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Phenotypic Variation and Diversity in Fruit, Leaf, Fatty Acid, and Their Relationships to Geoclimatic Factors in Seven Natural Populations of *Malania oleifera* Chun et S.K. Lee

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Abstract: *Malania oleifera* Chun et S.K. Lee is an endangered woody oil tree that produces nervonic acid, which is closely related to brain development. The phenotypic variation and diversity in some representative traits in natural populations are poorly understood. To investigate phenotypic variation, diversity and their relationship with geoclimatic factors, we evaluated 21 traits of 97 sampled individuals from seven natural populations. Coefficient of variation for 21 traits widely ranged from 0.05 (shape index of fruit) to 0.62 (water content of dry kernel), with an average of 0.18. The Shannon–Wiener indices ranged from 1.91 (water content of dry kernel) to 2.06 (leaf length and leaf width), with an average of 1.94. The variation of 20 traits was significantly influenced by the population effect ($p < 0.05$). The 21 traits were reduced to six principal components, which collectively accounted for 86.64% of the total phenotypic variation. The seven population was mainly divided into two groups: Group I, which included four populations distributed throughout the southern region and having wider and shorter leaves, larger fruit transverse diameters, and higher content of fatty acid and nervonic acid in the kernel, and Group II included the left populations with relative opposite characteristics to those in Group I. Trees with shorter leaves tend to produce oblate shape and heavier dried kernel, yielding more fatty acid and nervonic acids, and population-level pairwise kernel morphological distance were positively correlated with fatty acid distance (Mantel $r = 0.39$, $p < 0.05$). Annual average humidity was strongly positively correlated with leaf index (length/width, $r = 0.78$) but had a negative correlation with the kernel dry weight ($r = -0.84$). The shape of the kernel gradually altered from oblate to globose as longitude and rainfall increased. The environment became colder as latitude increased, resulting in a steady decrease in fatty acid content. No significant isolation by climatic and geographical distance was found for morphological and fatty acid variability. These results can have an important implication for genetic improvement, diversity conservation and resource management of the species in the future.

Keywords: *Malania oleifera*; nervonic acid; variation; diversity; natural populations; germplasm resources

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1. Introduction

Malania oleifera Chun et S.K. Lee, a monotypic species within the Olacaceae family, is an ancient evergreen tree species endemic to SW China (at the junction of Yunnan, Guangxi, and Guizhou provinces) [1]. It is naturally distributed with an extremely small population in a narrow, rocky desertification area and listed as Vulnerable (VU) on the IUCN Red List [2,3] and a Class II key protected species in China. *M. oleifera* is a famous woody oil plant for its kernels containing high oil content (60.5%), of which nervonic acid ($C_{24}H_{46}O_2$,

hereafter referred to as NAC) was the highest, accounting for 55.7–67.10% [4,5]. NAC is a monounsaturated fatty acid and has attracted considerable attention for its close relationship with brain development. There are merely about ten woody plants in China containing NAC content of more than 2% in kernels, with *M. oleifera* yielding the highest NAC and having the greatest commercial potential [6]. Given its significant economic value, so far, extensive effort in *M. oleifera* has been devoted to examining the content and composition of fatty acids [5,7], identifying the genes related to NAC biosynthesis through molecular biology methods [8], transcriptome analysis [9], genome sequencing [10], etc. In addition, genetic improvement of key traits is a conventional and promising approach in crops and trees. Furthermore, common gardens and trials established in a genetic improvement program are essential for ex situ conservation. However, genetic improvement of the levels of NAC in *M. oleifera* kernels is still in its infancy.

Characterization of the diversity and variation of valuable traits is crucial for genetic improvement and reasonable conservation. Generally, the diversity of germplasm resources is the expression of inherent genetic information in a variety of environments, reflecting the richness of genes within a species and its response to environmental changes [11]. In natural populations, forest trees generally harbor great diversity and variation due to inherent genetic-based differences, various environmental conditions and their interactions in adaptation across a range of habitats [12]. The inter- and intra-population diversity and variation have been extensively reported in tree improvement studies [13–18]. In most cases, geoclimatic factors of different populations significantly influenced the diversity level and have gained much attention. For instance, the phenotypic variation of leaves was influenced by climatic factors such as temperature and rainfall and changed along gradients of geographical factors like longitude, latitude, and altitude in *Tetracentron sinense* Oliv. [19]. Seed morphological characteristics such as mass and size were altered along latitudinal and longitudinal gradients in *Euptelea pleiosperma* J.D. Hooker et Thomson [17]. The fatty acid profile of *Xanthoceras sorbifolium* Bunge was affected by latitude, mean annual temperature and longitude [18]. In *M. oleifera*, Wang et al. investigated the morphological characterization of fruits and the concentration of seed oil and its fatty acid composition using fifty-three trees from two populations [20]. However, more comprehensive information on phenotypic variation, diversity and their relationship with geoclimatic factors at the population level is needed.

In this study, we sampled 97 individuals from seven natural populations, based on a survey of the main distribution regions of *M. oleifera* in China, and measured 21 phenotypic traits of fruits, kernels, leaves, fatty acid and nervonic acid content. Our main objectives were to (1) quantify the phenotypic variation and diversity; (2) test the relationships among phenotype traits to find if there were indicators of fatty acid and nervonic acid content; (3) examine the influence of geoclimatic factors on the phenotypic variability. The results will provide the foundations for subsequent genetic improvement, diversity conservation, afforestation and resource management of the species.

2. Materials and Methods

2.1. Plant Materials

From September to October 2021, we surveyed the primary distribution regions of *M. oleifera.* The mature fruits, branches, and leaves were collected from each sampled tree and brought back for measurement. The sample sizes differed due to the population size, the fruit availability and the sampling difficulty. A total of 97 individuals were sampled from seven populations, including Bama, Fengshan, Leye, Lingyun, Tianlin in Guangxi province, and Funing, Guangnan in Yunnan province (Figure 1). The altitude, latitude, and longitude of each population were documented by the global positioning systems (GPS). Annual mean temperature, annual mean rainfall, and relative humidity of each population were obtained from the China Meteorological Data Network (http://data.cma.cn/; 1981–2010, accessed on 1 April 2022). Basic information on the populations, including sample sizes and ages, as well as climate and geographic factors, is presented in Table 1.

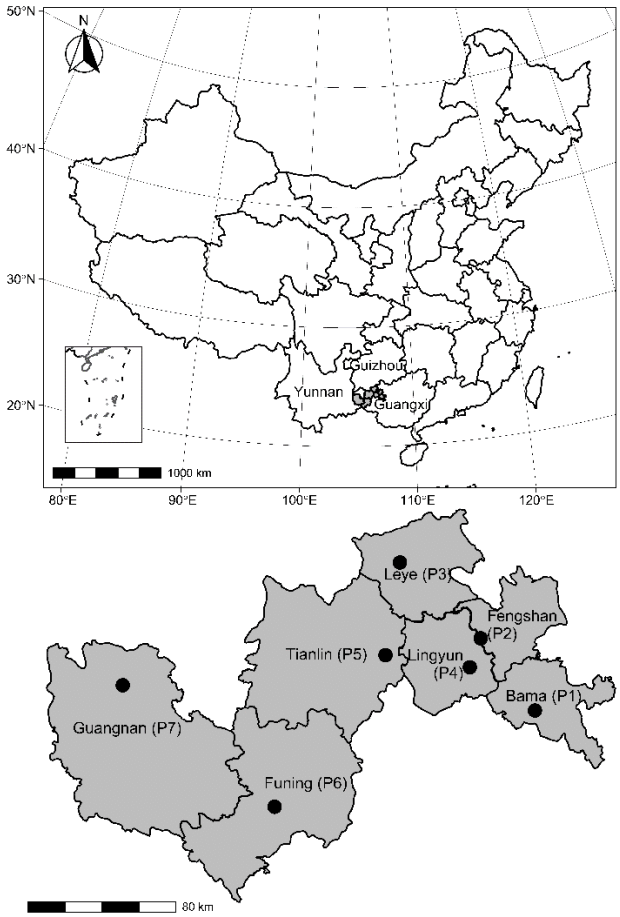

Figure 1. Locations of the seven populations sampled of M. oleifera. Upper panel: A map of China with grey indicating the studied area. Lower panels: The enlarged studied area, where solid black dots indicate the studied populations.

Table 1. Population, sample size, age, and geoclimatic factors for seven natural populations of *M. oleifera*.

Population	Sample Size	Sample Age	Annual Average Temperature (°C, AAT)	Annual Average Humidity (%, AAH)	Average Annual Precipitation (mm, AAP)	Latitude (° N, LAT)	Longitude (° E, LON)	Altitude (m, ALT)
Bama (P1)	6	22–46	20.7	80	1505.9	24.12	107.12	532.6–541.2
Fengshan (P2)	8	25–60	19.4	79	1509.3	24.47	106.83	698.2–953.2
Leye (P3)	20	30–80	16.8	82	1327.2	24.84	106.40	986.1–1027.8
Lingyun (P4)	3	30–100	20.4	77	1669.6	24.33	106.77	705.6–777.8
Tianlin (P5)	10	35–100	20.8	81	1193.3	24.39	106.32	600.8–955.0
Funing (P6)	13	30–80	19.8	78	1103.5	23.65	105.73	819.9–878.2
Guangnan (P7)	37	30–80	17.0	78	994.7	24.24	104.92	1217.1–1314.6

2.2. Trait Measurements

Morphological traits of the fruit, kernel, and leaf were measured with calipers (in mm). For each tree, twelve fruits were measured and then the measurements were averaged for each trait. The thickness of the pericarp is the difference between the transverse diameter of the fruit and kernel. The shape index of the fruit or kernel was calculated as the ratio of the vertical diameter to the transverse diameter of the fruit or kernel. The weight of the fruit and the kernel was measured with a portable electronic balance (in g). The water content of fresh kernels was determined after drying in an oven at 60 °C for 72 h to constant weight. The length, width, area, and perimeter of leaves were measured using the WSeen LA-S System (WSeen Testing Technology Co., Ltd., Hangzhou, China). For each tree, 20 leaves were measured and then the measurements were averaged for each trait. The leaf index was the length-to-width ratio of a leaf.

Quality traits of dried kernels consist of water, fatty, and nervonic acid content. The dried kernels were ground to powder in liquid nitrogen and subsequently analyzed by the Agricultural Products Quality Supervision, Inspection and Testing Center (Zhengzhou) of the Ministry of Agriculture and Rural Affairs in accordance with the following relevant national technical standards. The water content was determined by the direct drying method following the National food safety standard–Determination of moisture in foods (GB5009.3–2016). The determination of fatty acid and nervonic acid content was carried out using the A1-3 6890N gas chromatograph by the alkaline hydrolysis method following the National food safety standard–Determination of fatty acids in foods (GB5009.168–2016).

2.3. Statistical Analysis

All statistical analyses were performed using R software (version 4.0.2) [21]. The coefficients of phenotypic variation (CV) indicate the magnitude of variation and were calculated as

$$CV = \sigma / \overline{x} \tag{1}$$

where σ and $\overline{x}$ are standard deviation and arithmetic mean.

The Shannon–Wiener index (H) was used to evaluate the population diversity for each trait. The phenotypic trait was converted to a ten-grade variable: the values $< \overline{x} - 2\sigma$ were assigned to the first grade; the remaining grades were set in steps of 0.5σ [22].

$$H = -\sum p_i (ln p_i) \tag{2}$$

where p_i indicates the percentage of each grade.

Variance analysis combined with F-test was used to test the effects of population on each trait and was implemented using the R function 'aov'. Subsequently, multiple comparisons and the statistical significance at $p < 0.05$ were performed using the 'duncan.test' function in the R package agricolae (version 1.3-5) [23].

Principal component analysis (PCA) was used to reduce the variability and explore the trait structure and relationships among populations. Data standardization using the Z-score method was carried out prior to PCA. PCA was performed using the R function 'prcompt'.

To investigate the relationships among phenotype traits and their correlations with geoclimatic factors, firstly, Pearson's correlation coefficients and the statistical significance were obtained using the R function 'cor.test'. Next, the Mantel test [24] was carried out to test the correlations between fruit, kernel, and leaf morphology, chemical traits, as well as their variation patterns of isolation by isolation by climatic and geographical distance based on six different matrices of distances among the studied populations. The first five matrices were obtained using the 'vegdist' function in the R package vegan (version 2.6-2) [25]. Squared Mahalanobis distances between the studied populations were calculated to obtain the matrices of morphometric (fruit, kernel and leaf) and chemical distances. Climatic differences were calculated as the Euclidian distance for three climatic factors of the site of sample collection (Table 1). Geographic distances in the last matrix were calculated from the latitude and longitude of the site of sample collection using the 'distm' function with the Haversine method in the R package geosphere (version 1.5-14) [26]. The Mantel test was implemented in the R package vegan (version 2.6-2) [25] using the 'mantel' function. The Canberra distance, a weighted version of the Manhattan distance, was produced by the R function 'dist' and then was subjected to a clustering procedure (UPGMA method) using the 'upgma' function in the R package phangorn (version 2.7.0) [27].

3. Results

3.1. Phenotypic Variation and Diversity

The phenotypic variation for 21 traits of *M. oleifera* is shown in Table 2. CVs of the 21 traits ranged from 0.05 (shape index of fruit, FSI) to 0.62 (water content of dry kernel, WCF), with a mean value of 0.18. For fruit- and kernel-related traits, CVs of diameter and shape index ranged from 0.05 to 0.07, which was lower than the CV of weight (0.16–0.27).

For leaf-related traits, *CV*s ranged from moderate (0.12–0.18) to high (0.33). For fruit quality traits, *CV*s were moderate, at 0.18 and 0.11 for fatty acid content (FAC) and nervonic acid content (NAC), respectively.

Table 2. Variation and diversity of the 21 traits in seven natural populations of *M. oleifera* (* and ** denote significance for $p < 0.05$ and $p < 0.01$, respectively.).

Traits		Code	Mean	SD	Max	Min	Range	CV	H	*F* Value
Fruit	Fruit vertical diameter (mm)	FVD	36.77	44.16	26.28	2.58	17.88	0.07	1.96	5.81 **
	Fruit transverse diameter (mm)	FTD	41.37	45.81	25.85	2.75	19.96	0.07	1.86	2.18
	Shape index of fruit	FSI	0.89	1.06	0.70	0.05	0.36	0.05	1.74	13.15 **
	Fresh fruit weight (g)	FFW	36.19	46.66	10.21	5.89	36.45	0.16	1.93	2.44 *
	Pericarp thickness (mm)	PT	9.42	13.34	4.05	1.63	9.29	0.17	2.02	3.19 **
Kernel	Kernel vertical diameter (mm)	KVD	29.62	34.56	24.27	1.90	10.29	0.06	2.02	9.05 **
	Kernel transverse diameter (mm)	KTD	32.12	36.72	26.60	1.95	10.12	0.06	2.05	4.14 **
	Shape index of kernel	KSI	0.92	1.15	0.81	0.06	0.34	0.07	1.96	7.55 **
	Fresh kernel weight (g)	KFW	15.14	21.13	7.17	2.36	13.96	0.16	2.00	6.55 **
	Dry kernel weight (g)	KDW	8.84	13.42	2.02	2.39	11.40	0.27	2.00	20.82 **
	Water content of fresh kernel (g)	WCF	6.30	14.08	2.73	2.22	11.35	0.35	1.92	25.50 **
	Water content of dry kernel (%)	WCD	7.75	28.20	3.54	4.84	24.66	0.62	1.51	7.66 **
Leaf	Petiole length (mm)	PL	12.33	17.15	8.41	2.08	8.74	0.17	2.00	11.03 **
	Knot spacing (mm)	KS	1.22	2.44	0.49	0.41	1.95	0.33	2.04	5.93 **
	Leaf length (mm)	LL	19.12	27.80	10.50	3.45	17.30	0.18	2.06	10.88 **
	Leaf width (mm)	LW	5.81	8.06	3.85	0.96	4.21	0.17	1.98	5.71 **
	Leaf index	LI	3.32	4.28	2.35	0.40	1.93	0.12	2.06	8.33 **
	Leaf area (mm^2)	LA	70.67	128.92	27.74	23.35	101.18	0.33	2.04	9.99 **
	Leaf perimeter (mm)	LP	44.56	64.07	26.38	7.61	37.69	0.17	2.05	10.38 **
Fatty acid	Fatty acid content (%)	FAC	53.85	67.80	28.60	9.81	39.20	0.18	1.87	27.53 **
	Nervonic acid content (%)	NAC	45.02	51.40	21.70	4.87	29.70	0.11	1.69	3.91 **
Mean (SD)			-	-	-	-	-	0.18 (0.13)	1.94 (0.14)	

Shannon–Wiener index (*H*) of the 21 traits had a mean value of 1.94 and varied from 1.51 (water content of dry kernel, WCD) to 2.06 (leaf length, LL, and leaf width, LW). For fruit-related traits, the pattern of *H* was typically consistent with *CV*. Pericarp thickness (PT) had the highest *H* of 2.02, whereas FSI had the lowest at 1.74. For kernel-related traits, however, the opposite trend was observed for *H* related to *CV*. The largest *H* (2.05) was found in the kernel transverse diameter (KTD, while the lowest (1.51) was found in the WCD. For leaf-related traits, the maximum *H* was 2.06 for LL and LW, and the lowest *H* was 1.98 for LSI. For fruit quality traits, the *H* of FAC and NAC were low, at 1.87 and 1.5, respectively.

3.2. Differences among Seven Natural Populations

The population significantly affected the variation of all the traits except for FTD (Table 2). Table 3 shows the means, standard deviations, and multiple comparisons of the 21 traits at the population level. P1 (Bama) had globose-shaped fruits (FSI = 0.94, KSI = 0.96) and had the greatest NAC (48.52%). P2 (Fengshan) had narrow and long leaves, of which the LW was the smallest at 4.9 cm, and the LI was the largest at 3.6. P3 (Leye) had the highest WCF and KDW but the least FAC and NAC. P4 (Lingyun) had the largest fruit, kernel, and leaves. The measurements for all the traits of P5 (Tianlin) ranged from moderate to high. P6 (Funing) had oblate-shaped fruits (FSI = 0.86, KSI = 0.89) and had the greatest FAC (61.94%). P7 (Guangnan) yields the heaviest fruit (KFW = 16.34), with a similar fruit shape to P6 and the highest FAC and NAC. Therefore, P6, P7 and P1 were more advantageous in selecting trees with higher fatty acid and nervonic acid content for future genetic improvement, while the remaining populations were better suited to preserving genetic diversity.

In general, the two populations in Yunnan province had oblate-shaped fruit and kernel characteristics (the smallest shape index of fruit and kernel) and high fatty acid content; the five populations in Guangxi province had relatively rounder shapes of fruits and kernels and a lower fatty acid content than the Yunnan population.

Table 3. Mean value, standard deviation, and multiple comparison of 21 traits (Means with the same letter are not significantly different at the $p < 0.05$ level.).

Traits		P1	P2	P3	P4	P5	P6	P7
Fruit	FVD	38.22 ± 3.69 abc	36.68 ± 2.03 bcd	37.18 ± 2.63 bc	40.49 ± 0.59 a	38.98 ± 2.46 ab	34.61 ± 3.28 d	36.19 ± 1.2 cd
	FTD	40.91 ± 3.67 b	40.05 ± 3.78 b	40.53 ± 3.72 b	43.9 ± 2.32 a	42.12 ± 2.05 ab	40.28 ± 2.35 b	42.17 ± 1.64 ab
	FSI	0.94 ± 0.03 a	0.92 ± 0.05 a	0.92 ± 0.04 a	0.92 ± 0.04 a	0.92 ± 0.03 a	0.86 ± 0.06 b	0.86 ± 0.02 b
	FFW	35.76 ± 9.81 b	32.68 ± 8.23 b	34.15 ± 6.85 b	42.54 ± 4.38 a	37.97 ± 5.35 ab	34.37 ± 5.64 b	37.77 ± 3.19 ab
	PT	11.15 ± 1.37 a	9.26 ± 2.57 b	8.88 ± 1.26 b	11 ± 0.77 a	10.35 ± 1.04 ab	8.82 ± 1.85 b	9.29 ± 1.45 b
Kernel	KVD	28.64 ± 2.73 cd	29.09 ± 1.33 cd	31.35 ± 1.54 ab	32.34 ± 0.35 a	30.01 ± 1.42 bc	28.04 ± 1.92 d	29.18 ± 1.29 cd
	KTD	29.76 ± 2.51 c	30.79 ± 2.35 bc	32.42 ± 1.59 ab	32.9 ± 3.03 a	31.77 ± 1.18 ab	31.46 ± 2.44 abc	32.88 ± 1.37 a
	KSI	0.96 ± 0.04 a	0.95 ± 0.06 ab	0.97 ± 0.05 a	0.99 ± 0.1 a	0.94 ± 0.04 ab	0.89 ± 0.05 b	0.89 ± 0.06 b
	KFW	11.66 ± 3.42 c	14.03 ± 1.96 b	15.22 ± 1.68 ab	16.28 ± 3.39 a	14.96 ± 1.76 ab	13.77 ± 2.82 b	16.34 ± 1.6 a
	KDW	7.7 ± 2.54 c	8.03 ± 0.99 bc	5.72 ± 1.73 d	10.54 ± 1.62 a	9.01 ± 1.67 abc	9.47 ± 1.92 ab	10.49 ± 1.31 a
	WCF	3.96 ± 0.92 c	6 ± 1.26 b	9.5 ± 2.13 a	5.74 ± 2.26 b	5.95 ± 1.18 b	4.3 ± 1.24 c	5.85 ± 0.98 b
	WCD	7.61 ± 4.02 abc	9.14 ± 5.14 abc	11.8 ± 5.64 a	8.39 ± 0.18 abc	10.53 ± 7.5 ab	7.08 ± 2.77 bc	4.73 ± 0.96 c
Leaf	PL	15.02 ± 1.87 a	11.62 ± 1.79 cd	13.38 ± 1.93 abc	13.1 ± 1.38 bc	13.69 ± 1.82 ab	12.97 ± 2.32 bc	10.83 ± 0.95 d
	KS	1.41 ± 0.5 ab	1.21 ± 0.21 bc	1.21 ± 0.43 bc	1.68 ± 0.35 a	1.31 ± 0.22 abc	1.6 ± 0.43 ab	1 ± 0.31 c
	LL	19.63 ± 2.14 ab	17.25 ± 2.8 bc	21.99 ± 3.33 a	20.3 ± 2.9 a	21.15 ± 1.29 a	20.67 ± 2.36 a	16.7 ± 2.81 c
	LI	3.2 ± 0.25 bc	3.6 ± 0.41 a	3.56 ± 0.3 ab	2.9 ± 0.06 c	3.57 ± 0.3 ab	3.19 ± 0.54 bc	3.15 ± 0.33 c
	LW	6.15 ± 0.35 bc	4.9 ± 1.07 d	6.21 ± 0.91 ab	7.03 ± 1.12 a	5.97 ± 0.5 bc	6.6 ± 0.99 ab	5.32 ± 0.69 cd
	LA	74.02 ± 10.37 ab	53.93 ± 20.12 c	85.24 ± 24.65 a	92.72 ± 28.93 a	77.37 ± 9.51 a	89.2 ± 19.55 a	55.76 ± 16.45 bc
	LP	45.68 ± 4.7 ab	39.81 ± 6.32 b	50.6 ± 7.49 a	47.59 ± 6.51 a	48.45 ± 2.86 a	48.65 ± 5.52 a	39.41 ± 6.1 b
Fatty acid	FAC	55.08 ± 9.12 bc	50.52 ± 7.54 c	41.28 ± 6.87 d	41.7 ± 0.6 d	51.13 ± 7.99 c	61.94 ± 4.65 a	60.04 ± 4.55 ab
	NAC	48.52 ± 2.22 a	42.59 ± 7.76 b	41.89 ± 3.83 b	48.4 ± 0.72 a	44.46 ± 2.48 ab	44.31 ± 7.01 ab	46.79 ± 3.56 ab

3.3. Principal Components and Cluster Analysis of the 21 Traits

The principal components of the 21 traits are shown in Table 4. The proportion of the cumulative contribution of the principal components with six eigenvalues (eigenvalue > 1) was 86.64%. The first principal component (PC1) explained 28.44% of observed variability and was strongly positively with the leaf-related traits, FSI, KSI, WCD, and strongly negatively correlated with the fatty acid contents (Figure 2A). The second principal component (PC2) explained 21.80% of observed variability and mostly defined the fruit and kernel-related traits. The third principal component, in addition to predominantly defining leaf-related traits, also contributed to the FAC. The fourth principal component mainly described FVD, PT, and KSI. The fifth and sixth principal components had lower contributions, which primarily characterized LI, KDW, and FAC, respectively.

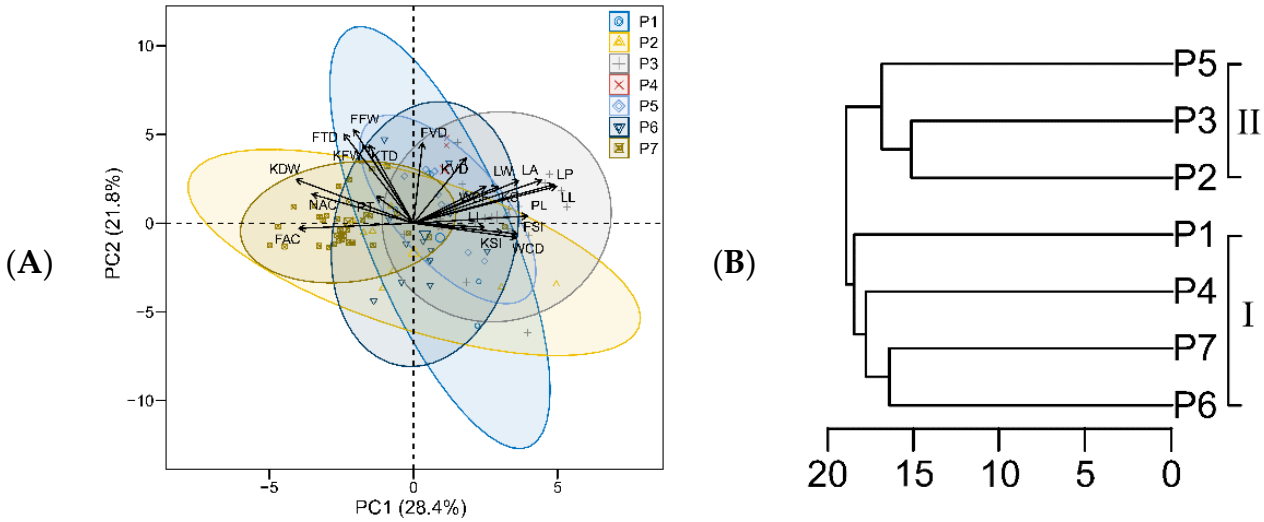

Figure 2. Biplot of the principal component analysis (**A**) and clustering relationship of the seven populations by UPGMA (**B**) based on 21 traits.

Table 4. Principal component analysis of the 21 traits.

Traits		1	2	3	4	5	6
Fruit	FVD	0.05	0.75	−0.28	0.50	0.00	−0.02
	FTD	−0.40	0.83	−0.02	0.17	0.21	−0.13
	FSI	0.59	−0.10	−0.38	0.44	−0.32	0.13
	FFW	−0.34	0.87	0.01	0.20	0.12	−0.01
	PT	−0.21	0.25	0.01	0.82	0.21	−0.21
Kernel	KVD	0.30	0.61	−0.59	0.11	−0.22	0.16
	KTD	−0.25	0.73	−0.21	−0.55	−0.05	−0.01
	KSI	0.51	−0.09	−0.38	0.62	−0.20	0.17
	KFW	−0.29	0.73	−0.30	−0.40	−0.19	0.20
	KDW	−0.67	0.41	0.30	0.02	−0.23	0.30
	WCD	0.41	0.34	−0.64	−0.44	0.04	−0.10
Leaf	PL	0.66	0.07	0.21	0.18	0.10	0.29
	KS	0.49	0.34	0.54	0.05	−0.10	−0.14
	LL	0.82	0.34	0.34	−0.09	0.20	0.12
	LI	0.41	−0.04	−0.26	−0.08	0.71	0.44
	LW	0.60	0.40	0.57	−0.05	−0.28	−0.20
	LA	0.73	0.40	0.50	−0.10	−0.07	−0.06
	LP	0.80	0.36	0.39	−0.10	0.15	0.10
	WCF	0.59	−0.14	−0.36	−0.12	0.03	−0.46
Fatty acid	FAC	−0.66	−0.05	0.50	0.11	−0.12	0.39
	NAC	−0.58	0.27	0.20	0.18	0.30	−0.33
	Eigen value	5.97	4.58	3.01	2.37	1.17	1.10
	Contribution	28.44	21.80	14.33	11.27	5.55	5.26
	Cumulative contribution	28.44	50.24	64.57	75.84	81.39	86.64

The seven populations were not well clustered by the first two principal components except P7 (Guangnan; Figure 2A). However, they could be divided into two groups based on cluster analysis with UPGMA and Canberra distance (Figure 2B). Group I included P6 (Funing), P7, P4 (Lingyun) and P1 (Bama). The Group I population, which consisted of four populations distributed in the southern region, had wider and shorter leaves, larger FTD, and higher fatty acid and nervonic acid content in kernels. Group II comprised P2 (Fengshan), P3 (Leye) and P5 (Tianlin), which had higher WCF and WCD, larger LI, longer and narrower leaves, and lower nervonic acid content.

3.4. Correlations among the 21 Traits and Their Relationship with Geoclimatic Factors

Figure 3 depicts the pairwise trait correlations and their variation patterns of isolation by isolation by climatic and geographical distance. In general, within the four trait types, fruit, kernel, leaf, and fatty acid, traits were all strongly correlated with each other; however, among the four trait types, there was no relationship between leaf-related traits with fruit and kernel traits. FAC and NAC were positively correlated with KDW (r = 0.67 and 0.42) and negatively correlated with the shape index of fruit and kernel (r = −0.41 and −0.36) and leaf-related traits except for KS and LW. PL and LL were positively correlated with FSI and KSI and negatively correlated with KDW. It is indicated that trees with shorter leaves tend to produce oblate-shaped and heavier dry kernels, which yield more fatty acid and nervonic acid. Population-level pairwise morphological distance of kernel were positively correlated with fatty acid distance (Mantel r = 0.39, $p < 0.05$) and morphological distance of fruit (Mantel r = 0.47, $p < 0.05$).

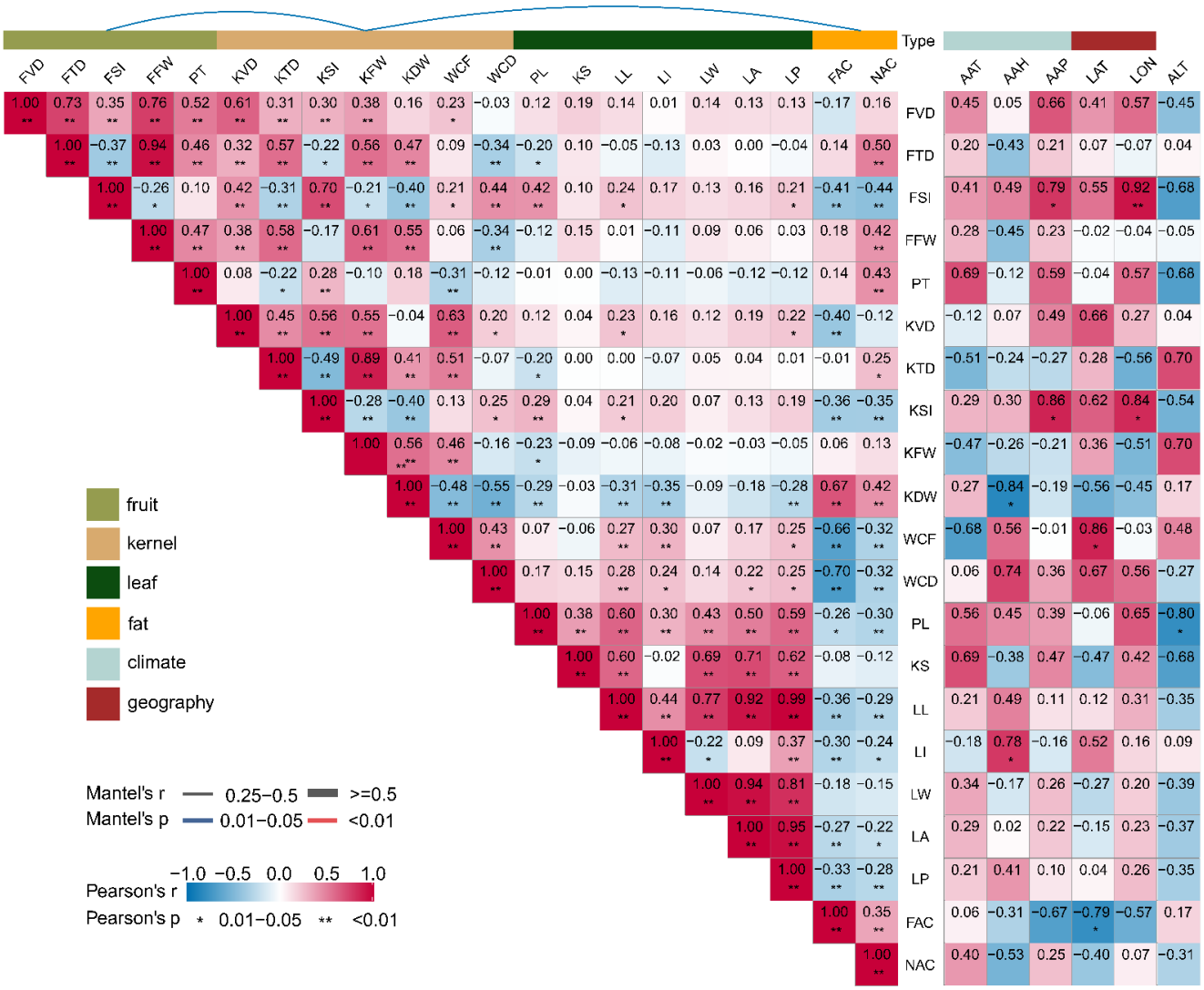

Figure 3. Correlations among the 21 traits and geoclimate factors and association between population and geographic distance.

Most of the relationships between the geographical and climate factors of the seven populations and the 21 traits were not significant (Figure 3). There was no correlation between AAT and the 21 traits. AAH was strongly positively correlated with LI ($r = 0.78$) and had a negative correlation with the KDW ($r = -0.84$), indicating the higher the humidity, the longer and narrower leaves and the smaller kernel dry weight. AAP and LON were highly positively correlated with FSI and KSI. From Yunnan to Guangxi province, with the increase in longitude and rainfall, the shape of the kernel gradually changed from oblate to globose. There was a negative correlation between ALT and PL ($r = -0.80$). LAT was found to be positively correlated with WCF ($r = 0.86$) and negatively correlated with FAC ($r = -0.79$), indicating that as latitude increased, the colder environment might reduce the fatty acid content. No significant isolation by climatic and geographical distance was found for morphological and fatty acid variability.

4. Discussion

Phenotypic diversity is affected by genetic and environmental factors and their interactions [28]. Phenotyping of desirable traits is the simplest and most direct method to survey and evaluate the diversity of forest germplasm resources, which is crucial for their reasonable conservation and genetic improvement. There is likely considerable phenotypic

variation and diversity in forest tree species for the underlying complex environmental conditions, long-term geographic isolation, natural selection, and also phenotypic plasticity [29,30]. In the present study, we comprehensively evaluated the phenotypic diversity and variation in 21 phenotypic traits of fruits, kernels, leaves, fatty acid and nervonic acid content of *M. oleifera* from seven natural populations. We also investigated the relationships between these traits and geoclimatic factors, as well as the influence of these factors on phenotypic variability.

The coefficient of variation (*CV*) reflects the variation discovered in *M. oleifera* germplasm resources for each trait. At the individual level, great differences in *CV* were observed across the 21 traits, ranging from 0.05 (shape index of fruit, FSI) to 0.62 (water content of dry kernel, WCF) (Table 2). The size (e.g., diameter) of kernel/seed typically has limited and stable variation, for instance, at 0.06 in this study, in agreement with a previous report of *M. oleifera* (0.04–0.10) [20], *Torreya grandis* Fort. ex Lindl. (0.10) [15], *Quercus variabilis* Blume (around 0.10) [17]. The *CV*s of fruit size-related traits were slightly higher than those of kernel/seed, for instance, 0.07 in this study, 0.10–0.21 in *Sorbus domestica* L. [31], 0.12–0.18 in *Actinidia eriantha* Benth. [32] and 0.16–0.19 in *Camellia oleifera* Abel. [33]. The *CV*s of weight, as well as water content, had higher fluctuations, 0.16–0.62 in this study, 0.37 in Sweet Chestnut (*Castanea sativa* Mill.) [34], 0.06–0.40 in *Sapindus mukorossi* Gaertn. [35], which could be affected by fruit maturity and maternal factors, such as the position of the fruit in the tree. The *CVs* for fatty acid content (FAC) and nervonic acid content (NAC) were 0.18 and 0.11, respectively (Table 2), lower or similar to those reported for other tree species, like 0.20 for crude fat in *S. domestica* L. [31], 0.25 for crude fat in *Castanea sativa* Mill. [34], 0.41 for oil content in *Acer truncatum* Bunge [36]. In forest tree species, traits with lower *CV* generally have higher heritability [37], indicating that they were mainly controlled by the additive genetic effect, and it was capable of capturing higher genetic gain through traditional breeding approaches [38] or dissecting the underlying genetic architecture using contemporary association studies. On the other hand, for the traits with high *CV*s, such as the water content of fresh and dry kernels, to a large extent, the variation was caused by the environment and genetic-environment interaction effects. It requires a multi-year measurement in natural populations or multi-site provenance-progeny trials to obtain the genetic components in the variation in the forward studies.

The Shannon–Wiener index (*H*) reflects the diversity or evenness of the traits. At the individual level, the *H* of the 21 traits ranged from 1.51 (water content of dry kernels) to 2.06 (leaf width), with an average value of 1.94. The average level of diversity was comparable to that of *Eucommia ulmoides* Oliv. ($H = 1.92$) [39], slightly lower than that of *Erythrophleum fordii* Oliv. ($H = 2.03$) [22], both of which are Class II key protected species in China. It was similar to that of *Populus cathayana* Rehd. ($H = 1.97$) [40] and *Catalpa fargesii* Bur. ($H = 1.92$) [41]. The estimates of *H* had no significant relationship with those corresponding *CVs*. The lower the *H*, the fewer grades concentratedly distributed by the measurements. This phenomenon can be explained by the measurements from a small number of individuals showing some departure from those taken by the majority. In this study, the low *H* of the fatty acid content (FAC, 1.87) and nervonic acid content (NAC, 1.5), along with the moderate *CV*, demonstrated that *M. oleifera* has a great deal of breeding potential for genetic improvement.

It is crucial to identify selection indicators for excellent resources in forest tree species. For example, high-quality feeds, leaf biomass and crude protein content can be considered indicators in the selection of excellent provenance in *Broussonetia papyrifera* (L.) L'Hér. ex Vent. [42]. In this study, the following relationship between the traits was found: the larger the fruit, especially in terms of the transverse diameter, the higher the content of nervonic acid; meanwhile, small and wide short leaves and petioles tend to correspond to higher fat and nervonic acid content. As a result, small leaves, short petioles, and large fruits can be used as key indicators for the selection and breeding of varieties of *M. oleifera* with high FAC and NAC. In addition, this conclusion was also further supported by the result that population-level pairwise kernel morphological distance were positively correlated with fatty acid distance (Mantel $r = 0.39$, $p < 0.05$) and morphological distance of fruit

(Mantel $r = 0.47$, $p < 0.05$). Leaves play a key role in effective water balance, light capture, and temperature regulation [43], as well as the petiole, which plays an important role in the adjustment of foliage and its inclination angles for optimal light capture [44]. Thus, leaf-related traits, such as leaf area development and leaf size, were robust indicators of biomass production in trees [45]. Similarly, seed size could be used as an indicator for the selection of seed sources [46]. Therefore, germplasm resources with high FAC and NAC could be selected simply based on the measurement of leaf and fruit characteristics to circumvent the time-consuming and costly chemical assays.

Longitude, latitude, and altitude, as key geographical factors, mainly affect plant traits through temperature and precipitation. With the increase in longitude and rainfall from west to east, the longitudinal diameters of fruits and kernels gradually increased, and their shape gradually changed from oblate to globose (Figure 3). With the increased humidity, the leaves tended to become narrower, and the dry kernel weight tended to decrease (Figure 3). The petiole tended to shorten with the gradual increase in altitude. As the latitude increased, the climate became colder, and the fresh kernel tended to become smaller with higher water content and lower FAC. Similar results have been documented in other species. In *Eucommia ulmoides* Oliver, latitude and precipitation were the key environmental factors that impacted the seed fatty acid composition [47]. The oil content was negatively correlated with latitude and annual rainfall in *A. truncatum* [36]. Latitude was the main effect factor in the variation of growth traits and nutritional traits [42]. The fruit length-to-width ratio showed a concave variation trend with longitude [17]. Therefore, annual average humidity and latitude should be considered when we introduce *M. oleifera* to the northern regions to expand its planting area.

With the increase of longitude and precipitation, the distribution area of *M. oleifera* expanded, which was consistent with the prediction that the distribution range would extend to the east and north under global climate change [48]. Historical research revealed that *M. oleifera* was dispersed in 22 counties (19 in Guangxi province and three in Yunnan province). Based on the climate data of 22 counties, Xie et al. [49] reported that the extreme minimum temperature for *M. oleifera* survival was between −5.8 °C and −0.3 °C, and the extreme maximum temperature was between 33.4 °C and 42.5 °C; heat and precipitation were the main factors affecting its distribution, and it was hard to adapt to high temperature and dry climate. According to Gong et al. [48], the coefficient of seasonal variation in temperature was the most important factor affecting the distribution and population scale of *M. oleifera*. Therefore, annual average humidity, latitude and temperature were the main limiting factors of *M. oleifera* distribution.

The clustering relationships and geographical distribution characteristics of the seven groups on 21 traits reflected, to some extent, the pattern of geographical variation dominated by latitudinal changes. The four populations distributed in the southern region had wider and shorter leaves, larger FTD, and higher fatty acid and nervonic acid content in kernels. At the same time, the three northern populations had higher WCF and WCD, larger LI, longer and narrower leaves, and lower NAC. The seven populations were not clustered strictly according to geographic distances, although the two populations in Yunnan province (Guangnan and Funing) were clustered together on the dendrogram (Figure 2B), indicating that geographic distance was not a critical factor in the differentiation of *M. oleifera*. Furthermore, the results of the Mantel test also suggested that morphological and fatty acid variability was not significantly associated with climatic and geographical distance (Figure 3). There was no significant correlation between the AAT and the 21 traits of the sample population. However, for *M. oleifera*, a small population with narrow distribution, temperature undoubtedly affects its species distribution and population size. Moreover, Gong et al. [48] used the maximum entropy model (MaxEnt) and 20 main environmental factors to predict the potential suitable range of *M. oleifera*: the longitude and latitude were 104°–107° E and 22°–26° N, respectively; the top four environmental factors were all temperature-related factors. The negligible contribution of AAT on population morphological diversity can be attributed to the little temperature difference resulting from the relatively narrow research region with a longitude spread of 2.2° and a latitude span of 1.22°.

5. Conclusions

There was abundant variation and diversity in 21 traits in seven natural populations of *Malania oleifera*. There is considerable breeding potential for genetic gain improvement of the fatty acid content and nervonic acid content in *M. oleifera*. Leaf length could be an indicator trait for superior tree selection because trees with shorter leaves tend to produce oblate shapes and heavier dry kernels, which yields more fatty acid and nervonic acid. The Yunnan province provenances had higher fatty acid content. Annual average humidity and latitude are key geographical factors affecting fatty acid content. Annual average humidity, latitude and temperature were the main limiting factors of *M. oleifera* distribution. Climatic and geographic distances were not critical factors in the morphological and fatty acid differentiation of *M. oleifera*.

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