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Natural Hybridization between *Bursera bicolor* × *B. glabrifolia* (Burseraceae) Complex: Molecular and Chemical Evidence

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Abstract: The hybridization phenomenon is recognized as an important evolutionary force that influences the diversification and evolution of different vascular plant groups. Hence, it is important to identify hybrid individuals. In Mexico, the dry tropical forest (DTF) is considered as the main center of diversification and endemism of the *Bursera* genus, containing 85% of the approximately 106 described species worldwide. In the Mexican DTF, a complex of *Bursera* species was identified, which is formed by two putative parental species *Bursera bicolor* (Will. ex Schltdl.) Engl. and *B. glabrifolia* (H.B.K) Engl. Their putative hybrids were analyzed using molecular (cpSSR) and chemical markers (monoterpenes, sesquiterpenes, and triterpenes) in two pure sites (one site for each parental species) and two hybrid zones. We tested whether individuals with atypical morphology between *B. bicolor* and *B. glabrifolia* from sympatric sites were the result of genetic flow between these two species. A total of 80 individuals were analyzed with 4 microsatellite diagnostic primers and 37 secondary metabolites (SMs). The genetic and chemical markers were highly coincident according to the statistical analyses performed, and they supported the hybridization hypothesis in this *Bursera* complex, with both species remaining distinct even in sympatric zones. α-Amyrin (b), β-amyrin (a), and β-amyrin (b) (triterpenes) were the SMs that most contributed to differentiating putative parental species according to SIMPER analysis. The putative hybrids registered the highest genetic diversity values along with the highest number of SMs, out of which 11 were novel and distributed as follows: triterpenes > monoterpenes > sesquiterpenes. Finally, the chemical markers of the three analyzed families give a framework for future studies to explore hybridization events between *Bursera* species.

Keywords: hybridization; microsatellites; *Bursera*; chemotaxonomy; monoterpenes; sesquiterpenes; triterpenes

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1. Introduction

Hybridization is a frequent phenomenon in plants [1] which is recognized as an important evolutionary force that influences the diversification of different plant groups [2,3], which may provoke high genetic diversity levels, new linages, adaptation transfer, as well as the colonization of new environments of the involved taxa [4].

In recent years, it has been recognized that hybrid plants may have a cascade effect on ecosystems, because hybridization promotes an increase in genetic diversity levels [5]

and changes in plant SM composition via changes in SM production or the creation of new ones [6]. Therefore, plant hybridization has been a topic of interest in ecology and evolution studies, being the main starting point for correct hybrid individual identification.

Morphologic and genetic variation generated by hybridization events as well as the wide phenotypic plasticity of some plant groups have generated taxonomical problems in species identification; as a result, in recent decades, different markers have been used to support taxonomical identification of plant species [7,8]. Specifically, molecular and chemical markers, such as simple sequence repeats (SSRs [5]); and SM profiles, have been used successfully in detection and delimitation of hybrid zones and hybrid individuals. SMs have a key role as defense mechanisms against herbivores and in plant signaling pathways [9–12]. By being under oligogenic control, they have a much more predictable inheritance in comparison with morphological characteristics, which are under polygenic control, so the occurrence of transgressive characteristics is frequent in hybridization events [13,14]. Hence, a combination of chemical and molecular markers is desirable and becomes necessary in hybrid individual identification [9,15,16].

The genus *Bursera* (Burseraceae) is one of the most diversified tree groups in the DTF, with 106 species distributed worldwide [17]. Mexico is considered one of the main centers of diversification of this genus [18–20], containing 100 species, out of which 85% are considered endemic. Despite the recent identification of hybrid zones and of hybridization events between *Bursera* species [21], there are no studies addressing the analysis of these hybrid zones with an integrative approach using molecular and chemical markers. In the present study, two *Bursera* species were selected, *B. bicolor* (Will. ex Schltdl.) Engl. and *B. glabrifolia* (H.B.K) Engl. These species show noticeable distinct morphological characteristics when they are distributed in pure allopatric sites [18]. However, intermediate individuals with atypic leaf morphology are observed when both species overlap spatially in sympatric sites, suggesting that interspecific hybridization events may explain these characteristics. In sympatric sites there are no other *Bursera* species that may be considered as possible parental species. Hybridization in vascular plants may promote profound genetic, chemical, and ecological changes in the involved species, and the correct identification of hybrid plants is an important step in understanding the processes that shape hybrid populations.

In the present study, we evaluated whether trees with atypical morphology between *B. bicolor* and *B. glabrifolia* are the result of interspecific gene flow between these two species. We describe and compare genetic diversity and chemical patterns of the *B. bicolor* × *B. glabrifolia* complex in allopatric and sympatric zones.

2. Materials and Methods

2.1. Study Sites

Bursera bicolor and *B. glabrifolia* belong to the Balsas River Basin (BRB), which is dominated by DTF and considered as one of the main diversification centers of the *Bursera* genus [17]. In Mexico, both species are distributed in the States of Michoacán, Guerrero, Morelos, Puebla, and Oaxaca [18,22]. *B. bicolor* is at an altitudinal range between 800 and 1600 m, while *B. glabrifolia* is distributed between 1000 and 2000 m a.s.l.

Two allopatric populations were chosen (one for each parental species) where each species is dominant, and no hybrid individuals were detected. The *B. bicolor* allopatric population is localized in Coatlán del Río (99°25′52.7″ W, 18°45′35.9″ N) at 1030 m a.s.l. surrounded by DTF, with warm subhumid climate. The *B. glabrifolia* allopatric population is localized in Tetela del Volcán (98°44′48.4″ W, 18°50′13.4″ N) in a transition zone between the dry tropical and temperate forests at an altitude of 1800 m [23] (Figure 1).

Geographic distribution of *B. bicolor* and *B. glabrifolia* overlaps in Morelos State, where trees with intermediate morphology between both species are present. Two sympatric zones were chosen in Morelos State: (1) La Tigra, in Puente de Ixtla municipality (99°16′25.13″ W, 18°30′50.0″ N) at 1100 m a.s.l., and (2) Quilamula in Tlaquiltenango municipality (99°00′11.8″ W, 18°29′39.1″ N) at 1083 m a.s.l., both sites surrounded by DTF [23] (Figure 1). In general, hybrid individuals are present in low frequency and are generally

close to their putative parental species in sites with high disturbance levels (agricultural and livestock sites). It is very uncommon to find trees with atypical morphology between *B. bicolor* and *B. glabrifolia* outside sympatric sites. Moreover, in sympatric sites there is no other *Bursera* species that may indicate hybridization events between the present study species and them.

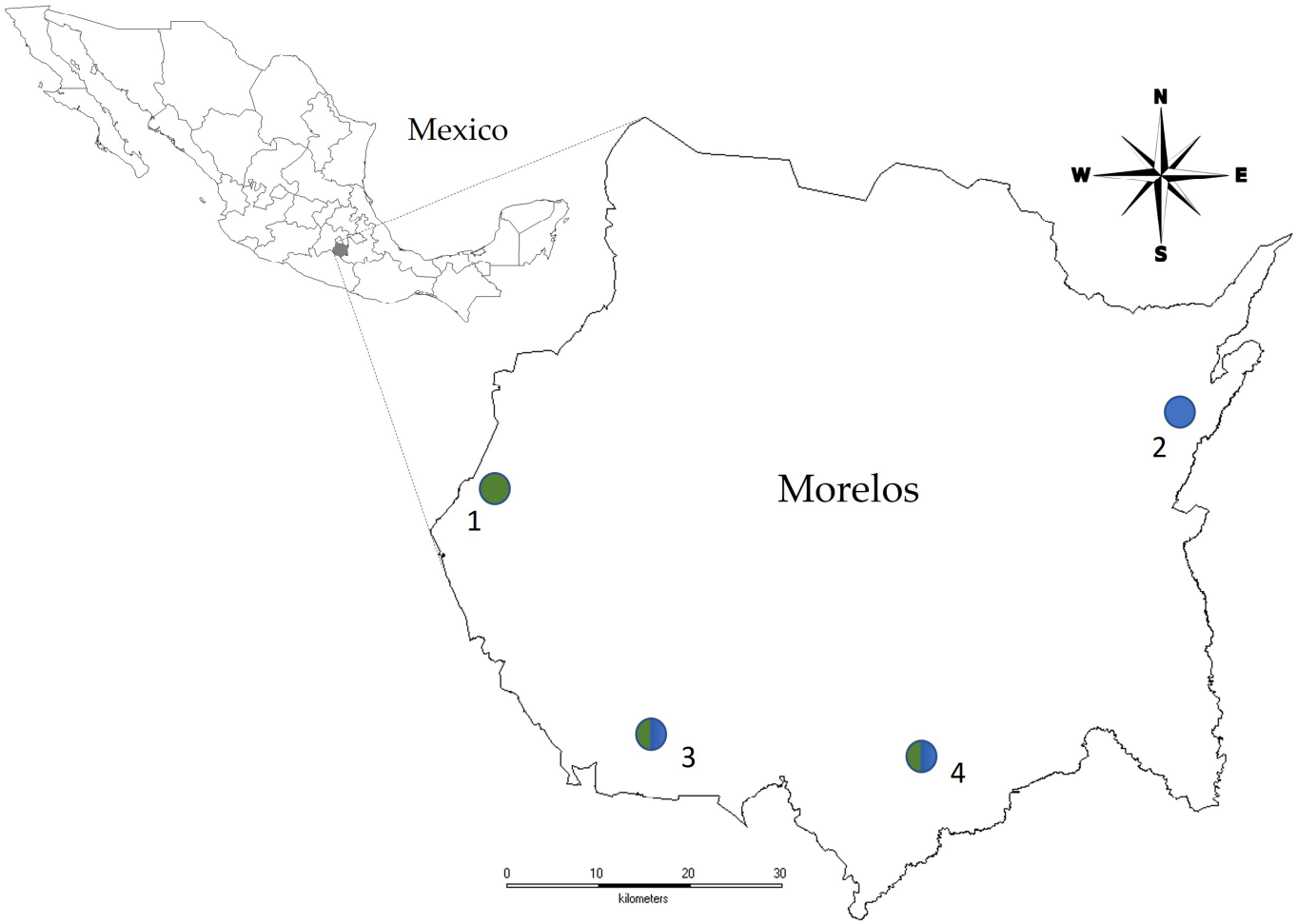

Figure 1. Map of sampled populations of *Bursera bicolor* (Will. ex Schltdl.) Engl. × *B. glabrifolia* (H.B.K) Engl. complex in the Morelos State, Mexico. The allopatric zones are represented by numbers: 1 = *B. bicolor*, Coatlán del Río and 2 = *B. glabrifolia*, Tetela del Volcán. The sympatric zones between *B. bicolor* and *B. glabrifolia* are represented by numbers: 3 = La Tigra, 4 = Quilamula.

2.2. Study Species

Bursera glabrifolia is recognized easily in the field by its imparipinnate leaves, oblong shape, (5)7 or 9(11) sessile or almost sessile folioles, suborbicular terminal, and glabrous and glossy upper surface and underside with sparse pubescence on veins [18,22]. On the other hand, the *B. bicolor* leaves present strong differences between upper (glabrous–glossy) and underside (stormy-white), imparipinnate leaves 18–25 cm long and ribbed petioles 2–4 (10) cm long, with 13–17 sessile leaflets and lanceolate to linear-lanceolate 4–8 (10) cm long and (3) 6–10 (15) mm wide [22].

2.3. Population Sampling

Four sites in Morelos State, Mexico, were studied (Figure 1). To minimize the environmental influences that can modify the genetic flow and SM chemical profile, four sites were chosen with common characteristics: vegetation type (DTF), geological history (formation processes of all sites began during the Quaternary–Pliocene [24]), climate (warm

sub-humid), type of soil (Feozem), and local disturbance by cultivated fields and livestock (rural zones). Botanical samples were identified by Gabriel Flores Franco MSc as *Bursera glabrifolia* and *B. bicolor* at the HUMO herbarium (Herbarium of the University of Morelos, Biodiversity and Conservation Research Center, Autonomous University of the Morelos State) and deposited with the following voucher numbers: 31,092, 31,093, 31,094, 31,095, 31,096, and 31,097.

2.4. Molecular Data

Foliar tissue with no apparent damage from herbivory were collected from 160 trees of *B. bicolor* (n = 60), *B. glabrifolia* (n = 60), and hybrids (n = 40), distributed in two allopatric zones (20 trees per zone) and two sympatric zones (20 trees per taxon/zone). The leaves were frozen in a container with liquid nitrogen and carried to the environmental research laboratory for DNA extraction. Genomic DNA was removed from 120 mg of fresh leaf tissue employing the cetyl trimethyl ammonium bromide (CTAB) technique with the variation of Doyle and Doyle [25]. DNA was quantified using fluorometric analysis (Eppendorf, Hamburg, Germany).

First, we verified microsatellite polymorphism with a group of individuals of both *B. bicolor* and *B. glabrifolia*, employing eight chloroplast microsatellite markers (ccmp1, ccmp2, ccmp3, ccmp4, ccmp7 [26], NTCP9, NTCP13, and NTCP18 [27]), and the polymerase chain reaction (PCR). Only four showed polymorphic loci for both *Bursera* species (ccmp2, ccmp4, ccmp7, and NTCP18). PCRs were carried out in a volume of 10 µL employing the specifications described in Paniego et al. [28]. We measured the length of the amplified SSR fragments by running an aliquot of each PCR product on an ABI 3100 automatic sequencer (Applied Biosystems, Foster City, CA, USA) at 35 W for 80–90 min using gene scan ROX-2500 (Applied Biosystems, CA, USA) as size standard. Gene Mapper software (ver. 3.7, Applied Biosystems, CA, USA) was used to evaluate the allele score.

2.5. Genetic Analysis

We characterized the genetic variation of *B. bicolor*, *B. glabrifolia*, and putative hybrids employing allele frequencies per locus in each population (four cpSSRs). Haplotypes represent a combination of unique four cpSSRs which were polymorphic. The haplotypic variation was estimated in each taxon using the unbiased genetic diversity following the methods of Nei [29], together with the number of haplotypes (*nh*), gene diversity (*Gd*), and Shannon diversity index (*H*). Shannon diversity index ($-\Sigma p_i \ln(p_i)$) [30] and gene diversity ($1 - \Sigma {p_i}^2$) [29] (where p_i is the frequency of the ith allele and $\Sigma {p_i}^2$ is the sum of the squared allele frequencies) were calculated in POPGEN version 1.13 and ARLEQUIN version 2000, respectively.

The software STRUCTURE 2.3 [31] was used to estimate to which genetic groups cpSSRs were assigned, further corroborated with the Evano method [32], where ΔK value refers to the probability that sampled alleles come from certain number of genetic groups involved in genetic inheritance. Subsequently, we employed the model proposed by Lepais et al. [33], in which all individuals with an assignment coefficient Q = 0.9 were identified as purebred of *B. bicolor* or *B. glabrifolia*.

2.6. Plant Material

Canopy leaves (base, middle, and top) with no apparent damage (e.g., fungi, herbivory) were collected from 80 trees of *B. bicolor* (n = 30), *B. glabrifolia* (n = 30), and hybrids (n = 20), distributed in two allopatric zones (10 trees per zone) and two sympatric zones (10 trees per taxon/zone).

2.7. Obtaining n-hexane Extracts

The leaves of each individual were dried at room temperature under shade and crushed until homogeneous particles were obtained. Subsequently, 15 g of each individual was extracted by maceration with 150 mL of n-hexane (1:10 weight/volume) after 72 h. The

organic phase was filtered and concentrated under reduced pressure in a rotary evaporator (BUCHI model R-100); the same process was repeated three times for every individual.

2.8. Analysis of the Extracts by GC/MS

All n-hexane extracts of *B. glabrifolia*, *B. bicolor*, and hybrids n = 80 were analyzed by a GC-MS Agilent GC 6890, MSD 5973N (Agilent Technologies, Santa Clara, CA, USA) to determine their chemical composition. The analysis was conducted with the column HP-5MS (30 mm × 0.25 mm × 0.25 µm). The carrier gas was helium with a gas flow rate of 1 mL/min and a linear velocity of 37 cm/s. The injector temperature was set to 250 °C Splitless. The initial oven temperature was 40 °C and increased to 250 °C for 5 min and 10 °C/min, and the final temperature was held at 285 °C for 20 min. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. The compounds were identified by comparison of retention times and fragmentation patter, with reference compounds in the NIST version 1.7a database. The relative percentage amounts were determined by integrating the peaks using GC ChemStation software version C.00.01. The composition is presented as a percentage of the total peak area.

2.9. Phytochemical Analysis

With the GC-MS chromatograms, supervision of all the compounds that each individual presented was carried out; from minute 6 to minute 45 of retention, the percentage of correlation between the theoretical and experimental defragmentation pattern was verified. Compounds with correlation greater than 80% were accepted as authentic. The abundance of each supervised SM was registered, obtaining a matrix of concentration and retention time for each individual species, the hybrids, and the different locations. Non-metric multidimensional scaling (NMDS) analysis was used to observe the differences in chemical composition between species and localities with the MS. These analyses were carried out by section according to the size of the molecules to determine the type of MS used for differentiation. The NMDS was performed to generate a dissimilarity matrix between species and localities (two allopatric, two sympatric) using the Bray–Curtis coefficient of dissimilarity [34]. ANOSIM bootstrap analysis was employed to test the differences in the chemical composition among species in the different locations, and 9999 random reassignments were made in the ANOSIM with the Bonferroni adjustment to determine whether there are significant differences between the groups by type of molecules, chemical composition, and locality. Finally, to ascertain which MS group contributed the most to the similarity or dissimilarity between parental species and hybrids, an analysis of similarity percentages (SIMPER) was employed. These analyses were performed with the PAST program version 4.12 c [35].

3. Results

3.1. Assignment of Parental Categories

3.1.1. Genetic Data

The 4 (ccmp3, ccmp4, ccmp7, and Ntcp18) cpSSR loci were polymorphic in 160 individuals of *B. bicolor*, *B. glabrifolia* and derived hybrids. The Bayesian clusters obtained applying the software STRUCTURE for sympatric and allopatric zones show two well-defined genetic groups that support the two pure phenotypic species previously identified as *B. bicolor* and *B. glabrifolia*. This finding was confirmed by the ΔK values, which indicate that K = 2 is the most likely number of genetic groups (Figure 2). Analyses employing STRUCTURE program showed a high percentage of ancestry ($Q > 0.9$) for individual trees from the allopatric reference populations. We detected that 62% of *B. bicolor* individuals were identified as purebred ($Q = 0.994 \pm 0.02$ (mean SD), and 50% of *B. glabrifolia* individual distributed in the allopatric zone were classified as purebred ($Q = 0.985 \pm 0.01$).

A total of 16 alleles and 24 haplotypes were found. *B. bicolor* and *B. glabrifolia* share alleles at all loci, but each harbored private allele was not found in the other species (*B. bicolor* = 10 and *B. glabrifolia* = 6). The test for homogeneity of allele distribution was

highly significant ($p < 0.001$) for all four loci. Considering the frequencies of all haplotypes for the analysis, we found that genetic diversity of parental species varies as follows: *B. bicolor* > *B. glabrifolia* (Table 1).

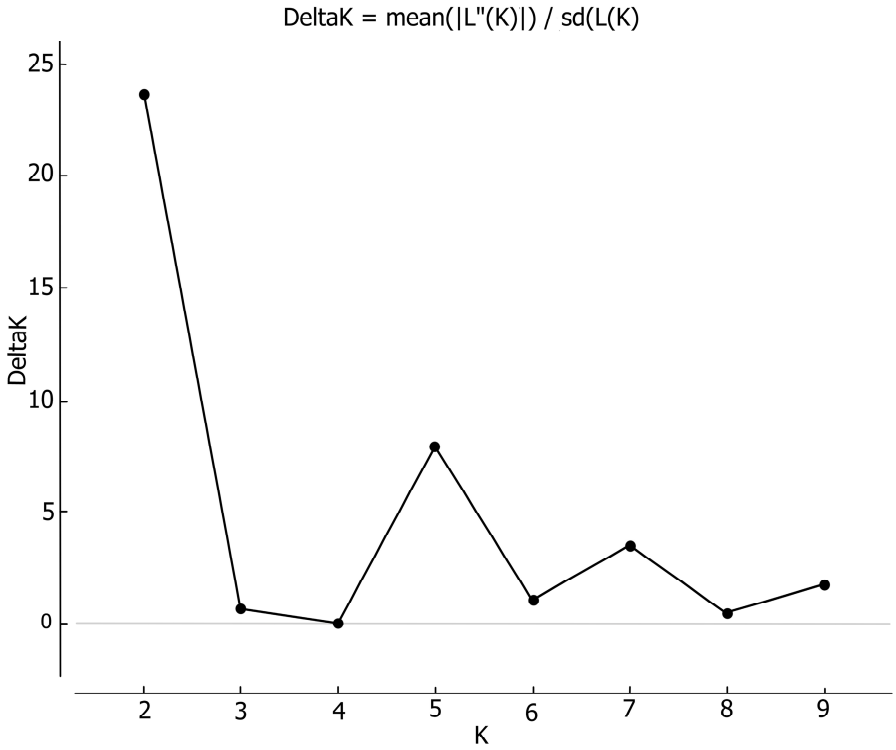

Figure 2. Cluster analysis to estimate genetic groups (*K*) with STRUCTURE program. Graph of the statistic delta *K* with respect to genetic grouping *K* (from 1 to 9). The peak shows the most probable number of genetic groups. Delta *K* of the genetic groups associated with the complex *B. bicolor* (Will. ex Schltdl.) Engl. × *B. glabrifolia* (H.B.K) Engl.

Table 1. Genetic diversity at cpDNA markers in four Mexican populations of *Bursera bicolor* (Will. ex Schltdl.) Engl. × *B. glabrifolia* (H.B.K) Engl. complex.

Taxa	Locality	N	No. Loci	*nh*	*H*	*Gd*
B. bicolor	Coatlán del Río	20	4	13	0.539	0.275
B. glabrifolia	Tetela del Volcán	20	4	11	0.412	0.201
B. bicolor × *B. glabrifolia*	La Tigra	20	4	14	0.750	0.314
B. bicolor × *B. glabrifolia*	Quilamula	20	4	15	0.846	0.379

Note: N, number of individuals; *nh*, number of haplotypes; *H*, Shannon diversity index *Gd*; gene diversity.

3.1.2. Chemical Data

In total, allopatric populations of *B. bicolor* and *B. glabrifolia* registered 26 SMs, belonging to the terpene family (monoterpenes, sesquiterpenes, and triterpenes) (see Supplementary Materials Table S1). Each parental specie reported three diagnostic secondary metabolites (DSMs) of terpenes (*B. bicolor*: cis-verbenol; myrtenol; bicyclo[3.1.1]hept-3-en-2-one,4,6,6-trimethyl- and *B. glabrifolia*: pinocarveol; benzemethanol,α,α,4-trimethyl-; bornyl acetate); eight DSMs of sesquiterpenes, one in *B. bicolor* (caryophyllene oxide) and seven in *B. glabrifolia* (spathulenol; tricyclo [5.2.2.0(1,6)] undecan-3-ol-2-methylene-6,8,8-trimethyl-; Naphthalenol,2,3,4,4a,5,6,7-octahydro-1,4a-dimethyl-7-(2-hydroxy-1-methylethyl)-; 7R, 8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3]undec-1-ene; platambin). Finally, *B. bicolor* registered D,α-tocopherol and six DSM-like triterpenes (β-amyrin (a); β-amyrin (b); α-amyrin (b); friedelin; 6a,14a-methanopicene,perhydro-1,2,4a,6b,9,9,12a-heptamethyl-

10hydroxy-; acetic acid,4,4,6a,8a,11,12,14b-octamethyl-14-oxo-1,2,3,4,4a,5,6,6a,7,8,8a,9,10,11,12,12a,14,14a,14b-eicosahydropicen-3-y ester), while *B. glabrifolia* record the triterpene α-amyrin (a) as the only DSM.

We detected significant differences in SM composition between putative parental species, regardless of the family of SM analyzed (monoterpenes, ANOSIM $R = 0.9727$, $p = 0.0002$, sesquiterpenes, $R = 0.9847$, $p = 0.0001$, triterpenes, $R = 1.0$, $p = 0.0001$, all SMs $R = 1.0$, $p = 0.0001$, Figure 3).

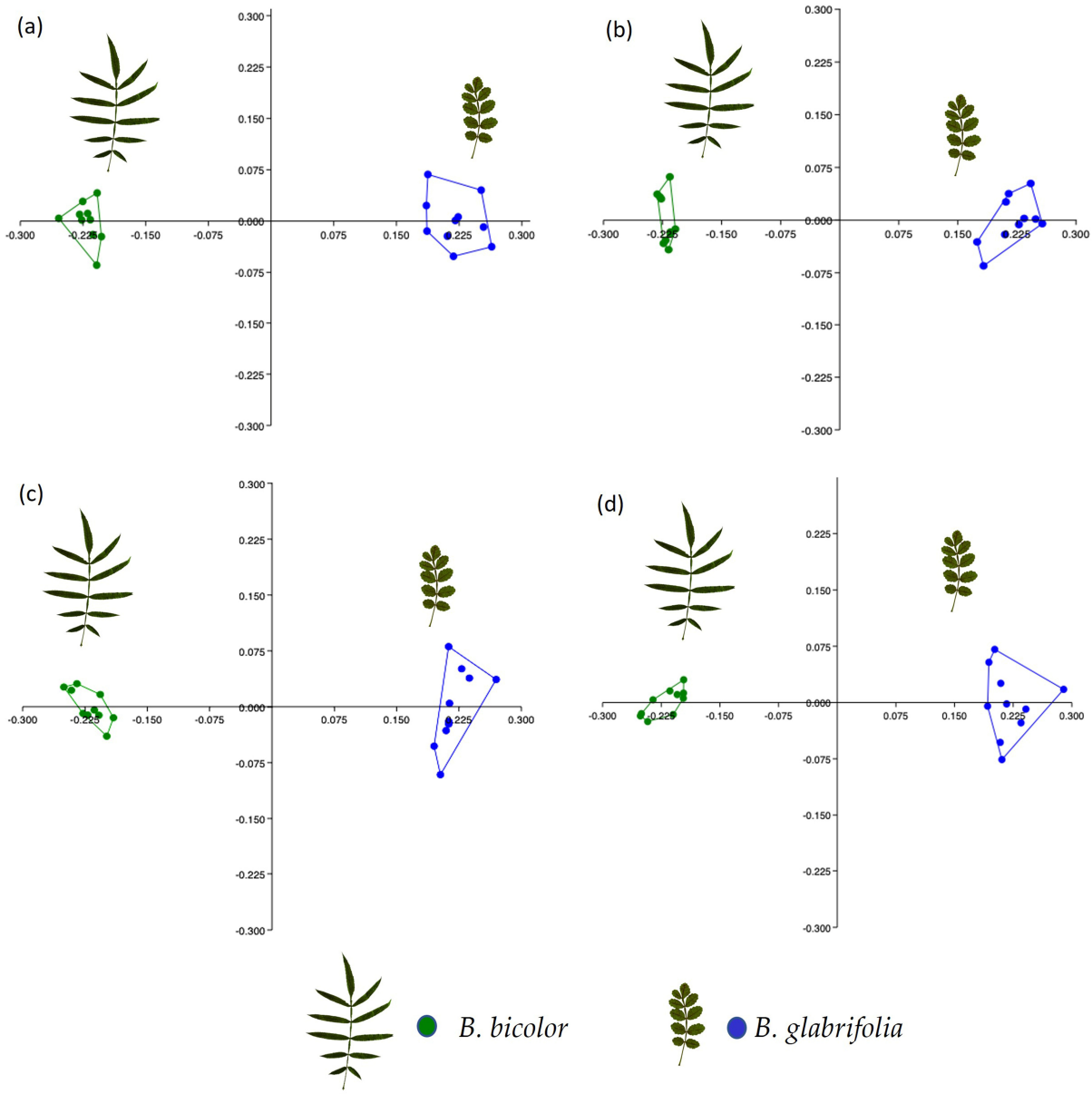

Figure 3. Differences between secondary metabolites (SMs) of *B. bicolor* (Will. ex Schltdl.) Engl. and *B. glabrifolia* (H.B.K) Engl., employing NMDS. Each point is a two-dimensional (NMDS 1 and NMDS 2) representation of SM composition (10 points per *Bursera* species). Distances between points show a dissimilarity matrix created using the Bray–Curtis dissimilarity coefficient. Points that are close together have SMs that are more similar in composition compared to points that are far apart. Data of monoterpenes (stress values: 0.1463; final instability: 0.0003), sesquiterpenes (stress values: 0.090; final instability: 0.0002), triterpenes (stress values: 0.1314; final instability: 0.0001), and all SMs (stress values: 0.1357; final instability: 0.0001) were used in this analysis. (**a**) monoterpenes, (**b**) sesquiterpenes, (**c**) triterpenes, and (**d**) all SMs.

On the other hand, the SIMPER analysis recognized that the three most important SMs in relation to abundance per family, that contributed to the dissimilarity between putative parental species, were as follows: monoterpenes (benzemethanol,α,α,4-trimethyl-; pinocarveol and trans-verbenol), sesquiterpenes (spathulenol; platambin and hexahydrofarnesyl acetone), and triterpenes (α-amyrin (b); β-amyrin (a) y β-amyrin (b)), all SMs (α-amyrin (b); β-amyrin (a); β-amyrin (b)) (see Supplementary Materials Table S2).

3.2. Assignment of Hybrid Categories

3.2.1. Genetic Data

Genetic analyses of 40 trees randomly sampled in two sympatric zones showed the presence of nine trees (20% of the total analyzed individuals) with signs of mixed ancestry. The results of Bayesian analysis showed that the number of hybrid and pure genotypes changes between the sympatric zones, with a hybridization of 25% in Quilamula and 21% in La Tigra. In general, the two sympatric zones showed a dominance of pure *B. bicolor* genotypes (Quilamula with 42.9% and La Tigra with 63.2%) and of pure *B. glabrifolia* genotypes which registered the following percentages: Quilamula 33.3% and La Tigra 15.8% (Figure 4).

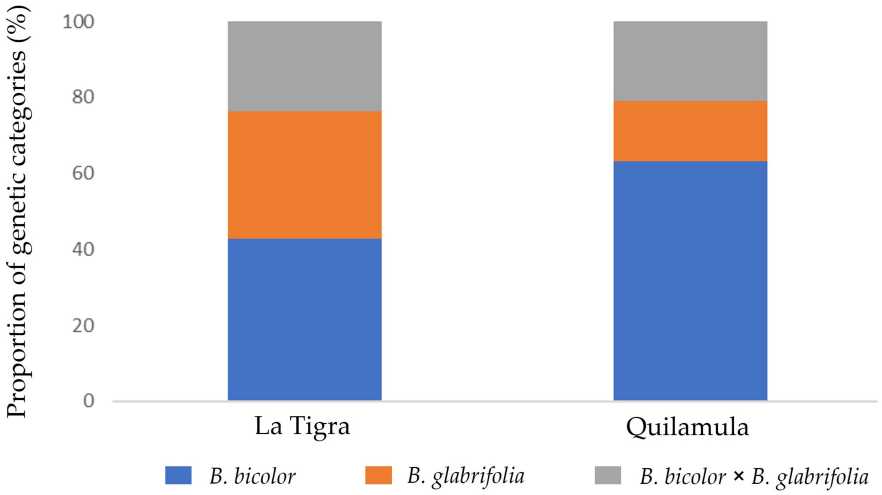

Figure 4. Proportion of the different genetic categories documented in each sympatric *Bursera bicolor* (Will. ex Schltdl.) Engl. and *B. glabrifolia* (H.B.K) Engl. population. Trees were assigned to each category (*B. bicolor* pure genotype, *B. glabrifolia* pure genotype, and *B. bicolor* × *B. glabrifolia* hybrid genotype), depending on their individual coefficient of admixture derived from STRUCTURE.

Hybrid populations had the highest levels of haplotype number (*nh*), gene diversity (*Gv*), and Shannon diversity index (*H*) in comparison with *B. bicolor* and *B. glabrifolia* (Table 1). A total of 29 haplotypes were found in sympatric zones. Private alleles were higher in hybrid populations (n = 19) in comparison to allopatric populations of *B. bicolor* (n = 10) and *B. glabrifolia* (n = 6).

3.2.2. Chemical Data

In general, 37 SMs belonging to the family of terpenes associated with the *B. bicolor* × *B. glabrifolia* complex were recorded (monoterpenes, sesquiterpenes, and triterpenes). Hybrid plants recorded 11 SMs that were not registered in the putative parental species. Two monoterpenes (3-cyclopentene-1-acetaldehyde,2,2,3-trimethyl-, bicyclo[2,2,1] hept-3-one,6,6-dimethyl-2-methylene), one sesquiterpene (caryophyllene) and eight triterpenes (ursa-9(11),12-dien-3-ol; ursa-9(11),12-dien-3-one; 4,4,6a,6b,8a,11,11,14b-Octametyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14b-octadecahydro-2H-picen-3-one; 9,19-Cyclolanostan-3-ol,24,24-epoxymethano-,acetate; 13,27-cycloursan-3-ol,acetate,(3β, 13β 14β)-; 9,19-Cyclolanostan-3-ol,24,24-epoxymethano-,acetate; acetic acid, 4,4,6a,8a,11,12,14b-octamethyl-

14-oxo-1,2,3,4,4a,5,6,6a,7,8,8a,9,10,11,12,12a,14,14a,14b-eicosahydropicen-3-y ester; acetic acid, 4,4,6a,8a,11,12,14b-octamethyl-14-oxo-1,2,3,4,4a,5,6,6a,7,8,8a,9,10,11,12,12a,14,14a,14b-eicosahydropicen-3-y ester) (see Supplementary Materials Table S1).

We detected significant differences in SM composition among three genotypes (*B. bicolor*, *B. glabrifolia*, and hybrids), independently of the terpene family analyzed in La Tigra hybrid zone (monoterpenes, ANOSIM R = 0. 5925, p = 0.0001, sesquiterpenes, R = 0.6639, p = 0.0001, triterpenes, R = 0.8678, p = 0.0001, all MS R = 0.8556, p = 0.0001, Figure 5).

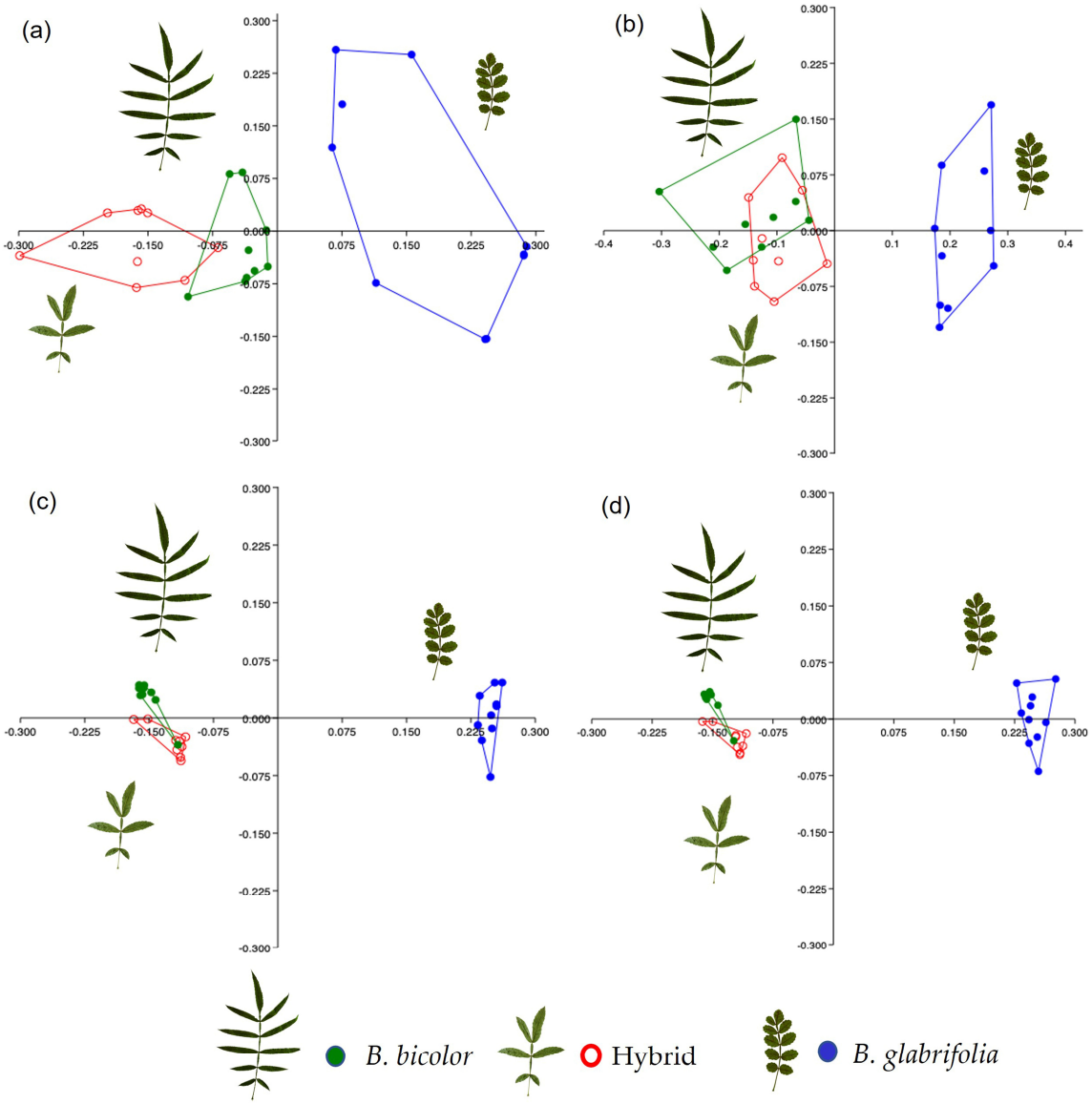

Figure 5. NMDS analysis of SM of the sympatric site La Tigra, *B. bicolor* (Will. ex Schltdl.) Engl. (green), *B. glabrifolia* (H.B.K) Engl. (blue), and hybrids (red); (**a**) monoterpenes, (**b**) sesquiterpenes, (**c**) triterpenes, (**d**) all SMs.

Based on the composition of SMs, the NMDS analysis showed the ordination of three chemical groups regardless of the analyzed SM family. These three chemical groups correspond to the three genotypes recognized with molecular markers (*B. bicolor*, *B. glabrifolia*, and hybrids) (Figure 5).

SIMPER analysis showed that the SMs that most contribute to the dissimilarity between hybrids and their putative parental species were as follows: monoterpenes: trans-verbenol and bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-; sesquiterpenes: caryophyl-

lene oxide and hexahydrofarnesyl acetone; triterpenes: hybrids vs. *B. bicolor* (Lupeol and α-amyrin (b)), hybrids vs. *B. glabrifolia* (α-amyrin (b) and β-amyrin (a)), all MS: hybrids vs. *B. bicolor* (Lupeol and α-amyrin (a)), hybrids vs. *B. glabrifolia* (α-amyrin (b) and β-amyrin (a)) (see Supplementary Materials Table S3).

In the sympatric site of Quilamula, the ANOSIM detected that the SM composition differs significantly among the three *Bursera* taxa, independently of the analyzed SM family (monoterpenes: $R = 0.4419$, $p = 0.0001$, sesquiterpenes $R = 0.7002$, $p = 0.0001$, triterpenes $R = 0.8692$, $p = 0.0001$, all SMs $R = 0.8661$, $p = 0.0001$).

On the other hand, the NMDS analysis showed the conformation of three groups, based on their chemical SM composition, independently of the analyzed SM family (Figure 6). The three groups correspond to the three genotypes groups (*B. bicolor*, *B. glabrifolia*, and hybrids) that were recognized with molecular markers (Figure 4).

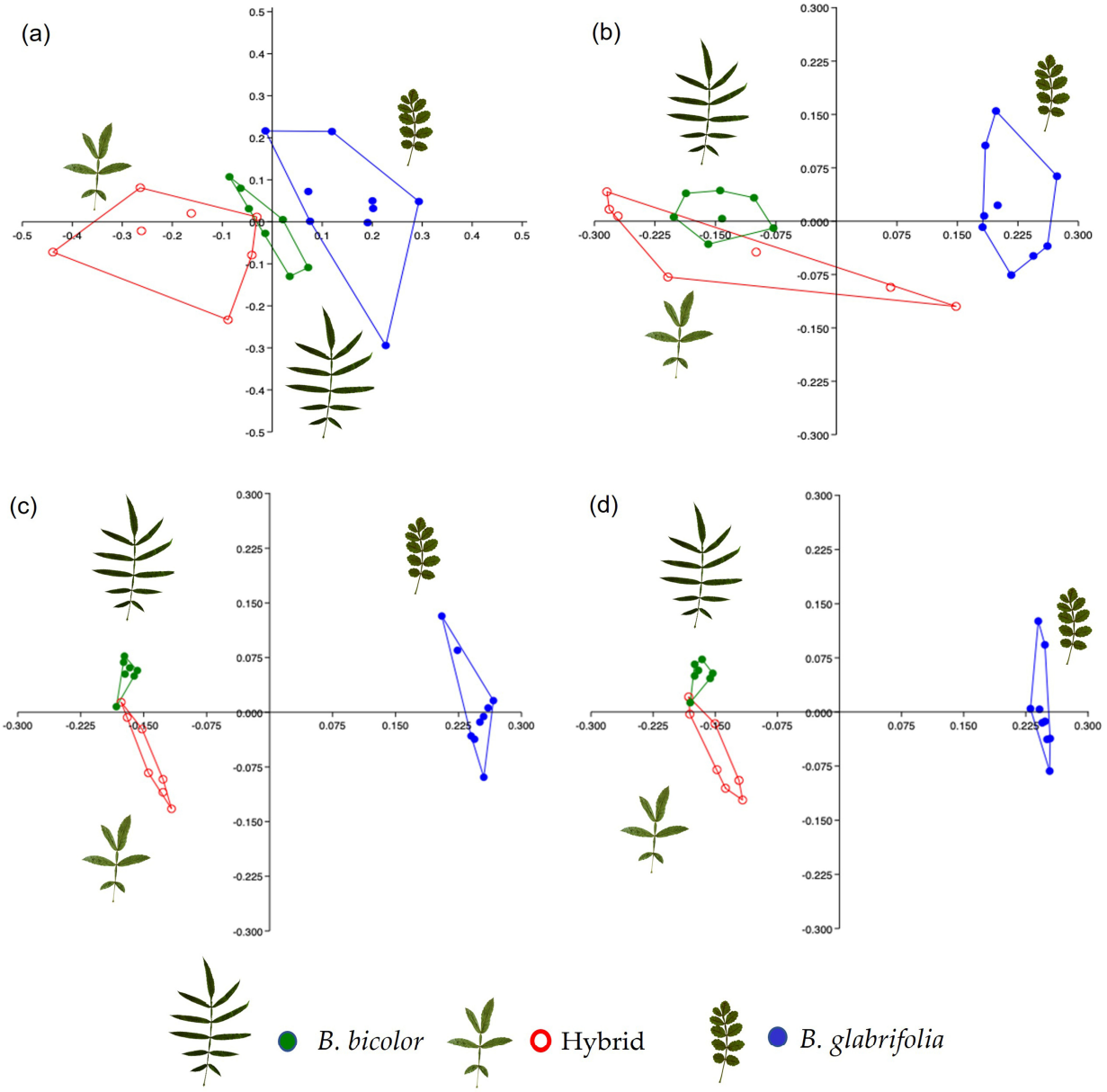

Figure 6. NMDS analysis of the sympatric site of Quilamula, *B. bicolor* (Will. ex Schltdl.) Engl. (green), *B. glabrifolia* (H.B.K) Engl. (blue), and hybrids (red); (**a**) monoterpenes, (**b**) sesquiterpenes, (**c**) triterpenes, (**d**) all SMs.

SIMPER analysis showed that the SMs that most contributed to the dissimilarity between hybrids and their putative parental species were as follows: monoterpenes: trans-verbenol and bicyclo[3.1.1]hept-3-en-2-one,4,6,6-trimethyl-; sesquiterpenes: hexahydro-farnesyl acetone and caryophyllene oxide: triterpenes: hybrids vs. *B. bicolor* (α-amyrin (b) and Lupeol), hybrids vs. *B. glabrifolia* (α-amyrin (a) and β-amyrin (b)), all SM: hybrids vs. *B. bicolor* (α-amyrin (b) and Lupeol), hybrids vs. *B. glabrifolia* (α-amyrin (a) and β-amyrin (b)) (see Supplementary Materials Table S4).

4. Discussion

4.1. Genetic and Chemical Assignment of Bursera bicolor and B. glabrifolia at Allopatric Sites

The field determination from the foliar morphological characteristics of *B. bicolor* and *B. glabrifolia* was confirmed by SSR analyses, which recognize two well-defined genetic groups in allopatric zones. Our study is supported by Centeno-Betanzos et al. [8], who documented with microsatellites that allopatric populations previously determined by morphological characteristics of *Zephyranthes alba* and *Z. fosteri* are two well-defined genetic groups, suggesting that these populations are useful as reference populations because they maintain their own morphological and genetic identities. Similarly, these markers were useful for identification of several *Vitis* species and interspecific hybrids [36] and *Rhizoctonia* species, fungal pathogens that diminish crop quality and yield of cotton (*Gossypium* sp.) [37]. In general, it has been recognized that the suitable number of SSRs for the acceptable detection of the number of genetic groups and for putative hybrid identification depends on their efficiency, exclusion power, and accuracy (e.g., [9,38]). We documented the capacity of the cpSSR set to differentiate between *B. bicolor* and *B. glabrifolia* genotypes produced with HybridLab. We supported our results with Vähä and Primmer [39] criterion, employing a threshold of $Q \geq 0.9$ to categorize trees as purebred, consistent with other studies (e.g., [9,40]).

Our results showed a significant percentage ($Q > 0.1$) of foreign genome in some trees within allopatric population, saccording to Vähä and Primmer [39]. The introduction of the foreign genome could be explained by agroecological management in *Bursera* species; for example, we observed in Tetela del Volcán and Coatlán del Río that *Bursera* trees are regularly used as living fences, and this management event can still have some influence on the genetic diversity of distant populations. Vegetative propagation of *Bursera* species from cutting is a common management practice in *Bursera* species [41,42] and may be utilized for restoration practices of TDF, where they are frequently absent, for commercial purposes, or in agroforestry activities.

Of the 37 SMs identified in this study, 57% (n = 21) were produced exclusively in the parental species (*B. bicolor*, n = 12; *B. glabrifolia*, n = 9), 13.5% (n = 5) were present in parental species and hybrids and all localities, and 30% (n = 11) of SMs are novel compounds in the hybrid trees (see Supplementary Materials Table S1). In general, hybrids of the *B. bicolor* × *B. glabrifolia* complex have chemical characteristics that are additive, novel, and like those of their parental species (see Supplementary Materials Table S1). Diverse studies have recognized that differences in SM production after hybridization events may be clarified by three processes: (i) polymorphism in the loci that regulate the production of the SMs in the parental species; (ii) changes in the biosynthetic pathways; and (iii) the extension of various phases of the metabolic pathway [13]. The synthesis of novel compounds in hybrid plants can explain the presence of novel compounds in the hybrid of the *B. bicolor* × *B. glabrifolia* complex. Although, at the phenotypic level, the effect of hybridization phenomenon on SM production is recognized, the genetic causes of the high occurrence of chemical additivity and the synthesis of novel SMs in hybrids remain basically unidentified [43].

In this study, the chemical analyses show that in the allopatric populations there are two well-defined groups, each group corresponding to each putative parental species, independently of SM family analyzed (monoterpene, sesquiterpene, triterpene). In general, NMDS, ANOSIM, and SIMPER analysis documented that triterpene compounds are the

SMs that provide the most information on the dissimilarity between species. These results are supported by the studies of Paul et al. [44] and Zhang et al. [45] on species of the genus *Boswellia*; the authors suggest that triterpene is a good chemical marker to discriminate between species of *Boswellia*. In addition, *Boswellia* is a genus phylogenetically close to the genus *Bursera* [46], which supports the idea of employing triterpene SM as a chemical marker to answer taxonomic problems among *Bursera* species. Therefore, it is to be expected that the changes resulting from hybridization will be less striking when the parental species share similar genomes than when the genomes of two phylogenetically more distant species are mixed.

In contrast, in this study, we registered the presence of only 13.5% (n = 5) of SMs (trans-verbenol; hexahydrofarnesyl acetone; †-sitosterol, spiro-10-(tricyclo[5.5.0.0(5,9)]decane-7,8-diol)-(oxirane),1-methyl-4-isopropyl-(8S)-; Lupeol) (see Supplementary Materials Table S1) that are produced in all taxa (hybrids and putative parental species) and at all study sites. These results may be due to the documented fact that terpene SMs have high heritability in the plants with which they are associated. It is possible that intraspecific variation is higher because the individuals derived by hybridization events between distance species have metabolic profiles that are different from both parental species [47,48]. In this sense, Becerra and Venable [49] showed that *B. bicolor* and *B. glabrifolia* are phylogenetically distant species using nuclear ribosomal DNA. It is thus reasonable that the variations caused by interspecific hybridization will be overexpressed when the parental species share different genomes than when the genomes of two phylogenetically more nearby species are mixed. This last statement can also clarify the high number of novel SMs produced in hybrid trees (n = 11) of this complex, which exceeds the percentage of novel SMs (from 5 to 18%) documented by Rieseberg and Ellstrand [50] and Cheng et al. [13].

4.2. Genetic and Chemical Evidence of Hybridization between Bursera bicolor and B. glabrifolia

The hybridization phenomenon documented with the microsatellite markers in this study supports the hypothesis that the atypical foliar morphology observed in individuals in sympatric zones between *B. bicolor* and *B. glabrifolia* can be promoted, in part, to interspecific genetic flow. Atypical foliar morphology at sympatric zones between tree species denotes the first signal of hybridization occurrences; therefore, it is considered a useful tool in the field [9,10,51,52].

The Bayesian analysis documented the occurrence of nine hybrid individuals that showed admixture between *B. bicolor* and *B. glabrifolia* involved in this complex in two sympatric populations. These results are congruent with a significant increase in genetic diversity levels in hybrid zones (number of haplotypes; gene diversity; Shannon diversity index) in relation to the parental species, indicating that there is introgressive hybridization in the sympatric zones [5,21,53,54]. Our results suggest that hybrid zones favor an increase in genetic diversity levels.

Clearly, the DTF in "Cuenca del Balsas" has been recognized as a hotspot for richness and endemism of the genus *Bursera*, where diverse maternal lineages combine while exhibiting high levels of diversity. Regardless of the variations noted in how species partition cpDNA diversity, they widely share the same haplotypes, even at a fine spatial scale. These last observations are supported by previous studies in Mexico between *B. schlechtendalli* × *B. morelensis* [55], *B. bipinnata* × *B. gracilis* [18], *B. bipinnata* × *B. cuneata* [56], and *B. cuneata*, *B. bipinnata*, and *B. palmeri* [21]. They support the view that introgressive hybridization was not limited to sporadic events during long periods of sympatry.

In both hybrid zones, the composition of SM data documented three well-defined groups; these groups correspond to *B. bicolor*, *B. glabrifolia*, and putative hybrids shown by microsatellites. Similarly, Hinge and colleagues [57] using several molecular markers (including microsatellites) documented those nine banana cultivars (*Mussa* sp.) with different genomic constitutions showing different volatile compound profiles. The above mentioned differences suggest that chemical characteristics showed genetic cohesivness of each taxon. These results agree with other reported studies since the chemical char-

acteristics are a powerful tool for discriminating among taxa involved in hybridization events [13]. Moreover, the locality is not a factor that modified the pattern of cohesive chemical characteristics; therefore, these results can be explained considering that both localities have common ecological and geological characteristics, for example geological history (the formation process of both localities began during the Quaternary–Pliocene [24]), climate (warm sub-humid), vegetation type (dry tropical forest), soil type (Feozem), and these areas present local disturbance by cultivated fields (rural zones). Therefore, ecological and geological similarity between localities can reduce the environment role in the expression of phytochemical traits [58,59].

Natural hybridization between *B. bicolor* and *B. glabrifolia* may have several evolutionary and ecological consequences. Hybridization has been documented as an evolutionary force that facilitates the combination of genetic variants among involved species, facilitating the expression of new traits and the segregation of transgressive traits, a requirement that can promote speciation and adaptation events [60]. For instance, both species exhibit a wide geographic distribution and can be established in populations of disturbed habitats. Anthropogenic disturbance (e.g., agriculture, forest fires, road construction, and logging) may create new opportunities for *B. bicolor* and *B. glabrifolia* to attain sympatry. Anthropogenic activities may increase the hybridization events as they modify reproductive barriers [61]. In this sense, use and management of *Bursera* species may promote the overlap of species in time and space and can have some impact on hybridization events.

The high genetic diversity in hybrid trees can display phenotypic novelty, including SM chemical profiles. They may influence changes in the abundance, distribution, and diversity of herbivores due to their novel SM chemical profiles. In this sense, the *B. bicolor* × *B. glabrifolia* complex documented a high number ($n = 9$) of novel SMs. Phytotoxic activity for terpenes has been documented; for example, caryophyllene compound (sesquiterpene) presents antipredator activity in larvae of *Blepharida flacostovea* (Chrysomelidae) [62]. On the other hand, Raffa et al. [63] documented that a high concentration of monoterpene increases the mortality percentage of *Ips pini* beetle (adults) (Curculionidae). Therefore, further studies are necessary to elucidate the phytotoxic activities and fitness of *Bursera* hybrids.

In general, the genus *Bursera* is distributed mainly in DTF, and it is characterized by the high frequency of hybridization in natural conditions, and Mexico is considered the main diversification center of the genus. Our results suggest that DTF with high *Bursera* species richness co-dominated by *B. bicolor* and *B. glabrifolia* may facilitate an increase in hybridization events with other *Bursera* species, promoting the establishment of species complexes. From a conservation perspective, the high genetic diversity generated by hybridization events can have cascading effects that extend to their associated communities or in ecosystem processes, for example nutrient cycling due to the SM composition of hybrids [6]. Moreover, it has recently been recognized that climate changes promote the movement of species far from their original distribution range, which has promoted contact between species that increase the probability of hybridization. Finally, our study shows the importance of considering the genetic and chemical information of the species to develop effective management and conservation strategies to maintain the biodiversity of DTF.

5. Conclusions

Hybrid populations showed the highest levels of haplotype number, genetic diversity, and total genetic diversity in comparison with sympatric populations. NMDS and ANOSIM analyses showed significant differences in SM composition between parental species, regardless of the family of SMs analyzed. Hybrid individuals registered 11 novel SMs, that were not registered in the parental species and were distributed as follows: triterpenes > monoterpenes > sesquiterpenes. Triterpenes were the SMs that most contributed to differentiating putative parental species according to SIMPER analyses. The hybrids registered the highest genetic diversity values along with the highest number of SMs. Based on the composition of SMs, we registered three well-defined chemical groups regardless of

the analyzed SM family according to ANOSIM and NMDS analyses. These three chemical groups correspond to the three genotypes recognized with molecular markers. Hence, the genetic and chemical markers were highly coincident, and they supported the hybridization hypothesis in this *Bursera* complex, with both species remaining distinct even in sympatric zones.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14071382/s1, Table S1: Table of compounds reported by CG-MS in the complex *Bursera bicolor* × *B. glabrifolia*. Table S2: Summary of SIMPER results: average concentration of discriminating species in allopatric sites, their contribution (%) to dissimilarity between groups, and the accumulative total (%) of contribution (75% cutoff). Table S3: Summary of SIMPER results: average concentration of discriminating taxa in La Tigra sympatric zone, their contribution (%) to dissimilarity between groups, and the accumulative total (%) of contribution (75% cutoff). Table S4: Summary of SIMPER results: average concentration of discriminating taxa in Quilamula sympatric zone, their contribution (%) to dissimilarity between groups, and the accumulative total (%) of contribution (75% cutoff).

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References

1. Whitney, K.D.; Ahern, J.R.; Campbell, L.G.; Albert, L.P.; King, M.S. Patterns of hybridization in plants. Perspect. *Plant Ecol. Evol. Syst.* **2010**, *12*, 175–182. [CrossRef]
2. Beddows, I.; Rose, L.E. Factors determining hybridization rate in plants: A case study in Michigan. Perspect. *Plant Ecol. Evol. Syst.* **2018**, *34*, 51–60. [CrossRef]
3. Mitchell, N.; Campbell, L.G.; Ahern, J.R.; Paine, K.C.; Giroldo, A.B.; Whitney, K.D. Correlates of hybridization in plants. *Evol. Lett.* **2019**, *3*, 570–585. [CrossRef]
4. Rieseberg, L.H.; Wendel, J.F. Introgression and its consequences in plants. In *Hybrid Zones and the Evolutionary Process*; Harrison, R.G., Ed.; Oxford University Press: New York, NY, USA, 1993; pp. 70–109.
5. Valencia-Cuevas, L.; Piñero, D.; Mussali-Galante, P.; Valencia-Ávalos, S.; Tovar-Sánchez, E. Effect of a red oak species gradient on genetic structure and diversity of *Quercus castanea* (Fagaceae) in Mexico. *Tree Genet. Genomes* **2014**, *10*, 641–652. [CrossRef]
6. Driebe, E.M.; Whitham, T.G. Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. *Oecologia* **2000**, *123*, 99–107. [CrossRef]
7. Arnold, M.L. *Evolution through Genetic Exchange*; Oxford University Press: New York, NY, USA, 2007.
8. Centeno-Betanzos, L.Y.; López-Caamal, A.; Cortés Rendon, N.; León Santiago, M.; Osorio, E.; Bastida-Armengol, J.; Cano-Santana, Z.; Reyes-Chilpa, R.; Tovar-Sánchez, E. Microsatellites, morphological, and alkaloids characterization of *Zephyranthes fosteri* and *Z. alba* (Amaryllidaceae): Allopatric populations. *Biochem. Syst. Ecol.* **2022**, *101*, 104398. [CrossRef]
9. López-Caamal, A.; Reyes-Chilpa, R.; Tovar-Sánchez, E. Hybridization between *Tithonia tubaeformis* and *T. rotundifolia* (Asteraceae) evidenced by nSSR and secondary metabolites. *Plant Syst. Evol.* **2018**, *304*, 313–326. [CrossRef]
10. Zobel, B. Oleoresin composition as a determinant of Pine hybridity. *Bot. Gaz.* **1951**, *113*, 221–227. [CrossRef]
11. Castillo-Mendoza, E.; Salinas-Sánchez, D.; Valencia-Cuevas, L.; Zamilpa, A.; Tovar-Sánchez, E. Natural hybridisation among *Quercus glabrescens*, *Q. rugosa* and *Q. obtusata* (Fagaceae): Microsatellites and secondary metabolites markers. *Plant Biol.* **2019**, *21*, 110–121. [CrossRef]
12. Wink, M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* **2003**, *64*, 3–19. [CrossRef]

13. Cheng, D.; Vrieling, K.; Klinkhamer, P.G. The effect of hybridization on secondary metabolites and herbivore resistance: Implications for the evolution of chemical diversity in plants. *Phytochem. Rev.* **2011**, *10*, 107–117. [CrossRef]
14. López-Caamal, A.; Mussali-Galante, P.; Valencia-Cuevas, L.; Ramírez, J.J.; Flores, K.V.; Tovar-Sánchez, E. Transgressive character expression in hybrid zones between the native invasives *Tithonia tubaeformis* and *Tithonia rotundifolia* (Asteraceae) in Mexico. *Plant Syst. Evol.* **2013**, *299*, 1781–1792. [CrossRef]
15. Georgescu, L.; Stefanakis, M.K.; Kokkini, S.; Katerinopoulos, H.E.; Pirintsos, S.A. Chemical and genetic characterization of *Phlomis* species and wild hybrids in Crete. *Phytochemistry* **2016**, *122*, 91–102. [CrossRef]
16. López-Caamal, A.; Tovar-Sánchez, E. Genetic, morphological, and chemical patterns of plant hybridization. *Rev. Chil. Hist. Nat.* **2014**, *87*, 16. [CrossRef]
17. De-Nova, J.A.; Medina-Lemos, R.; Montero, J.C.; Weeks, A.; Rosell, J.A.; Olson, M.E.; Eguiarte, L.E.; Magallon, S. Insights into the historical construction of species-rich Mesoamerican seasonally dry tropical forests: The diversification of *Bursera* (Burseraceae, Sapindales). *New Phytol.* **2012**, *193*, 276–287. [CrossRef] [PubMed]
18. Rzedowski, J.; Medina-Lemos, R.; Calderón de Rzedowski, G. Inventario del conocimiento taxonómico, así como de la diversidad y del endemismo regionales de las especies mexicanas de *Bursera* (Burseraceae). *Acta Bot. Mex.* **2005**, *70*, 85–111. [CrossRef]
19. Becerra, J.X. Timing the origin and expansion of the Mexican tropical dry forest. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 10919–10923. [CrossRef]
20. Gámez, N.; Escalante, T.; Espinosa, D.; Eguiarte, L.E.; Morrone, J.J. Temporal dynamics of areas of endemism under climate change: A case study of Mexican *Bursera* (Burseraceae). *J. Biogeogr.* **2014**, *41*, 871–881. [CrossRef]
21. Quintero-Melecio, E.; Rico, Y.; Lira-Noriega, A.; González-Rodríguez, A. Molecular evidence and ecological niche modeling reveal an extensive hybrid zone among three *Bursera* species (Section *Bullockia*). *PLoS ONE* **2021**, *16*, e0260382. [CrossRef] [PubMed]
22. Toledo-Manzur, C.A. El Género *Bursera* (Burseraceae) en el Estado de Guerrero. Tesis de Licenciatura, Facultad de Ciencias, Universidad Nacional Autónoma de México, México City, Mexico, 1982.
23. INEGI. Clima. 2022. Available online: https://cuentame.inegi.org.mx/monografias/informacion/mor/territorio/clima.aspx?tema=me&e=17 (accessed on 22 November 2022).
24. Gómez-Tuena, A.; Orozco-Esquivel, M.T.; Ferrari, L. Igneous petrogenesis of the Trans-Mexican Volcanic Belt. In *Geology of Mexico: Celebrating the Centenary of the Geological Society of Mexico*; Alaniz-Álvarez, S.A., Nieto-Samaniego, Á.F., Eds.; Geological Society of America: Boulder, CO, USA, 2007; p. 422. [CrossRef]
25. Doyle, J.J.; Doyle, J.L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **1987**, *19*, 11–15.
26. Wills, D.M.; Hester, M.L.; Liu, A.; Burke, J.M. Chloroplast SSR polymorphisms in the Compositae and the mode of organellar inheritance in *Helianthus annuus*. *Theor. Appl. Genet.* **2005**, *110*, 941–947. [CrossRef] [PubMed]
27. Bryan, G.J.; McNicoll, J.; Ramsay, G.; Meyer, R.C.; De Jong, W.S. Polymorphic simple sequence repeats markers in chloroplast genomes of Solanaceous plants. *Theor. Appl. Genet.* **1999**, *99*, 859–867. [CrossRef]
28. Paniego, N.; Echaide, M.; Muñoz, M.; Fernández, L.; Torales, S.; Faccio, P.; Fuxan, I.; Carrera, M.; Zandomeni, R.; Suárez, E.Y.; et al. Microsatellite isolation and characterization in sunflower (*Helianthus annuus* L.). *Genome* **2002**, *45*, 34–43. [CrossRef]
29. Nei, M. *Molecular Evolutionary Genetics*; Columbia University Press: New York, NY, USA, 1989.
30. Excoffier, L.; Lischer, H.E.L. Arlequin Suite Ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* **2010**, *10*, 564–567. [CrossRef]
31. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [CrossRef] [PubMed]
32. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software structure: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef]
33. Lepais, O.; Petit, R.J.; Guichoux, E.; Lavabre, J.E.; Alberto, F.; Kremer, A.; Gerber, S. Species relative abundance and direction of introgression in oaks. *Mol. Ecol.* **2009**, *18*, 2228–2242. [CrossRef]
34. Faith, D.; Minchin, P.; Belbin, L. Compositional dissimilarity as a robust measure of ecological distance. *Vegetatio* **1987**, *69*, 57–68. [CrossRef]
35. Hammer, O.; Harper, D.; Ryan, P. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 1–9.
36. Upadhyay, A.; Kadam, U.; Chacko, P.; Aher, L.; Karibasappa, G.M. Microsatellite and RAPD analysis of grape (*Vitis* spp.) accession and identification of duplicates/misnomers in germplasm collection. *Indian J. Hortic.* **2010**, *67*, 8–15.
37. Chavhan, R.L.; Sable, S.; Narwade, A.V.; Hinge, V.R.; Kalbande, B.B.; Mukherjee, A.K.; Chakrabarty, P.K.; Kadam, U.S. Multiplex molecular marker-assisted analysis of significant pathogens of cotton (*Gossypium* sp.). *Biocatal. Agric. Biotechnol.* **2023**, *47*, 102557. [CrossRef]
38. Soto, Á.; Rodríguez-Martínez, D.; López De Heredia, U. SIMHYB: A simulation software for the study of the evolution of hybridizing populations. Application to *Quercus ilex* and *Q. suber* suggests hybridization could be underestimated. *Iforest-Biogeosci. For.* **2018**, *11*, 99. [CrossRef]
39. Vähä, J.-P.; Primmer, C.R. Efficiency of model-based bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of Loci: Genetic detection of hybrids. *Mol. Ecol.* **2005**, *15*, 63–72. [CrossRef]
40. Valencia-Cuevas, L.; Mussali-Galante, P.; Piñero, D.; Castillo-Mendoza, E.; Rangel-Altamirano, G.; Tovar-Sánchez, E. Hybridization of *Quercus castanea* (Fagaceae) across a red oak species gradient in Mexico. *Plant Syst. Evol.* **2015**, *301*, 1085–1097. [CrossRef]

41. Castellanos-Castro, C.; Bonfil, C. Propagación de tres especies de *Bursera* a partir de estacas. *Bot. Sci.* **2013**, *91*, 217–224. [CrossRef]
42. Siles, P.; Martínez Rayo, J.; Rugama, F.; Molina, L. Diversidad arbórea en cercas vivas y dos fragmentos de bosque en la comunidad de Santa Adelaida, *Estelí*. *Encuentro* **2013**, *96*, 60–76. [CrossRef]
43. Caseys, C.; Stritt, C.; Glauser, G.; Blanchard, T.; Lexer, C. Effects of hybridization and evolutionary constraints on secondary metabolites: The genetic architecture of phenylpropanoids in European *Populus* species. *PLoS ONE* **2015**, *10*, e0128200. [CrossRef]
44. Paul, M.; Brüning, G.; Bergmann, J.; Jauch, J. A thin-layer chromatography method for the identification of three different olibanum resins (*Boswellia serrata*, *Boswellia papyrifera* and *Boswellia carterii*, respectively, *Boswellia sacra*). *Phytochem. Anal.* **2012**, *23*, 184–189. [CrossRef]
45. Zhang, Y.; Ning, Z.; Lu, C.; Zhao, S.; Wang, J.; Liu, B.; Xu, X.; Liu, Y. Triterpenoid resinous metabolites from the genus *Boswellia*: Pharmacological activities and potential species-identifying properties. *Chem. Cent. J.* **2013**, *7*, 153. [CrossRef]
46. Cole, T.; Gonzalez, A. Filogenia de las Burseraceae (Español). IBONE. 2018. Available online: https://www.researchgate.net/publication/322993034_Filogenia_de_las_Burseraceae_espanol (accessed on 22 November 2022).
47. Bangert, R.K.; Lonsdorf, E.V.; Wimp, G.M.; Shuster, S.M.; Fischer, D.; Schweitzer, J.A.; Allan, G.J.; Bailey, J.K.; Whitham, T.G. Genetic structure of a foundation species: Scaling community phenotypes from the individual to the region. *Heredity* **2008**, *100*, 121–131. [CrossRef] [PubMed]
48. Bangert, R.K.; Turek, R.J.; Rehill, B.; Wimp, G.M.; Schweitzer, J.A.; Allan, G.J.; Bailey, J.K.; Martinsen, G.D.; Keim, P.; Lindroth, R.L.; et al. A genetic similarity rule determines arthropod community structure. *Mol. Ecol.* **2006**, *15*, 1379–1391. [CrossRef]
49. Becerra, J.X.; Venable, D.L. Nuclear ribosomal DNA phylogeny and its implications for evolutionary trends in Mexican *Bursera* (Burseraceae). *Am. J. Bot.* **1999**, *86*, 1047–1057. [CrossRef] [PubMed]
50. Rieseberg, L.H.; Ellstrand, N.C.; Arnold, M. What can molecular and morphological markers tell us about plant hybridization? *Crit. Rev. Plant Sci.* **1993**, *12*, 213–241. [CrossRef]
51. Weeks, A.; Simpson, B.B. Molecular genetic evidence for interspecific hybridization among endemic hispaniolan *Bursera* (Burseraceae). *Am. J. Bot.* **2004**, *91*, 976–984. [CrossRef]
52. Peñaloza-Ramírez, J.M.; González-Rodríguez, A.; Mendoza-Cuenca, L.; Caron, H.; Kremer, A.; Oyama, K. Interspecific gene flow in a multispecies oak hybrid zone in the sierra Tarahumara of Mexico. *Ann. Bot.* **2010**, *105*, 389–399. [CrossRef]
53. Tovar-Sánchez, E.; Mussali-Galante, P.; Esteban-Jiménez, R.; Piñero, D.; Arias, D.M.; Dorado, O.; Oyama, K. Chloroplast DNA polymorphism reveals geographic structure and introgression in the *Quercus crassifolia* × *Quercus crassipes* hybrid complex in Mexico. *Botany* **2008**, *86*, 228–239. [CrossRef]
54. Zhang, R.; Hipp, A.L.; Gailing, O. Sharing of chloroplast haplotypes among red oak species suggests interspecific gene flow between neighboring populations. *Botany* **2015**, *93*, 691–700. [CrossRef]
55. Rzedowski, J.; Ortiz, E. Estudios quimiotaxonómicos de *Bursera* (Burseraceae). II. Una especie nueva de origen híbrido de la barranca de Tolantongo, estado de Hidalgo. *Acta Bot. Mex.* **1988**, *1*, 11–19. [CrossRef]
56. Rico, Y.; Reyes-Estanislao, L. Pollen viability and germinability of putative *Bursera* hybrids (Section *Bullockia*; Burseraceae) in Mexico. *Acta Bot. Mex.* **2018**, *126*, 1–10. [CrossRef]
57. Hinge, V.R.; Shaikh, I.M.; Chavhan, R.L.; Deshmukh, A.S.; Shelake, R.M.; Ghuge, S.A.; Dethe, A.M.; Suprasanna, P.; Kadam, U.S. Assessment of genetic diversity and volatile content of commercially grown banana (*Musa* spp.) cultivars. *Sci. Rep.* **2022**, *12*, 7979. [CrossRef]
58. Xu, S.; Zhou, W.; Baldwin, I.T. The rapidly evolving associations among herbivore associated elicitor-induced phytohormones in *Nicotiana*. *Plant Signal. Behav.* **2015**, *10*, e1035850. [CrossRef] [PubMed]
59. Glassmire, A.E.; Jeffrey, C.S.; Forister, M.L.; Parchman, T.L.; Nice, C.C.; Jahner, J.P.; Wilson, J.S.; Walla, T.R.; Richards, L.A.; Smilanich, A.M.; et al. Intraspecific phytochemical variation shapes community and population structure for specialist caterpillars. *New Phytol.* **2016**, *212*, 208–219. [CrossRef] [PubMed]
60. Arnold, M.L. Natural hybridization and the evolution of domesticated, pest and disease organisms. *Mol. Ecol.* **2004**, *13*, 997–1007. [CrossRef]
61. Tovar-Sánchez, E.; Oyama, K. Natural hybridization and hybrid zones between *Quercus crassifolia* and *Quercus crassipes* (Fagaceae) in Mexico: Morphological and molecular evidence. *Am. J. Bot.* **2004**, *91*, 1352–1363. [CrossRef] [PubMed]
62. Becerra, J.X.; Venable, D.L.; Evans, P.H.; Bowers, W.S. Interactions between chemical and mechanical defenses in the plant genus *Bursera* and their implications for herbivores. *Am. Zool.* **2001**, *41*, 865–876.
63. Raffa, K.F. Terpenes tell different tales at different scales: Glimpses into the chemical ecology of conifer-bark beetle-microbial interactions. *J. Chem. Ecol.* **2014**, *40*, 1–20. [CrossRef] [PubMed]