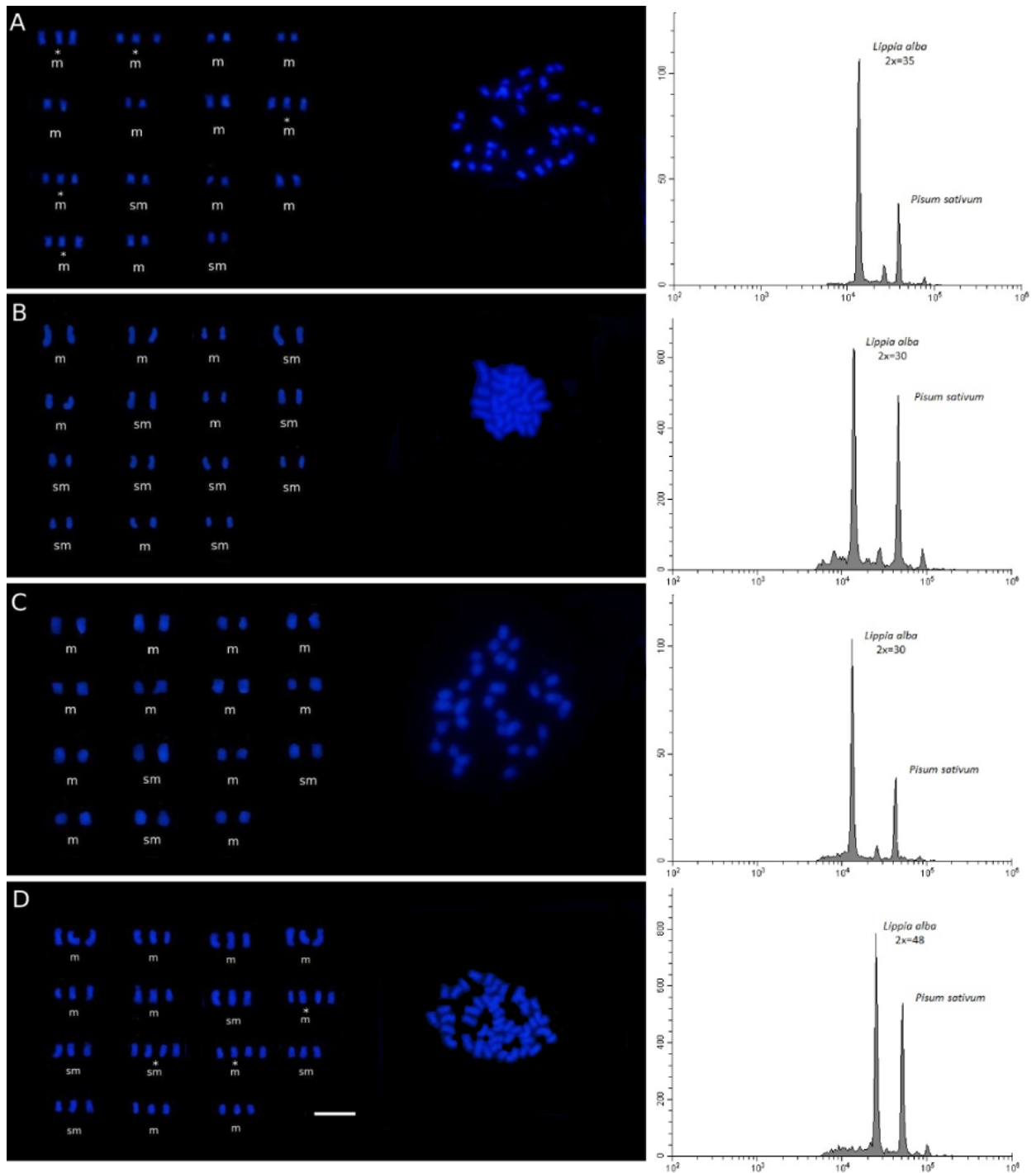


Figure 30- Karyogram and DNA content of the offspring individuals in *Lippia alba*. A: offspring with $2n=35$ from the artificial crossing between the diploid BGEN-77 and the triploid BGEN-59; B: offspring with $2n=30$ from open pollination of the diploid BGEN-77; C: offspring with $2n=30$ from open pollination of the tetraploid BGEN-64; D: offspring with $2n=48$ from controlled pollination of the triploid BGEN-59. The asterisk indicates the chromosomes with extra copies. Bar=5 μ m.

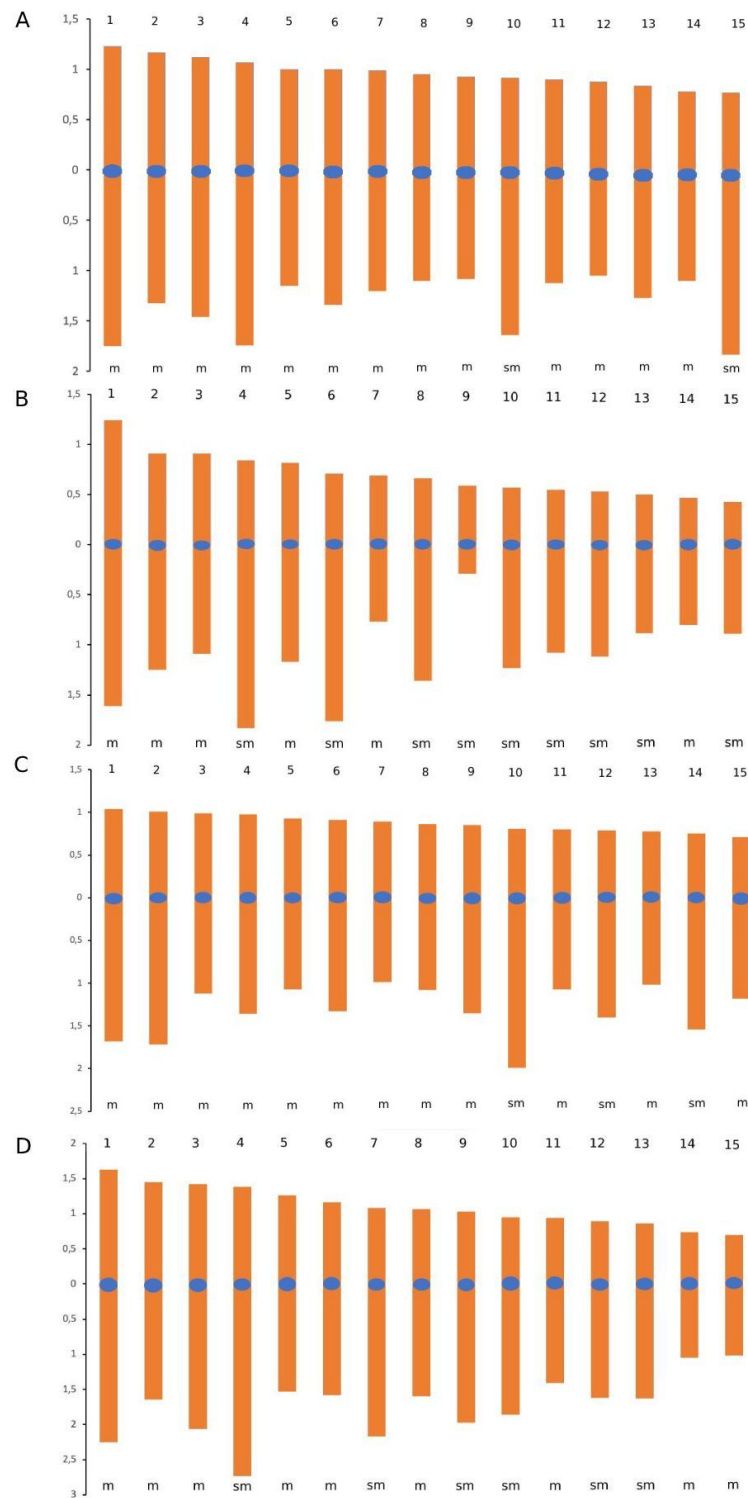


Figure 31- Ideograms of the offspring individuals in *Lippia alba*. A: offspring with $2n=35$ from the artificial crossing between the diploid BGEN-77 and the triploid BGEN-59; B: offspring with $2n=30$ from open pollination of the diploid BGEN-77; C: offspring with $2n=30$ from open pollination of the tetraploid BGEN-64; D: offspring with $2n=48$ from controlled pollination of the triploid BGEN-59.

Table 10. Morphometric data of the offspring with $2n=35$ from the artificial crossing between the diploid BGEN-77 and the triploid BGEN-59 accessions of *Lippia alba*. The asterisk indicates the chromosomes with extra copies.

Chrom.	sa	la	Total	Ratio (la/sa)	Relative size	Classification
*I	1.23	1.75	2.98	1.42	8.59	m
*II	1.17	1.32	2.49	1.13	7.17	m
III	1.12	1.46	2.58	1.31	7.43	m
IV	1.07	1.74	2.81	1.63	8.1	m
V	1.00	1.15	2.15	1.16	6.19	m
VI	1.00	1.34	2.33	1.34	6.71	m
VII	0.99	1.20	2.19	1.21	6.31	m
*VIII	0.95	1.10	2.05	1.16	5.91	m
*IX	0.93	1.08	2.01	1.16	5.79	m
X	0.92	1.64	2.56	1.78	7.38	sm
XI	0.9	1.12	2.02	1.24	5.82	m
XII	0.88	1.05	1.93	1.19	5.56	m
*XIII	0.84	1.27	2.11	1.52	6.08	m
XIV	0.78	1.10	1.87	1.41	5.39	m
XV	0.77	1.83	2.60	2.37	7.49	sm

sa=short arm; la= long arm.

Table 11. Morphometric data of the offspring with $2n=30$ from open pollination of the diploid BGEN-77.

Chrom.	sa	la	Total	Ratio (la/sa)	Relative size	Classification
I	1.24	1.61	2.85	1.30	10.00	m
II	0.91	1.25	2.16	1.37	7.58	m
III	0.91	1.09	2.00	1.20	7.02	m
IV	0.84	1.83	2.67	2.17	9.35	sm
V	0.82	1.17	1.99	1.43	6.98	m
VI	0.71	1.76	2.47	2.47	8.65	sm
VII	0.69	0.77	1.46	1.11	5.10	m
VIII	0.66	1.36	2.02	2.05	7.07	sm
IX	0.59	1.29	1.88	2.19	6.59	sm
X	0.57	1.23	1.80	2.16	6.31	sm
XI	0.55	1.08	1.63	1.96	5.72	sm
XII	0.53	1.12	1.65	2.10	5.77	sm
XIII	0.5	0.88	1.38	1.75	4.82	sm
XIV	0.47	0.80	1.27	1.69	4.44	m
XV	0.43	0.89	1.32	2.06	4.61	sm

sa=short arm; la= long arm.

Table 12. Morphometric data of the offspring with $2n=30$ from open pollination of the tetraploid BGEN-64.

Chrom.	sa	la	Total	Ratio (la/sa)	Relative size	Classification
I	1.04	1.68	2.72	1.62	8.25	m
II	1.01	1.72	2.73	1.70	8.28	m
III	0.99	1.12	2.11	1.13	6.4	m
IV	0.98	1.36	2.34	1.38	7.1	m
V	0.93	1.07	2.00	1.15	6.06	m
VI	0.91	1.33	2.24	1.46	6.79	m
VII	0.89	0.99	1.88	1.11	5.7	m
VIII	0.86	1.08	1.94	1.26	5.88	m
IX	0.85	1.35	2.20	1.58	6.67	m
X	0.81	1.99	2.80	2.45	8.49	sm
XI	0.8	1.07	1.87	1.34	5.67	m
XII	0.79	1.40	2.19	1.77	6.64	sm
XIII	0.78	1.02	1.80	1.30	5.46	m
XIV	0.75	1.54	2.29	2.05	6.94	sm
XV	0.71	1.18	1.89	1.66	5.73	m

sa=short arm; la= long arm.

Table 13. Morphometric data of the offspring with $2n=48$ from controlled pollination of the triploid BGEN-59. The asterisk indicates the chromosomes with extra copies.

Chrom.	sa	la	Total	Ratio (la/sa)	Relative size	Classification
I	1.63	2.25	3.88	1.38	9.10	m
II	1.45	1.65	3.10	1.14	7.26	m
III	1.42	2.06	3.48	1.45	8.16	m
IV	1.38	2.73	4.11	1.98	9.64	sm
V	1.26	1.53	2.79	1.21	6.54	m
VI	1.16	1.58	2.74	1.37	6.42	m
VII	1.08	2.17	3.25	2.02	7.61	sm
*VIII	1.06	1.60	2.66	1.50	6.23	m
IX	1.03	1.97	3.00	1.92	7.02	sm
*X	0.95	1.86	2.81	1.96	6.58	sm
*XI	0.94	1.41	2.35	1.49	5.50	m
XII	0.89	1.62	2.51	1.81	5.88	sm
XIII	0.86	1.63	2.49	1.89	5.83	sm
XIV	0.74	1.05	1.79	1.43	4.20	m
XV	0.7	1.02	1.72	1.45	4.02	m

sa=short arm; la= long arm.

Table 14. Karyotypic asymmetry, chromosome number and DNA content of *Lippia alba* offspring.

Individual	A	Chromosome number	DNA amount (pg)
Artificial crossing	0.15	2n=35	3.16
O.D.O.P.	0.27	2n=30	2.74
O.T.O.P	0.19	2n=30	2.84
O.T.C.P.	0.22	2n=48	4.49

O.D.O.P= offspring of diploid from open pollination.

O.T.O.P= offspring of tetraploid from open pollination.

O.T.C.P= offspring of triploid from controlled pollination.

4.4.2 Essential oil constituents

Regarding the essential oil, figure 32A shows the citral (geranial+neral) as the major component of essential oil in three of the four offspring analyzed: the $2n=35$ from the artificial crossing between diploid and triploid with 21,97% of its terpenes composed of citral; the $2n=30$ of a diploid from open pollination with 86,26% and the $2n=30$ of a tetraploid from open pollination with 68,1%. The individual $2n=48$ of a triploid from controlled pollination in turn, presented linalool as major component of its essential oil, representing 26,67% of all components (Figure 32B). The chromatograms of the different offspring with emphasis on the major component are shown in the figure 33.

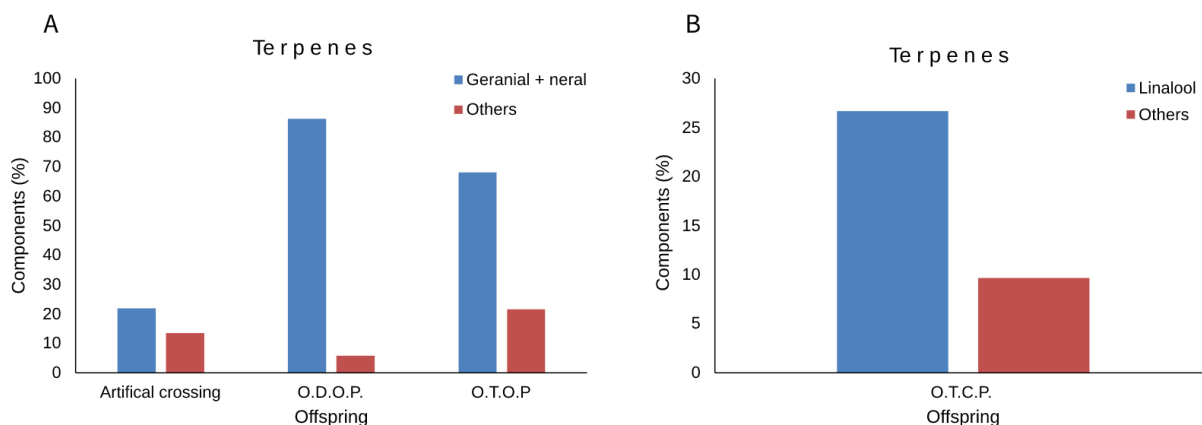


Figure 32- Terpenes found in the analyzed offspring of *Lippia alba*. A: $2n=35$ from artificial crossing; $2n=30$ of a diploid from open pollination and $2n=30$ of a tetraploid from open pollination. B: $2n=48$ of a triploid from controlled pollination.

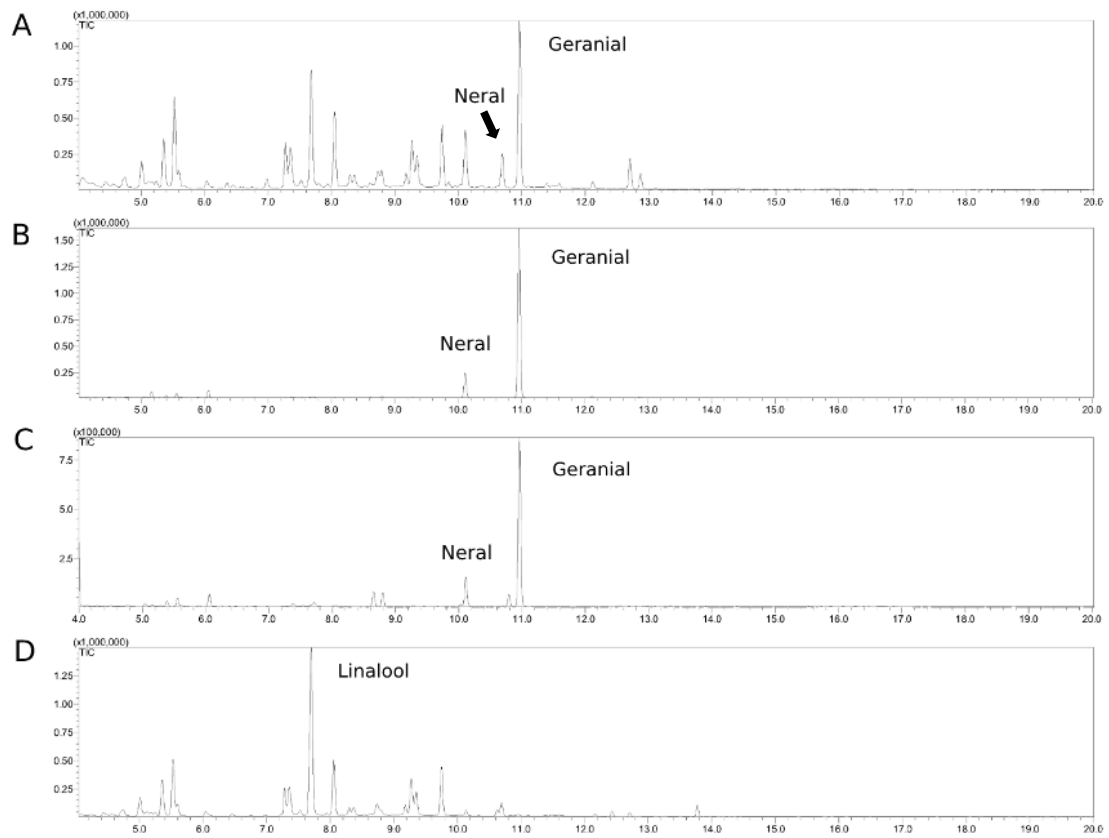


Figure 33- Chromatograms from offspring individuals. A: Artificial crossing ($2n=35$) with geranial as the majority component. B: Offspring ($2n=30$) from diploid of open pollination with geranial as major component. C: Offspring ($2n=30$) from tetraploid of open pollination with geranial as major component. D: Offspring ($2n=48$) from triploid of controlled pollination with linalool as major component.

4.5- Microsatellite analysis

The samples analyzed by microsatellite comprise 12 individuals: individuals 1, 2 and 3 respectively comprise, respectively, the diploid mother plant BGEN-77 from the artificial crossing, the aneuploid offspring $2n=35$ from the artificial crossing and the triploid paternal parent BGEN-59. The individual 3 is also the triploid mother plant of the offspring 4 (same individual of $2n=48$ analyzed in the case study) coming from controlled pollination. Individual 5 is the triploid mother plant BGEN-27 of the offspring 6, 7 and 8, which came from controlled pollination. Individual 9, in turn, is the triploid mother plant BGEN-10 of the offspring 10, 11 and 12 from controlled pollination (Figure 34). Samples coming from controlled pollination were analyzed due to the possible confirmation of the occurrence of self-fertilization and/or apomixis.

For a better understanding of the results obtained in the microsatellite analysis, the scheme of figure 35 was created, in which it is possible to observe a list of primers with their respective detected alleles and the presence or absence of these same alleles (represented by balls of different colors) in different individuals (samples 1 to 12). The samples that show an “X” presented an error in their analysis (Figure 35).

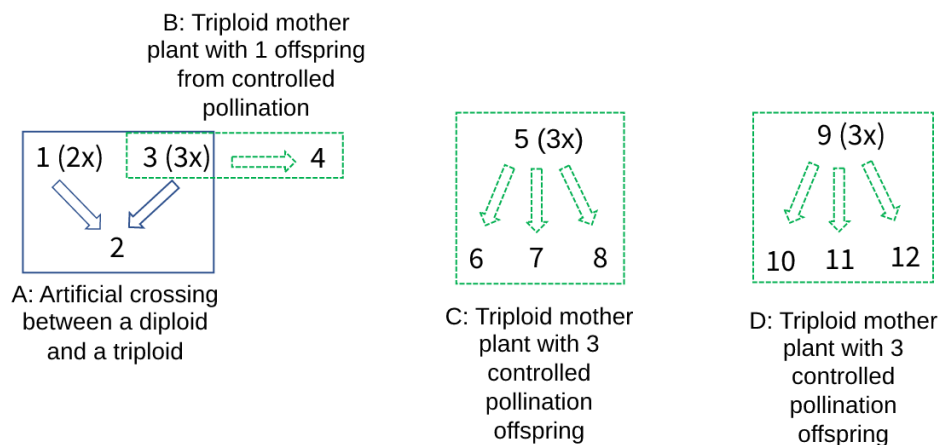


Figure 34- Representative scheme of the samples analyzed by the SSR technique. (A) The numbers 1, 2 and 3, respectively, comprise the diploid mother plant (BGEN-77) of the artificial cross; the offspring $2n=35$ of the artificial cross and the triploid paternal genitor (BGEN-59) of the artificial cross; (B) The number 4 is the $2n=48$ offspring from controlled pollination of the sample 3 (BGEN-59); (C) The number 5 is a triploid (BGEN-27) while its offspring from controlled pollination comprises the numbers 6, 7 and 8; (D) The number 9 is a triploid (BGEN-10) while its offspring from controlled pollination comprises the numbers 10, 11 and 12.

Primers					Samples							
					5	6	7	8	9	10	11	12
p229												
147	x	x	x	●	●	●	●	x	●	●	●	●
150	x	x	x		●			x	●		●	●
163	x	x	x		●		●	x	●			
169	x	x	x	●	●	●	●	x	●	●	●	●
p270												
113		●	●	●	x	●	●	●	●	●	●	●
115	●	●	●	●	x	●	●	●	●	●	●	●
120	●	●	●	●	x	●	●	●	●	●	●	●
122			●	●	x			●	●			
124	●	●	●		x		●		●	●	●	
126		●	●	●	x	●	●	●	●	●	●	●
p283												
189	●	●	●	●	●	●	●	●	●	●	●	●
190	●	●										
p294												
148	x	●	●	●	●	●	●	●	●	●	●	●
173	x	●	●	●	●	●	●	●	●	●	●	●
176	x	●										
180	x	●	●	●	●	●	●	●	●	●	●	
p484												
167	●	●										
170	●	●										
172	●	●	●	●	●	●	●	●	●	●	●	●
173	●	●	●	●	●	●		●	●	●	●	●
LAC4												
263	●	●	●	●	●	●	●	●	●	●	●	x
264		●	●	●	●	●	●	●	●	●	●	x
267	●	●	●	●	●	●	●	●	●	●	●	x
270	●	●										x
276		●	●	●	●	●	●	●	●	●	●	x
p77												
179		●	●	●	●	●	●	●	●	●	●	●
199	●											
209	●	●										
p249												
186	●	●	x		●	●	x	●	●	●		
187			x	●	●	●	x	●		●		
218	●	●	x	●	●	●	x	●	●			●
227		x	●		●		x	●	●	●	●	
p370												
173	●	●	●	●	●	●	●	●	●	●	●	●
273	●	●			●	●	●	●	●	●	●	●
274			●	●	●	●	●	●	●	●	●	●
277		●	●	●	●	●	●	●	●	●	●	●
279		●	●		●		●	●	●	●	●	●

Figure 35- Representative scheme of the allele pattern in 14 primers in the different analyzed individuals of *Lippia alba*. The numbers 1 to 12 represent the samples and different colors were chosen for each allele.

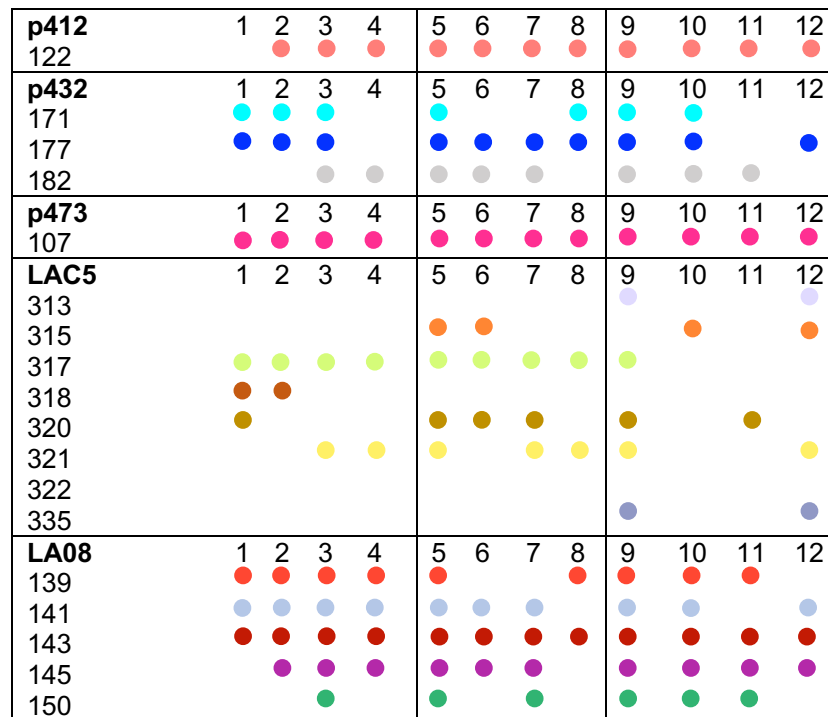


Figure 35- Representative scheme of the allele pattern in 14 primers in the different analyzed individuals of *Lippia alba*. The numbers 1 to 12 represent the samples and different colors were chosen for each allele (cont.).

Regarding the artificial crossing, in 6 primers (p283, LAC4, p412, p473, LAC5 and LA08) it was possible to observe alleles of the offspring that appear only in the male-parent. The figure 36 exemplify this situation. The red arrow shows an allele of the offspring that does not appear on the mother plant but appears on the male-parent. As the maternal progenitor is certain due to the experimental design and manipulation of the material, we can assume that this offspring comes from the crossing between this maternal individual (diploid BGEN-77) and the triploid BGEN-59. The microsatellite technique confirms the successful of the artificial crossing carried out in the species.

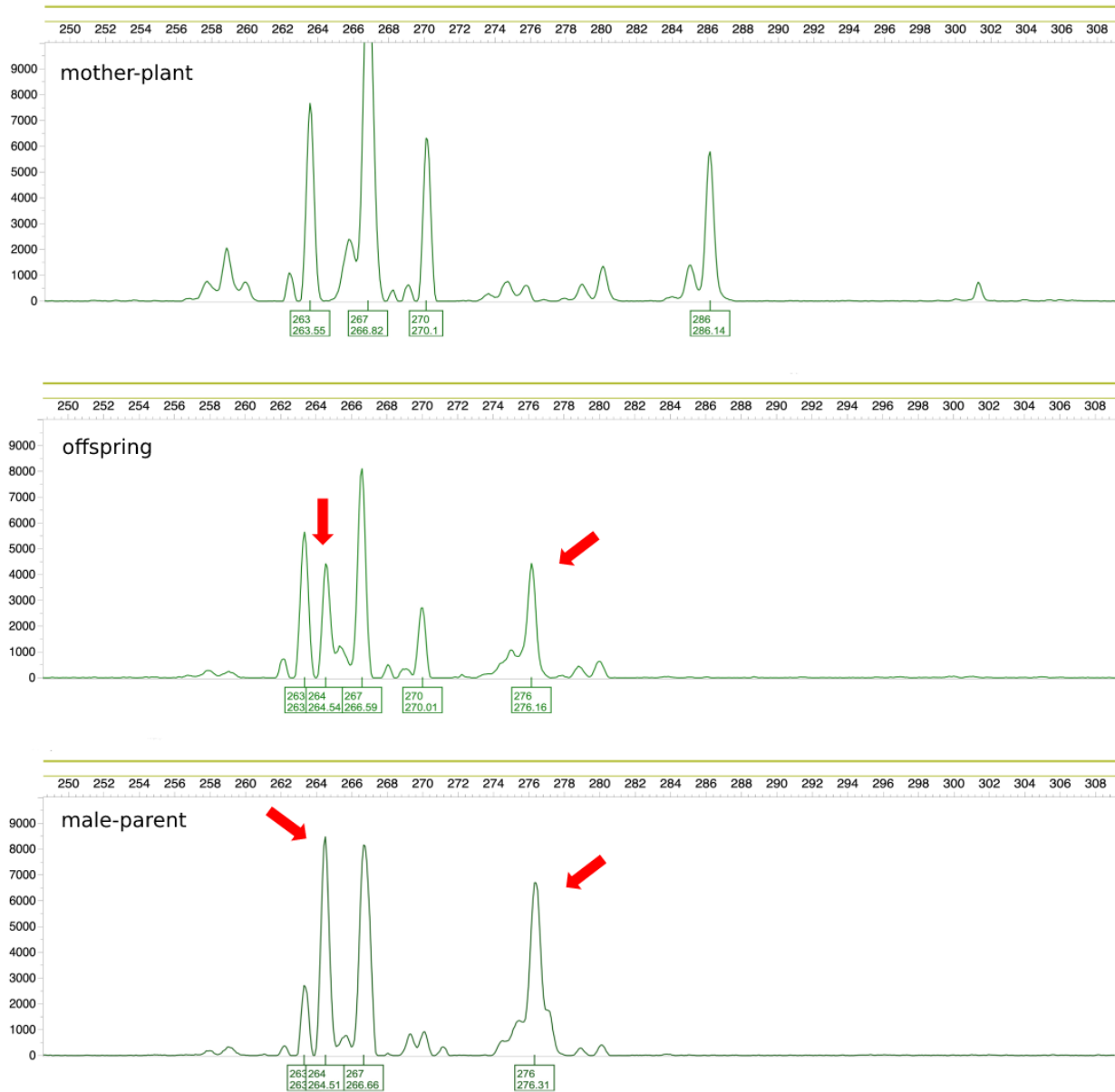


Figure 36- Representative electropherograms of primer LAC4 of three individuals of *Lippia alba*. The sequence of samples corresponds to the diploid mother plant BGEN-77, the offspring with $2n=35$ (from artificial crossing) and the male-parent triploid BGEN-59.

The microsatellite analysis also revealed, as shown in the table 15, that out of the 14 primers, in 8 of them it was possible to observe at least in one offspring a pattern of bands different from the pattern observed in its mother plant, suggesting self-fertilization instead of apomixis.

Table 15- Offspring samples of *Lippia alba* that showed a pattern of bands different from its mother plant (selfing), under controlled pollination. Each plant was associated with the used primers.

Sample	Primers
4	p432
6	p229, p249, p432
7	p484, p432
8	p229, p412, LA08
10	p229, p249, p412
11	p412, p432, LAC5
12	p294, p249, p412, p432

On the other hand, some individuals showed a pattern of bands identical to the mother plant, suggesting the possibility of an apomixis or a self-fertilization in which the alleles remained the same as the mother. In fact, despite these identical bands, based on the data in table 15, it was possible to conclude that all samples from controlled pollination (4, 6, 7, 8, 10, 11, 12) came from self-pollination, reinforcing once again the occurrence of the reproductive process in *Lippia alba* under controlled pollination.

The Figures 37, 38 and 39 exemplifies that information.

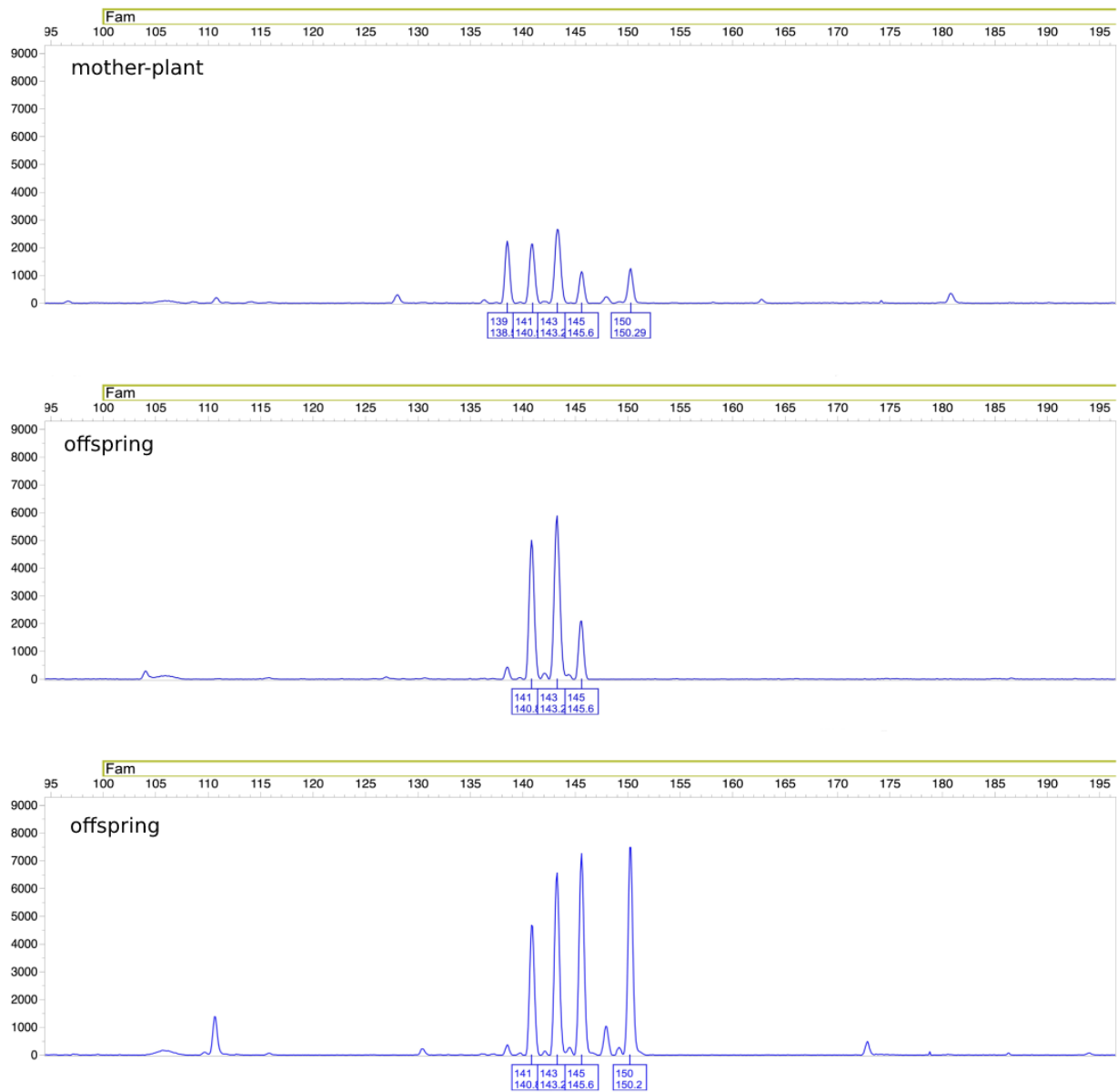


Figure 37- Representative electropherograms of primer LA08 of three individuals of *Lippia alba*. The sequence of samples corresponds to the triploid mother plant BGEN-27 (sample 5) and two offspring (sample 6 and 7) from controlled pollination.

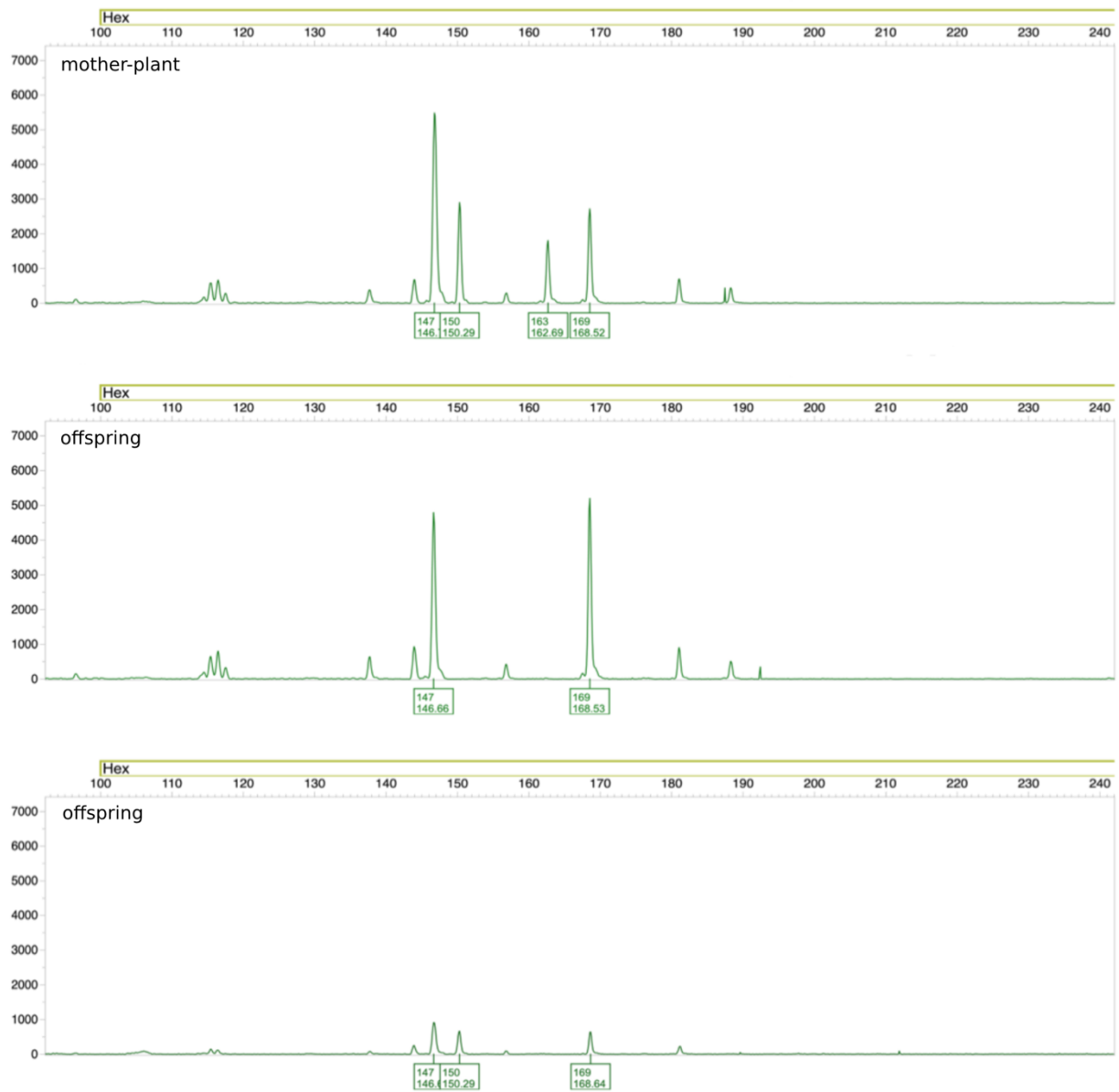


Figure 38- Representative electropherograms of primer p229 of three individuals of *Lippia alba*. The sequence of samples corresponds to the triploid mother plant BGEN-10 (sample 9) and two offspring (samples 10 and 12) from controlled pollination.

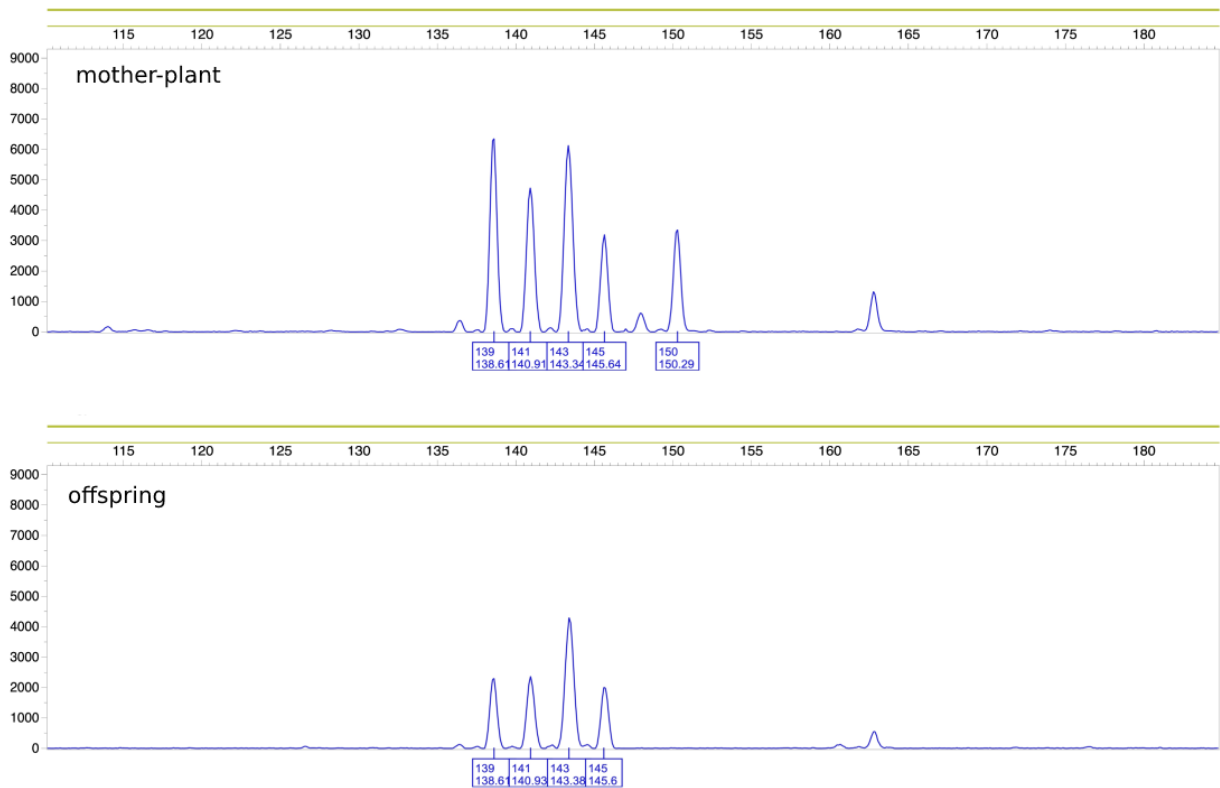


Figure 39- Representative electropherograms of primer LA08 of two individuals of *Lippia alba*. The sequence of samples corresponds to the triploid mother plant BGEN-59 (sample 3) and its offspring (sample 4) from controlled pollination.

4.6- Pollen analysis

In order to investigate the production of unreduced pollens, pollen measurements were carried out in 29 diploid individuals. The average of pollen size, including the minimum and maximum values can be seen in table 16.

To analyze the data distribution of each individual, the values observed between the lowest and the highest were classified in quartiles. As an example, the table 17 illustrates how the minimum (23.17 μ m) and the maximum value (47.44 μ m) of pollen measurements of BGEN-38. The difference between the highest and lowest values was divided into 4 groups: 23.17 μ m - 29.24 μ m (quartile 1); 29.25 μ m - 35.32 μ m (quartile 2); 35.33 μ m - 41.4 μ m (quartile 3) and 41.5 μ m - 47.44 μ m (quartile 4). As an example, table 16 shows the number of pollen measurements observed in each quartile. In the case of BGEN-38, it was possible to observe 90 measurements in the 1st quartile range, 699 in the 2nd quartile, 179 in the 3rd quartile and 32 in the 4th quartile, totaling 1000 measurements. The objective of dividing the measurements into quartiles is to facilitate the visualization of the measurement's distribution. Regarding the calculated quartiles, most of the accessions presented the largest number of measures concentrated in the second and third quartiles, in contrast of the first and fourth quartiles, that cover the extreme values (the smallest and the largest ones). In other words, even when the individual had a very small minimum measurement or a very large maximum measurement, most of the data obtained are not part of these extremes.

The lowest average observed was 29.47 μ m from BGEN-57 individual, while the highest was 43.83 μ m from BGEN-75. The smallest pollen measure found in the species, in turn, was 14.99 μ m from BGEN-89, while the highest was 60.23 μ m from BGEN-12.

Table 16- Pollen measurement data from 29 diploid individuals of *Lippia alba*

Individual	Q1	Q2	Q3	Q4	Minimum value	Maximum value	Average
BGEN-11	2	202	589	207	18.27	49.22	38.03
BGEN-12	317	377	300	6	24.78	60.23	38.28
BGEN-13	18	441	381	160	19.10	49.43	35.57
BGEN-17	516	334	133	17	26.87	56.59	35.80
BGEN-21	412	77	110	1	29.32	43.92	33.75
BGEN-38	90	699	179	32	23.17	47.44	33.17
BGEN-40	99	150	621	130	24.72	53.17	41.14
BGEN-43	72	65	336	27	22.79	44.87	32.67
BGEN-46	146	249	533	72	28.48	55.41	42.01
BGEN-48	339	572	73	16	27.94	50.44	35.03
BGEN-55	2	594	399	5	19.56	48.71	33.95
BGEN-56	186	6	293	115	28.36	49.11	37.90
BGEN-57	431	460	97	12	23.46	43.53	29.47
BGEN-58	11	888	72	29	20.32	58.56	35.73
BGEN-65	242	667	87	4	24.08	57.05	35.52
BGEN-67	647	280	45	28	23.84	55.77	32.15
BGEN-68	183	462	308	47	24.85	48.30	34.87
BGEN-72	83	430	336	151	24.42	48.34	36.55
BGEN-73	37	444	514	5	21.72	55.73	38.44
BGEN-75	30	210	701	59	27.76	55.92	43.83
BGEN-77	291	445	215	49	25.82	43.64	32.64
BGEN-78	549	192	190	69	26.15	54.22	35.60
BGEN-80	181	467	241	111	22.40	55.60	36.98
BGEN-81	278	604	113	5	29.62	45.87	35.16
BGEN-89	34	500	337	129	14.99	58.40	36.96
BGEN-90	48	196	535	221	24.98	46.75	38.22
BGEN-98	276	534	143	47	23.74	51.72	34.09
Planta F	163	688	144	5	25.51	40.01	30.89
Planta D	72	682	171	75	21.58	54.31	35.92

Q1=quartile 1; Q2=quartile 2; Q3=quartile 3; Q4=quartile 4

Table 17- Number of pollen measurements for a diploid accession (BGEN-38) of *Lippia alba*.

Quartiles	Measurements (μm)	Number of measurements
1	23.17 - 29.24	90
2	29.25 - 35.32	699
3	35.33 - 41.4	179
4	41.5 - 47.44	32
Total		1000

Among the diploid individuals, it was possible to observe differences in pollen measurement. Some of them showed greater variation (BGEN-58 and BGEN-89, Figure 40A and 40B), while others showed less variation such as BGEN-21 and individual F (Figure 40C and 40D). Below, the scatter plots illustrate the distribution of pollen measurements.

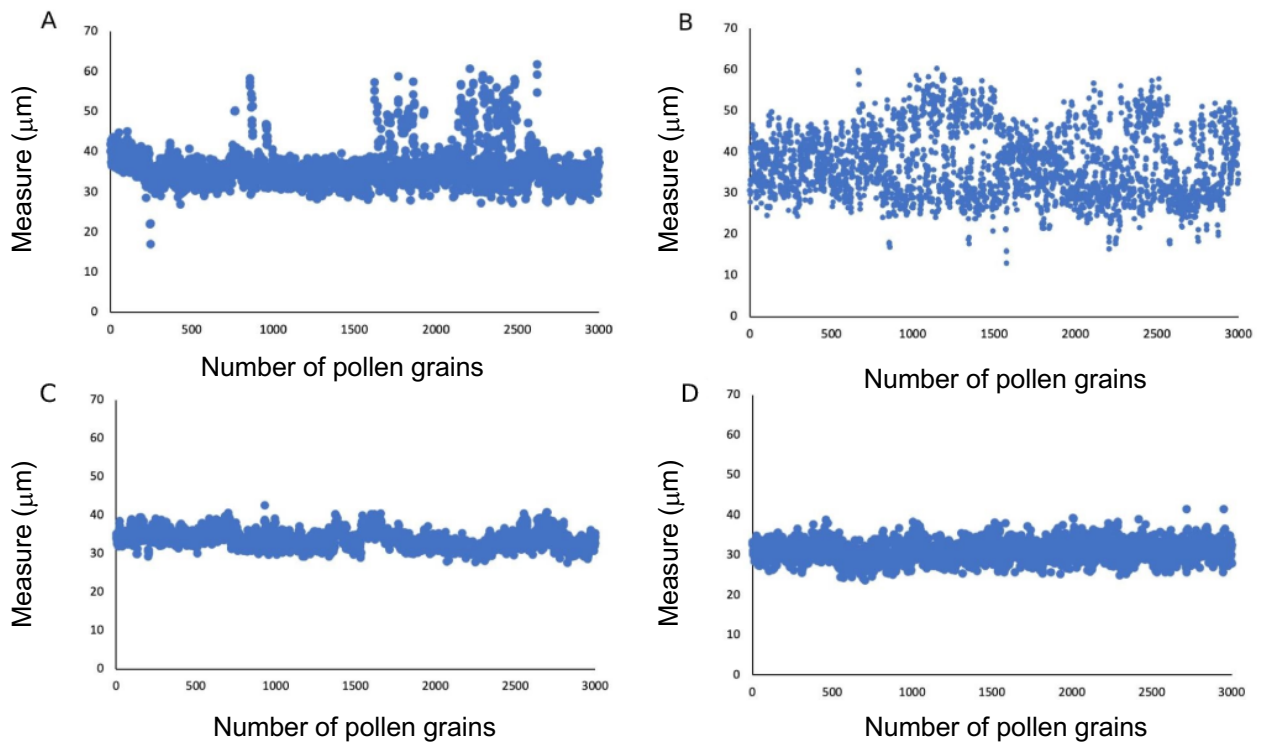


Figure 40- Representative scatter plots of pollen grains showing wide variations in two diploid individuals of *Lippia alba* (A: BGEN-58; B: BGEN-89) and less variations (C: BGEN-21; D: Planta F).

In Figure 41, it is possible to observe pollen grains of these same individuals: A: BGEN-58, B: BGEN-89, C: BGEN-21 and D: Planta F, representing the largest and smallest variations, respectively. Red arrows highlight this variation. Red arrows highlight this variation.

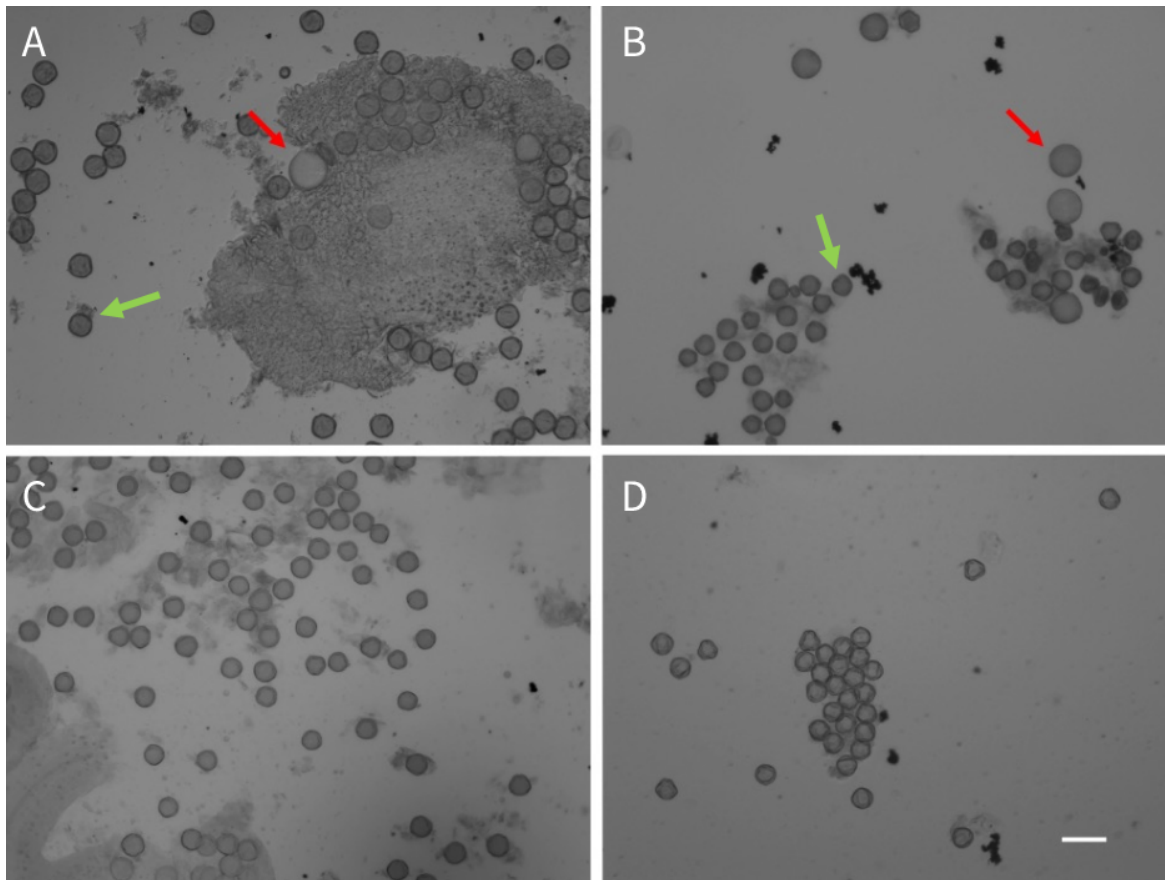


Figure 41- Pollen grains from different diploid individuals of *Lippia alba*. A and B: individuals with greater variation. C and D: individuals with lesser variation. Red arrows highlight bigger pollens and green arrows highlight smaller pollens. Bar=50 μ m.

4.7- Comparative anatomy of *Lippia alba* anthers and ovules

4.7.1- Pollen grains and microgametophytes

In summary, in diploid individuals (Figure 42A-E), well-formed pollen grains showed rounded median contour and high density in the protoplast, largely prevail. The generative cell has an ellipsoid shape, is long and has tapered ends. All anthers sampled contained germinating pollen grains, with evident protrusion of pollen tubes.

In triploid individuals (Figure 42F-J), there are numerous well-formed pollen grains, with a rounded median contour and high density in the protoplast. However, there are several aborted grains, both with irregular (very lobed) contour and others with typical contour, but hyaline protoplast in comparison to the well-formed grains. Less often, tetrads were observed whose grains were not individualized, always with evidence of abortion. In viable grains, the generative cell is similar in shape to that described for most diploid grains; however, pollen grains were observed with a more vacuolated vegetative cell and a generative cell without the characteristic contour, with well-curved ends, indicative of unviability. Pollen grains with protrusion of pollen tubes were also observed.

In tetraploid individuals (Figure 42K-N), it is noteworthy that the anther is clearly smaller than those from diploid and triploid individuals. In tetraploids, aborted pollen grains prevail, with a very lobed contour and without content. Few well-formed pollen grains are recognized in the samples.

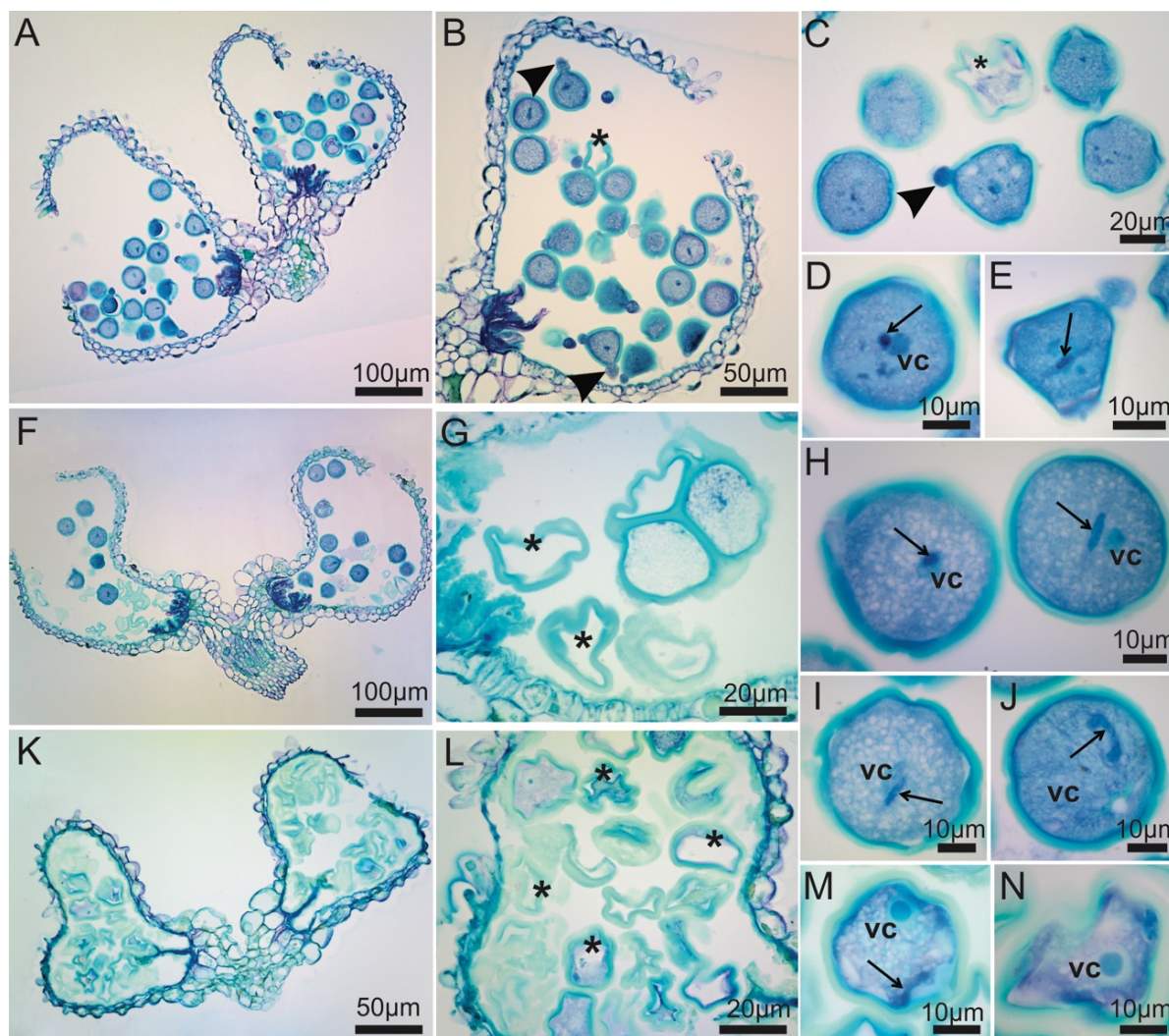


Figure 42- Mature anthers of *Lippia alba* in transversal sections stained with Toluidine Blue. **A-E.** Diploid individuals. **A.** General view of anther in dehiscence, showing the two theca. **B.** Detail of a theca; note the density of most pollen grains. **C-E.** Pollen grain details; in **D-E**, typical pollen grains in different section planes, with the ellipsoid generative cell, long and tapered, sectioned transversally (**D**) and longitudinally (**E**). **F-J.** triploid individuals. **F.** General view of anther in dehiscence, showing the two theca with several dense and typical shaped grains and others more hyaline, with an atypical contour that indicates their inviability. **G.** Detail of a theca; note pollen grains presented in monads or tetrads, all of which are not viable in this field. **H-J.** Pollen details. **H.** Pollen grains with typical characteristics. **I.** Grain with slightly lobed contour and protoplast more hyaline than typical grains. **J.** Grains with typical contour, but the protoplast is more hyaline than that seen in **H** and the generative cell does not have the characteristic contour, but rather curved ends. **K-N.** tetraploid individuals. **K.** General view of the anther at the beginning of the dehiscence, showing the two theca with several hyaline grains and with an atypical contour, aspects that indicate their inviability; observe that the anther is clearly smaller than those from diploid and triploid individuals. **L.** Detail of a theca; to notice nonviable pollen grains, with variable contours. **M-N.** Details of pollen that has some obvious content. **M.** Bicellular pollen grain, with very vacuolated vegetative cell and atypical generative cell, with curved ends. **N.** Grain with lobed contour, protoplast more hyaline than typical grains and only the vegetative cell formed. (asterisk: non-viable pollen, with irregular and atypical wall contour, without evident content; arrowhead: pollen grains emitting pollen tube; arrow: generative cell; vc: vegetative cell).

4.7.2- Ovules and Megagametophytes

In the ovules of flowers in anthesis of diploid individuals (Figure 43A-F), the presence of a unitegumented ovule is observed, with phenolic compounds deposited in the chalazal cells (characterizing hypostasis) and in the inner epidermis of the integument (characterizing the endothelium), that delimits the mature megagametophyte. The ovules are tenuicellate and, with the megagametophyte already constituted, show few nucellar residues, which are barely conspicuous and rarely recognizable (Figure 43D). The polygonum-type megagametophyte is well differentiated, being possible, in different sections of the longitudinal series, to recognize all seven cells that compose it (the two synergids and the egg in the micropylar pole, the three antipodes in the chalazal pole and the broad and vacuolated central cell, characteristically located between the two poles).

In triploid individuals (Figure 43G-I), the main variation is the increase in the presence of phenolic compounds, both superficially to the ovule and in the tegument (Figure 43G-H) and even bordering the megagametophyte (Figure 43I), an aspect not verified in diploids. Nucellar residues are more frequently observed, both close to the micropylar pole and laterally (Figure 43G-H). Although the antipodes were not recognized in the analyzed samples, the oospheric apparatus is sometimes well formed and accompanied by the central cell (Figure 43I), so that there may be megagametophyte viability.

In tetraploid individuals (Figure 43J-L), the megagametophytes did not fully develop and show immaturity even in the flower at anthesis. The greater persistence of the nucellus is highlighted (Figure 43K-L).

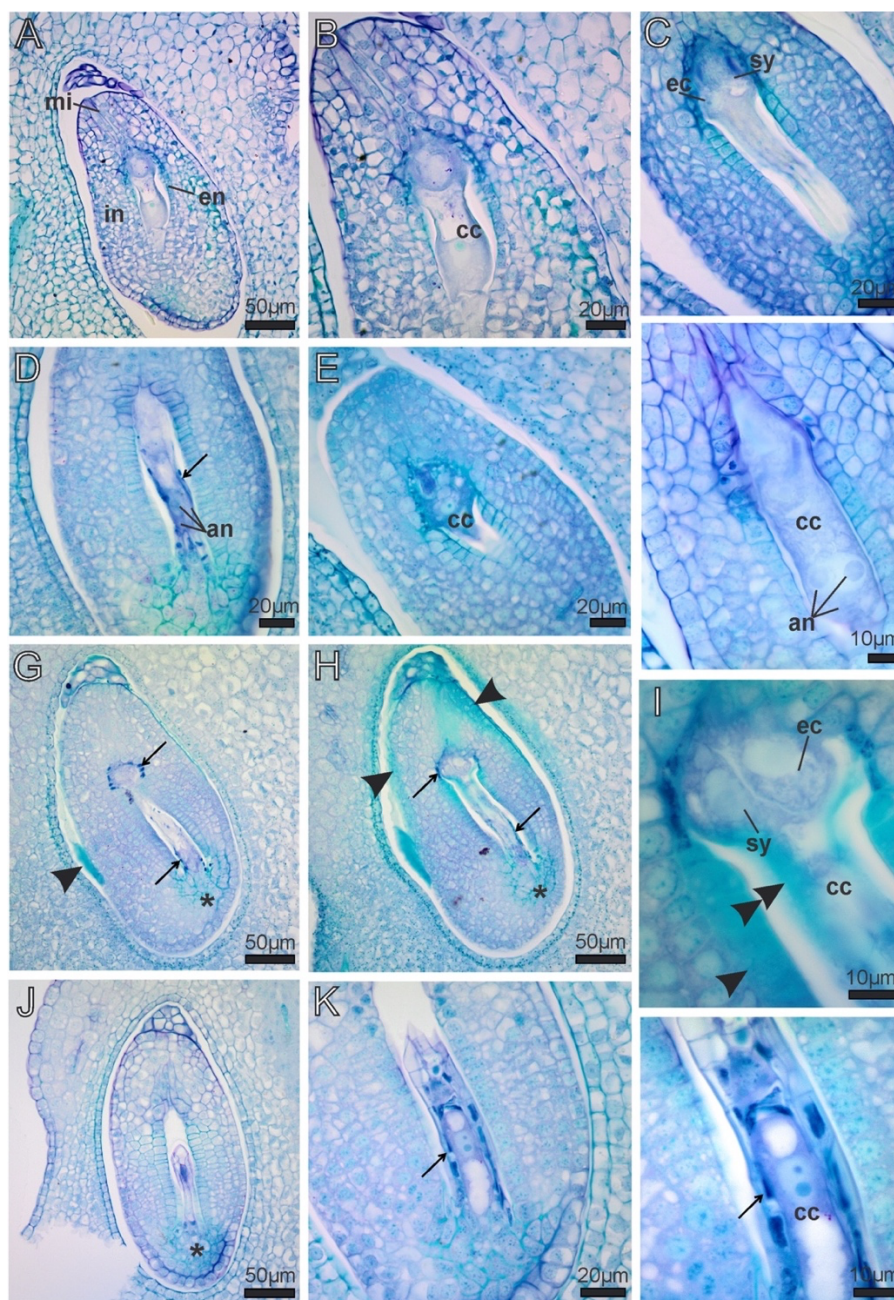


Figure 43- *Lippia alba* flower ovules in anthesis, seen in longitudinal sections stained with Toluidine Blue. **A-F** Diploid individuals. **A**. General view of the unitegumented ovule, with phenolic compounds deposited in the chalazal cells (characterizing hypostasis) and in the inner epidermis of the integument (endothelium), which delimits the megagametophyte. **B-F**. Ovules details showing different views of the megagametophyte. **G-I**. Triploid individuals; note the expansion of phenolic deposits, both tegumentary and bordering the megagametophyte (the latter in detail in I). **J-L**. Tetraploid individuals; note the immature-looking megagametophyte, accompanied by the more evident nucellus. **K-L**. Details of megagametophyte in which only the central cell is recognized. (asterisk: hypostasis; arrowhead: deposition of phenolic compounds in the tegument; double arrowhead: deposition of phenolic compounds in the megagametophyte; arrow: nucellar residues; an: antipode; cc: central cell; ec: oosphere; en: endothelium; in: tegument; mi: micropyle; sy: synergid).

5- DISCUSSION

The present work aimed to contribute on the understanding of the unprecedented description of the reproductive behavior of different accessions of *Lippia alba*. This investigation has particular importance considering the variation in the ploidal level previously reported for the species (REIS et al., 2104). Studies of this nature including an intraspecific variation of wild species are rare in the literature.

According to previous studies, *Lippia alba* has an excellent vegetative propagation (BIASI, 2003). Schocken (2007), suggests that the species is preferentially allogamous. The hypothesized that an allelic series may occur in the population that is linked to self-incompatibility, demonstrating a tendency to cross-fertilization hindering an alternative reproduction way. Pierre et al. (2011), indicated that sexual reproduction among three chemotypes is unlikely due to different chromosome numbers and suggests the beginning of the reproductive isolation in the species. Budeguer et al. (2013), in turn, claim that the presence of rhizomes could be an evolutionary consequence of the low viability of the seeds.

It is important to remember that *Lippia alba* is a polyploid complex with five different chromosome numbers (REIS et al., 2014) and that none of the reproductive studies so far addressed the presence of these different ploidal levels in the species. In addition, any systematic study on the reproduction of *Lippia alba* has been performed so far.

Based on our results it is possible to infer that *Lippia alba* presents different reproductive behaviors. The occurrence of plant species that have only one reproductive system is rare in nature. Pollination, in turn, has a great impact on the organization of sexual reproductive systems, and it has been verified that plants pollinated by animals (as *Lippia alba*) end up to a continuous distribution between the two types of reproductive system including forms with preferential autogamy and intermediate forms to preferentially allogamous individuals (VOGLER and KALISZ, 2001).

5.1- Diploids and polyploids of *Lippia alba* show different behavior regarding seed production and germination

The seed production analysis was performed during approximately six years. The samples were randomly collected according to the availability of material and the general result was similar throughout that time, allowing a robust conclusion. The data indicates an interesting behavioral difference between diploids and polyploids when subjected to controlled pollination. While some diploids are not capable of producing any seeds under the controlled pollination, all polyploids were capable to produce seeds. Clearly, the species has a preference for cross fertilization, especially the diploids.

Regarding seed production and germination from open pollination, statistical analysis showed that diploids are more efficient than polyploids, as expected, considering that the whole genome duplications impose major changes in cellular architecture and gene expression, and cause epigenetic instability and difficulties in mitosis and meiosis (HOLLISTER, 2015; DOYLE and COATE, 2019; SIOPA et al., 2020). The fact that diploids had a higher germination rate in open pollination can be associated to the data previously reported in *L. alba* by Reis et al. (2014; 2021), showing that these individuals hold the lower index of meiotic irregularity in contrast to polyploids. These irregularities are closely related to the polyploidization event due to the increase of structural chromosomal alterations such as inversion, deletion, and translocation, which can affect the karyotypic structure and even the fertility of the individual (MA and GUSTAFSON, 2005; WEISS-SCHNEEWEISS et al., 2013).

Reis et al. (2016), also observed in a tetraploid individual of *L. alba*, the phenomenon called cytomixis, which consists of the migration of nuclear or cytoplasmic material between adjacent cells (LATTOO et al., 2006; SINGHAL and KUMAR 2008; GUAN et al., 2012; MURSALIMOV et al., 2013; MURSALIMOV and DEINEKO 2015). Often, hybrid, aneuploid, polyploid and apomixis individuals are related to this phenomenon (DE NITTANCOURT and GRANT 1964; GOTTSCHALK, 1970; LI et al., 2009; PIERRE and SOUSA, 2011). Regardless of how cytomixis occurs, its genetic consequences are serious and can interfere with gametogenesis and fertility, producing unbalanced gametes due to an increase or decrease in DNA content (MURSALIMOV et al., 2013; MURSALIMOV and DEINEKO, 2015).

The seed size from open pollination revealed that polyploids had greater size and variation. Known as the gigas effect (STEBBINS, 1971; RAMSEY and RAMSEY, 2014), genome duplication can change the size of some fundamental organs, often because of an increase in cell size (MELARAGNO et al., 1993; LEVIN, 2002; DOYLE and COATE, 2019). Thus, it can be inferred that this greater variation in seed size observed in *L. alba* polyploids may be a consequence of this process.

Interestingly, the seed size data from controlled pollination showed that the seed average of diploid individuals decreased compared to its seeds from open pollination, while those from polyploids maintained or had higher average when the inflorescence were isolated. Considering the possibility of selfing especially in polyploid individuals, the extremely irregular meiosis could lead to an embryo with the same or larger DNA amount than its progenitor, leading to a larger seed size.

The controlled pollination also revealed lower germination of seeds from polyploid plants (especially tetraploids). This could be explained also by the meiosis instability of these individuals. Assuming the occurrence of self-fertilization, meiosis tends to be more regular in diploid than polyploids individuals. The most curious data regarding seed germination, both from controlled and open pollination, occurred in triploids. Although these individuals had the highest rates of meiotic irregularity and pollen unviability (reaching 78%) (Reis et al., 2022), they have an intermediate germination rate between diploids and tetraploids. Besides, the seed weight of these individuals comprises the largest average among ploidal levels.

The size of seeds observed in some polyploids of *L. alba*, both from controlled and open pollination, may also be associated with their germination difficulty. According to Haig and Westobi (1991), the imbalance of gene dosage in the endosperm of maternal and paternal origin is strongly linked to failures in the crossing between different ploidal levels of the same species. The authors also suggest that seed size can be influenced by this imbalance through gene expression being differentiated depending on which parent (maternal or paternal) it comes from. When the inheritance is maternal, the seeds tend to be smaller while the seeds that have received the gene by the paternal parent, tend to be larger. It is important to note, in turn, that when compared within the same ploidal level, diploids seeds from controlled pollination had smaller dimensions when compared to open

pollination ones. Considering the two possibilities found in the condition of reproductive isolation (apomixis and selfing), we have that both can be related to this wrong proportion in the progeny since the maternal and paternal material come from the same individual or one gives rise to an individual with only maternal genetic material. Although no evidence has been found so far, the asexual process cannot be ruled out. In this context, seeds from cross-fertilization would have a mixture of maternal and paternal material, and the paternal material could be more expressed and thus providing a greater dimension to the seeds.

5.2- Selfing as an alternative way of reproduction in *Lippia alba*

According to Venâncio et al. (2016), *Lippia alba*, which has hermaphrodite flowers, has a stigma receptivity period of 72 h. The anthers, in turn, release pollen grains until the third day of flowering, when the stigma is still receptive, allowing self-fertilization. When analyzing the morphology of the flowers, the anthers are positioned above the stigma what might facilitate selfing. Additionally, it was also observed that the floral longevity of the species is greater than that found in other species of the same family, such as *Lantana camara*, *Stachytarpheta glabra* and *Stachytarpheta maximiliani* (BARROS et al., 2001; ANTONINI et al., 2005; BARBOLA et al. al., 2006) increasing the attraction of pollinators that would contribute to cross-fertilization.

Despite the preference for crossing, the seed flow cytometry from controlled pollination suggests that self-fertilization is occurring in cases where the inflorescences were isolated. Considering individuals that were kept under controlled pollination and perform sexual reproduction, it was possible to infer that, somehow, the self-incompatibility system is not effective in some individuals, especially in polyploids. This fact was reinforced by the index of self-incompatibility.

Analyzing the index of self-incompatibility (ISI), it was possible to notice that diploids, triploids and tetraploids do not show the same behavior. The breakdown of self-incompatibility has been described as a direct result of genome duplications (BARRINGER et al., 2007; HUSBAND et al., 2008; ROBERTSON et al., 2011; SUTHERLAND et al., 2018; ZENIL-FERGUSON et al., 2019). The strength of the self-incompatibility system varies within and between populations (COOK and SOLTIS, 1999).

2000; MABLE, 2004; BRENNAN et al., 2005; COSTA et al., 2017). Thus, instead of a complete shift to self-compatibility as a necessary condition at initial stages of polyploid emergence, partial self-compatibility could be enough to reduce reproductive problems in newly arisen polyploid individuals (MABLE, 2004; HUSBAND et al., 2008). Besides, following polyploidization, colonizing new habitats or even within the progenitor's population, neopolyploid individuals are subjected to limited availability of mates of the same ploidy, thus giving rise to selection for reproductive assurance and the evolution of self-fertilization (BAKER, 1955; PANNELL and BARRETT, 1998; BROCHMANN et al., 2004; SIOPA et al., 2020).

Selfing provides the opportunity to escape mate limitation, enabling the production of new individuals, and increasing the likelihood of fixation of new polyploid lineages (LEVIN, 1975; RAUSCH and MORGAN, 2005; BUGGS and PANNELL, 2006). Although common (ALLEN and HISCOCK, 2008), losses of the self-incompatibility process have been documented when selection favors the ability to self-fertilize (BUSCH AND SCHOEN, 2008; IGIC et al., 2008). Newly formed neotetraploids, in sympatry with their diploid counterparts, are subjected to frequency-dependent selection, having difficulties in persisting and in establishing viable populations (LEVIN, 1975; THOMPSON and LUMARET, 1992; HUSBAND, 2000). Under this scenario, some levels of self-compatibility will enable the production of viable offspring. Selfing may thus ameliorate the negative effects associated with minority cytotype disadvantage. By reducing the fitness costs of intercytotype mating due to neotetraploid minority status, self-compatibility opens the possibility for selfing, increasing the probability of neotetraploid establishment within diploid populations (SIOPA et al., 2020).

Another important data regarding selfing as a reproduction mode is the offspring from the triploid BGEN-59 ($2n=45$) from controlled pollination, which has the chromosome number of $2n=48$. It is possible to rule out in this case the presence of asexual reproduction (apomixis) since the offspring chromosome number is not the same as the mother plant. Assuming that the meiosis of the triploid individual tends to be extremely irregular, infinite possibilities of gamete combinations may have been carried out in this process during the self-fertilization, such as $n=23$ plus $n=25$, or $n=20$ plus $n=28$, for instance.

The allele comparison data by microsatellite also reveal, in some primers, difference between the mother plant and its offspring from controlled pollination, suggesting the occurrence of the self-fertilization in the species.

Severe population bottlenecks can also occur after polyploidization (HUSBAND et al., 2008). When tetraploids arise in a diploid population through genome duplication, any reproduction between cytotypes is likely to result in triploid hybrid embryos, which are subject to severe fitness deficits due to low viability and fertility (MILLER and VENABLE, 2000; MABLE et al., 2004; HUSBAND et al., 2008). This creates substantial reproductive isolation between diploids and polyploids. Under such conditions, polyploids experience strong selection against the self-incompatibility system (SABARA et al., 2013). In fact, most polyploids emerging in diploid populations are transient individuals that fall victim to minority cytotype exclusion because most mating attempts result in inappropriate hybrids, and only those individuals that are self-compatible tend to persist (HUSBAND, 2000; PANNELL, 2000; PANNELL et al., 2004). This selective pressure for loss of self-incompatibility is quite prevalent as changes in the mating system have been correlated with changes in ploidy (ENTANI et al., 1999). In addition to strong selection against this process, polyploids can also be pre-adapted for loss of this self-incompatibility (HAUCK, 2006; TAO and IEZZONI, 2010). Genome duplication has been shown to reduce the efficiency of these self-incompatible systems, particularly gametophytic self-incompatibility systems, in which self-recognition is mediated by the haploid pollen genotype (HAUCK et al., 2006; MILLER et al., 2008).

Siopa et al (2020) report through pollination experiments that the self-incompatible *Jasione maritima* var. *maritima* has an intermediate breakdown of the SI system in some individuals favoring neopolyploid establishment and enabling the formation of some viable offspring, while maintaining the capacity for outcrossing (LLOYD, 1992).

5.3- Fitness and adaptability under different scenarios

Analyzing the adaptive value (“*fitness*”) of the different individuals of *L. alba*, it was possible to observe, as expected, that in an open pollination environment, without any type of external (human) interference, diploid individuals have the highest rates of seed production and germination. As already mentioned, whole genome duplications impose major changes that may cause instability and difficulties in mitosis and meiosis (RISSO-PASCOTTO et al., 2005; HOLLISTER, 2015; DOYLE and COATE, 2019).

Even producing seeds, unexpectedly, few diploids have lower fitness than other polyploids. Reis et al. (2022), already mentioned a remarkable variation within the diploid individuals of the species. Through data of non-homology found in satDNA regions within diploids, the authors suggested that molecular mechanisms might be responsible to control chromosome pairing/homology: unequal crossing-over, amplifications of satellite DNA by transposons activity; recombination between non-homologous carrying the same satDNA family and also eliminations of repetitive sequences. In general, together with the data on meiotic irregularity, it was possible to observe some extremely irregular diploid individuals. Considering natural variation and selection, even some diploids may have difficulty in adapting and prevailing when compared to polyploids. Possibly, diploids are also paleopolyploids and some individuals are not well adapted to this “new” scenario.

The isolated material (controlled pollination) from polyploids revealed an opposite behavior: in an environment where cross-pollination is somehow block, polyploids show better adaptability. Siopa et al. (2020) observed in *J. maritima* var. *maritima* that, contrarily to diploids that presented a significant reduction in the fitness of selfed progeny relative to outcrossed one, the offspring from selfing and outcrossing in neotetraploid presented similar fitness performances. The neotetraploids of the species might have benefited from selfing at initial stages after their formation. Genome duplications resulted in leaky self-incompatibility enabling the production of offspring under minority cytotype disadvantage, with selfed offspring having similar fitness as outcrossed ones. Their work supports theoretical assumptions that selfing might be important in neopolyploid establishment phase, although changes in self-incompatibility might not be abrupt and variability in the expression of self-incompatibility is expected.

The polyploid are known to be usually more adapted to environmental stress than diploids (SOLTIS et al., 2014; IANNICELLI et al., 2016; SHMEIT et al., 2020), which is supported by the observation that present-day polyploids often occur at increased frequencies in newly created, disrupted or harsh environments (RAMSEY. J., 2011; MADLUNG. A., 2013; DIALLO et al., 2016; VAN DE PEER et al., 2017). Also, it is widely accepted that, compared with diploids, polyploids have an increased potential for specific adaptation with a higher tolerance for a broader range of ecological and environmental conditions and environmental robustness. These properties might reduce their risk of extinction during periods of environmental turmoil (VAN DE PEER et al., 2009; CROW et al., 2006; VAN DE PEER et al., 2017). The increase of genetic variation, and the restoration of sexual reproduction through selfing or apomixis could have been advantageous for exploiting new and changed ecosystems (TE BEEST et al., 2012; HOVICK and WHITNEY, 2014; BOCK et al., 2015).

In *Lippia alba*, Nascimento (2020), analyzed the effect of abiotic stresses on growth, physiology, essential oil production and expression of genes related to the monoterpene pathway, in *in vitro* accessions of diploid, triploid and tetraploid accessions of *L. alba*. As result, polyploid accessions presented, in general, greater growth and metabolic tolerance under stresses, when compared to diploid accessions.

In *Lippia alba* polyploid complex, it is evident that polyploids are better seeds producers comparing to diploids, when their inflorescences were isolated. Despite the large meiotic irregularity of these individuals, the production of large amounts of seed in controlled pollination (especially in tetraploids), may indicate an "alternative" way of perpetuating the species. As self-incompatibility (SI) system may be broken on polyploids, the ability to produce seeds by self-fertilization may contribute to the adaptation of these individuals.

5.4- Artificial crossing and the role of triploids and aneuploids in the formation of the *L.alba* polyploid complex

As is already known, newly formed tetraploid individuals in a diploid population may face difficulties in establishing due to the inviability of its triploid offspring. However, in some cases, this triploid block is overcome, when triploid plants are not completely sterile, yield some viable gametes and, producing viable diploid, triploid, or tetraploid progeny from self-crossings or from crossings with diploids or tetraploids (BURTON and HUSBAND, 2000).

This process, called triploid bridge, involves the fusion of a reduced gamete from tetraploids with an unreduced gamete of diploid parent plants producing triploid offspring. In turn, these triploids might generate tetraploid offspring through selfing or through crossing with either diploid progenitors or other triploids, depending on their fertility (HUSBAND 2004; KOHLER et al., 2010; MASON and PIRES. 2015). Our results of flow cytometry suggests that both diploid and triploid can produce a tetraploid offspring, even in small amount.

In some plant species, the parental chromosome dosage in the endosperm seems to be critical for seed development and fertility. Deviations from the usual 2:1 ratio between maternal and paternal chromosome contributions disorganize the expression of parentally imprinted genes necessary for normal endosperm development (KOHLER et al., 2010; LU et al., 2012; HAIG, 2013). Seeds with vestigial endosperm tend to abort or are mostly infertile (BRINK and COOPER, 1947). Hence, the sexual fusion of a haploid reduced with a diploid unreduced gamete result in a seed with a triploid embryo but imbalanced endosperm that probably blocks triploid seed formation (RAMSEY and SCHEMSKE, 1998; SPIELMANN et al., 2003). Moreover, in case of triploid seeds do survive, the unequal chromosome number and severe difficulties regarding chromosomal pairing and segregation in meiosis make triploids gametophytically unstable, producing a variety of euploid ($1n$, $2n$, or $3n$) and aneuploid gametes (HUSBAND, 2004). Despite these information, Ramsey and Schemske (1998) evaluated that viable triploid in natural populations produced a surprisingly large amount of fertile euploid gametes. However, this statement refers primarily to pollen, for which a mean fertility of 31.9% is reported in triploid angiosperms.

Here we described the first report of an artificial crossing carried out in *L. alba* species. Probably due to the small size of the inflorescences and the fact that a polyploid series were just recently reported in *Lippia alba* (Reis et al., 2014), this type of data does not exist in the literature so far. The cross between a diploid and a triploid individual raises important questions about the role of triploids and aneuploids in the formation of the polyploid complex.

The success of the cross, the seed viability and individual development, points out the possible fertility of an euploid gamete coming from a triploid. Despite that, it is curious that the triploid used as pollen provider has a pollen unviability reaching 74% (REIS et al., 2021). Regardless of reliable figures for female gametes are scarce (BICKNELL and KOLTUNOW, 2004; PARISOD and BESNARD, 2007; SHARBEL et al., 2009; SCHINKEL et al., 2016; WANG et al., 2016) and need to be further investigated, this same extremely irregular triploid can produce viable offspring being the provider of female gametes. As an important example is an offspring with $2n=48$ that came from this triploid controlled pollination.

Kovalsky et al. (2017) studying *Turnera sidoides* autopolyploid complex, prove that despite frequent abnormalities in chromosome pairing and of unbalanced chromosome segregation during the first or second meiotic division, triploids individuals of *T. sidoides* can form some viable male and female gametes that possess euploid or mainly aneuploid chromosome numbers (ZHOU et al., 2008). Besides, cytogenetic analyses revealed that the meiotic behavior, percentage of normal and irregular sporads as well as pollen viability vary among triploids. Triploid originating by the fusion of $2n \times n$ gametes of the same taxon show a more regular meiotic behavior and higher fertility than triploids from the contact zone of diploids and tetraploids and triploids of hybrid origin. The finding of triploids in the progeny of $2x \times 4x$ experimental crosses, suggest that the triploid block may be overcome in this species complex as well in natural populations of *T. sidoides*. So far, our results regarding *L. alba* suggest that the triploid block is also overcome since diploids and tetraploids can produce viable seeds with DNA content of triploid individuals. Also, the triploids that already exist naturally in the species, can produce viable seeds.

The triploid bridge is reported in several studies making the triploids a key cytotype for incrementing intraspecific variation (RAMSEY and SCHEMSKE, 1998; SCHINKEL et

al., 2017; DOYLE and COATE, 2019). Our results corroborate with those of Reis et al. (2021), suggesting that in *L. alba*, the triploids play an important role in the formation of new cytotypes.

Regarding the aneuploids role in *L. alba* polyploid complex, Lopes et al. (2022) hypothesized through molecular, cytogenetic, reproductive and chemical approaches that in the species, the aneuploids originate from diploids and triploids, being one particular group of diploids the possible genitor of aneuploids. Interestingly, genetic, chemical and karyotypical data revealed differences among the aneuploids, despite having the same chromosome number ($2n=38$). All three aneuploids analyzed have chemical profile of essential oil closely associated to the genetic similarity index and chromosome composition.

The emergence of the aneuploid individual with $2n=35$ coming from the artificial crossing confirms the possibility of aneuploids formation from the cross between diploids and triploids, considering that the chromosome number is close to $2n=38$. Besides, the chemical profile indicates citral as major component of essential oil. The individual with $2n=35$ obtained artificially raises some questions related to the appearance and prevalence of aneuploids with different chromosome numbers in nature. Why so far only aneuploids of $2n=38$ chromosomes have been recorded naturally? What factors are behind the survival and prevalence of these individuals? Despite several possibilities of aneuploidy, why is “magic number” of $2n=38$ seems to be more viable? If the individual $2n=35$ was placed in nature along with the other cytotypes, what would be its chances of survival?

Other questions regarding the chemical profile arisen from these data. Why some $2n=38$ individuals have citral as major component while the other $2n=38$ has linalool? Why the $2n=35$ also has citral as the major component? What mechanisms of gene expression can be related to the characteristics and variations in the major component of the essential oil of the species?

Lopes et al (2022) also suggest that this chromosome number might be related to gene dosage since, somehow it seems to be more stable allowing those plants to survive. Further, the authors report viable seeds/offspring with an intermediary DNA amount from $3x$ individuals corroborating that the majority of aneuploids arise from triploid accessions.

The “triploid bridge” in turn, seems to be a reality in the species since the triploid state is considered as an intermediate state in the origin of other cytotypes in *Lippia alba*.

5.5- Crosses between cytotypes and the emergence of different chromosomal numbers

It is important to highlight that the individuals analyzed in this study came from a population of samples collected in all Brazilian regions, not constituting, in principle, a natural population. Despite of this fact, the different accessions have shared the same environment for approximately 13 years, allowing the investigation of gene flow and new individuals formation (CHAPMAN and ABBOTT, 2010; ARNOLD et al., 2015; HÜLBER et al., 2015). Certner et al. (2017) point out that the mixed-ploidy populations serve as a convenient microcosm for the study of factors affecting the origin and establishment of polyploid derivatives (HUSBAND, 2004; HANZL et al., 2014).

As previously mentioned, the evolutionary consequences of polyploidy are numerous and dynamic (COMAI, 2005; OTTO, 2007; WENDEL, 2015; BARKER et al., 2016; SOLTIS et al., 2016b) and immediately after this phenomenon, newly formed polyploids (neopolyploids) experience strong reproductive isolation from their diploid progenitors (VAN DE PEER et al., 2017). Flow cytometry data indicate that diploid individuals of *L. alba* tend to produce only diploid individuals with few exceptions. Based on these results, it is possible to suggest that this scenario may occur in *L. alba* if we consider a relatively recent complex behavior (neopolyploids). Somehow, it seems that the female structure of diploid plants “accepts” mainly gametes from other diploids, thus resulting in most diploid individuals. The analyzed offspring of $2n=30$ from a diploid is also an example of the tendency of diploids to give rise to diploids. From all the individuals available on the field, the male gamete that fertilized this individual was probably from another diploid.

Regarding the flow cytometry data, it was possible to detect that few diploid individuals produced aneuploid/triploid individuals. As is already known, the frequencies of unreduced gametes ($2n$) in natural populations are important to determine both the rate of neopolyploid formation and the probability of their establishment. Higher frequencies of

these $2n$ gametes result in higher rates of neopolyploid formation, regardless unilateral or bilateral pathway (FELBER, 1991; KREINER et al., 2017).

The triploids, in turn, give rise to the greatest variation in the amount of DNA, in other words, they give rise to individuals with different ploidies, from possible diploids, aneuploids and triploids to tetraploids. This result is already expected since these individuals have the highest rates of meiotic irregularity (REIS et al., 2021), possibly allowing the formation of gametes with different chromosomal numbers.

The tetraploids give rise in most cases to aneuploids, followed by diploids and triploids. What needs most attention in tetraploids is the fact that these individuals, who have $2n=60$, can originate theoretically diploid individuals ($2n=30$) by crossing. Assuming that meiosis of diploid individuals is more regular than that of the tetraploid, we can suggest that the male gamete that fertilized the tetraploid would be a $n=15$ coming from a diploid. It is interesting to note that although diploids indicate a possible reproductive isolation, crossings with higher ploidies can occur. Our best example of this viable crossing is the $2n=30$ offspring of a tetraploid from open pollination. Reis et al. (2014), analyzed the karyotype of the tetraploid BGEN-64, which is the same mother plant of this $2n=30$ offspring. Interestingly, comparing the karyotypes of the mother plant and the offspring, some differences can be observed such as the chromosome morphometry. While the mother plant has 7 submedian and 8 median chromosomes, the offspring has 3 submedian and 12 median chromosomes. As the progeny came from an open pollination, it is not known exactly who is the paternal parent, but we can infer that some of the submetacentric chromosomes were not inherited, indicating a possible sexual reproduction.

Sousa et al. (2009) classified 15 chromosome pairs as 10 median and 5 submedian chromosomes. Reis et al. (2014), in turn, reported 11 pairs of median and 4 submedian chromosomes also from a diploid accession. These data further evidence the karyotypic and genomic variation in *L. alba*. In the same study, the triploid cytotype ($2n=45$) had only 2 median and 13 submedian chromosomes. As diploids have more median chromosomes in their karyotypic formula, we can raise the hypothesis that the paternal inheritance of this individual with $2n=30$ that came from a tetraploid, is a diploid individual, since its karyotypic formula comprises a greater number of median chromosomes. At the same

time, it is difficult to confirm this hypothesis using only the karyotypic formula since due to the similarity between the chromosomes, small differences in the measurements can lead to different chromosome classifications.

Regardless of which paternal origin this individual has, this tetraploid individual was able to produce a viable individual with $2n=30$ through crossing.

Sutherland and Galloway (2021) analyzing *Campanula rotundifolia* autopolyploid complex observed ploidy-mediated differences in reproductive barriers and the apparent asymmetrical gene flow. Apparently, parentally imprinted small RNAs that regulate the development of endosperm can be involved on these mechanisms. In diploid–tetraploid crosses, imbalance of these RNAs results in aberrant seed development and poor viability, but the effect tends to be less severe when the maternal parents has the higher ploidy (HAIG and WESTOBY, 1988; SCOTT et al., 1998; STOUTE et al., 2012). A similar mechanism may be behind the formation of this $2n=30$ offspring in *L. alba*. Also, despite the number of 30 chromosomes, this individual could be a “compensated aneuploid”(CHESTER et al., 2012). Johnson et al. (2020) used a dosage series of maize long arm of chromosome 1 (1L) to compare relative global gene expression in different types and degrees of aneuploidy aiming to understand how the magnitude of genomic imbalance as well as hypoploidy affects global gene expression. In general, the results of their study illustrate that the greater the genomic imbalance, the greater are the modulations of gene expression across the genome.

Lopes et al., (2020) analyzed the genetic relationships and polyploid origins in the *Lippia alba* complex using molecular, chemical, and morphological data. The patterns of genetic and chemical variation were not related to geographical distribution. However, there was an association between ploidal levels and morphological characters. Here, we also observed an association between ploidal level and seed traits supporting the relationship between morphological data and DNA content. The authors also confirmed, as described by Viccini et al. (2014) that triploids are linalool producers while diploids and tetraploids produce citral. Observing our chemical data, it was possible to observe that chromosome number was associated with the major component of the essential oil. In other words, the offspring with $2n=30$ from diploid and tetraploids under open pollination and also an offspring with $2n=35$ from an artificial crossing showed citral as the major

component while the offspring with $2n=48$ from an isolated triploid, showed linalool as the major component. Also, Julião et al (2020) obtained an artificial triploid that presented linalool as a marjoritary component, reinforcing this differentiated characteristic of triploids. As already reported, these data raise questions regarding the relationship between the chromosome number and the essential oil composition in those individuals.

Lopes et al. (2020) also suggested that one particular group of diploids is a distinct lineage based on its unique DNA sequences and SSR markers. Also, there is only one origin of triploids from a single group of diploid accessions corroborating morphological and chemical data. The tetraploids in turn, showed from plastid sequence data, the maternal origin from diploid accessions.

Reis et al. (2014, 2021) suggest that *L. alba* complex shows a mixture of characteristics typical of young and older polyploids with individuals showing different stages of genomic organization. Naturally formed, the complex presents typical features of reticulated polyploids, which are still under genome stabilization. Taken the scheme proposed by Reis et al. (2014) as reference (Figure 44) for the origin of different ploidal levels in *Lippia alba*, we analyzed below different scenarios, adding some reproductive biology aspects on the origin of each cytotype.

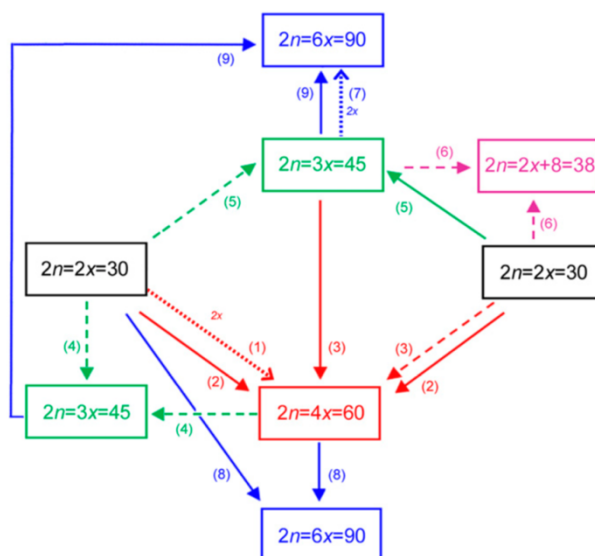


Figure 44- Representative scheme proposed by Reis et al (2014) for the origin of the different cytotypes in *L. alba*. Each cytotype is depicted by a different color. Continuous arrows represent unreduced gametes; dashed arrows represent reduced gametes; dotted arrows represent chromosome duplication (2x).

✓ Tetraploid ($2n = 60$) formation by chromosome duplication: it is rare in the literature and until now nothing that we have points to that possibility.

✓ Tetraploid ($2n = 60$) formation by bilateral cross between diploids: our data revealed that the diploid BGEN-75 produced a unique case of an offspring with DNA amount compatible or close the one of a tetraploid. The same diploid accession also produces larger pollen grains compatible with unreduced pollen. As the diploids tend to cross with diploids, it is possible that the paternal origin is another unreduced diploid gamete.

✓ Tetraploid ($2n = 60$) formation by cross between diploid and triploid: flow cytometry also revealed that the triploids can produce a tetraploid offspring, even in small amount. Consequently, some crossings possibilities can be suggested: a diploid reduced pollen ($n=15$) with a triploid unreduced female gamete ($n=45$); or a diploid unreduced pollen ($n=30$) with a female irregular triploid gamete ($n=30$).

✓ Triploid formation ($2n = 45$) by cross between diploid and tetraploid and also between diploids individuals: The seed and seedlings flow cytometry data show that diploids and tetraploids are capable of producing triploids. As our data shows a tendency to diploids produce only diploids, we can hypothesize that the triploid offspring from the diploids came from crossing between two diploids: one with unreduced gamete ($n=30$) and the other with a reduced one ($n=15$). The tetraploids in turn, as confirmed by diploid progeny from open pollination, are capable of receive gametes from diploid individuals. Taking this into account, we can infer that it is possible to have a cross involving a diploid reduced pollen ($n=15$) and a tetraploid female reduced gamete ($n=30$), giving rise to a triploid individual.

✓ Aneuploid ($2n = 38$) formation from crossing involving diploid ($n = 15$) and triploid ($n = 23$): our offspring analysis confirmed this possibility. Both diploids and triploids are capable to produce progeny with intermediate DNA amount between diploids and triploids. The artificial crossing confirms this variation of chromosome number possibilities among aneuploids, once the cross between a diploid and a triploid

gave rise to an individual with $2n=35$ chromosomes. As the number of chromosomes in these aneuploids can vary, it is not yet clear why the chromosome number $2n=38$ is the only one found naturally in the species so far.

✓ Hexaploid ($2n = 90$) formation by genome duplication of triploids, cross between diploid and tetraploid cytotypes, or alternatively bilateral cross between triploids cytotypes: our data revealed that tetraploids are possible hexaploid cytotype formers. Some tetraploids produce aneuploid offspring with an amount of DNA close to the natural hexaploid individual. While $2n=6x=90$ has around 6.90pg of DNA, the maximum observed so far was an offspring of 6.34pg of DNA, from a tetraploid accession. This data corroborate with those found by Lopes et al. (2020), that suggested, using molecular analysis, that the hexaploid cytotype possibly comes from tetraploids. Also, the lack of molecular correspondence between triploids and hexaploid accessions excluded the suggestion that the hexaploid arose from duplication of the triploid accessions. Instead, they suggest that the hexaploid ($2n = 6x = 90$) could be formed from reduced ($n = 30$) and unreduced ($n = 60$) gametes of tetraploids.

In addition to these hypotheses raised by Reis et al. (2014) regarding the formation of different cytotypes in the species, the data from the present work allow the addition of two additional ways based on the formation of smaller ploidies from larger ones.

✓ Diploid formation by crossing between a diploid and a tetraploid and also between a diploid and a triploid: flow cytometry data suggests the formation of possible diploid individuals from both triploid and tetraploid mother plant. As the diploids are represented by the largest number of individuals in the field and as they tend to produce pollen of $n=15$, we can infer that the paternal provider of these cross tends to be a diploid individual. As already mentioned, with flow cytometry alone it is not possible to confirm the chromosome number of the offspring. Even so, the individual $2n=30$ coming from an open-pollinated tetraploid provides us important evidence of these crosses.

Considering $31.51\mu\text{m}$ of diameter as a reference value for a normal pollen grain ($n=x$) and considering 25% of increase in size as an indication of non-gametic reduction (KOVALSKY and NEFFA, 2012), it was possible to observe that all individuals showed some possible non-reduced pollens. When we consider the parameter $40.04\mu\text{m}$ as a measure, the number of individuals decreases a little, but still corresponds to 48% of the total, indicating that even at a lower rate than the “normal” measures, non-reduced pollens are constantly produced in the species. As an example, the observation of unreduced microspores in the polyploid complex *Turnera sidoides* subsp. *carnea* (KOVALSKY and NEFFA, 2012) provide evidence that support the hypothesis of sexual polyploidization as one of the most probable mechanisms involved in the origin of polyploids within this species complex.

The frequency of these $2n$ gametes also affect the relative frequencies of different types of neopolyploids formed (e.g. $3x$ vs $4x$), thus influencing the relative rates of formation via single (one unreduced gamete) or dual (both gametes unreduced) pathways. Furthermore, for the polyploids establishment, these unreduced gametes must be generated at a sufficient rate taken into account the expected frequency-dependent mating disadvantage (minority cytotype exclusion). Higher production of $2n$ gametes by diploids not only generates neotetraploids at a faster rate (formation phase), but also increases the likelihood that neotetraploids will produce tetraploid progeny when they mate with diploids (establishment phase) (KREINER et al., 2017).

Observing the results of diploids individuals from seed flow cytometry, it was possible to notice that even in small proportion, the presence of 3:4 ratio (embryo/endosperm) what indicates a fusion between a normal meiotically reduced haploid egg cell of a diploid mother plant with an unreduced $2n$ pollen (SCHINKEL et al., 2017). Tetraploids typically produce reduced pollen with a diploid chromosome set ($2x$). Events whereby diploid pollen fertilize the egg of a sexual diploid would also result in triploid embryos accompanied by a tetraploid endosperm (3:4). Nonetheless, our results point out a partial isolation of diploids so far, in which diploid mother plants accept pollens only from diploid individuals, being the second option (tetraploids providing the diploid pollen) discarded at first.

In autopolyploids, the unreduced gamete formation should be directly correlated with the rate of autopolyploid formation in sexually reproducing organisms, because, unlike allopolyploids, they do not depend on a contact zone between two different taxa in order to form (SPOELHOF et al., 2017). Both effects of environmental factors (especially extreme heat and cold) and genotype on unreduced gamete formation are well characterized (reviewed in MASON and PIRES, 2015). Besides, many specific genes were identified with either directly or indirectly impact on meiotic reduction (reviewed in BROWNFIELD and K  HLER, 2011; CRISMANI et al., 2013; SPOELHOF et al., 2017). Unreduced gametes production also increases in the cases of relaxed selection of gametic viability, as in organisms with asexual reproductive strategies (KREINER et al., 2017).

As already reported, despite the unreduced egg play a large role in the formation of new autopolyploids, it is much easier to isolate and quantify unreduced pollen than eggs. In some cases, as reported from experimental crosses in *Achillea borealis*, the variation in the rate of unreduced pollen production had no statistical impact on the ploidy of the offspring (RAMSEY, 2007). The triploid progeny of *Dioscorea alata* and *Turnera sidoides* in turn, were only produced in cases where the egg was unreduced (NEMORIN et al., 2013; KOVALSKY and NEFFA, 2016). These findings are particularly notable due to the strong influence of triploids in the formation of autopolyploid lineages (i.e., the 'triploid bridge'). Also, triploids often have reduced fertility (K  HLER et al., 2010), but they frequently produce high proportions of unreduced gametes what may increase the probability of forming new tetraploids (RAMSEY and SCHEMSKE, 1998). Stable autotriploid lineages also occur in ferns and angiosperms and are frequently characterized by asexual reproductive habits (WHITTON et al., 2008).

In the histological sections of the microgametophyte and megagametophyte analysis of *L.alba*, it was not possible to observe a difference in the two pollination conditions (open and closed). It was interesting to observe, in turn, the germination of pollens occurring still in the anther, at all ploidy levels. As expected, the highest number of aborted pollens was seen in tetraploid individuals, followed by triploids. In the formation of the megagametophyte it was also possible to observe problems in the tetraploids

female gametes formation suggesting a difficulty of these individuals in the normal production of these structures.

Despite of the importance of unreduced pollen analysis, it is important to emphasize the important contribution of obtaining data regarding the amount of unreduced female gametes produced in the different cytotypes of *L. alba* in order to better understand the formation of different chromosome numbers in the species.

5.7- The importance of asexual reproduction in *Lippia alba*

The combination of sexual and asexual reproduction is found in many flowering plants, contributing to enormous diversity in reproductive systems (FRYXELL, 1957; BARRET, 2015). The vegetative reproduction, also referred as clonal growth or vegetative propagation, is the most common type of asexual reproduction and it has been estimated that ~80% of angiosperms reproduce by this way (KLIMES et al., 1997; VALLEJO-MARIN et al., 2010). Clonal propagation is also used widely in horticulture, agriculture, and forestry to replicate desirable varieties of domesticated cultivars and produce uniform performance.

As already highlighted in the literature, *Lippia alba* presents a great facility of clonal propagation when compared to other species of the genus *Lippia* (PIMENTA et al., 2007). Besides, the presence of rhizomes could be an adaptation to the low viability of seeds (BUDEGUER et al., 2013). Although asexual reproduction by seeds (apomixis) has not been confirmed in *L. alba* so far, clonal reproduction seems to be an important factor of dispersion in the species.

When sexual reproduction is not possible or efficient, clonal reproduction enables newly formed polyploids to persist by forming sufficiently large populations. Also, in facultatively sexual species, clonality may increase the degree of foraging and pollination within the same ploidy, thus reducing the strength of minority cytotype disadvantage. In addition, the prevalence of vegetative propagation may allow neopolyploids to persist long enough for additional sexually compatible polyploid(s) to be formed within a population. Although the link between polyploidy and apomixis is widely explored (e.g. WHITTON et al., 2008), only few studies addressed the association between karyological properties

and the intensity of vegetative reproduction. While some comparisons at intra-specific level failed to find any inter-ploidy differences (e.g. KEELER, 2004; BALDWIN and HUSBAND, 2013), others reported greater vegetative reproduction in (high) polyploids as compared with their diploid/lower polyploid counterparts (ECKERT et al., 2003; DUCHOSLAV and STANKOVA, 2015).

It is interesting to mention that some new individuals emerge in our experimental area through the time but not in large quantities evidencing that asexual still represent an important way of reproduction in *Lippia alba*.

Despite of many reports on the nature about the relationship between asexual reproduction by seed (apomixis) and polyploidy and its influence on the formation of polyploid complexes, little is known about the relationship between vegetative asexual reproduction and polyploidy opening another important aspect to be explored, especially in *Lippia alba* polyploid complex.

5.8- Final considerations

Here we described the first systematic study on the reproductive biology of *Lippia alba* integrating morphological, chromosomal, molecular and chemical data. The lack of any previous information on this theme, the small size of the floral structure – hindering artificial crossings in large amount - constitute a huge challenge for a definitive conclusion on the species' reproduction mode. The use of different approaches here employed, aimed to obtain a set of data that, together, allowed the elaboration of a working hypothesis.

Despite the important conclusions obtained in this work, it is necessary to continue to obtain data in order to acquire the greatest number of information and possibilities about the formation of different cytotypes in the species. The use of different techniques aiming to deepen the analysis of the offspring obtained as well as to carry out new artificial crosses between the individuals, can bring important contributions.

Our study points to an even greater complexity involved in the formation of the *Lippia alba* polyploid complex. Furthermore, the data obtained in this study add

information to the species database, aiming at not only understand the evolutionary process in tropical species, but also to contribute in the genetic improvement of the species.

6- CONCLUSION

With the data obtained so far, it is possible to conclude that:

- *Lippia alba* has a preferentially allogamous reproductive behavior;
- Diploid individuals showed a differentiated reproductive behavior when compared to polyploid individuals;
- In addition to cross-fertilization, self-fertilization is also observed, especially in polyploids;
- Possible break of the self-incompatibility system was observed in polyploids;
- Diploids showed partial reproductive isolation comparing to other ploidal levels;
- Diploids can be formed from triploids and tetraploids;
- The production of unreduced gametes from diploids might represent a source of new polyploid individuals;
- Triploids seems to be important in the formation of different ploidal levels.
- In addition to allogamy, *Lippia alba* polyploid complex has alternative reproduction pathways, expanding the possibilities to generate new polyploids.

7- APPENDIX

APPENDIX A- Identification, ploidal level, DNA content, registration number and origin of different individuals of *Lippia alba*

Identification	Ploidal level	2n	DNA (pg)	Herbarium number	Origin
BGEN-01	3x	45	3.89	48375	ABC / DF
BGEN-02	2x	30	2.56	48374	Araguaina/ TO
BGEN-03	4x	60	4.77	48373	Atibaia/SP
BGEN-06	4x	60	4.82	48370	Brazilândia I/DF (Chácara das Corujas)
BGEN-07	4x	60	4.83	48386	Brazilândia II/ DF (Chácara das Corujas)
BGEN-09	2x	30	2.61	48393	Ceasa de Brasília/DF
BGEN-10	3x	45	3.93	48391	Cruzeiro/Grande/DF
BGEN-11	2x	30	2.62	48390	Cruzeiro/Médio/DF
BGEN-12	2x	30	2.66	48389	Cruzeiro/Pequena/DF
BGEN-13	2x	30	2.70	48400	Cruzeiro Sem./Grande/DF
BGEN-17	2x	30	2.57	48396	Florianópolis/SC
BGEN-19	2x	30	2.67	48398	GDF/FZDF/ DF
BGEN-20	2x	30	2.65	48456	Goiânia/GO
BGEN-21	2x	30	2.61	48458	Ilhéus/BA
BGEN-23	3x	45	4.00	48460	Lavras/MG
BGEN-24	3x	45	3.90	48462	Luminárias/MG
BGEN-26	4x	60	4.85	48463	Manaus/AM
BGEN-27	3x	45	3.99	48464	Muzambinho/MG
BGEN-30	2x	30	2.57	48467	Planaltina de Goiás/GO
BGEN-31	4x	60	4.77	48468	Posse/GO
BGEN-38	2x	30	2.66	48382	Curitiba/PR
BGEN-40	2x	30	2.65	63827	Botucatu/SP
BGEN-43	2x	30	2.63	63799	Juiz de Fora/MG
BGEN-45	4x	60	4.82	63743	Atibaia/SP
BGEN-46	2x	30	2.57	63744	Brazilândia I/DF (Chácara das Corujas)
BGEN-47	4x	60	4.86	63745	Brazilândia/Cafuringa/ DF
BGEN-48	2x	30	2.58	63746	Ceasa de Brasília/DF
BGEN-49	3x	45	3.94	63747	Cruzeiro/Grande/DF
BGEN-56	2x	30	2.63	63755	IBAMA/SEDE/DF
BGEN-57	2x	30	2.57	63751	Ilhéus/BA
BGEN-58	2x	30	2.56	63752	Lago Norte/Brasília/DF
BGEN-59	3x	45	3.90	63753	Lavras/MG
BGEN-60	3x	45	3.80	63798	Luziânia/GO
BGEN-62	3x	45	3.95	63756	Piracicaba/SP
BGEN-64	4x	60	4.84	63758	Posse/GO
BGEN-65	2x	30	2.58	63759	Recife/Boa Viagem/PE
BGEN-67	2x	30	2.47	63761	Santa Maria da Vitória/BA
BGEN-68	2x	30	2.61	63762	São Paulo/Ceasa/SP
BGEN-70	4x	60	4.74	63764	Brasília/DF
BGEN-71	2x	30	2.59	63765	Brasília Bairro Sudoeste/DF
BGEN-72	2x	30	2.59	63766	Uberlândia/MG
BGEN-73	2x	30	2.48	63767	Estação Biológica UNB/DF
BGEN-74	2x	30	2.64	63768	Faz. Água Limpa UNB/DF
BGEN-75	2x	30	2.54	63770	Curitiba/PR
BGEN-77	2x	30	2.57	63772	Bairro Vicente Pires/ DF
BGEN-78	2x	30	2.53	63773	Viçosa/ MG
BGEN-80	2x	30	2.56	63774	Aracaju/ Parque da Sementeira/ SE
BGEN-81	2x	30	2.63	63775	Aracaju/ Aruana/ SE

APPENDIX A- Identification, ploidal level, DNA content, registration number and location of origin of different individuals of *Lippia alba* (cont.).

Identification	Ploidal level	2n	DNA (pg)	Herbarium number	Origin
BGEN-86	4x	60	4.81	63779	Rio Real/3ª travessa da Creche Betel/ BA
BGEN-87	4x	60	4.9	63780	Rio Real/Rua joaquim Matos/ BA
BGEN-88	2x	30	2.33	63781	Rio Real/ Rua Joaquim Matos/ BA
BGEN-89	2x	30	2.53	63782	Juiz de Fora/MG
BGEN-90	2x	30	2.52	63783	Rio Real/ Povoado salgado / BA
BGEN-96	2x	30	2.55	62382	Santana do S. Francisco/Assent. Samanbira/ SE
BGEN-98	2x	30	2.51	63789	Santana do S. Francisco/Pov. Saúde/ SE
BGEN-109	3x	45	3.9	64027	Lost identification

APPENDIX B- Fitness (W) and coefficient of selection (s) regarding average of seed production under open pollination in different ploidal levels of *Lippia alba*.

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-30	2x	1.000	0
BGEN-20	2x	0.907	0.093
BGEN-78	2x	0.842	0.158
BGEN-09	2x	0.819	0.181
BGEN-12	2x	0.771	0.229
BGEN-98	2x	0.759	0.241
BGEN-13	2x	0.744	0.256
BGEN-77	2x	0.735	0.265
BGEN-96	2x	0.732	0.268
BGEN-21	2x	0.724	0.276
BGEN-56	2x	0.703	0.297
BGEN-48	2x	0.702	0.298
BGEN-80	2x	0.680	0.320
BGEN-89	2x	0.675	0.325
BGEN-46	2x	0.655	0.345
BGEN-57	2x	0.646	0.354
BGEN-58	2x	0.643	0.357
BGEN-71	2x	0.605	0.395
BGEN-81	2x	0.582	0.418
BGEN-74	2x	0.566	0.434
BGEN-75	2x	0.565	0.435
BGEN-72	2x	0.559	0.441
BGEN-73	2x	0.547	0.453
BGEN-90	2x	0.545	0.455
BGEN-87	4x	0.536	0.464
BGEN-17	2x	0.518	0.482
BGEN-40	2x	0.517	0.483
BGEN-31	4x	0.511	0.489
BGEN-88	2x	0.498	0.502
BGEN-62	3x	0.494	0.506
BGEN-65	2x	0.491	0.509
BGEN-43	2x	0.469	0.531
BGEN-67	2x	0.444	0.556
BGEN-47	4x	0.429	0.571
BGEN-07	4x	0.409	0.591
BGEN-86	4x	0.393	0.607
BGEN-45	4x	0.371	0.629
BGEN-64	4x	0.347	0.653

APPENDIX B- Fitness (W) and coefficient of selection (s) regarding average of seed production under open pollination in different ploidal levels of *Lippia alba* (cont.).

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-06	4x	0.339	0.661
BGEN-11	2x	0.265	0.735
BGEN-27	3x	0.265	0.735
BGEN-49	3x	0.261	0.739
BGEN-03	4x	0.242	0.758
BGEN-59	3x	0.161	0.839
BGEN-24	3x	0.138	0.862
BGEN-01	3x	0.129	0.871
BGEN-02	2x	0.049	0.951

APPENDIX C- Fitness (W) and coefficient of selection (s) regarding average of seed production under controlled pollination in different ploidal levels of *Lippia alba*.

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-62	3X	1.000	0.000
BGEN-87	4X	0.896	0.104
BGEN-45	4X	0.667	0.333
BGEN-86	4X	0.639	0.361
BGEN-31	4X	0.614	0.386
BGEN-06	4X	0.603	0.397
BGEN-07	4X	0.587	0.413
BGEN-64	4X	0.557	0.443
BGEN-47	4X	0.546	0.454
BGEN-03	4X	0.428	0.572
BGEN-96	2X	0.413	0.587
BGEN-80	2X	0.234	0.766
BGEN-59	3X	0.194	0.806
BGEN-75	2X	0.181	0.819
BGEN-81	2X	0.170	0.830
BGEN-67	2X	0.111	0.889
BGEN-13	2X	0.085	0.915
BGEN-43	2X	0.069	0.931
BGEN-12	2X	0.055	0.945
BGEN-98	2X	0.049	0.951
BGEN-27	3X	0.046	0.954
BGEN-72	2X	0.045	0.955
BGEN-24	3X	0.035	0.965
BGEN-49	3X	0.029	0.971
BGEN-01	3X	0.020	0.980
BGEN-65	2X	0.020	0.980
BGEN-73	2X	0.019	0.981
BGEN-89	2X	0.014	0.986
BGEN-57	2X	0.012	0.988
BGEN-78	2X	0.009	0.991
BGEN-02	2X	0.008	0.992
BGEN-48	2X	0.007	0.993
BGEN-71	2X	0.006	0.994
BGEN-88	2X	0.003	0.997
BGEN-46	2X	0.002	0.998
BGEN-21	2X	0.002	0.998
BGEN-58	2X	0.001	0.999
BGEN-09	2X	0.001	0.999

APPENDIX C- Fitness (W) and coefficient of selection (s) regarding average of seed production under controlled pollination in different ploidal levels of *Lippia alba* (cont.).

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-77	2X	0.001	0.999
BGEN-40	2X	0.000	1.000
BGEN-56	2X	0.000	1.000
BGEN-74	2X	0.000	1.000
BGEN-30	2X	0.000	1.000
BGEN-20	2X	0.000	1.000
BGEN-90	2X	0.000	1.000
BGEN-11	2X	0.000	1.000
BGEN-17	2X	0.000	1.000

APPENDIX D- Fitness (W) and coefficient of selection (s) regarding seed length under open pollination in different ploidal levels of *Lippia alba*.

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-59	3X	1	0.000
BGEN-27	3X	0.841	0.159
BGEN-01	3X	0.817	0.183
BGEN-65	2X	0.795	0.205
BGEN-40	2X	0.760	0.240
BGEN-43	2X	0.756	0.244
BGEN-74	2X	0.748	0.252
BGEN-09	2X	0.729	0.271
BGEN-77	2X	0.721	0.279
BGEN-86	4X	0.705	0.295
BGEN-75	2X	0.698	0.302
BGEN-98	2X	0.690	0.310
BGEN-78	2X	0.690	0.310
BGEN-21	2X	0.686	0.314
BGEN-48	2X	0.682	0.318
BGEN-64	4X	0.674	0.326
BGEN-89	2X	0.671	0.329
BGEN-12	2X	0.671	0.329
BGEN-72	2X	0.667	0.333
BGEN-81	2X	0.667	0.333
BGEN-62	3X	0.667	0.333
BGEN-07	4X	0.667	0.333
BGEN-13	2X	0.659	0.341
BGEN-68	2X	0.641	0.359
BGEN-57	2X	0.640	0.360
BGEN-17	2X	0.640	0.360
BGEN-46	2X	0.636	0.364
BGEN-45	4X	0.636	0.364
BGEN-53	2X	0.635	0.365
BGEN-111	2X	0.632	0.368
BGEN-71	2X	0.628	0.372
BGEN-06	4X	0.628	0.372
BGEN-70	4X	0.627	0.373
BGEN-02	2X	0.624	0.376
BGEN-56	2X	0.616	0.384
BGEN-67	2X	0.616	0.384
BGEN-80	2X	0.612	0.388
BGEN-24	3X	0.605	0.395

APPENDIX D- Fitness (W) and coefficient of selection (s) regarding seed length under open pollination in different ploidal levels of *Lippia alba* (cont.).

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-73	2X	0.601	0.399
BGEN-96	2X	0.589	0.411
BGEN-58	2X	0.581	0.419
BGEN-11	2X	0.578	0.422
BGEN-60	3X	0.574	0.426
BGEN-90	2X	0.570	0.430

APPENDIX E- Fitness (W) and coefficient of selection (s) regarding seed width under open pollination in different ploidal levels of *Lippia alba*.

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-59	3X	1.000	0.000
BGEN-72	2X	0.965	0.035
BGEN-58	2X	0.940	0.060
BGEN-27	3X	0.940	0.060
BGEN-12	2X	0.881	0.119
BGEN-30	2X	0.860	0.140
BGEN-43	2X	0.856	0.144
BGEN-64	4X	0.821	0.179
BGEN-86	4X	0.818	0.182
BGEN-96	2X	0.796	0.204
BGEN-24	3X	0.782	0.218
BGEN-03	4X	0.775	0.225
BGEN-81	2X	0.768	0.232
BGEN-90	2X	0.768	0.232
BGEN-87	4X	0.768	0.232
BGEN-70	4X	0.766	0.234
BGEN-56	2X	0.761	0.239
BGEN-65	2X	0.747	0.253
BGEN-45	4X	0.747	0.253
BGEN-111	2X	0.734	0.266
BGEN-40	2X	0.730	0.270
BGEN-48	2X	0.730	0.270
BGEN-17	2X	0.730	0.270
BGEN-46	2X	0.723	0.277
BGEN-11	2X	0.723	0.277
BGEN-88	2X	0.719	0.281
BGEN-74	2X	0.709	0.291
BGEN-09	2X	0.695	0.305
BGEN-77	2X	0.695	0.305
BGEN-62	3X	0.695	0.305
BGEN-75	2X	0.670	0.330
BGEN-80	2X	0.656	0.344
BGEN-98	2X	0.646	0.354
BGEN-21	2X	0.646	0.354
BGEN-89	2X	0.646	0.354
BGEN-73	2X	0.642	0.358
BGEN-53	2X	0.632	0.368
BGEN-02	2X	0.621	0.379

APPENDIX E- Fitness (W) and coefficient of selection (s) regarding seed width under open pollination in different ploidal levels of *Lippia alba* (cont.).

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-78	2X	0.607	0.393
BGEN-68	2X	0.602	0.398
BGEN-71	2X	0.600	0.400
BGEN-49	3X	0.600	0.400
BGEN-60	3X	0.544	0.456
BGEN-67	2X	0.530	0.470

APPENDIX F- Fitness (W) and coefficient of selection (s) regarding seed weight under open pollination in different ploidal levels of *Lippia alba*.

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-27	3X	1.000	0.000
BGEN-86	4X	0.810	0.190
BGEN-01	3X	0.746	0.254
BGEN-65	2X	0.707	0.293
BGEN-81	2X	0.657	0.343
BGEN-62	3X	0.657	0.343
BGEN-59	3X	0.632	0.368
BGEN-07	4X	0.629	0.371
BGEN-72	2X	0.620	0.380
BGEN-43	2X	0.618	0.382
BGEN-64	4X	0.604	0.396
BGEN-17	2X	0.590	0.410
BGEN-24	3X	0.568	0.432
BGEN-40	2X	0.565	0.435
BGEN-78	2X	0.563	0.437
BGEN-70	4X	0.561	0.439
BGEN-98	2X	0.558	0.442
BGEN-45	4X	0.554	0.446
BGEN-68	2X	0.540	0.460
BGEN-48	2X	0.538	0.462
BGEN-77	2X	0.531	0.469
BGEN-21	2X	0.531	0.469
BGEN-89	2X	0.524	0.476
BGEN-75	2X	0.522	0.478
BGEN-73	2X	0.513	0.487
BGEN-13	2X	0.510	0.490
BGEN-96	2X	0.510	0.490
BGEN-46	2X	0.503	0.497
BGEN-71	2X	0.501	0.499
BGEN-12	2X	0.487	0.513
BGEN-06	4X	0.471	0.529
BGEN-111	2X	0.455	0.545
BGEN-09	2X	0.442	0.558
BGEN-57	2X	0.435	0.565
BGEN-53	2X	0.414	0.586
BGEN-67	2X	0.412	0.588
BGEN-58	2X	0.403	0.597

APPENDIX F- Fitness (W) and coefficient of selection (s) regarding seed weight under open pollination in different ploidal levels of *Lippia alba* (cont.).

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-02	2X	0.366	0.634
BGEN-80	2X	0.364	0.636
BGEN-56	2X	0.343	0.657
BGEN-60	3X	0.323	0.677

APPENDIX G- Fitness (W) and coefficient of selection (s) regarding seed germination under open pollination in different ploidal levels of *Lippia alba*.

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-75	2X	1	0
BGEN-09	2X	1	0
BGEN-80	2X	1	0
BGEN-02	2X	1	0
BGEN-71	2X	1	0
BGEN-65	2X	1	0
BGEN-98	2X	1	0
BGEN-78	2X	1	0
BGEN-81	2X	1	0
BGEN-12	2X	1	0
BGEN-43	2X	0.95	0.05
BGEN-72	2X	0.95	0.05
BGEN-67	2X	0.95	0.05
BGEN-57	2X	0.95	0.05
BGEN-48	2X	0.95	0.05
BGEN-77	2X	0.9	0.1
BGEN-56	2X	0.9	0.1
BGEN-73	2X	0.9	0.1
BGEN-17	2X	0.9	0.1
BGEN-53	2X	0.9	0.1
BGEN-40	2X	0.85	0.15
BGEN-111	2X	0.85	0.15
BGEN-46	2X	0.8	0.2
BGEN-21	2X	0.8	0.2
BGEN-13	2X	0.8	0.2
BGEN-58	2X	0.8	0.2
BGEN-89	2X	0.8	0.2
BGEN-68	2X	0.8	0.2
BGEN-59	3X	0.75	0.25
BGEN-90	2X	0.7	0.3
BGEN-24	3X	0.6	0.4
BGEN-64	4X	0.5	0.5
BGEN-60	3X	0.45	0.55
BGEN-07	4X	0.45	0.55
BGEN-27	3X	0.4	0.6
BGEN-86	4X	0.4	0.6
BGEN-06	4X	0.3	0.7

APPENDIX G- Fitness (W) and coefficient of selection (s) regarding seed germination under open pollination in different ploidal levels of *Lippia alba* (cont.).

Individual	Ploidal level	Fitness (W)	Selection coefficient (s)
BGEN-70	4X	0.3	0.7
BGEN-01	3X	0.25	0.75

APPENDIX H.1- Fitness (W) and selection coefficient (s) regarding average of seed production within diploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-30	1.000	0.000
BGEN-20	0.907	0.093
BGEN-78	0.842	0.158
BGEN-09	0.819	0.181
BGEN-12	0.771	0.229
BGEN-98	0.759	0.241
BGEN-13	0.744	0.256
BGEN-77	0.735	0.265
BGEN-96	0.732	0.268
BGEN-21	0.724	0.276
BGEN-56	0.703	0.297
BGEN-48	0.702	0.298
BGEN-80	0.680	0.320
BGEN-89	0.675	0.325
BGEN-46	0.655	0.345
BGEN-57	0.646	0.354
BGEN-58	0.643	0.357
BGEN-71	0.605	0.395
BGEN-81	0.582	0.418
BGEN-74	0.566	0.434
BGEN-75	0.565	0.435
BGEN-72	0.559	0.441
BGEN-73	0.547	0.453
BGEN-90	0.545	0.455
BGEN-17	0.518	0.482
BGEN-40	0.517	0.483
BGEN-88	0.498	0.502
BGEN-65	0.491	0.509
BGEN-43	0.469	0.531
BGEN-67	0.444	0.556
BGEN-11	0.265	0.735
BGEN-02	0.049	0.951

APPENDIX H.2- Fitness (W) and selection coefficient (s) regarding average of seed production within triploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-62	1.000	0.000
BGEN-27	0.537	0.463
BGEN-49	0.529	0.471
BGEN-59	0.325	0.675
BGEN-24	0.279	0.721
BGEN-01	0.261	0.739

APPENDIX H.3- Fitness (W) and selection coefficient (s) regarding average of seed production within tetraploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-87	1.000	0.000
BGEN-31	0.952	0.048
BGEN-47	0.799	0.201
BGEN-07	0.762	0.238
BGEN-86	0.733	0.267
BGEN-45	0.692	0.308
BGEN-64	0.646	0.354
BGEN-06	0.632	0.368
BGEN-03	0.452	0.548

APPENDIX I.1- Fitness (W) and selection coefficient (s) regarding average of seed production within diploids under controlled pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-96	1.000	0.000
BGEN-80	0.566	0.434
BGEN-75	0.438	0.562
BGEN-81	0.412	0.588
BGEN-67	0.270	0.730
BGEN-13	0.206	0.794
BGEN-43	0.168	0.832
BGEN-12	0.134	0.866
BGEN-98	0.120	0.880
BGEN-72	0.109	0.891
BGEN-65	0.049	0.951
BGEN-73	0.047	0.953
BGEN-89	0.034	0.966
BGEN-57	0.028	0.972
BGEN-78	0.022	0.978
BGEN-02	0.019	0.981
BGEN-48	0.017	0.983
BGEN-71	0.013	0.987
BGEN-88	0.007	0.993
BGEN-46	0.004	0.996
BGEN-21	0.004	0.996
BGEN-58	0.003	0.997
BGEN-09	0.002	0.998
BGEN-77	0.002	0.998
BGEN-40	0.000	1.000
BGEN-56	0.000	1.000
BGEN-74	0.000	1.000
BGEN-30	0.000	1.000
BGEN-20	0.000	1.000
BGEN-90	0.000	1.000
BGEN-11	0.000	1.000
BGEN-17	0.000	1.000

APPENDIX I.2- Fitness (W) and selection coefficient (s) regarding average of seed production within triploids under controlled pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-62	1.000	0.000
BGEN-59	0.194	0.806
BGEN-27	0.046	0.954
BGEN-24	0.035	0.965
BGEN-49	0.029	0.971
BGEN-01	0.020	0.980

APPENDIX I.3- Fitness (W) and selection coefficient (s) regarding average of seed production within tetraploids under controlled pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-87	1.000	0.000
BGEN-45	0.744	0.256
BGEN-86	0.714	0.286
BGEN-31	0.685	0.315
BGEN-06	0.673	0.327
BGEN-07	0.655	0.345
BGEN-64	0.621	0.379
BGEN-47	0.609	0.391
BGEN-03	0.478	0.522

APPENDIX J.1- Fitness (W) and selection coefficient (s) regarding seed length within diploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-65	1.000	0.000
BGEN-40	0.956	0.044
BGEN-43	0.951	0.049
BGEN-74	0.941	0.059
BGEN-09	0.917	0.083
BGEN-77	0.907	0.093
BGEN-75	0.878	0.122
BGEN-98	0.868	0.132
BGEN-78	0.868	0.132
BGEN-21	0.863	0.137
BGEN-48	0.859	0.141
BGEN-89	0.844	0.156
BGEN-12	0.844	0.156
BGEN-72	0.839	0.161
BGEN-81	0.839	0.161
BGEN-13	0.829	0.171
BGEN-68	0.807	0.193
BGEN-57	0.805	0.195
BGEN-17	0.805	0.195
BGEN-46	0.800	0.200
BGEN-53	0.799	0.201
BGEN-111	0.795	0.205
BGEN-71	0.790	0.210
BGEN-02	0.785	0.215
BGEN-56	0.776	0.224
BGEN-67	0.776	0.224
BGEN-80	0.771	0.229
BGEN-73	0.756	0.244
BGEN-96	0.741	0.259
BGEN-58	0.732	0.268
BGEN-11	0.727	0.273
BGEN-90	0.717	0.283

APPENDIX J.2- Fitness (W) and selection coefficient (s) regarding seed length within triploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-59	1.000	0.000
BGEN-27	0.841	0.159
BGEN-01	0.817	0.183
BGEN-62	0.667	0.333
BGEN-24	0.605	0.395
BGEN-60	0.574	0.426

APPENDIX J.3- Fitness (W) and selection coefficient (s) regarding seed length within tetraploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-86	1.000	0.000
BGEN-64	0.956	0.044
BGEN-07	0.945	0.055
BGEN-45	0.901	0.099
BGEN-06	0.890	0.110
BGEN-70	0.889	0.111

APPENDIX K.1- Fitness (W) and selection coefficient (s) regarding seed width within diploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-72	1.000	0.000
BGEN-58	0.975	0.025
BGEN-12	0.913	0.087
BGEN-30	0.891	0.109
BGEN-43	0.887	0.113
BGEN-96	0.825	0.175
BGEN-81	0.796	0.204
BGEN-90	0.796	0.204
BGEN-56	0.789	0.211
BGEN-65	0.775	0.225
BGEN-111	0.761	0.239
BGEN-40	0.756	0.244
BGEN-48	0.756	0.244
BGEN-17	0.756	0.244
BGEN-46	0.749	0.251
BGEN-11	0.749	0.251
BGEN-88	0.745	0.255
BGEN-74	0.735	0.265
BGEN-09	0.720	0.280
BGEN-77	0.720	0.280
BGEN-75	0.695	0.305
BGEN-80	0.680	0.320
BGEN-98	0.669	0.331
BGEN-21	0.669	0.331
BGEN-89	0.669	0.331
BGEN-73	0.665	0.335
BGEN-53	0.655	0.345
BGEN-02	0.644	0.356
BGEN-78	0.629	0.371
BGEN-68	0.624	0.376
BGEN-71	0.622	0.378
BGEN-67	0.549	0.451

APPENDIX K.2- Fitness (W) and selection coefficient (s) regarding seed width within triploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-59	1.000	0.000
BGEN-27	0.940	0.060
BGEN-24	0.782	0.218
BGEN-62	0.695	0.305
BGEN-49	0.600	0.400
BGEN-60	0.544	0.456

APPENDIX K.3- Fitness (W) and selection coefficient (s) regarding seed width within tetraploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-64	1.000	0.000
BGEN-86	0.996	0.004
BGEN-03	0.944	0.056
BGEN-87	0.936	0.064
BGEN-70	0.933	0.067
BGEN-45	0.910	0.090

APPENDIX L.1- Fitness (W) and selection coefficient (s) regarding seed weight within diploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-65	1.000	0.000
BGEN-81	0.929	0.071
BGEN-72	0.877	0.123
BGEN-43	0.874	0.126
BGEN-17	0.835	0.165
BGEN-40	0.799	0.201
BGEN-78	0.796	0.204
BGEN-98	0.790	0.210
BGEN-68	0.764	0.236
BGEN-48	0.761	0.239
BGEN-77	0.751	0.249
BGEN-21	0.751	0.249
BGEN-89	0.741	0.259
BGEN-75	0.738	0.262
BGEN-73	0.725	0.275
BGEN-13	0.722	0.278
BGEN-96	0.722	0.278
BGEN-46	0.712	0.288
BGEN-71	0.709	0.291
BGEN-12	0.689	0.311
BGEN-111	0.644	0.356
BGEN-09	0.625	0.375
BGEN-57	0.615	0.385
BGEN-53	0.586	0.414
BGEN-67	0.583	0.417
BGEN-58	0.570	0.430
BGEN-02	0.518	0.482
BGEN-80	0.515	0.485
BGEN-56	0.485	0.515

APPENDIX L.2- Fitness (W) and selection coefficient (s) regarding seed weight within triploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-27	1.000	0.000
BGEN-01	0.746	0.254
BGEN-62	0.657	0.343
BGEN-59	0.632	0.368
BGEN-24	0.568	0.432
BGEN-60	0.323	0.677

APPENDIX L.3- Fitness (W) and selection coefficient (s) regarding seed weight within tetraploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-86	1.000	0.000
BGEN-07	0.777	0.223
BGEN-64	0.746	0.254
BGEN-70	0.692	0.308
BGEN-45	0.684	0.316
BGEN-06	0.582	0.418

APPENDIX M.1- Fitness (W) and selection coefficient (s) regarding seed germination index within diploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-75	1.000	0.000
BGEN-09	1.000	0.000
BGEN-80	1.000	0.000
BGEN-02	1.000	0.000
BGEN-71	1.000	0.000
BGEN-65	1.000	0.000
BGEN-98	1.000	0.000
BGEN-78	1.000	0.000
BGEN-81	1.000	0.000
BGEN-12	1.000	0.000
BGEN-43	0.950	0.050
BGEN-72	0.950	0.050
BGEN-67	0.950	0.050
BGEN-57	0.950	0.050
BGEN-48	0.950	0.050
BGEN-77	0.900	0.100
BGEN-56	0.900	0.100
BGEN-73	0.900	0.100
BGEN-17	0.900	0.100
BGEN-53	0.900	0.100
BGEN-40	0.850	0.150
BGEN-111	0.850	0.150
BGEN-46	0.800	0.200
BGEN-21	0.800	0.200
BGEN-13	0.800	0.200
BGEN-58	0.800	0.200
BGEN-89	0.800	0.200
BGEN-68	0.800	0.200
BGEN-90	0.700	0.300

APPENDIX M.2- Fitness (W) and selection coefficient (s) regarding seed germination index within triploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-59	1.000	0.000
BGEN-24	0.800	0.200
BGEN-60	0.600	0.400
BGEN-27	0.533	0.467
BGEN-01	0.333	0.667

APPENDIX M.3- Fitness (W) and selection coefficient (s) regarding seed germination index within tetraploids under open pollination in *Lippia alba*.

Individual	Fitness (W)	Selection coefficient (s)
BGEN-64	1.000	0.000
BGEN-07	0.900	0.100
BGEN-86	0.800	0.200
BGEN-06	0.600	0.400
BGEN-70	0.600	0.400

APPENDIX N.1- DNA amount of diploids offsprings in *Lippia alba* under open pollination.

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Ofspring ploidal level
BGEN-40	2x	2.62	2x
BGEN-40	2x	2.55	2x
BGEN-40	2x	2.52	2x
BGEN-40	2x	2.38	2x
BGEN-40	2x	2.50	2x
BGEN-40	2x	2.35	2x
BGEN-40	2x	2.50	2x
BGEN-40	2x	2.50	2x
BGEN-40	2x	2.57	2x
BGEN-40	2x	2.56	2x
BGEN-40	2x	2.55	2x
BGEN-40	2x	2.62	2x
BGEN-40	2x	2.60	2x
BGEN-40	2x	2.43	2x
BGEN-40	2x	2.60	2x
BGEN-40	2x	2.52	2x
BGEN-40	2x	2.56	2x
BGEN-40	2x	2.46	2x
BGEN-40	2x	2.73	2x
BGEN-40	2x	2.45	2x
BGEN-40	2x	2.60	2x
BGEN-40	2x	2.49	2x
BGEN-40	2x	2.40	2x
BGEN-40	2x	2.46	2x
BGEN-02	2x	2.55	2x
BGEN-02	2x	2.55	2x
BGEN-02	2x	2.52	2x
BGEN-02	2x	2.38	2x
BGEN-02	2x	2.39	2x
BGEN-02	2x	2.41	2x
BGEN-02	2x	2.45	2x
BGEN-02	2x	2.40	2x
BGEN-02	2x	2.34	2x
BGEN-02	2x	2.19	2x
BGEN-02	2x	2.31	2x
BGEN-02	2x	2.27	2x
BGEN-02	2x	2.63	2x
BGEN-02	2x	2.48	2x

APPENDIX N.1- DNA amount of diploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Offspring ploidal level
BGEN-02	2x	2.48	2x
BGEN-02	2x	2.45	2x
BGEN-02	2x	2.43	2x
BGEN-02	2x	2.48	2x
BGEN-02	2x	2.54	2x
BGEN-02	2x	2.56	2x
BGEN-02	2x	2.58	2x
BGEN-02	2x	2.55	2x
BGEN-02	2x	2.54	2x
BGEN-02	2x	2.52	2x
BGEN-02	2x	2.57	2x
BGEN-02	2x	2.58	2x
BGEN-02	2x	2.35	2x
BGEN-02	2x	2.40	2x
BGEN-02	2x	2.39	2x
BGEN-02	2x	2.14	2x
BGEN-09	2x	2.62	2x
BGEN-09	2x	2.64	2x
BGEN-09	2x	2.66	2x
BGEN-09	2x	2.65	2x
BGEN-77	2x	2.60	2x
BGEN-77	2x	2.65	2x
BGEN-12	2x	2.35	2x
BGEN-12	2x	2.67	2x
BGEN-12	2x	2.11	2x
BGEN-12	2x	2.19	2x
BGEN-12	2x	2.32	2x
BGEN-12	2x	2.29	2x
BGEN-12	2x	2.37	2x
BGEN-12	2x	2.27	2x
BGEN-12	2x	2.30	2x
BGEN-12	2x	2.32	2x
BGEN-12	2x	2.29	2x
BGEN-12	2x	2.22	2x
BGEN-12	2x	2.22	2x
BGEN-12	2x	2.12	2x
BGEN-12	2x	2.19	2x
BGEN-12	2x	2.32	2x

APPENDIX N.1- DNA amount of diploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Ofspring ploidal level
BGEN-12	2x	2.17	2x
BGEN-12	2x	2.26	2x
BGEN-12	2x	2.26	2x
BGEN-12	2x	2.22	2x
BGEN-12	2x	2.21	2x
BGEN-12	2x	2.20	2x
BGEN-12	2x	2.23	2x
BGEN-12	2x	2.14	2x
BGEN-12	2x	2.17	2x
BGEN-12	2x	2.23	2x
BGEN-12	2x	2.25	2x
BGEN-12	2x	2.21	2x
BGEN-12	2x	2.26	2x
BGEN-12	2x	2.32	2x
BGEN-12	2x	2.22	2x
BGEN-12	2x	2.33	2x
BGEN-12	2x	2.24	2x
BGEN-12	2x	2.67	2x
BGEN-13	2x	2.51	2x
BGEN-13	2x	2.47	2x
BGEN-13	2x	2.44	2x
BGEN-13	2x	2.42	2x
BGEN-13	2x	2.49	2x
BGEN-13	2x	2.41	2x
BGEN-13	2x	2.37	2x
BGEN-13	2x	2.59	2x
BGEN-13	2x	2.71	2x
BGEN-13	2x	2.45	2x
BGEN-13	2x	2.37	2x
BGEN-13	2x	2.48	2x
BGEN-13	2x	2.37	2x
BGEN-13	2x	2.32	2x
BGEN-13	2x	2.44	2x
BGEN-13	2x	2.46	2x
BGEN-13	2x	2.44	2x
BGEN-13	2x	2.52	2x
BGEN-13	2x	2.35	2x
BGEN-13	2x	2.46	2x

APPENDIX N.1- DNA amount of diploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Ofspring ploidal level
BGEN-13	2x	2.20	2x
BGEN-67	2x	2.38	2x
BGEN-67	2x	2.42	2x
BGEN-67	2x	2.30	2x
BGEN-67	2x	2.45	2x
BGEN-67	2x	2.39	2x
BGEN-67	2x	2.21	2x
BGEN-67	2x	2.41	2x
BGEN-67	2x	2.42	2x
BGEN-67	2x	2.34	2x
BGEN-67	2x	2.29	2x
BGEN-67	2x	2.16	2x
BGEN-67	2x	2.27	2x
BGEN-67	2x	1.89	2x
BGEN-67	2x	2.25	2x
BGEN-67	2x	2.33	2x
BGEN-67	2x	2.04	2x
BGEN-67	2x	2.05	2x
BGEN-67	2x	2.77	2x
BGEN-67	2x	2.23	2x
BGEN-67	2x	2.36	2x
BGEN-67	2x	2.07	2x
BGEN-21	2x	2.61	2x
BGEN-21	2x	2.51	2x
BGEN-21	2x	2.38	2x
BGEN-21	2x	2.41	2x
BGEN-21	2x	2.40	2x
BGEN-21	2x	2.40	2x
BGEN-21	2x	2.30	2x
BGEN-21	2x	2.26	2x
BGEN-21	2x	2.41	2x
BGEN-21	2x	2.63	2x
BGEN-21	2x	2.39	2x
BGEN-21	2x	2.58	2x
BGEN-21	2x	2.47	2x
BGEN-21	2x	2.53	2x
BGEN-21	2x	2.57	2x
BGEN-21	2x	2.94	ANEU

APPENDIX N.1- DNA amount of diploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Offspring ploidal level
BGEN-21	2x	2.41	2x
BGEN-21	2x	2.37	2x
BGEN-21	2x	2.12	2x
BGEN-21	2x	2.78	2x
BGEN-21	2x	2.27	2x
BGEN-21	2x	2.27	2x
BGEN-68	2x	2.63	2x
BGEN-68	2x	2.71	2x
BGEN-68	2x	2.63	2x
BGEN-68	2x	2.61	2x
BGEN-68	2x	2.66	2x
BGEN-68	2x	2.64	2x
BGEN-68	2x	2.66	2x
BGEN-68	2x	2.64	2x
BGEN-68	2x	2.55	2x
BGEN-68	2x	2.55	2x
BGEN-68	2x	2.65	2x
BGEN-68	2x	2.65	2x
BGEN-68	2x	2.64	2x
BGEN-68	2x	2.59	2x
BGEN-68	2x	2.70	2x
BGEN-68	2x	2.69	2x
BGEN-68	2x	2.61	2x
BGEN-68	2x	2.61	2x
BGEN-68	2x	2.76	2x
BGEN-68	2x	2.66	2x
BGEN-81	2x	2.50	2x
BGEN-81	2x	2.65	2x
BGEN-81	2x	2.58	2x
BGEN-81	2x	2.60	2x
BGEN-81	2x	2.28	2x
BGEN-81	2x	2.48	2x
BGEN-81	2x	2.62	2x
BGEN-81	2x	2.62	2x
BGEN-81	2x	2.53	2x
BGEN-81	2x	2.59	2x
BGEN-81	2x	2.58	2x
BGEN-81	2x	2.59	2x

APPENDIX N.1- DNA amount of diploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Ofspring ploidal level
BGEN-81	2x	2.65	2x
BGEN-81	2x	2.59	2x
BGEN-81	2x	2.19	2x
BGEN-81	2x	2.53	2x
BGEN-48	2x	2.34	2x
BGEN-48	2x	2.31	2x
BGEN-48	2x	2.28	2x
BGEN-48	2x	2.39	2x
BGEN-48	2x	2.46	2x
BGEN-48	2x	2.23	2x
BGEN-48	2x	2.31	2x
BGEN-48	2x	2.26	2x
BGEN-48	2x	2.23	2x
BGEN-48	2x	2.44	2x
BGEN-48	2x	2.33	2x
BGEN-48	2x	2.40	2x
BGEN-48	2x	2.39	2x
BGEN-48	2x	2.33	2x
BGEN-48	2x	2.35	2x
BGEN-48	2x	2.32	2x
BGEN-48	2x	2.22	2x
BGEN-48	2x	2.26	2x
BGEN-48	2x	2.22	2x
BGEN-48	2x	2.39	2x
BGEN-48	2x	2.58	2x
BGEN-48	2x	2.38	2x
BGEN-48	2x	2.38	2x
BGEN-48	2x	2.41	2x
BGEN-48	2x	2.37	2x
BGEN-48	2x	2.36	2x
BGEN-48	2x	2.34	2x
BGEN-48	2x	2.32	2x
BGEN-48	2x	2.45	2x
BGEN-48	2x	2.67	2x

APPENDIX N.1- DNA amount of diploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Offspring ploidal level
BGEN-56	2x	2.54	2x
BGEN-56	2x	2.57	2x
BGEN-56	2x	2.59	2x
BGEN-56	2x	2.55	2x
BGEN-72	2x	2.72	2x
BGEN-72	2x	2.66	2x
BGEN-72	2x	2.68	2x
BGEN-72	2x	2.66	2x
BGEN-72	2x	2.60	2x
BGEN-72	2x	2.57	2x
BGEN-72	2x	2.56	2x
BGEN-72	2x	2.62	2x
BGEN-72	2x	2.69	2x
BGEN-72	2x	2.66	2x
BGEN-58	2x	2.40	2x
BGEN-58	2x	2.33	2x
BGEN-58	2x	2.37	2x
BGEN-58	2x	2.46	2x
BGEN-58	2x	2.67	2x
BGEN-58	2x	2.43	2x
BGEN-58	2x	2.41	2x
BGEN-58	2x	2.34	2x
BGEN-58	2x	2.19	2x
BGEN-58	2x	2.33	2x
BGEN-58	2x	2.52	2x
BGEN-58	2x	2.38	2x
BGEN-58	2x	2.90	ANEU
BGEN-58	2x	2.34	2x
BGEN-58	2x	2.48	2x
BGEN-58	2x	2.53	2x
BGEN-58	2x	2.29	2x
BGEN-58	2x	2.28	2x
BGEN-58	2x	2.09	2x
BGEN-58	2x	2.04	2x
BGEN-73	2x	2.37	2x
BGEN-73	2x	2.35	2x
BGEN-73	2x	2.37	2x
BGEN-73	2x	2.36	2x

APPENDIX N.1- DNA amount of diploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Offspring ploidal level
BGEN-73	2x	2.41	2x
BGEN-73	2x	2.33	2x
BGEN-73	2x	2.27	2x
BGEN-73	2x	2.43	2x
BGEN-73	2x	2.27	2x
BGEN-73	2x	2.32	2x
BGEN-73	2x	2.43	2x
BGEN-73	2x	2.38	2x
BGEN-73	2x	2.30	2x
BGEN-73	2x	2.49	2x
BGEN-73	2x	2.54	2x
BGEN-73	2x	2.32	2x
BGEN-73	2x	2.41	2x
BGEN-73	2x	2.36	2x
BGEN-73	2x	2.43	2x
BGEN-98	2x	2.36	2x
BGEN-98	2x	2.33	2x
BGEN-98	2x	2.39	2x
BGEN-98	2x	2.35	2x
BGEN-98	2x	1.95	2x
BGEN-98	2x	2.42	2x
BGEN-98	2x	2.53	2x
BGEN-98	2x	2.34	2x
BGEN-98	2x	2.31	2x
BGEN-98	2x	2.39	2x
BGEN-98	2x	2.35	2x
BGEN-98	2x	2.34	2x
BGEN-98	2x	2.33	2x
BGEN-98	2x	2.31	2x
BGEN-98	2x	2.46	2x
BGEN-98	2x	2.40	2x
BGEN-98	2x	2.43	2x
BGEN-98	2x	2.49	2x
BGEN-98	2x	2.50	2x
BGEN-98	2x	2.43	2x
BGEN-98	2x	2.36	2x
BGEN-98	2x	2.36	2x
BGEN-98	2x	2.25	2x

APPENDIX N.1- DNA amount of diploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Ofspring ploidal level
BGEN-98	2x	2.45	2x
BGEN-98	2x	2.52	2x
BGEN-98	2x	2.58	2x
BGEN-98	2x	2.41	2x
BGEN-98	2x	2.46	2x
BGEN-98	2x	2.44	2x
BGEN-98	2x	2.44	2x
BGEN-98	2x	2.61	2x
BGEN-98	2x	2.77	2x
BGEN-80	2x	2.63	2x
BGEN-80	2x	2.61	2x
BGEN-80	2x	2.56	2x
BGEN-80	2x	2.62	2x
BGEN-80	2x	2.68	2x
BGEN-80	2x	2.64	2x
BGEN-80	2x	2.61	2x
BGEN-80	2x	2.57	2x
BGEN-80	2x	2.67	2x
BGEN-80	2x	2.74	2x
BGEN-80	2x	2.39	2x
BGEN-80	2x	2.36	2x
BGEN-80	2x	2.42	2x
BGEN-80	2x	2.46	2x
BGEN-80	2x	2.40	2x
BGEN-80	2x	2.42	2x
BGEN-80	2x	2.39	2x
BGEN-80	2x	2.84	ANEU
BGEN-80	2x	2.39	2x
BGEN-80	2x	2.42	2x
BGEN-80	2x	2.38	2x
BGEN-80	2x	2.37	2x
BGEN-80	2x	2.47	2x
BGEN-80	2x	2.30	2x
BGEN-80	2x	2.36	2x
BGEN-80	2x	2.34	2x
BGEN-80	2x	2.35	2x
BGEN-80	2x	2.45	2x

APPENDIX N.1- DNA amount of diploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Offspring ploidal level
BGEN-96	2x	2.52	2x
BGEN-96	2x	2.20	2x
BGEN-96	2x	2.17	2x
BGEN-96	2x	2.20	2x
BGEN-96	2x	2.31	2x
BGEN-96	2x	2.11	2x
BGEN-96	2x	2.11	2x
BGEN-96	2x	2.14	2x
BGEN-96	2x	2.22	2x
BGEN-96	2x	2.47	2x
BGEN-96	2x	2.79	2x
BGEN-96	2x	2.22	2x
BGEN-96	2x	2.15	2x
BGEN-96	2x	2.41	2x
BGEN-96	2x	2.38	2x
BGEN-96	2x	2.66	2x
BGEN-96	2x	1.83	2x
BGEN-96	2x	2.60	2x
BGEN-75	2x	2.74	2x
BGEN-75	2x	2.57	2x
BGEN-75	2x	2.58	2x
BGEN-75	2x	2.54	2x
BGEN-75	2x	2.62	2x
BGEN-75	2x	4.54	ANEU
BGEN-75	2x	2.80	2x
BGEN-75	2x	2.61	2x
BGEN-75	2x	2.62	2x
BGEN-75	2x	2.59	2x
BGEN-75	2x	2.69	2x
BGEN-38	2x	2.64	2x
BGEN-38	2x	2.59	2x

APPENDIX N.2 - DNA amount of triploids offsprings in *Lippia alba* under open pollination.

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Ofspring ploidal level
BGEN-27	3x	3.01	ANEU
BGEN-27	3x	3.25	ANEU
BGEN-27	3x	3.85	3x
BGEN-27	3x	3.98	3x
BGEN-27	3x	3.12	ANEU
BGEN-27	3x	3.13	ANEU
BGEN-27	3x	3.02	ANEU
BGEN-27	3x	3.95	3x
BGEN-27	3x	3.87	3x
BGEN-27	3x	3.27	ANEU
BGEN-27	3x	3.09	ANEU
BGEN-27	3x	3.02	ANEU
BGEN-27	3x	2.72	2x
BGEN-27	3x	2.99	ANEU
BGEN-27	3x	3.11	ANEU
BGEN-27	3x	3.10	ANEU
BGEN-27	3x	3.03	ANEU
BGEN-27	3x	2.71	2x
BGEN-27	3x	2.84	ANEU
BGEN-27	3x	3.65	ANEU
BGEN-27	3x	3.14	ANEU
BGEN-27	3x	3.13	ANEU
BGEN-27	3x	2.94	ANEU
BGEN-27	3x	3.35	ANEU
BGEN-27	3x	3.31	ANEU
BGEN-27	3x	3.36	ANEU
BGEN-27	3x	3.32	ANEU
BGEN-27	3x	3.12	ANEU
BGEN-27	3x	3.44	ANEU
BGEN-59	3x	3.99	3x
BGEN-59	3x	3.11	ANEU
BGEN-59	3x	3.09	ANEU
BGEN-59	3x	2.96	ANEU
BGEN-59	3x	3.15	ANEU
BGEN-59	3x	3.24	ANEU
BGEN-59	3x	3.20	ANEU

APPENDIX N.2 - DNA amount of triploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Offspring ploidal level
BGEN-59	3x	3.03	ANEU
BGEN-59	3x	3.78	ANEU
BGEN-59	3x	3.25	ANEU
BGEN-59	3x	3.78	ANEU
BGEN-59	3x	3.16	ANEU
BGEN-59	3x	3.19	ANEU
BGEN-59	3x	2.96	ANEU
BGEN-59	3x	3.26	ANEU
BGEN-59	3x	3.26	ANEU
BGEN-59	3x	3.31	ANEU
BGEN-59	3x	3.82	3x
BGEN-59	3x	3.38	ANEU
BGEN-59	3x	2.90	ANEU
BGEN-59	3x	3.26	ANEU
BGEN-60	3x	3.01	ANEU
BGEN-60	3x	3.13	ANEU
BGEN-60	3x	3.11	ANEU
BGEN-60	3x	2.79	2x
BGEN-60	3x	2.75	2x
BGEN-60	3x	2.94	ANEU
BGEN-60	3x	2.94	ANEU
BGEN-109	3x	3.06	ANEU
BGEN-109	3x	3.16	ANEU
BGEN-109	3x	3.33	ANEU
BGEN-109	3x	3.71	ANEU
BGEN-109	3x	3.04	ANEU
BGEN-109	3x	3.16	ANEU
BGEN-109	3x	3.32	ANEU
BGEN-109	3x	3.42	ANEU
BGEN-109	3x	3.42	ANEU
BGEN-109	3x	3.35	ANEU
BGEN-109	3x	2.99	ANEU
BGEN-109	3x	2.57	2x
BGEN-109	3x	2.70	2x
BGEN-109	3x	2.88	ANEU
BGEN-109	3x	2.77	2x
BGEN-109	3x	4.60	4x
BGEN-109	3x	2.80	2x

APPENDIX N.2 - DNA amount of triploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Ofspring ploidal level
BGEN-109	3x	2.70	2x
BGEN-109	3x	2.92	ANEU
BGEN-109	3x	3.04	ANEU
BGEN-23	3x	3.50	ANEU
BGEN-23	3x	3.00	ANEU
BGEN-23	3x	3.99	3x
BGEN-23	3x	3.26	ANEU
BGEN-23	3x	3.08	ANEU
BGEN-23	3x	2.95	ANEU
BGEN-23	3x	3.09	ANEU
BGEN-23	3x	3.12	ANEU
BGEN-23	3x	3.16	ANEU
BGEN-23	3x	3.83	3x
BGEN-23	3x	3.10	ANEU
BGEN-23	3x	4.27	ANEU
BGEN-23	3x	2.86	ANEU
BGEN-23	3x	2.98	ANEU
BGEN-23	3x	3.13	ANEU
BGEN-23	3x	3.24	ANEU
BGEN-23	3x	2.83	ANEU
BGEN-23	3x	3.39	ANEU
BGEN-23	3x	3.22	ANEU

APPENDIX N.3 - DNA amount of tetraploids offsprings in *Lippia alba* under open pollination.

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Ofspring ploidal level
BGEN-64	4x	3.82	3x
BGEN-64	4x	2.60	2x
BGEN-64	4x	3.67	ANEU
BGEN-64	4x	3.69	ANEU
BGEN-64	4x	3.71	ANEU
BGEN-64	4x	3.72	ANEU
BGEN-64	4x	3.60	ANEU
BGEN-64	4x	3.42	ANEU
BGEN-64	4x	3.72	ANEU
BGEN-64	4x	3.28	ANEU
BGEN-64	4x	3.59	ANEU
BGEN-64	4x	3.50	ANEU
BGEN-64	4x	3.55	ANEU
BGEN-64	4x	3.69	ANEU
BGEN-64	4x	3.45	ANEU
BGEN-64	4x	3.59	ANEU
BGEN-64	4x	3.19	ANEU
BGEN-64	4x	3.53	ANEU
BGEN-64	4x	3.28	ANEU
BGEN-64	4x	3.62	ANEU
BGEN-64	4x	3.77	ANEU
BGEN-64	4x	3.33	ANEU
BGEN-64	4x	3.39	ANEU
BGEN-64	4x	2.58	2x
BGEN-64	4x	3.72	ANEU
BGEN-64	4x	3.54	ANEU
BGEN-64	4x	2.16	2x
BGEN-64	4x	3.85	3x
BGEN-64	4x	2.62	2x
BGEN-64	4x	3.78	ANEU
BGEN-64	4x	3.75	ANEU
BGEN-64	4x	3.53	ANEU
BGEN-64	4x	3.45	ANEU
BGEN-64	4x	3.32	ANEU
BGEN-64	4x	3.34	ANEU
BGEN-64	4x	3.51	ANEU
BGEN-64	4x	3.54	ANEU
BGEN-64	4x	3.17	ANEU

APPENDIX N.3 - DNA amount of tetraploids offsprings in *Lippia alba* under open pollination (cont.).

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Ofspring ploidal level
BGEN-64	4x	3.20	ANEU
BGEN-64	4x	3.33	ANEU
BGEN-86	4x	2.65	2x
BGEN-86	4x	3.63	ANEU
BGEN-86	4x	2.67	2x
BGEN-86	4x	3.58	ANEU
BGEN-07	4x	3.76	ANEU
BGEN-07	4x	2.62	2x
BGEN-07	4x	3.08	ANEU
BGEN-07	4x	3.69	ANEU
BGEN-07	4x	3.23	ANEU
BGEN-07	4x	3.61	ANEU
BGEN-07	4x	3.98	3x
BGEN-07	4x	3.63	ANEU
BGEN-07	4x	3.46	ANEU
BGEN-07	4x	3.02	ANEU
BGEN-07	4x	3.73	ANEU
BGEN-07	4x	3.50	ANEU

APPENDIX O - DNA amount of triploids offsprings in *Lippia alba* under controlled pollination.

Mother-plant	Mother-plant ploidal level	Offspring DNA amount (pg)	Offspring ploidal level
BGEN-10	3x	4,40	ANEU
BGEN-10	3x	3,72	ANEU
BGEN-27	3x	4,74	4x
BGEN-27	3x	4,20	ANEU
BGEN-27	3x	3,96	3x
BGEN-27	3x	4,09	3x
BGEN-59	3x	4,49	ANEU

8- REFERENCES

- ALIX, K.; JOETS, J.; RYDER, C. D.; MOORE, J.; BARKER, G. C.; BAILEY, J. P.; KING, G.J.; HESLOP-HARRISON, J. S. The CACTA transposon Bot1 played a major role in *Brassica* genome divergence and gene proliferation. *The Plant Journal*. v. 56(6), pp. 1030-1044.2008.
- ANTONINI, Y.; SOUZA, H. G.; JACOBI, C. M. & MURY, F. B. Diversidade e comportamento dos insetos visitantes florais de *Stachytarpheta glabra* Cham. (Verbenaceae), em uma área de campo ferruginoso, Ouro Preto, MG. *Neotropical Entomology*. v. 34(4), pp. 555-564.2005.
- ARAÚJO, M. S.; COSTA-PEREIRA, R. Latitudinal gradients in intraspecific ecological diversity. *Biology letters*. v. 9(6), pp. 20130778.2013.
- ARMBRUSTER, W. S. Floral specialization and angiosperm diversity: phenotypic divergence, fitness trade-offs and realized pollination accuracy. *AoB plants*. v. 6, 2014.
- ARNOLD, B.; KIM, S.T.; BOMBLIES, K. Single geographic origin of a widespread autotetraploid *Arabidopsis arenosa* lineage followed by interploidy admixture. *Molecular Biology and Evolution*. v. 32, pp. 1382–1395.2015.
- ARRIGO, N.; BARKER, M. S. Rarely successful polyploids and their legacy in plant genomes. *Current opinion in plant biology*. v. 15(2), pp. 140-146.2012.
- ASHMAN, T.L.; KWOK, A.; HUSBAND, B.C. Revisiting the dioecy–polyploidy association: alternate pathways and research opportunities. *Cytogenetic and Genome Research*. v. 140, pp. 241–255.2013.
- AVISE, J. Clonality: the genetics, ecology, and evolution of sexual abstinence in vertebrate animals. Oxford University Press on Demand.2008.
- AZIZ, M. A.; MEHEDI, M.; AKTER, M. I.; SAJON, S. R.; MAZUMDER, K.; RANA, M. S. In vivo and in silico evaluation of analgesic activity of *Lippia alba*. *Clinical Phytoscience*. v. 5(1), pp. 1-9.2019.
- BAKER, H. G. Self-compatibility and establishment after'long-distance'dispersal. *Evolution*. v. 9(3), pp. 347-349.1955.
- BALDWIN, S. J., & HUSBAND, B. C. The association between polyploidy and clonal reproduction in diploid and tetraploid *Chamerion angustifolium*. *Molecular ecology*. v. 22(7), pp. 1806-1819.2013.
- BARBOLA, I. D. F., LAROCA, S., ALMEIDA, M. C. D., & NASCIMENTO, E. A. D. Floral biology of *Stachytarpheta maximiliani* Scham.(Verbenaceae) and its floral visitors. *Revista Brasileira de Entomologia*. v. 50(4), pp. 498-504.2006.

BARCACCIA, G.; ALBERTINI, E. Apomixis in plant reproduction: a novel perspective on an old dilemma. *Plant reproduction*. v. 26(3), pp. 159-179.2013.

BARCACCIA, G.; PALUMBO, F.; SGORBATI, S.; ALBERTINI, E.; PUPILLI, F.A. reappraisal of the evolutionary and developmental pathway of apomixis and its genetic control in angiosperms. *Genes*. v. 11(8), pp. 859.2020.

BARRETT, S. C. Influences of clonality on plant sexual reproduction. *Proceedings of the National Academy of Sciences*. v. 112(29), pp. 8859-8866.2015.

BARROS, M. G.; RICO-GRAY, V. & DÍAZ-CASTELAZO, C. Sincronia de floração entre *Lantana camara* L.(Verbenaceae) e *Psittacanthus calyculatus* (DC.) G. Don (Loranthaceae) ocorrentes nas dunas de La Mancha, Veracruz, México. *Acta Botanica Mexicana*. v. 57, pp. 1-14.2001.

BARKE, B. H.; DAUBERT, M.; HÖRANDL, E. Establishment of apomixis in diploid F2 hybrids and inheritance of apospory from F1 to F2 hybrids of the *Ranunculus auricomus* complex. *Frontiers in plant science*. p. 1111.2018.

BARKER, M.S.; ARRIGO, N.; BANIAGA, A.E.; LI, Z.; LEVIN, D.A. On the relative abundance of autopolyploids and allopolyploids. *New Phytologist*. v. 210, pp. 391–398. 2015.

BARRINGER, B.C. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany*. v. 94, pp. 1527–1533.2007.

BIASI, L. A.; COSTA, G. Propagação vegetativa de *Lippia alba*. *Ciência rural*. v. 33, pp. 455-459.2003.

BICKNELL, R. A.; KOLTUNOW, A. M. Understanding apomixis: recent advances and remaining conundrums. *The Plant Cell*.v.16, pp. 228-245.2004.

BIERZYCHUDEK, P. Patterns in plant parthenogenesis. *Experientia*. v. 41(10), pp. 1255-1264.1985.

BOCK, D. G.; CASEYS, C.; COUSENS, R. D.; HAHN, M. A.; HEREDIA, S. M.; HÜBNER, S.; TURNER, K.G.; RIESEBERG, L. H. What we still don't know about invasion genetics. *Molecular ecology*. v. 24(9), pp. 2277-2297.2015.

BODANESE-ZANETTINI, M.H.; CAVALLI, S.S. Variabilidade genética em função do modo de reprodução. *Genética e Evolução Vegetal*. 1a ed. Editora UFRGS. Porto Alegre.pp. 177-188. 2003.

BOLNICK, D. I.; AMARASEKARE, P.; ARAÚJO, M. S.; BÜRGER, R.; LEVINE, J. M.; NOVAK, M.; RUDOLF, V.H.W.; SCHREIBER, S.J.; URBAN, M.C.; VASSEUR, D. A. Why intraspecific trait variation matters in community ecology. *Trends in ecology & evolution*. v. 26(4), pp. 183-192.2011.

BOROWSKA-ZUCHOWSKA, N.; SENDEROWICZ, M.; TRUNOVA, D.; KOLANO, B. Tracing the Evolution of the Angiosperm Genome from the Cytogenetic Point of View. *Plants*. v. 11(6), pp. 784.2022.

BOSE, R. B.; CHOUDHURY, J. K. Cytological studies in *L. alba* (Mill) NE Br. Bulletin of the Botanical Society of Bengal. v.14, pp. 71-72.1960.

BRANDÃO, A.D.; VICCINI, L.F.; SALIMENA, F.R.G.; VANZELA, A.L.L.; RECCO PIMENTEL, S.M. Cytogenetic characterization of *Lippia alba* and *Lantana camara* (Verbenaceae) from Brazil. *Journal of Plant Research*. v. 120, pp. 317-321.2007.

BRENNAN, A. C.; HARRIS, S. A.; HISCOCK, S. J. Modes and rates of selfing and associated inbreeding depression in the self-incompatible plant *Senecio squalidus* (Asteraceae): a successful colonizing species in the British Isles. *New Phytologist*. v. 168(2), pp. 475-486.2005.

BRINK, R. A.; COOPER, D. C. The endosperm in seed development. *The Botanical Review*. v. 13(9), pp. 479-541.1947.

BROCHMANN, C.; BRYSTING, A. K.; ALSOS, I. G.; BORGÉN, L.; GRUNDT, H. H.; SCHEEN, A. C.; ELVEN, R. Polyploidy in arctic plants. *Biological journal of the Linnean society*. v. 82(4), pp. 521-536.2004.

BROWNFIELD, L.; KÖHLER, C. Unreduced gamete formation in plants: mechanisms and prospects. *Journal of Experimental Botany*. v. 62(5), pp. 1659-1668.2011.

BUDEGUER, C. J.; NASIF, A.; MARTÍNEZ PULIDO, L.; PASTORIZA, A.; ANDRADA MANSILLA, B. Cytogenetics of *Lippia alba* (Mill.) Brown from Lules, Tucumán. *Revista Agronómica del Noroeste Argentino*. v. 33(1), pp. 11-14.2013.

BUGGS, R. J.; PANNELL, J. R. Rapid displacement of a monoecious plant lineage is due to pollen swamping by a dioecious relative. *Current Biology*. v. 16(10), pp. 996-1000.2006.

BURTON, T. L.; HUSBAND, B. Fitness differences among diploids, tetraploids, and their triploid progeny in *Chamerion angustifolium*: mechanisms of inviability and implications for polyploid evolution. *Evolution*. v. 54(4), pp. 1182-1191.2000.

BUSCH, J. W.; SCHOEN, D. J. The evolution of self-incompatibility when mates are limiting. *Trends in plant science*. v. 13(3), pp. 128-136.2008.

CAMPOS, J.M.S.; SOUSA, S.M.; SILVA, P.S.; PINHEIRO, L.C.; SAMPAIO, F.; VICCINI, L.F. Chromosome numbers and DNA C values in the genus *Lippia* (Verbanaceae). *Plant Systematic and Evolution*. v. 291, pp.133-140.2011.

CARDOSO, J.C.; VIANA, M.L.; MATIAS, R.; FURTADO, M.T.; CAETANO, A.P.; CONSOLARO, H.; BRITO, V.L. Towards a unified terminology for angiosperm reproductive systems. *Acta Botanica Brasilica*. v. 32, pp. 329-48.2018.

CARMONA, F.; ANGELUCCI, M. A.; SALES, D. S.; CHIARATTI, T. M.; HONORATO, F. B.; BIANCHI, R. V.; PEREIRA, A. *Lippia alba* (Mill.) NE Brown hydroethanolic extract of the leaves is effective in the treatment of migraine in women. *Phytomedicine*. v. 20(10), pp. 947-950.2013.

CARVALHO, C.R.; SARAIVA, L.S. A new heterochromatin banding pattern revealed by modified HKG banding technique in maize chromosomes. *Heredity*. v. 70, pp.515-519.1993.

CASTRO, M.; CASTRO, S.; LOUREIRO, J. Genome size variation and incidence of polyploidy in Scrophulariaceae sensu lato from the Iberian Peninsula. *AoB Plants*.2012.

CHAPMAN, M. A., ABBOTT, R. J. Introgression of fitness genes across a ploidy barrier. *New Phytologist*. v. 186(1), pp. 63-71.2010.

ČERTNER, M., FENCLOVÁ, E., KÚR, P., KOLÁŘ, F., KOUTECKÝ, P., KRAHULCOVÁ, A., SUDA, J. Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids. *Annals of botany*. v. 120(2), pp. 303-315.2017.

CHEN, Z. J. Molecular mechanisms of polyploidy and hybrid vigor. *Trends in plant science*. v. 15(2), pp. 57-71.2010.

CHESTER, M.; GALLAGHER, J. P.; SYMONDS, V. V.; DA SILVA, A. V. C.; MAVRODIEV, E. V.; LEITCH, A. R.; SOLTIS, P.S.; SOLTIS, D. E. Extensive chromosomal variation in a recently formed natural allopolyploid species, *Tragopogon miscellus* (Asteraceae). *Proceedings of the National Academy of Sciences*. v. 109(4), pp. 1176-1181.2012.

COMAI, L. The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*. v. 6, pp. 836–846.2005.

COOK, L. M.; SOLTIS, P. S. Mating systems of diploid and allotetraploid populations of *Tragopogon* (Asteraceae). II. Artificial populations. *Heredity*. v. 84(4), pp. 410-415.2000.

CORREA, J.R.C.; MING, L.C.; SCHEFFER, M. Cultivo de plantas medicinais, condimentares e aromáticas (2ed). FUNEP, Jaboticabal. p.151.1994.

COSTA, J., FERRERO, V.; CASTRO, M.; LOURIERO, J.; NAVARRO, L.; CASTRO, S. 2017. *Variation in the incompatibility reactions in tristylous Oxalis pes-caprae: large-scale screening in South African native and Mediterranean basin invasive populations*. *Perspectives in Plant Ecology, Evolution, and Systematics*. v. 24, pp. 5– 36.

COSTA, P. S.; OLIVEIRA, S. S.; SOUZA, E. B.; BRITO, E. H. S.; CAVALCANTE, C. S. P.; MORAIS, S. M.; LEAL, A. L. A. B.; BARRETO, H. M.; TEIXEIRA, A. M. R.; NOGUEIRA, C. E. S.; FONTENELLE, R. O. S.; SANTOS, H. S. Antifungal activity and synergistic effect of essential oil from *Lippia alba* against *Trichophyton rubrum* and *Candida spp.* Revista Virtual de Química. v. 12, pp. 1529-1540.2020.

CRISMANI, W.; GIRARD, C.; MERCIER, R. Tinkering with meiosis. Journal of experimental botany. v. 64(1), pp. 55-65.2013.

CROW, K. D.; WAGNER, G. P. Tri-National Young Investigators. Proceedings of the SMBE Tri-National Young Investigators' Workshop 2005. What is the role of genome duplication in the evolution of complexity and diversity? Molecular biology and evolution. v. 23, pp. 887–892.2006.

DALL'AGNOL, M.; SCHIFINO-WITTMANN, M. Apomixia, genética e melhoramento de plantas. Current Agricultural Science and Technology. v.11(2).2005.

DE FREITAS SOUZA, C.; BALDISSERA, M. D.; BIANCHINI, A. E.; DA SILVA, E. G.; MOURÃO, R. H. V.; DA SILVA, L. V. F.; SCHMIDT, D.; HEINZMANN, B.M.; BALDISSEROTTO, B. Citral and linalool chemotypes of *Lippia alba* essential oil as anesthetics for fish: a detailed physiological analysis of side effects during anesthetic recovery in silver catfish (*Rhamdia quelen*). Fish Physiology and Biochemistry. v. 44(1), pp. 21-34.2018.

DE NITTANCOURT, D.; GRANT, W. F. La cytogenetique de *Lotus (Leguminosae)* III. Un cas de cytomixie dans un hybride interspecifique. Cytologia. v. 29, pp. 191–195.1964.

DE STORME, N.; GEELEN, D. Sexual polyploidization in plants—cytological mechanisms and molecular regulation. New Phytologist. v. 198(3), pp. 670-684.2013.

DELGADO, L.; GALDEANO, F.; SARTOR, M. E.; QUARIN, C. L.; ESPINOZA, F.; ORTIZ, J. P. A. Analysis of variation for apomictic reproduction in diploid *Paspalum rufum*. Annals of Botany. v. 113(7), pp. 1211-1218.2014.

DIALLO, A. M.; NIELSEN, L. R.; KJAER, E. D.; PETERSEN, K. K.; RAEBILD, A. Polyploidy can confer superiority to West African *Acacia senegal* (L.) Willd. trees. Frontiers in Plant Science. v. 7, p. 821.2016.

DODSWORTH, S.; CHASE, M.W.; LEITH, A.R. Is post-polyploidization diploidization the key to the evolutionary success of angiosperms? *Botanical Journal of the Linnean Society*. v. 180(1), pp.1–5.2016.

DOS SANTOS, A. P. M.; FRACASSO, C. M.; LUCIENE DOS SANTOS, M.; ROMERO, R.; SAZIMA, M.; OLIVEIRA, P. E. Reproductive biology and species geographical distribution in the Melastomataceae: a survey based on New World taxa. Annals of Botany. v. 110(3), pp. 667-679.2012.

- DOUHOVNIKOFF, V.; DODD, R.S. Epigenetics: a potential mechanism for clonal plant success. *Plant ecology*. v. 216(2), pp. 227-233.2015.
- DOYLE, J. J. Isolation of plant DNA from fresh tissue. *Focus*. v.12, pp.13-15.1990.
- DOYLE, J.J.; FLAGEL, L.E.; PATERSON, A.H.; RAPP, R.A.; SOLTIS, D.E.; SOLTIS, P.S.; WENDEL, J.F. Evolutionary genetics of genome merger and doubling in plants. *Annual Review of Genetics*.v. 42, pp. 443–461.2008.
- DOYLE, J.J.; SHERMAN-BROYLES, S. Double trouble: Taxonomy and definitions of polyploidy. *New Phytologist*. v. 213, pp. 487–493.2017.
- DOYLE, J. J.; COATE, J.E. Polyploidy, the nucleotype, and novelty: the impact of genome doubling on the biology of the cell. *International Journal of Plant Sciences*. v. 180, pp. 1–52.2019.
- DUCHOSLAV, M.; STAŇKOVÁ, H. The population genetic structure and clonal diversity of *Allium oleraceum* (Amaryllidaceae), a polyploid geophyte with common asexual but variable sexual reproduction. *Folia Geobotanica*. v. 50, pp. 123–136.2015.
- ECKERT, C. G.; LUI, K.; BRONSON, K.; CORRADINI, P.; BRUNEAU, A. Population genetic consequences of extreme variation in sexual and clonal reproduction in an aquatic plant. *Molecular Ecology*. v. 12(2), pp. 331-344.2003.
- EDGER, P.P.; HEIDEL-FISCHER, H.M.; BEKAERT, M.; ROTA, J.; GLOECKNER, G.; PLATTS, A.E.; HECKEL, D.G.; DER, J.P.; WAFULA, E.K.; TANG, M.; HOFBERGER, J.A. The butterfly plant arms-race escalated by gene and genome duplications. *Proceedings of the National Academy of Sciences USA*. v.112, pp. 8362–8366.2015.
- ENTANI, T.; TAKAYAMA, S.; IWANO, M.; SHIBA, H.; CHE, F.S.; ISOGAI, A. Relationship between polyploidy and pollen self-incompatibility phenotype in *Petunia hybrida* Vilm. *Bioscience, Biotechnology, and Biochemistry*. v. 63, pp. 1882–1888.1999.
- FEI, X.; SHI, J.; LIU, Y.; NIU, J.; WEI, A. The steps from sexual reproduction to apomixis. *Planta*. v. 249(6), pp. 1715-1730.2019.
- FELBER, F. Establishment of a tetraploid cytotype in a diploid population: effect of relative fitness of the cytotypes. *Journal of evolutionary biology*. v. 4(2), pp. 195-207.1991.
- FAZ, S.; WANG, X.; YOUNAS, A.; ALHARTHI, B.; RIAZ, A.; ALI, H. Apomixis and strategies to induce apomixis to preserve hybrid vigor for multiple generations. *GM Crops & Food* v. 12(1), pp. 57-70.2021.
- FINIGAN, P.; TANURDZIC, M.; MARTIENSSEN, R. A. Origins of novel phenotypic variation in polyploids. In *Polyploidy and genome evolution*. pp. 57-76. Springer, Berlin, Heidelberg.2012.

FLAGEL, L.E.; WENDEL, J.F. Gene duplication and evolutionary novelty in plants. *New Phytologist*. v. 183, pp. 557–564.2009.

FRYXEL, P. Mode of reproduction of higher plants. *Botanical Review*. v. 23, pp. 135-233.1957.

GLICK, L.; SABATH, N.; ASHMAN, T.L.; GOLDBERG, E.; MAYROSE, I. Polyploidy and sexual system in angiosperms: is there an association? *American Journal of Botany*. v. 103, pp. 1223–1235.2016.

GOMES, A. F.; ALMEIDA, M. P.; LEITE, M. F.; SCHWAIGER, S.; STUPPNER, H.; HALABALAKI, M.; AMARAL, J.G.; DAVID, J. M. Seasonal variation in the chemical composition of two chemotypes of *Lippia alba*. *Food chemistry*. v. 273, pp. 186-193.2019.

GOTTSCHALK, W. Chromosome and nucleus migration during microsporogenesis of *Pisum sativum*. *Nucleus*. v. 13, pp.1–9.1970.

GUAN, J.Z.; WANG, J.J.; CHENG, Z.H.; LIU, Y.; LI, Z.Y. Cytomixis and meiotic abnormalities during microsporogenesis are responsible for male sterility and chromosome variations in *Houttuynia cordata*. *Genetics and Molecular Research*. v. 11, pp. 121–130.2012.

GUSTAFSSON, A. Polyploidy, life-form and vegetative reproduction. *Hereditas*. v. 34, pp. 1–22.1948.

HAIG, D.; WESTOBY, M. Genomic imprinting in endosperm: its effect on seed development in crosses between species, and between different ploidies of the same species, and its implications for the evolution of apomixis. *Philosophical Transactions of the Royal Society B: Biological Sciences*. v. 333(1266), pp: 1-13.1991.

HAIG, D.; WESTOBY, M. On limits to seed production. *The American Naturalist*. v. 131, pp. 757– 759.1988.

HAIG, D. Kin conflict in seed development: an interdependent but fractious collective. *Annual review of cell and developmental biology*. v. 29, pp. 189-211.2013.

HAMMER, Ø.; HARPER, D.A.T.; RYAN, P.D. PAST v.2.17c: PAleontological STatistics Software Package for Education and data analysis. *Paleontol. Elec.* 4(1): 1-9, 2001.

HANZL, M.; KOLÁŘ, F.; NOVÁKOVÁ, D.; SUDA, J. Nonadaptive processes governing early stages of polyploid evolution: insights from a primary contact zone of relict serpentine *Knautia arvensis* (Caprifoliaceae). *American Journal of Botany*. v. 101, pp. 935–945.2014.

HARDER, L. D.; BARRETT, S. C. H. (Eds.). *Ecology and evolution of flowers*. Oxford: Oxford University Press. pp. 61-80.2006.

HAUCK, N. R. Accumulation of nonfunctional s-haplotypes results in the breakdown of gametophytic self-incompatibility in tetraploid *Prunus*. *Genetics*. v. 172, pp. 1191–1198.2006.

HERBEN, T.; SUDA, J.; KLIMEŠOVÁ, J. Polyploid species rely on vegetative reproduction more than diploids: a re-examination of the old hypothesis. *Annals of Botany*. v. 120, pp. 341–349.2017.

HOJSGAARD, D. H.; MARTÍNEZ, E. J.; QUARIN, C. L. Competition between meiotic and apomictic pathways during ovule and seed development results in clonality. *New Phytologist*. v. 197(1), pp. 336–347.2013.

HOJSGAARD, D.; KLATT, S.; BAIER, R.; CARMAN, J. G.; HÖRANDL, E. Taxonomy and biogeography of apomixis in angiosperms and associated biodiversity characteristics. *Critical Reviews in Plant Sciences*. v. 33(5), pp. 414–427.2014.

HOJSGAARD, D.; HÖRANDL, E. The rise of apomixis in natural plant populations. *Frontiers in plant science*. v. 10, p. 358.2019.

HOLLISTER, J. D. Polyploidy: adaptation to the genomic environment. *New Phytologist*. v. 205, pp. 1034–1039.2015.

HÖRANDL, E. The complex causality of geographical parthenogenesis. *New Phytologist*. v. 171(3), pp. 525–538.2006.

HÖRANDL, E.; COSENDI, A. C.; TEMSCH, E. M. Understanding the geographic distributions of apomictic plants: a case for a pluralistic approach. *Plant Ecology & Diversity*. v. 1(2), pp. 309–320.2008.

HÖRANDL, E.; HOJSGAARD, D. The evolution of apomixis in angiosperms: a reappraisal. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*. v. 146(3), pp. 681–693.2012.

HÖRANDL, E.; PAUN, O. Patterns and sources of genetic diversity in apomictic plants: implications for evolutionary potentials. *Apomixis: Evolution, mechanisms and perspectives*. ARG Gantner Verlag KG, Lichtenstein. pp. 169–194.2007.

HOVICK, S. M.; WHITNEY, K. D. Hybridisation is associated with increased fecundity and size in invasive taxa: meta-analytic support for the hybridisation-invasion hypothesis. *Ecology Letters*. v. 17, pp. 1464–1477.2014.

HÜLBER, K.; SONNLEITNER, M.; SUDA, J.; KREJČÍKOVÁ, J.; SCHÖNSWETTER, P.; SCHNEEWEISS, G. M.; WINKLER, M. Ecological differentiation, lack of hybrids involving diploids, and asymmetric gene flow between polyploids in narrow contact zones of *Senecio carniolicus* (syn. *Jacobaea carniolica*, Asteraceae). *Ecology and Evolution*. v. 5(6), pp. 1224–1234.2015.

HUSBAND, B.C.; SCHEMSKE, D.W. Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium*. *Journal of Ecology*. v. 88, pp. 689–701.2000.

HUSBAND, B.C. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. *Biological Journal of the Linnean Society*. v. 82, pp. 537–546.2004.

HUSBAND, B.C.; OZIMEC, B.; MARTIN, S.L.; POLLOCK, L. Mating consequences of polyploid evolution in flowering plants: current trends and insights from synthetic polyploids. *International Journal of Plant Sciences*. v. 169, pp. 195–206.2008.

HUSBAND, B.C.; BALDWIN, S.J.; SUDA, J. The incidence of polyploidy in natural plant populations: Major patterns and evolutionary processes. In: Leitch IJ, Greilhuber J, Dolezel J, Wendel J eds. *Plant genome diversity*. v. 2. Vienna: Springer. pp. 255–276. 2013.

IANNICELLI, J.; ELECHOSA, M. A.; JUÁREZ, M. A.; MARTÍNEZ, A.; BUGALLO, V.; BANDONI, A. L.; ESCANDÓN, A. S.; VAN BAREN, C. M. Effect of polyploidization in the production of essential oils in *Lippia integrifolia*. *Industrial Crops and Products*. v. 81, p. 20–29, 2016.

IGIC, B.; LANDE, R.; KOHN, J. R. Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences*. v. 169(1), pp.93-104.2008.

JIAO, Y.; WICKETT, N.J.; AYYAMPALAYAM, S.; CHANDERBALI, .S.; LANDHERR, L.; RALPH, P.E.; TOMSHO, L.P.; HU, Y.; LIANG, H.; SOLTIS, P.S.; SOLTIS,D.E.; CLIFTON, S.W.; SCHLARBAUM, S.E.; SCHUSTER, S.C.; MA, H.; LEEBENS- MACK, J.; DEPAMPHILIS, C.W. Ancestral polyploidy in seed plants and angiosperms.*Nature*. v. 473(7345), pp. 97-100. 2011.

JOHNSON, A. F.; HOU, J.; YANG, H.; SHI, X.; CHEN, C.; ISLAM, M. S.; JI, T.; CHENG, J.; BIRCHLER, J. A. Magnitude of modulation of gene expression in aneuploid maize depends on the extent of genomic imbalance. *Journal of Genetics and Genomics*. v. 47(2), pp. 93-103.2020.

JOACHIMIAK, A. J.; HASTEROK, R.; SLIWINSKA, E.; MUSIAŁ, K.; GRABOWSKA-JOACHIMIAK, A. FISH-aimed karyotype analysis in *Aconitum* subgen. *Aconitum* reveals excessive rDNA sites in tetraploid taxa. *Protoplasma*. v. 255(5), pp. 1363-1372.2018.

JULIÃO, S. A.; RIBEIRO, C. D. V.; LOPES, J. M. L.; MATOS, E. M. D.; REIS, A. C.; PEIXOTO, P. H. P.; MACHADO, M.A.; AZEVEDO, A.L.S.; GRAZUL, R.M.; CAMPOS, J.M.S.; VICCINI, L. F. Induction of synthetic polyploids and assessment of genomic stability in *Lippia alba*. *Frontiers in plant science*. v. 11, pp. 292.2020.

KHANDAY, I.; SKINNER, D.; YANG, B.; MERCIER, R.; SUNDARESAN, V. A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature*. v. 565, pp.91–95.2019.

KARNOVSKY, M.J. A formaldehyde glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology*. v. 27, pp. 137A-138A.1965.

KARUNARATHNE, P.; SCHEDLER, M.; MARTÍNEZ, E. J.; HONFI, A. I.; NOVICHKOVA, A.; HOJSGAARD, D. Intraspecific ecological niche divergence and reproductive shifts foster cytotype displacement and provide ecological opportunity to polyploids. *Annals of botany*. v. 121(6), pp. 1183-1196.2018.

KEARNEY, M. Hybridization, glaciation and geographical parthenogenesis. *Trends in ecology & evolution*. v. 20(9), pp. 495-502.2005.

KEELER, K. H. Impact of intraspecific polyploidy in *Andropogon gerardii* (Poaceae) populations. *The American Midland Naturalist* v. 152(1), pp. 63-74.2004.

KLIMEŠ, L.; KLIMEŠOVA, J.; HENDRIKS, R. J. J.; VAN GROENENDAEL, J. M. Clonal plant forms: classification, distribution and phylogeny. *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden.1997.

KÖHLER, C.; SCHEID, O. M.; ERILOVA, A. The impact of the triploid block on the origin and evolution of polyploid plants. *Trends in Genetics*. v. 26(3), pp. 142-148.2010.

KOLTUNOW, A. M.; GROSSNIKLAUS, U. Apomixis: a developmental perspective. *Annual review of plant biology*. v. 54(1), pp. 547-574.2003.

KOVALSKY, I. E.; SOLÍS NEFFA, V. G. Evidence of 2n microspore production in a natural diploid population of *Turnera sidoides* subsp. *carnea* and its relevance in the evolution of the *T. sidoides* (Turneraceae) autopolyploid complex. *Journal of plant research*. v. 125(6), pp. 725-734.2012.

KOVALSKY, I. E.; ROGGERO LUQUE, J. M.; ELÍAS, G.; FERNÁNDEZ, S. A.; SOLÍS NEFFA, V. G. The role of triploids in the origin and evolution of polyploids of *Turnera sidoides* complex (Passifloraceae, Turneroideae). *Journal of plant research*. v. 131(1), pp. 77-89.2018.

KREINER, J.M.; KRON, P.; HUSBAND, B.C. Frequency and maintenance of unreduced gametes in natural plant populations: Associations with reproductive mode, life history and genome size. *New Phytologist* .v. 214, pp: 879–889.2017.

LANDIS, J. B.; SOLTIS, D. E.; LI, Z.; MARX, H. E.; BARKER, M. S.; TANK, D. C.; SOLTIS, P. S. Impact of whole-genome duplication events on diversification rates in angiosperms. *American journal of botany*. v. 105(3), pp. 348-363.2018.

LATTOO, S.K.; KHAN, S.; BAMOTRA, S.; DHAR, A.K. Cytomixis impairs meiosis and influences reproductive success in *Chlorophytum comosum* (Thunb) Jacq.—an additional strategy and possible implications. *Journal of Biosciences*. v. 31, pp. 629–637.2006.

LEGGIO, A.; LEOTTA, V.; BELSITO, E. L.; DI GIOIA, M. L.; ROMIO, E.; SANTORO, I.; TAVERNA, D.; SINDONA, G.; LIGUORI, A. Aromatherapy: Composition of the gaseous phase at equilibrium with liquid bergamot essential oil. *Chemistry Central Journal*. v. 11(1), pp. 1-11.2017.

LEI, X.; NING, Y.; EID ELESAWI, I.; YANG, K.; CHEN, C.; WANG, C.; LIU, B. Heat stress interferes with chromosome segregation and cytokinesis during male meiosis in *Arabidopsis thaliana*. *Plant Signaling & Behavior*. v. 15(5), p. 1746985.2020.

LEÓN-MARTÍNEZ, G.; VIELLE-CALZADA, J. P. Apomixis in flowering plants: Developmental and evolutionary considerations. *Current topics in developmental biology*. v.131, pp. 565-604.2019.

LEVAN, A.; FREDGA, K.; SANDBERG, A. A. Nomenclature for centromeric position on chromosomes. *Hereditas*. v. 52(2), pp. 201-220.1964.

LEVIN, D.A. Polyploidy and novelty in flowering plants. *The American Naturalist*.v. 122, pp. 1–25.1983.

LEVIN, D. A. The role of chromosomal change in plant evolution. Oxford University Press.2002.

LI, X.F.; SONG, Z.Q.; FENG, D.S.; WANG, H.G. Cytomixis in *Thinopyrum intermedium*, *Thinopyrum ponticum* and its hybrids with wheat. *Cereal Res Commun*. v. 37, pp.353–361.2009.

LISTON, A.; CRONN, R.; ASHMAN, T.L. *Fragaria*: a genus with deep historical roots and ripe for evolutionary and ecological insights. *American Journal of Botany*. v. 101, pp. 1686–1699.2014.

LLOYD, D. G. Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). *Contributions from the Gray Herbarium of Harvard University*. v. 195, pp. 3–134.1965.

LOPES, J. M.; DE CARVALHO, H. H.; ZORZATTO, C.; AZEVEDO, A. L. S.; MACHADO, M. A.; SALIMENA, F. R. G.; GRAZUL, R.M.; GITZENDANNER, M.A.; SOLTIS, D.E.; SOLTIS, P.S.; VICCINI, L. F. Genetic relationships and polyploid origins in the *Lippia alba* complex. *American Journal of Botany*. v. 107(3), pp. 466-476.2020.

LOPES, J. M.L.; MATOS, E. M. D.; ZORZATTO, C.; AZEVEDO, A. L. S.; MACHADO, M. A.; CHESTER, M.; VICCINI, L. F. Development of microsatellite markers for *Lippia alba* and related *Lippia* species. *Molecular Biology Reports*. v. 47(6), pp. 4911-4915.2020.

LOUREIRO, J.; RODRIGUEZ, E.; DOLEŽEL, J.; SANTOS, C. Two New Nuclear Isolation Buffers for Plant DNA Flow Cytometry: A Test with 37 Species. *Annals of Botany*. v. 100(4), pp. 875–888, 2007.

LU, J.; ZHANG, C.; BAULCOMBE, D. C.; CHEN, Z. J. Maternal siRNAs as regulators of parental genome imbalance and gene expression in endosperm of Arabidopsis seeds. *Proceedings of the National Academy of Sciences*. v. 109(14), pp. 5529-5534.2012.

LYNCH, M. Gene duplication and evolution. In: Moya A, Font E eds. *Evolution: From molecules to ecosystems*. Oxford: Oxford University Press. pp. 33– 47.2004.

MA, X. F.; GUSTAFSON, J. P. Genome evolution of allopolyploids: a process of cytological and genetic diploidization. *Cytogenetic and genome research*. v. 109(1-3), pp. 236-249.2005.

MABLE, B. K. Polyploidy and self-compatibility: Is there an association? *New Phytologist*. v. 162, pp. 803– 811.2004.

MANDÁKOVÁ, T.; LYSÁK, M. A. Post-polyploid diploidization and diversification through dysploid changes. *Current Opinion in Plant Biology*. v. 42, pp. 55-65.2018.

MANGLA, Y.; CHAUDHARY, M.; GUPTA, H.; THAKUR, R.; GOEL, S.; RAINA, S. N.; TANDON, R. Facultative apomixis and development of fruit in a deciduous shrub with medicinal and nutritional uses. *AoB Plants*. v. 7.2015.

MASON, A. S.; PIRES, J. C. Unreduced gametes: meiotic mishap or evolutionary mechanism?. *Trends in Genetics*. v. 31(1), pp. 5-10.2015.

MASON, A.S.; WENDEL, J.F. Homoeologous Exchanges, Segmental Allopolyploidy, and Polyploid Genome Evolution. *Frontiers in Genetics*. p. 1014.2020.

MATZK, F.; MEISTER, A.; SCHUBERT, I. An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *Plant Journal*. v. 21, pp.97–108.2000.

MELARAGNO, J. E.; MEHROTRA, B.; COLEMAN, A. W. Relationship between endopolyploidy and cell size in epidermal tissue of Arabidopsis. *The Plant Cell*. v. 5(11), pp. 1661-1668.1993.

MILLER, J. S.; VENABLE, D.L. Polyploidy and the evolution of gender dimorphism in plants. *Science*. v. 289, pp. 2335–2338.2000.

MILLER, J. S.; LEVIN, R.A.; FELICIANO, N.M. A tale of two continents: Baker's rule and the maintenance of self-incompatibility in *Lycium* (Solanaceae). *Evolution*. v. 62, pp. 1052–1065.2008.

MOGIE, M. *The evolution of asexual reproduction in plants*. London: Chapman and Hall.1992.

MUNTZING, A. The evolutionary significance of autopolyploidy. *Hereditas*.v. 21,pp. 263–378.1936.

MURSALIMOV, S.R.; SIDORCHUK, Y.V.; DEINEKO, E.V.New insights into cytomixis: specific cellular features and prevalence in higher plants. *Planta*. v. 238, pp. 415–423.2013.

MURSALIMOV, S. R.; DEINEKO, E. V. How cytomixis can form unreduced gametes in tobacco. *Plant Systematics and Evolution*. v. 301(4), pp. 1293-1297.2015.

NASCIMENTO, L.S.Q. Respostas morfofisiológicas e expressão gênica no complexo poliploide *Lippia alba* sob condições de estresse. Dissertação de mestrado. Universidade Federal de Juiz de Fora. Programa de pós-graduação em Ciências Biológicas: Imunologia e Genética.2020.

NAKAMURA, M.; NANAMI, S.; OKUNO, S.; HIROTA, S. K.; MATSUO, A.; SUYAMA, Y.; TOKUMOTO, H.; YOSHIHARA, S.; ITOH, A. Genetic diversity and structure of apomictic and sexually reproducing *Lindera* species (Lauraceae) in Japan. *Forests*. v. 12(2), pp. 227.2021.

NÉMORIN, A.; DAVID, J.; MALEDON, E.; NUDOL, E.; DALON, J.; ARNAU, G. Microsatellite and flow cytometry analysis to help understand the origin of *Dioscorea alata* polyploids. *Annals of Botany*. v. 112(5), pp. 811-819. 2013.

NOGLER, G. A.Gametophytic apomixis. *Embryology of angiosperms*. pp. 475-518.1984.

NUNES, M.R.; CASTILHO, M.D.S.M.; DE LIMA VEECK, A.P.; DA ROSA, C.G.; NORONHA, C.M.; MACIEL, M.V.; BARRETO, P.M., Antioxidant and antimicrobial methylcellulose films containing *Lippia alba* extract and silver nanoparticles. *Carbohydrate polymers*. v. 192, pp. 37-43.2018.

O'BRIEN, T.P.; FEDER, N.; MCCULLY, M.E. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma*. v.59, pp.368–373. 1964.

OSBORN, T. C.; PIRES, J. C.; BIRCHLER, J. A.; AUGER, D. L.; CHEN, Z. J.; LEE, H. S.; COMAI, L.; MADLUNG, A.; DOERGE, R.W.; COLOT, V.; MARTIENSSEN, R. A. Understanding mechanisms of novel gene expression in polyploids. *Trends in genetics*. v.19(3), pp. 141-147.2003.

OTTO, S.P.; WHITTON, J. Polyploid incidence and evolution. *Annual Review of Genetics*. v. 34, pp. 401–437.2000.

OZIAS-AKINS, P.; VAN DIJK, P. J. Mendelian genetics of apomixis in plants. *Annual Review of Genetics*. v. 41, pp. 509-537.2007.

PAIVA, E.A.S.; DE PINHO, S.Z.; OLIVEIRA, D.M.T. Large plant samples: how to process for GMA embedding? In: Chiarini-Garcia H, Melo RCN (eds) Light microscopy: methods and protocols, Springer/H. New York, pp. 37–49.2011.

PANNELL, J. R.; BARRETT, S. C. Baker's law revisited: reproductive assurance in a metapopulation. *Evolution*. v. 52(3), pp. 657-668.1998.

PANNELL, J. R. A hypothesis for the evolution of androdioecy: the joint influence of reproductive assurance and local mate competition in a metapopulation. *Evolutionary Ecology*.v. 14(3), pp. 195-211.2000.

PANNELL, J. R.; OBBARD, D.J.; BUGGS, R. Polyploidy and the sexual system: What can we learn from *Mercurialis annua*? *Biological Journal of the Linnean Society*. v. 82, pp. 547–560.2004.

PARISOD, C.; ALIX, K.; JUST, J.; PETIT, M.; SARILAR, V.; MHIRI, C.; AINOUCHE, M.; CHALHOUB, B.; GRANDBASTIEN, M.A. Impact of transposable elements on the organization and function of allopolyploid genomes. *New Phytologist*. v. 186, pp. 37–45.2010.

PEIXOTO, M. G.; COSTA-JÚNIOR, L. M.; BLANK, A. F.; DA SILVA LIMA, A.; MENEZES, T. S. A.; DE ALEXANDRIA SANTOS, D.; DE FÁTIMA ARRIGONI- BLANK, M. Acaricidal activity of essential oils from *Lippia alba* genotypes and its major components carvone, limonene, and citral against *Rhipicephalus microplus*. *Veterinary parasitology*. v. 210(1), pp. 118-122.2015.

PELLICER, J.; POWELL, R. F.; LEITCH, I. J. The application of flow cytometry for estimating genome size, ploidy level endopolyploidy, and reproductive modes in plants. In *Molecular Plant Taxonomy*, pp. 325-361.2021.

PIERRE, P. M.O. Caracterização citogenética e molecular de três acessos de *Lippia alba* (Mill.) N.E.Br. (Verbenaceae). 80p. Dissertação (Mestrado em Genética e melhoramento de Plantas) – Universidade Federal de Lavras, Lavras, MG.2004.

PIERRE, P.M.O. Poliploida em acessos de *Lippia alba* (Mill.) N.E.Br (Verbenaceae). Tese (Doutorado em genética e melhoramento de plantas)- Universidade Federal de Lavras, Lavras, MG.2008.

PIERRE, P.M.O.; SOUSA, S.M; DAVIDE, L. C.; MACHADO, M.A.; VICCINI, L.F. Karyotype analysis, DNA content and molecular screening in *Lippia alba* (Verbenaceae). *Annals of Brazilian Academy of Science*. v. 83(3), pp.993- 1005.2011.

PIERRE, P. M. O.; DE SOUSA, S. M. Citomixia em plantas: causas, mecanismos e consequências. *Revista Brasileira de Biociências*. v. 9(2).2011.

- PIMENTA, M. R.; FERNANDES, L. S.; PEREIRA, U. J.; GARCIA, L. S.; LEAL, S. R.; LEITÃO, S. G.; SALIMENA, F.R.G.; VICCINI, L.V., PEIXOTO, P. H. Floração, germinação e estaquia em espécies de *Lippia* L.(Verbenaceae). Brazilian Journal of Botany. v. 30(2), pp. 211-220.2007.
- RAMSEY, J.; SCHEMSKE, D. W. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annual Review of Ecology and Systematics. pp. 467-501.1998.
- RAMSEY, J.; SCHEMSKE, D.W. Neopolyploidy in flowering plants. Annual Review of Ecology and Systematics. v. 33, pp. 589 – 639.2002.
- RAMSEY, J. Unreduced gametes and neopolyploids in natural populations of *Achillea borealis* (Asteraceae). Heredity. v. 98(3), pp. 143-150.2007.
- RAMSEY, J.; RAMSEY, T. S. Ecological studies of polyploidy in the 100 years following its discovery. Philosophical Transactions of the Royal Society B: Biological Sciences. v. 369(1648), p. 20130352.2014.
- RAUSCH, J. H., & MORGAN, M. T. The effect of self-fertilization, inbreeding depression, and population size on autopolyploid establishment. Evolution. v. 59(9), pp. 1867-1875.2005.
- RAY, A.; SUMANGALA, R.C.; RAVIKANTH, G.; SHAANKER, R.U.; QUADER, S. Isolation and characterization of polymorphic microsatellite loci from the invasive plant *Lantana camara* L. Conservation Genetics Resources. v. 4, pp. 171–173.2012.
- REIS, A. C.; SOUSA, S. M.; VALE, A. A.; PIERRE, P. M.; FRANCO, A. L.; CAMPOS, J. M. S.; VICCINI, L. F. *Lippia alba* (Verbenaceae): A new tropical autopolyploid complex?. American journal of botany. v. 101(6), pp. 1002- 1012.2014.
- REIS, A.C.; SOUSA, S.M.; VICCINI, L.F. High frequency of cytomixis observed at zygotene in tetraploid *Lippia alba*. Plant Systematics and Evolution. v. 302, pp. 121–127.2016.
- REIS, A. C.; CHESTER, M.; DE SOUSA, S. M.; CAMPOS, V. R.; DE QUEIROZ NASCIMENTO, L. S.; PACHECO JUNIOR, S.; FRANCO, A.L.; VICCINI, L. F. Chromosomal view of *Lippia alba*, a tropical polyploid complex under genome stabilization process. Protoplasma. v. 259(1), pp. 33-46.2022.
- RISSO-PASCOTTO, C.; PAGLIARINI, M. S.; DO VALLE, C. B. Meiotic behavior in interspecific hybrids between *Brachiaria ruziziensis* and *Brachiaria brizantha* (Poaceae). Euphytica. v.145(1), pp. 155-159.2005.
- ROBERTSON, K.; GOLDBERG, E.E.; IGIĆ, B. Comparative evidence for the correlated evolution of polyploidy and self-compatibility in Solanaceae. Evolution. v. 65, pp. 139–155.2011.

SABARA, H. A.; KRON, P.; HUSBAND, B.C. Cytotype coexistence leads to triploid hybrid production in a diploid-tetraploid contact zone of *Chamerion angustifolium* (Onagraceae). *American Journal of Botany*. v. 100, pp. 962– 970.2013.

SALIMENA, F. R. G.Revisão taxonômica de *Lippia* sect. *Rhodolippia* Schauer (Verbenaceae).Dissertação de doutorado.2000.

SANTOS, F. R.; LIMA, P.F.;PRIOLLI, R.H.; SIQUEIRA, W.J.;Colombo, C.A. Isolation and characteristics of eight novel polymorphic microsatellite loci in *Lippia alba* (Verbenaceae). *American Journal of Botany*. v. 99, pp. 301–303.2012.

SATTLER, M.C.; CARVALHO, C.R; CLARINDO, W.R. The polyploidy and its key role in plant breeding. *Planta*.v. 243(2), pp. 281-296.2016.

SCHINKEL, C.C.F.; KIRCHHEIMER, B.; DELLINGER, A.S.; KLATT. S.; WINKLER, M.; DULLINGER, S.; HO`RANDL, E. Correlations of polyploidy and apomixis with elevation and associated environmental gradients in an alpine plant. *AoB Plants*. v. 8.2016.

SCHMIDT, A.; SCHMID, M. W.; GROSSNIKLAUS, U. Plant germline formation: common concepts and developmental flexibility in sexual and asexual reproduction. *Development*. v. 142(2), pp. 229-241.2015.

SCHOCKEN, N.R.L.Obtenção de quimiotipos híbridos de *Lippia alba* (Mill.).N.E.Brown.Dissertação de mestrado. Universidade estadual de Campinas, Campinas.2007.

SCOTT, R. J.; SPIELMAN, M.; BAILEY, J.; DICKINSON, H. G. Parent-of-origin effects on seed development in *Arabidopsis thaliana*. *Development*. v. 125, pp. 3329– 3341.1998.

SHARBEL, T.F.; VOIGT, M.L.; CORRAL, J.M.; THIEL, T.; VARSHNEY, A.; KUMLEHN, J.; VOGEL, H.; ROTTER, B. Molecular signatures of apomictic and sexual ovules in the *Boechera holboellii* complex. *PI J* v. 58, pp.870–882.2009.

SHARBEL, T. F.; VOIGT, M. L.; CORRAL, J. M.; GALLA, G.; KUMLEHN, J.; KLUKAS, C., SCHREIBER, F.; VOGEL, H.; ROTTER, B. Apomictic and sexual ovules of *Boechera* display heterochronic global gene expression patterns. *The Plant Cell*. v. 22(3), pp. 655-671.2010.

SHMEIT, Y. H.; FERNANDEZ, E.; NOVYB, P.; KLOUCEKB, P.; OROSZB, M.; KOKOSKAA, L. Autopolyploidy effect on morphological variation and essential oil content in *Thymus vulgaris* L. *Scientia Horticulturae*. v. 263, p. 1-7, 2020.

SILVA, P. S.; VICCINI, L. F.; SINGULANI, J. L.; SIQUEIRA, E. P. D.; ZANI, C. L.; ALVES, T. Composição química do óleo essencial e fração hexânica de espécies de *Lippia* e *Lantana*. *Revista Brasileira de Farmacognosia*. v. 20(6), pp. 843-849.2010.

SILVERTOWN, J. The evolutionary maintenance of sexual reproduction: evidence from the ecological distribution of asexual reproduction in clonal plants. *International Journal of Plant Sciences*. v. 169.1, pp. 157-168.2008.

SINGHAL, V.K.; KUMAR, P. Impact of cytomixis on meiosis, pollen viability and pollen size in wild populations of Himalayan poppy (*Meconopsis aculeata* Royle). *Journal of Biosciences*. v. 33, pp. 371–380.2008.

SIOPA, C.; DIAS, M. C.; CASTRO, M.; LOUREIRO, J.; CASTRO, S. Is selfing a reproductive assurance promoting polyploid establishment? Reduced fitness, leaky self-incompatibility and lower inbreeding depression in neotetraploids. *American Journal of Botany*. v. 107(3), pp. 526-538.2020.

SOARES, B. V.; NEVES, L. R.; OLIVEIRA, M. S. B.; CHAVES, F. C. M.; DIAS, M. K. R.; CHAGAS, E. C. & TAVARES-DIAS, M. Antiparasitic activity of the essential oil of *Lippia alba* on ectoparasites of *Colossoma macropomum* (tambaqui) and its physiological and histopathological effects. *Aquaculture*. v. 452, pp. 107-114.2016.

SOLTIS D.E.; SOLTIS, P.S. Polyploidy: Recurrent formation and genome evolution. *Trends in Ecology and Evolution*. v. 14, pp. 348–352.1999.

SOLTIS, D.E.; ALBERT, V.A.; LEEBENS-MACK, J.; BELL, C.D.; PATERSON, A.H.; ZHENG, C.; SANKOFF, D.; WALL, P.K.; SOLTIS, P.S. Polyploidy and angiosperm diversification. *American Journal of Botany*. v. 96, pp. 336– 348.2009.

SOLTIS, P.S.; LIU, X.; MARCHANT, D.B.; VISGER, C.J.; SOLTIS, D.E. Polyploidy and novelty: Gottlieb's legacy. *Philosophical Transactions of the Royal Society B: Biological Sciences*. v. 369, p. 20130351. 2014.

SOLTIS, P.S.; MARCHANT, D.B.; VAN DE PEER, Y.; SOLTIS, D.E. Polyploidy and genome evolution in plants. *Current Opinion in Genetics & Development*. v. 35, pp. 119–125.2015.

SOLTIS, P.S.; SOLTIS, D.E. Ancient WGD events as drivers of key innovations in angiosperms. *Current Opinion in Plant Biology*. v.30, pp. 159–165.2016.

SOLTIS, P. S.; FOLK, R. A.; SOLTIS, D. E. Darwin review: angiosperm phylogeny and evolutionary radiations. *Proceedings of the Royal Society B*. v. 286(1899), 20190099.2019.

SONNLEITNER, M.; HÜLBER, K.; FLATSCHER, R.; GARCÍA, P. E.; WINKLER, M.; SUDA, J.; SCHÖNSWETTER, P.; SCHNEEWEISS, G. M. Ecological differentiation of diploid and polyploid cytotypes of *Senecio carniolicus sensu lato* (Asteraceae) is stronger in areas of sympatry. *Annals of Botany*. v. 117(2), pp. 269-276.2016.

SOUSA, S. M.; SILVA, P. S.; TORRES, G. A.; VICCINI, L. F. Chromosome banding in *Lippia alba* (Verbenaceae). *Biologia* (Bratislava). v. 64 (4), pp. 711-715.2009.

SOUSA, D. G.; SOUSA, S. D. G.; SILVA, R. E. R.; SILVA-ALVES, K. S.; FERREIRA-DA-SILVA, F. W.; KERNTOPF, M. R.; BARBOSA, R. Essential oil of *Lippia alba* and its main constituent citral block the excitability of rat sciatic nerves. *Brazilian Journal of Medical and Biological Research*. v. 48, p. 697-702. 2015.

SPIELMANN, M.; VINKENOOG, R.; SCOTT, R.J. Genetic mechanisms of apomixis. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*. v. 358, pp.1095–1103.2003.

SPILLANE, C.; CURTIS, M. D.; GROSSNIKLAUS, U. Apomixis technology development—virgin births in farmers' fields?. *Nature biotechnology*. v. 22(6), pp. 687-691.2004.

SPOELHOF, J.P.; SOLTIS, P.S.; SOLTIS, D.E. Pure polyploidy: Closing the gaps in autopolyploid research. *Journal of Systematics and Evolution*. v. 55, pp. 340–352.2017.

STEBBINS, G.L. Variation and evolution in plants. Columbia University Press, New York.1950.

STEBBINS, G.L. Chromosomal evolution in higher plants. Reading: Addison- Wesley. p. 216 .1971.

STEFANINI, M.B.; RODRIGUES, S.D.; MING, L.C. Ação de fitorreguladores no crescimento da erva-cidreira-brasileira. *Horticultura Brasileira*. v. 20, pp.18-23.2001.

STOUTE, A. I.; VARENKO, V.; KING, G. J.; SCOTT, R. J.; KURUP, S. Parental genome imbalance in *Brassica oleracea* causes asymmetric triploid block. *The Plant Journal*. v. 71, pp. 503– 516.2012.

SUTHERLAND, B. L.; QUARLES, B. M.; GALLOWAY, L. F. Intercontinental dispersal and whole-genome duplication contribute to loss of self-incompatibility in a polyploid complex. *American Journal of Botany*. v. 105(2), pp.249-256.2018.

SUTHERLAND, B.L.; GALLOWAY, L.F. Variation in heteroploid reproduction and gene flow across a polyploid complex: One size does not fit all. *Ecology and evolution*. v. 11(14), pp. 9676-9688.2021.

TANK, D.C.; EASTMAN, J.M.; PENNELL, M.W.; SOLTIS, P.S.; SOLTIS, D.E.; HINCHLIFF, C.E.; BROWN, J.W.; SESSA, E.B.; HARMON, L.J. Nested radiations and the pulse of angiosperm diversification: Increased diversification rates often follow whole genome duplications. *New Phytologist* . v. 207, pp. 454–467.2015.

TAO, R.; IEZZONI, A.F. The S-RNase-based gametophytic self- incompatibility system in *Prunus* exhibits distinct genetic and molecular features. *Scientia Horticulturae*. v. 124, pp. 423–433.2010.

TE BEEST, M.; LE ROUX, J. J.; RICHARDSON, D. M.; BRYSTING, A. K.; SUDA, J.; KUBEŠOVÁ, M.; PYŠEK, P. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of botany*. v. 109(1), pp. 19-45.2012.

The Plant List. <http://www.theplantlist.org>. Accessed in 2022.

THOMPSON, J. D.; LUMARET, R. The evolutionary dynamics of polyploid plants: origins, establishment and persistence. *Trends in ecology & evolution*. v. 7(9), pp.302-307.1992.

TORRES, E.; LOPEZ, N. Phenotypic plasticity of *Lippia alba* and *Lippia origanoides* (Verbenaceae) in response to availability of light. *Acta Biológica Colombiana*. v. 12, pp.91-102.2007.

VALE, A.A. Plasticidade fenotípica em acessos de *Lippia alba* (Verbenaceae). Dissertação (Mestrado em Biologia), Universidade Federal de Juiz de Fora.2010.

VALLEJO-MARÍN, M.; DORKEN, M. E.; BARRETT, S. C. The ecological and evolutionary consequences of clonality for plant mating. *Annual Review of Ecology, Evolution, and Systematics*. v. 41, pp. 193-213.2010.

VAN DEN BERGH, E.; HOFBERGER, J.A.; SCHRANZ, M.E. Flower power and the mustard bomb: Comparative analysis of gene and genome duplications in glucosinolate biosynthetic pathway evolution in Cleomaceae and Brassicaceae. *American Journal of Botany*. v. 103, pp. 1212–1222. 2016.

VAN DE PEER, Y.; MAERE, S.; MEYER, A. The evolutionary significance of ancient genome duplications. *Nature Reviews Genetics*. v. 10, pp. 725–732.2009.

VAN DE PEER, Y.; MIZRACHI, E.; MARCHAL, K. The evolutionary significance of polyploidy. *Nature Reviews Genetics*. v. 18(7), pp. 411-424.2017.

VAN DRUNEN, W. E.; HUSBAND, B. C. Immediate vs. evolutionary consequences of polyploidy on clonal reproduction in an autopolyploid plant. *Annals of Botany*. v. 122(1), pp. 195-205.2018.

VENÂNCIO, D. D. F. A.; VICCINI, L. F.; LUIZI-PONZO, A. P. & PREZOTO, F. Flower-Visiting Insects and Phenology of *Lippia alba* (Lamiales: Verbenaceae): Floral Color Changes and Environmental Conditions as Cues for Pollinators. *Environmental entomology*. v. 45(3), pp. 685-693.2016.

VERHOEVEN, K. J.; PREITE, V. Epigenetic variation in asexually reproducing organisms. *Evolution*. v. 68(3), pp. 644-655.2014.

VICCINI, L. F.; SILVEIRA, R. S.; DO VALE, A. A.; DE CAMPOS, J. M. S.; REIS, A. C.; DE OLIVEIRA SANTOS, M.; GRAZUL, R. M. Citral and linalool content has been correlated to DNA content in *Lippia alba* (Mill.) NE Brown (Verbenaceae). *Industrial Crops and Products*. v. 59, pp. 14-19.2014.

VISGER, C. J.; GERMAIN-AUBREY, C. C.; PATEL, M.; SESSA, E. B.; SOLTIS, P. S.; SOLTIS, D. E. Niche divergence between diploid and autotetraploid *Tolmiea*. *American Journal of Botany*. v. 103(8), pp. 1396-1406.2016.

VOGLER, D.W.; KALISZ, S. Sex among the flowers: the distribution of plant mating systems. *Evolution*. v. 55(1), pp. 202-204.2001.

WANG, X.; CHENG, Z-M.; ZHI, S.; XU, F. Breeding Triploid Plants: A Review. *Czech Journal of Genetics and Plant Breeding*. v. 52, pp. 41–54.2016.

WANG, Y.; COPENHAVER, G. P. Meiotic recombination: mixing it up in plants. *Annual Review of Plant Biology*. v. 69, pp. 577-609.2018.

WATANABE, K.; YAHARA, T.; DENDA, T.; KOSUGE, K. Chromosomal evolution in the genus *Brachyscome* (Asteraceae, Astereae): statistical tests regarding correlation between changes in karyotype and habit using phylogenetic information. *Journal of Plant Research*. v. 112(2), pp. 145-161.1999.

WEISS-SCHNEEWEISS, H.; EMADZADE, K., JANG, T. S. & SCHNEEWEISS, G. M. Evolutionary consequences, constraints and potential of polyploidy in plants. *Cytogenetic and genome research*. v. 140(2-4),pp. 137-150. 2013.

WELLBORN, G. A.; LANGERHANS, R. B. Ecological opportunity and the adaptive diversification of lineages. *Ecology and evolution*. v. 5(1), pp. 176-195.2015.

WHITTON, J.; SEARS, C. J.; BAACK, E. J.; OTTO, S. P. The dynamic nature of apomixis in the angiosperms. *International Journal of Plant Sciences*. v. 169(1), pp. 169-182.2008.

YASMIN, K.; ARULBALACHANDRAN, D.; JOTHIMANI, K.; SOUNDARYA, V.; VANMATHI, S. Insights of Novel Breeding Strategies in Sustainable Crop Production. In *Sustainable Agriculture towards Food Security*. pp. 29-55. Springer, Singapore.2017.

ZENIL-FERGUSON, R.; BURLEIGH, J.G.; FREYMAN, W.A.; IGIĆ, B.; MAYROSE, I.; GOLDBERG, E.E. Interaction among ploidy, breeding system and lineage diversification. *New Phytologist*. v. 224, pp. 1252–1265. 2019.

ZHANG, K.; WANG, X.; CHENG, F. Plant polyploidy: origin, evolution, and its influence on crop domestication. *Horticultural Plant Journal*. v. 5(6), pp. 231-239.2019.

ZHOU, S.; BARBA-GONZALEZ, R.; LIM, K. B.; RAMANNA, M. S.; VAN TUYL, J. M. Interspecific hybridization in lily (*Lilium*): Interploidy crosses involving interspecific F1

hybrids and their progenies. Floriculture, Ornamental and Plant Biotechnology. v. 5, pp. 152-156.2008.