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Comparative Study of the Fatty Acid Composition of the *Acer truncatum* Bunge from Different Producing Areas

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Abstract: *Acer truncatum* Bunge is a new type of economic forest tree species that produces nervonic acid. Since it was developed as a woody oil tree species, its oil value has attracted increasing attention. However, new germplasm resources with oil-type characteristics are still lacking. In this study, we studied the differences in the oil content and fatty acid composition of the seed kernel oil of *A. truncatum* from 11 natural forest-producing areas. The Kashi city of Xinjiang and Yangling city of Shaanxi Province can be used as the high-oil germplasm. The oil content of these two areas is more than 50%. The highest relative content of nervonic acid was 9.92% in the Chifeng city of Inner Mongolia, and Jianping city of Liaoning Province was the second, accounting for 9.84%. These two areas can be used as germplasm for the high nervonic acid. Finally, high-quality germplasms with a high oil content and high relative content of nervonic acid were selected from Yangling city of Shaanxi Province and Chifeng city of Inner Mongolia. The relative content of nervonic acid in the kernel oil content in fatty acids from 11 different producing areas of *A. truncatum* plants was negatively correlated with the content of each fatty acid, whereas the relative content of nervonic acid was extremely significantly positively correlated with the relative content of erucic acid. However, the correlations between the kernel oil content, relative nervonic acid content and environmental factors did not reach an extremely significant level, and only the oil content showed a significant positive correlation with the longitude and frost-free period. The high content of nervonic acid in this study is the first report of the highest relative content of nervonic acid in *A. truncatum* kernel oil. We believe that the *A. truncatum* double-high characteristic oil-type germplasm resources obtained by this screening provide a scientific basis for breeding, development and utilization in the *A. truncatum*.

Keywords: *Acer truncatum*; oil content; fatty acid composition; nervonic acid; producing area

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1. Introduction

Acer truncatum is an economic forest tree species that integrates ecological, ornamental, edible and nutritional health values [1–6], and its comprehensive utilization value is extremely high. At present, *A. truncatum*, as a particular woody oil tree species in China [7,8], contains more than 5% nervonic acid, which can be used as a valuable resource for obtaining nervonic acid [9–12]. Because of its excellent oil yield and oil quality, it is also the main source of commercially available natural nervonic acid [11]. As the main natural component of brain nerve cells, nervonic acid can repair nerve cells in the brain, promote the regeneration of brain nerve fibers and be used to prevent and treat diseases such as nervous system disorders. Its resources are extremely scarce and precious [13,14]. In China, *A. truncatum* resources are abundant and widely distributed. Now, a large-scale planting base of *A. truncatum* has been formed. Moreover, the individual plants of *A. truncatum* have a large seed setting and high yield [15], which provides sufficient raw materials for the development of *A. truncatum* oil products, and its development potential is large.

Since the development of *A. truncatum* as a high-quality and high-efficiency economic forest species, some seedlings have been cultivated in various places, and excellent varieties and new varieties of ornamental and afforestation have been bred [16]. However, research on the collection, excavation and screening of oil-type germplasm resources is still insufficient [17]. Studies on the content of kernel oil and fatty acid compositions in *A. truncatum* mainly focus on the oil and fatty acid compositions in the fruit-ripening process and the fruit-ripening period in the single producing areas [18–21], the change in oil yield and fatty acid composition of kernel oil in different natural producing areas and genotypes [22–24], the variation in oil and fatty acid compositions among plants in different natural populations and the selection of superior oil-type individual plants among populations [25]. Although there have been relevant reports of individual plants with a high oil content and high nervonic acid production being selected separately, there has been no report on superior germplasm resources with a double-characteristic composition and both a high oil content and high nervonic acid yield. At present, there is still a lack of new lines with high-yield, stable-yield, high-oil and high-nervonic acid yields, which seriously restricts the development of the oil-use *A. truncatum* industry. Moreover, there were great differences in the appearance and chemical composition of fruit in *A. truncatum* from different regions.

Therefore, in this study, the differences in the composition and content of fatty acids and kernel oil of *A. truncatum* from 11 natural forest-producing areas were studied, and then high-quality oil-type germplasms with high oil and high nervonic acid contents were preliminarily screened. This study provides a theoretical basis and data support for the breeding, development and utilization of oil-use *A. truncatum* germplasm resources. It has important guiding significance for the preservation and genetic improvement of the excellent traits of *A. truncatum*, as well as the production, popularization and utilization of the excellent oil-type genotype.

2. Materials and Methods

2.1. Experimental Materials

From October to mid-November 2018, 33 samples of high-quality natural forest germplasm resources of *A. truncatum* were collected through field surveys in 11 regions of 4 provinces (autonomous regions) in Inner Mongolia, Liaoning, Xinjiang and Shaanxi in China. For each germplasm resource, 1–2 kg of samara with strong and consistent growth and complete maturity were randomly selected from multiple locations of each tree. After the samara were picked, they were put in a net bag and sent back to the laboratory. Then, they were placed in a ventilated and cool place to dry and were dried and stored at room temperature. At the same time, the GPS locator was used to record the latitude, longitude and altitude of each collection site, and the meteorological data of the collection sites were obtained according to the China Meteorological Administration Network (Table S1).

2.2. Sample Pretreatment

After collecting mature samara in natural forest production areas of *A. truncatum*, such as Horqin Right Wing Middle Banner (YZ), Wudantala Forest Farm (WD), Wengniuteqi Pine mountain Forest Farm (WN) and Chifeng city (CF) of Inner Mongolia, Zhangwu (ZW), Fuxin (FX), Jianping (JP) and Kazuo (KZ) city of Liaoning, Kashi (KS) city of Xinjiang and Fufeng (FF) and Yangling (YL) city of Shaanxi, the samara were intensively treated by manual removal of fruit wings and seed coat, after which, the obtained seed kernel was ground and crushed, sieved with a pore diameter of 0.600 mm (30 mesh) and dried in a 60°C oven to constant weight for standby.

2.3. Oil Extraction Using the Soxhlet Method

The Soxhlet extraction method was used to extract the seed kernel oil of *A. truncatum*. First, 5 g of kernel powder was accurately weighed, dried to constant weight in advance and recorded as M_1. Then, it was added to the prenumbered filter paper cylinder, and the total weight of the kernel powder and filter paper cylinder was accurately weighed after sealing and recorded as M_2. Then, the sealed filter paper cylinder was placed into a Soxhlet extraction cylinder, and an appropriate amount of petroleum ether was added for extraction. The extraction device was placed in a water bath at 80 °C and refluxed for 6–8 h. After the oil was completely leached, the resulting mixture was rotary evaporated at 40 °C for approximately 30 min using a rotary evaporator to recover petroleum ether. Afterwards, the remaining solution was dried in an oven at 50 °C, and the yellow oily product obtained at this time was *A. truncatum* seed kernel oil. The obtained oil was placed in a ventilated place for approximately 15 min, transferred into a centrifuge tube and stored in a 4 °C refrigerator away from light. Finally, the total weight of the extracted filter paper cylinder and the residual powder was accurately weighed and recorded as M_3. Three replicates were set for each germplasm sample in each region, and the formula for calculating the oil content of the kernels of *A. truncatum* was as follows:

$$\text{the oil content (x)\%} = (M_2 - M_3)/M_1 \times 100\% \tag{1}$$

2.4. Fatty Acid Composition and Content Determination

The oil obtained in the above experiments was first subjected to methyl esterification treatment, and then the fatty acid composition and content of the oil were determined and analyzed by gas chromatography–mass spectrometry (GC–MS) (Thermo Fisher Scientific, San Jose, CA, USA). The specific operations are as follows.

Methyl esterification treatment. One milliliter of *A. truncatum* kernel oil was placed into a 10 mL centrifuge tube, 2 mL of n-heptane was added and the two were mixed evenly. Then, 2 mL of KOH-CH3OH solution with a mass fraction of 0.5% was added, and a plug was added and vibrated evenly. After that, it was placed in a water bath at 40 °C for 10 min and then left to stand. When the layering phenomenon appeared, the supernatant was aspirated with a syringe. The supernatant was diluted 50 times (the specific dilution ratio was diluted according to the reagent concentration requirements determined by the instrument) and filtered through a 0.22 μm microporous organic filter membrane into a sample bottle. Finally, GC–MS analysis was performed, and 3 repetitions were performed.

GC–MS analysis. The chromatographic column was a TG-WAXMS (30 m × 0.25 mm × 0.25 μm) capillary column. High-purity helium (99.999%) was used as the carrier gas, and the temperature in the oven was 50–250 °C. The heating process of the column incubator was as follows: the initial temperature was 80.0 °C, and the hold time was 1 min. Ramp 01 rate: 50 °C/min; after, the final temperature rise reached 175 °C, and the hold time was 1 min. Ramp 02 rate: 5 °C/min; after, the final temperature rise reached 200 °C, and the hold time was 1 min. Ramp 03 rate: 2 °C/min; after, the final temperature rise reached 210 °C, and the hold time was 1 min. Ramp 04 rate: 5 °C/min; after, the final temperature rise reached 240 °C, and the hold time was 10 min. The column flow was controlled at 1 mL/min, and the injection volume was 1 μL; the split injection mode was selected as 20:1, and the temperature of the injection port and the GC/MS interface was kept at 250 °C. Mass spectrometry conditions: the temperature of the MS transfer line was 240 °C, the temperature of the ion source was set to 250 °C, the ionization mode was EI, the ionization voltage was 70 eV and the scanning range was controlled to be 40–460 aum. The Xcalibur system was used for data processing, and then the peak area normalization method was used to analyze the fatty acid composition and its relative content of seed kernels according to the test report.

2.5. Statistical Analysis

Microsoft office 2016 and SAS 8.0 software (http://www.sas.com, accessed on 17 August 2020) were used for data sorting and statistical analysis. One-way analysis of variance was used to test the significant difference in oil content and fatty acid content in the kernels of different sources, and the significance level was $\alpha = 0.05$.

3. Results

3.1. Oil Content of A. truncatum from Different Producing Areas

The research results are shown in Table 1. The oil content of 33 *A. truncatum* kernels ranged from 30.59% to 51.83%, with an average of 40.67%. The oil content was significantly different among the 11 producing areas ($p < 0.01$), and the oil content of the seed kernel in the 7 producing areas exceeded 40.00%. Among them, the areas with high kernel oil contents were KS (51.22%) and YL (50.54%), followed by ZW (43.35%), WD (42.77%) and YZ (42.52%). The oil content of intermediate kernels included FF (40.33%) and WN (40.17%), followed by KZ (38.52%) and CF (38.13%). FX (36.54%) and JP (32.10%) were the lowest. For these natural *A. truncatum* germplasm resources, from the single index of oil content, the two producing areas of KS and YL were the high-quality germplasm resources with a very high oil content (>50%), and these excellent germplasms have great potential in developing new oil-used germplasm resources.

Table 1. Oil content of *A. truncatum* kernel from different producing areas.

Producing Areas	Oil Content (X ± SD)/%	Variation Range/%	Coefficient of Variation/%
YZ	42.52 ± 1.36 B	41.55–43.48	3.21
WD	42.77 ± 1.31 B	40.82–43.85	3.06
WN	40.17 ± 2.01 C	37.29–42.97	5.01
CF	38.13 ± 3.48 D	35.32–42.02	9.12
ZW	43.35 ± 0.00 B	-	-
FX	36.54 ± 0.00 E	-	-
JP	32.10 ± 1.95 F	30.59–34.30	6.08
KZ	38.52 ± 1.67 D	37.34–39.70	4.33
KS	51.22 ± 0.86 A	50.61–51.83	1.68
FF	40.33 ± 2.46 C	35.98–42.73	6.09
YL	50.54 ± 0.76 A	50.00–51.08	1.51
Mean	40.67	30.39–51.83	11.36

Note: X represents mean value, SD represents standard deviation, coefficient of variation = standard deviation/mean value, there is significant difference in the same column marked with different capital letters ($p < 0.05$), - represents no variation.

3.2. Fatty Acid Composition and Relative Content of A. truncatum Oil from Different Producing Areas

Gas chromatography–mass spectrometry was used to detect and record each composition at each time point by using the different adsorption capacities of the adsorbent for different compositions of the mixed fatty acid methyl esters. Afterwards, the peak area normalization method was used to analyze the possibility of fatty acid detection results, and the retention time of the same fatty acid components in different producing areas was roughly the same. Figure 1 shows the ion chromatogram of the CF-2 (Chifeng, Inner Mongolia) sample, in which, some fatty acid methyl esters were not marked due to their low peak area.

A total of 14 fatty acids were detected from *Acer truncatum* kernel oil, which were palmitic acid (C16:0); hexadecenoic acid (C16:1); heptadecanoic acid (C17:0); stearic acid (C18:0); oleic acid (C18:1); linoleic acid (C18:2); linolenic acid (C18:3); arachidic acid (C20:0); *cis*-11-eicosenoic acid (C20:1); *cis*-11,14-eicosadienoic acid (C20:2); behenic acid (C22:0); erucic acid (C22:1); lignoceric acid (C24:0); nervonic acid (C24:1). Detailed identification information for CF-2 (Chifeng, Inner Mongolia) is shown in Table 2.

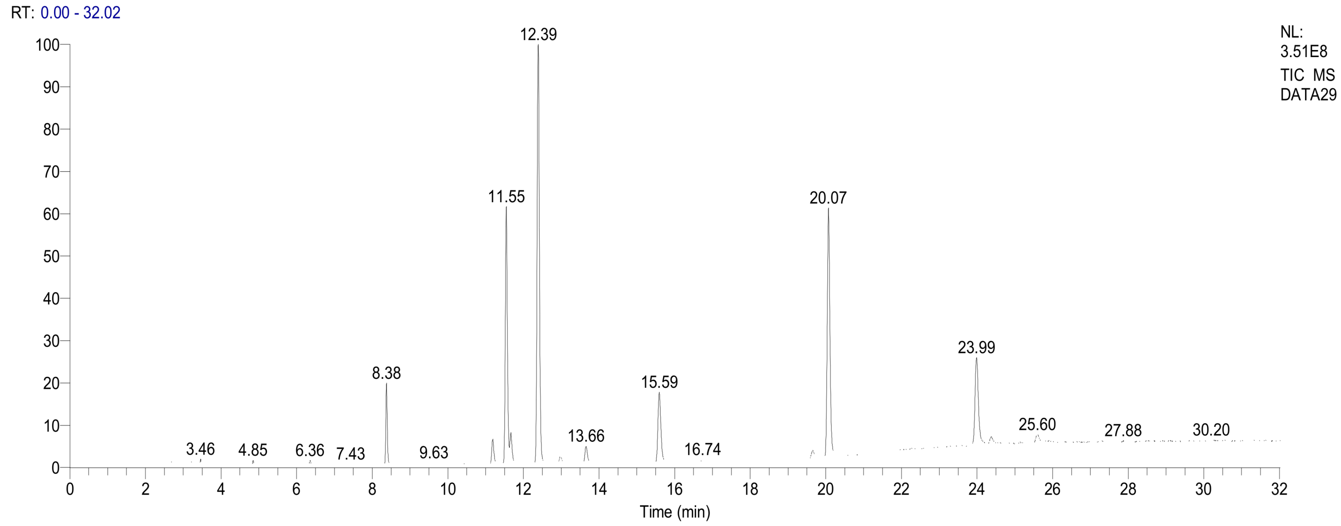

Figure 1. The total ion chromatograms of fatty acid methyl ester (CF-2) of *A. truncatum* seed oil. Note: 8.38 min—methyl palmitate (C16:0), 8.71 min—methyl hexadecenoate (C16:1), 9.63 min—methyl heptadecanoate (C17:1) 19 min—methyl stearate (C18:0), 11.55 min—methyl oleate (C18:1), 12.39 min—methyl linoleate (C18:2), 13.66 min—methyl linolenate (C18:3), 15.13 min—methyl arachidonate (C20:0), 15.59 min—cis-11-eicosenoic acid methyl ester(C20:1), 16.74 min—cis-11, 14-eicosadienoic acid methyl ester (C20:2), 19.65 min—behenic acid methyl ester (C22:0), 20.07 min—erucic acid methyl ester (C22:1), 23.51 min—lignoceric acid methyl ester (C24:0), 23.99 min—nervonic acid methyl ester (C24:1).

Table 2. Fatty acid methyl esters identified after methyl esterification of *the A. truncatum* oil.

Peak Number	Chemical Compound	Retention Time/Min	Molecular Formula	Molecular Weight	Possibility	Cas# Number	Area/%
1	Methyl palmitate	8.38	$C_{17}H_{34}O_2$	270	75.82	112-39-0	3.68
2	Methyl hexadecenoate	8.71	$C_{17}H_{32}O_2$	268	8.16	1120-25-8	0.02
3	Methyl heptadecanoate	9.65	$C_{18}H_{36}O_2$	284	34.75	1731-92-6	0.01
4	Methyl stearate	11.19	$C_{19}H_{38}O_2$	298	73.02	112-61-8	1.58
5	Methyl oleate	11.55	$C_{19}H_{36}O_2$	296	11.61	112-62-9	17.31
6	Methyl linoleate	12.39	$C_{19}H_{34}O_2$	294	24.42	112-63-0	32.13
7	Methyl linolenate	13.66	$C_{19}H_{32}O_2$	292	45.86	301-00-8	1.19
8	Methyl arachidonate	15.13	$C_{21}H_{42}O_2$	326	34.72	1120-28-1	0.03
9	*Cis*-11-eicosenoic acid methyl ester	15.59	$C_{21}H_{40}O_2$	324	26.02	2390-09-2	6.64
10	*Cis*-11,14-eicosadienoic acid methyl ester	16.74	$C_{21}H_{38}O_2$	322	24.05	2463-02-7	0.15
11	Methyl behenate	19.65	$C_{23}H_{46}O_2$	354	72.97	929-77-1	0.59
12	Methyl erucate	20.07	$C_{23}H_{44}O_2$	352	23.76	1120-34-9	21.85
13	Methyl lignocerate	23.51	$C_{25}H_{50}O_2$	382	77.30	2442-49-1	0.17
14	Methyl neurate	23.99	$C_{25}H_{48}O_2$	380	47.05	2733-88-2	9.92

Note: Fatty acids in oil can be detected by demethylation of fatty acid methyl ester, Cas# number represents CAS Registry Number.

Table 3 lists the changes in the main fatty acid composition with a relative content above 1.00%. From the test results, a total of eight kinds of fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, *cis*-11-eicosenoic acid, erucic acid and nervonic acid) were the main fatty acid compositions in the kernel oil. At the level of $p < 0.05$, the differences in fatty acid compositions and contents of *A. truncatum* from 11 producing areas showed that there was no significant difference in the relative contents of palmitic acid, stearic acid, oleic acid, linolenic acid and erucic acid among the different producing areas. One of the relative contents of linoleic acid in YL (24.14%) was significantly lower than that in other areas. The relative contents of *cis*-11-eicosenoic acid and nervonic acid were significantly different among different producing areas, among which, the relative content of *cis*-11-eicosenoic acid in KS was the highest. The relative content of nervonic acid was the highest in the Chifeng area of Inner Mongolia (CF), with CF-2 up to 9.92%, and Jianping in Liaoning Province (JP), with JP-1 up to 9.84%, and the average relative content of nervonic acid in the two areas was more than 8.60%, which can be used as an effective germplasm for producing nervonic acid resources. In addition, the relative content of unsaturated fatty acids in each production area fluctuated by approximately 88.00%, and the relative contents of total fatty acids and unsaturated fatty acids showed extremely significant differences, all of which were significantly lower in the Yangling area of Shaanxi (YL) than in other areas.

3.3. Relative Content of Total Nervonic Acid in the Kernel Oil of A. truncatum from Producing Areas

To determine the relative content of total nervonic acid in the fatty acids of *A. truncatum* kernel oil from different origins, we multiplied the results of the oil content and the relative percentage of nervonic acid in the oil to obtain the relative content of total nervonic acid in the seed kernel oil of each origin (Table 4). The relative contents of total nervonic acid in different producing areas were as follows from high to low: YL > CF > WD > JP > KS > ZW > WN > FF > YZ > KZ > FX. Among them, the relative content of total nervonic acid was the highest in YL (3.46%), followed by CF (3.35%). The two lowest areas were in KZ and FX, with an average content of 2.67%. In addition, by comparing the two provenances (FF and YL) in Shaanxi Province, it was found that there was no significant difference in the relative content of nervonic acid, but there were extremely significant differences in the oil content and total nervonic acid content, and the composition and content in the YL-producing area were significantly higher than those in the FF-producing area. However, the geographical locations of the two producing areas were close, and there was little difference in ecological factors between them. The reason for the difference in composition and content was most likely the genetic background and genotype of *A. truncatum* germplasm. Therefore, the natural forest of *A. truncatum* in the YL and CF areas can be selected as the oil-type strains with high levels of total nervonic acid content. This result also further refined and classified the samara from different producing areas according to the oil and fatty acid quality and provided theoretical guidance for formulating nervonic acid content in high-yield planting areas of *A. truncatum* fruits.

In addition, the analysis found that the oil yield and total nervonic acid content of the YL area were the best quality resources, and that the Chifeng area was the best strain in terms of the nervonic acid content and total nervonic acid content. This conclusion provides a reference for the subsequent breeding of *A. truncatum* seedlings.

Table 3. Relative content of main fatty acids in kernel oil of *A. truncatum* from different producing areas. The natural forest production areas: Horqin Right Wing Middle Banner (YZ), Wudantala Forest Farm (WD), Wengniuteqi Pine mountain Forest Farm (WN) and Chifeng city (CF) of Inner Mongolia, Zhangwu (ZW), Fuxin (FX), Jianping (JP) and Kazuo (KZ) city of Liaoning, Kashi (KS) city of Xinjiang, Fufeng (FF) and Yangling (YL) city of Shaanxi.

Producing Areas	**Relative Content of Fatty Acids/%**						
	C16:0	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**C20:1**	**C22:1**
YZ	3.51 ^{AB}	1.99 ^{B}	22.33 ^{AB}	32.19 ^{AB}	1.65 ^{A}	8.31 ^{ABC}	18.64 ^{B}
WD	3.30 ± 0.31 ^{B}	1.97 ± 0.17 ^{B}	21.64 ± 1.03 ^{ABC}	30.10 ± 1.36 ^{AB}	1.20 ± 0.40 ^{A}	8.25 ± 0.26 ^{ABC}	19.75 ± 0.78 ^{AB}
WN	3.50 ± 0.27 ^{AB}	1.97 ± 0.14 ^{B}	22.76 ± 1.36 ^{AB}	31.04 ± 2.22 ^{AB}	0.87 ± 0.16 ^{A}	8.12 ± 0.34 ^{BC}	19.68 ± 1.03 ^{AB}
CF	3.74 ± 0.05 ^{AB}	1.84 ± 0.23 ^{B}	17.52 ± 1.02 ^{C}	32.86 ± 1.37 ^{AB}	1.14 ± 0.16 ^{A}	6.93 ± 0.26 ^{D}	21.84 ± 0.04 ^{A}
ZW	3.46 ^{AB}	1.92 ^{B}	22.62 ^{AB}	32.48 ^{AB}	1.54 ^{A}	7.81 ^{CD}	18.04 ^{B}
FX	3.51 ^{AB}	1.46 ^{B}	20.91 ^{ABC}	34.15 ^{A}	1.31 ^{A}	8.07 ^{ABC}	18.34 ^{B}
JP	3.99 ± 0.54 ^{A}	1.87±0.42 ^{B}	20.28 ± 1.27 ^{ABC}	30.74 ± 2.52 ^{AB}	1.54 ± 0.92 ^{A}	7.68 ± 0.61 ^{CD}	19.32 ± 1.78 ^{AB}
KZ	4.15 ± 0.22 ^{A}	2.03 ± 0.11 ^{B}	24.16 ± 2.49 ^{A}	28.65 ± 1.15 ^{BC}	0.92 ± 0.05 ^{A}	6.89±0.06 ^{D}	19.70 ± 0.92 ^{AB}
KS	3.37 ^{AB}	2.85 ^{A}	21.57 ^{ABC}	31.51 ^{AB}	1.88 ^{A}	9.04A	19.36 ^{AB}
FF	3.98 ± 0.20 ^{AB}	1.78 ± 0.34 ^{B}	22.54 ± 2.32 ^{AB}	30.09 ± 1.59 ^{AB}	1.51 ± 0.34 ^{A}	7.68 ± 0.47 ^{CD}	18.80 ± 1.72 ^{B}
YL	3.80 ^{AB}	2.06 ^{B}	19.24 ^{BC}	24.14 ^{C}	1.81	8.85 ^{AB}	19.42 ^{AB}
Mean	3.66	1.93	21.68	30.67	1.25	7.89	19.56
Variation range	2.87–4.61	1.35–2.85	16.62–25.97	24.14–34.44	0.63–2.60	6.64–9.04	16.12–21.87
Coefficient of variation/%	10.30	15.01	9.88	6.39	35.27	7.56	6.92

Producing areas	**Relative content of fatty acids/%**					
	C24:1	**TFA**	**SFA**	**UFA**	**MUFA**	**PUFA**
YZ	6.47 ^{CD}	96.00 ^{AB}	6.4 ^{ABC}	89.60 ^{A}	55.76 ^{A}	33.84 ^{AB}
WD	7.52 ± 0.23 ^{B}	94.84 ± 2.27 ^{B}	6.26 ± 0.48 ^{ABC}	88.58 ± 2.52 ^{A}	57.18 ± 1.75 ^{A}	31.40 ± 1.63 ^{ABC}
WN	7.22 ± 0.94 ^{BC}	96.24 ± 0.60 ^{AB}	6.44 ± 0.30 ^{ABC}	89.80 ± 0.72 ^{A}	57.80 ± 2.49 ^{A}	32.00 ± 2.31 ^{AB}
CF	8.83 ± 1.01 ^{A}	95.78 ± 0.45 ^{AB}	6.49 ± 0.38 ^{ABC}	89.29 ± 0.08 ^{A}	55.14 ± 1.50 ^{A}	34.15 ± 1.49 ^{AB}
ZW	6.75 ^{BC}	95.37 ^{AB}	6.06 ^{BC}	89.31 ^{A}	55.22 ^{A}	34.09 ^{AB}
FX	7.30 ^{BC}	95.84 ^{AB}	5.58 ^{C}	90.26 ^{A}	54.64 ^{A}	35.62 ^{A}
JP	8.73 ± 1.23 ^{A}	95.29 ± 0.71 ^{AB}	6.84 ± 1.36 ^{AB}	88.46 ± 2.07 ^{A}	56.05 ± 1.30 ^{A}	32.41 ± 1.72 ^{AB}
KZ	6.93 ± 0.54 ^{BC}	94.21 ± 0.29 ^{AB}	6.95 ± 0.40 ^{ABC}	87.26 ± 0.11 ^{A}	57.70 ± 1.00 ^{A}	29.56 ± 1.10 ^{BC}
KS	5.84 ^{D}	97.19 ^{A}	7.78 ^{A}	89.41 ^{A}	55.84 ^{A}	33.57 ^{AB}
FF	6.94 ± 1.02 ^{BC}	94.08 ± 1.14 ^{AB}	6.47 ± 0.47 ^{ABC}	87.61 ± 0.69 ^{A}	56.00 ± 1.60 ^{A}	31.62 ± 1.79 ^{AB}
YL	6.84 ^{BC}	88.32 ^{C}	7.80 ^{A}	80.52 ^{B}	54.40 ^{A}	26.12 ^{C}
Mean	7.40	95.30	6.49	88.56	56.56	32.00
Variation range	5.20–9.92	88.32–97.19	5.58–8.41	84.27–91.00	53.24–61.98	26.12–35.85
Coefficient of variation/%	14.19	1.47	9.15	1.73	3.37	6.39

Note: the data in the table are the mean ± standard deviation, and some data only show the mean value; coefficient of variation = standard deviation/mean; lists the changes in the main fatty acid composition with a relative content above 1.00%; there is significant difference in labeling different letters in the same column ($p < 0.05$).

Table 4. Kernel oil content, the relative content of nervonic acid and total nervonic acid in kernel oil of *A. truncatum* from different producing areas.

Producing Areas	Oil Content (X ± SD)/%	Nervonic Acid Content/%	Total Nervonic Acid Content/%
YZ	42.52 ± 1.36 B	6.47 CD	2.75 CD
WD	42.77 ± 1.31 B	7.52 ± 0.23 B	3.22 ± 0.16 AB
WN	40.17 ± 2.01 C	7.22 ± 0.94 BC	2.90 ± 0.39 BCD
CF	38.13 ± 3.48 D	8.83 ± 1.01 A	3.35 ± 0.15 A
ZW	43.35 ± 0.00 B	6.75 BC	2.93 BCD
FX	36.54 ± 0.00 E	7.30 BC	2.67 D
JP	32.10 ± 1.95 F	8.73 ± 1.23 A	2.81 ± 0.47 ABC
KZ	38.52 ± 1.67 D	6.93 ± 0.54 BC	2.67 ± 0.32 D
KS	51.22 ± 0. 86 A	5.84 D	2.99 BCD
FF	40.33 ± 2.46 C	6.94 ± 1.02 BC	2.80 ± 0.45 CD
YL	50.54 ± 0.76 A	6.84 BC	3.46 A

Note: X represents mean value, SD represents standard deviation, coefficient of variation = standard deviation/mean value, there is significant difference in the same column marked with different letters ($p < 0.05$).

3.4. Correlation Analysis between Oil Content and Main Fatty Acids in Kernel Oil of A. truncatum from Different Producing Areas

In the correlation analysis results of the composition of *A. truncatum* from different producing areas (Table 5), the oil content was significantly negatively correlated with the relative contents of palmitic acid (C16:0; $p < 0.05$, −0.385) and nervonic acid (C24:1; $p < 0.01$, −0.473) and significantly positively correlated with the relative contents of stearic acid (C18:0; $p < 0.05$, 0.426), *cis*-11-eicosenoic acid (C20:1; $p < 0.01$, 0.548) and behenic acid (C22:0; $p < 0.01$, 0.526). The relative content of palmitic acid (C16:0) was only significantly negatively correlated with the relative content of *cis*-11-eicosenoic acid (C20:1; $p < 0.05$, −0.353) but did not reach a significant level with other major fatty acids. Stearic acid (C18:0) only showed an extremely significant positive correlation with *cis*-11-eicosenoic acid (C20:1; $p < 0.01$, 0.463) and behenic acid (C22:0; $p < 0.01$, 0.658), indicating that the presence of stearic acid synthesized twenty and twenty-one carbon fatty acids more, which promoted the continuous extension of the carbon chain. Oleic acid (C18:1) was extremely significantly negatively correlated with erucic acid (C22:1; $p < 0.01$, −0.522). Behenic acid (C22:0) was extremely significantly negatively correlated with linoleic acid (C18:2; $p < 0.01$, −0.484) and positively correlated with linolenic acid (C18:3; $p < 0.05$, 0.376) and *cis*-11-eicosenoic acid (c20:1; $p < 0.01$, 0.534), and both reached a significant level. The results showed that the synthesis of linolenic acid promoted the formation of high-carbon fatty acids such as *cis*-11-eicosenoic acid and behenic acid.

Table 5. Correlation between kernel oil content and fatty acids of *A. truncatum* from different areas.

Index	Oil Content	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid	*Cis*-11-Eicosenoic Acid	Behenic Acid	Erucic Acid
Oil content	1								
Palmitic acid	−0.385 *	1							
Stearic acid	0.426 *	−0.025	1						
Oleic acid	0.027	0.024	0.106	1					
Linoleic acid	−0.241	−0.238	−0.062	−0.303	1				
Linolenic acid	0.143	0.284	0.319	−0.209	−0.110	1			
Cis-11-eicosenoic acid	0.548 **	−0.353 *	0.463 **	0.254	−0.193	0.267	1		
Behenic acid	0.526 **	−0.057	0.658 **	−0.143	−0.484 **	0.376 *	0.534 **	1	
Erucic acid	0.033	−0.276	−0.186	−0.522 **	−0.025	−0.239	−0.269	0.202	1
Nervonic acid	−0.473 **	−0.075	−0.513 **	−0.541 **	−0.003	−0.214	−0.541 **	−0.193	0.614 **

* Indicates that the correlation is significant at the 0.05 level (two-tailed); ** indicates that the correlation is significant at the 0.01 level (two-tailed). The same below.

The study found that nervonic acid (C24:1) has a negative correlation with the oil content of the seed kernel and the content of all main fatty acids, but it has a very significant positive correlation with erucic acid (C22:1), and the correlation coefficient is 0.614. It is well-confirmed that the synthesis of nervonic acid is affected by the relative content of erucic acid, and it is possible that the conversion of erucic acid to nervonic acid has an inherent proportion, which requires further investigation.

3.5. Correlation Analysis of Relative Contents of Kernel Oil and Nervonic Acid and Geographical and Ecological Factors in *A. truncatum* from Different Producing Areas

From the correlation analysis (Table 6), it can be seen that the correlation between the oil content, the relative content of nervonic acid and various environmental factors was weak, and none of them reached an extremely significant level. In terms of the overall trend, the correlation between oil content and environmental factors was opposite to that between the relative content of nervonic acid and environmental factors. The oil content showed a significant negative correlation with longitude ($p < 0.05$, −0.652) and a significant positive correlation with the frost-free period ($p < 0.05$, 0.633), indicating that the growth cycle of *A. truncatum* seeds was approximately long, and the oil content of seeds also increased. The correlation between the oil content and other ecological factors was not significant, among which, it was negatively correlated with latitude, annual rainfall and annual average sunshine hours, and positively correlated with altitude and annual mean temperature. These data suggest that planting *A. truncatum* in high-temperature and high-altitude areas is beneficial for increasing the oil content of its seeds, especially in high-temperature and high-altitude areas such as Northwest China (KS and YL). The correlation between the relative content of nervonic acid and eco-environmental factors was not significant, but it could be seen that the relative content of nervonic acid showed a weak negative correlation with altitude and annual mean temperature and a weak positive correlation with annual rainfall.

Table 6. Correlation between geographical and ecological factors and contents of kernel oil and nervonic acid in *A. truncatum* from different areas.

Index	Latitude/°N	Longitude/°E	Altitude/m	Annual Mean Temperature/°C	Annual Rainfall/mm	Frost Free Period/d	Annual Average Sunshine Hours/h
Oil content	−0.452	−0.652 *	0.162	0.490	−0.432	0.633 *	−0.048
Nervonic acid	0.195	0.444	−0.144	−0.287	0.383	−0.429	0.079

* Indicates that the correlation is significant at the 0.05 level (two-tailed).

3.6. Hierarchical Cluster Analysis and Principal Component Analysis of *A. truncatum* from Different Producing Areas

To evaluate the possible similar relationship among different producing areas, in this study, based on the oil content and the relative contents of various fatty acids, such as palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, *cis*-11-eicosenoic acid, erucic acid and nervonic acid, a total of nine components were analyzed by hierarchical cluster analysis (Figure 2). The results showed that the 11 natural producing areas of *A. truncatum* for the test investigation were classified into three groups. The first group consists of six producing areas: YZ, ZW, WN, FF, WD and KZ, all of which were rich in high contents of oleic acid (the average content was 22.68%) and linoleic acid (the average content was 32.58%). CF, FX and JP from the producing areas were classified into the second group because of their high contents of nervonic acid (7.30–9.39%) and erucic acid (18.34–21.84%). The third group was characterized by a high oil content (average content was 50.88%) and high relative content of linolenic acid (average content was 1.85%), and its producing areas were KS and YL. In addition, the analysis found that high oil and nervonic acid contents did not appear in the same group, which also confirmed that the high oil- and nervonic acid-producing areas in the previous results did not coincide in the same region.

To deeply explore the interrelationship among the populations in various producing areas of *A. truncatum*, a principal component analysis was conducted on the basis of cluster analysis (Figure 3). The same nine component indexes were used for the analysis. The results showed that PC1 separated the difference in origin and that PC2 separated the difference in fatty acids, which accounted for 42.50% and 20.29% of the variance in all analyzed parameters, respectively. The first PC (PC1) and second PC (PC2) components together accounted for 62.79% of the total variance. Similarly, the producing areas can also be classified into three categories, similar to the same cluster analysis; the difference was that FX was classified into the first group. Additionally, FF and YL were not classified into the same group, which further confirmed the previously mentioned results of genetic and genotypic differences in *A. truncatum* germplasm between the two areas. The results of hierarchical clustering and principal component analysis classified the composition of fatty acids into producing areas, which provided a basis for the purposeful screening of *A. truncatum* germplasm.

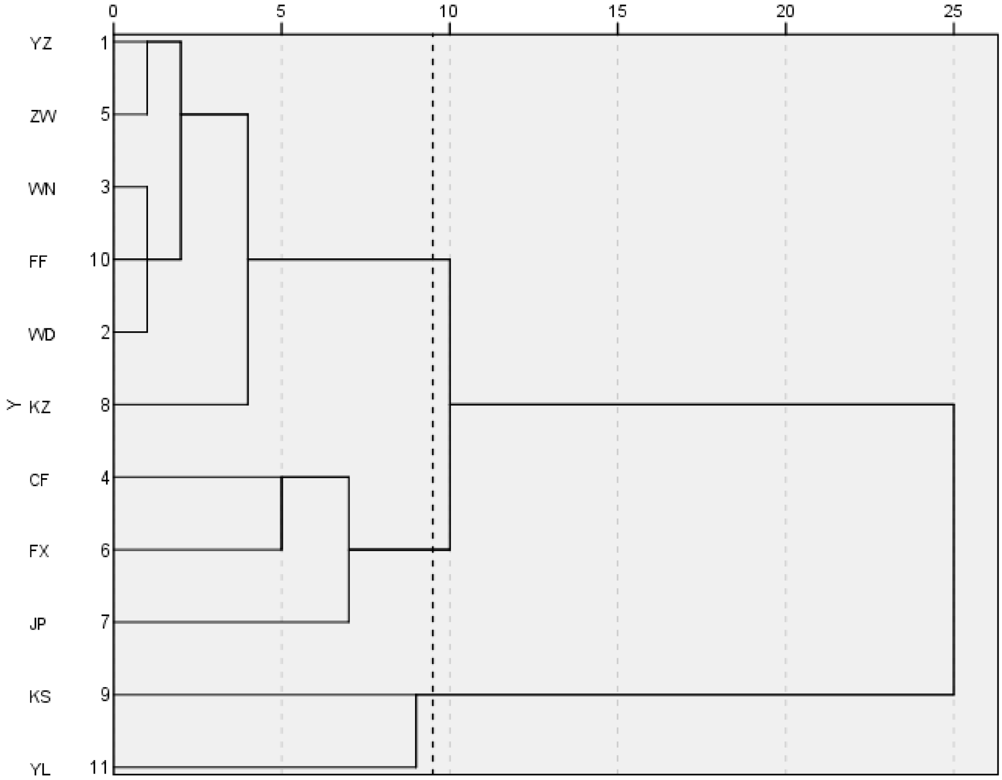

Figure 2. Clustering results of seed oil indexes of *A. truncatum* from different areas. The natural forest production areas: Horqin Right Wing Middle Banner (YZ), Wudantala Forest Farm (WD), Wengniuteqi Pine mountain Forest Farm (WN) and Chifeng city (CF) of Inner Mongolia, Zhangwu (ZW), Fuxin (FX), Jianping (JP) and Kazuo (KZ) city of Liaoning, Kashi (KS) city of Xinjiang, Fufeng (FF) and Yangling (YL) city of Shaanxi.

To analyze the transformation relationship of oil and fatty compositions among different groups of producing areas, we conducted a correlation analysis again for the fatty acid compositions of the first group and the second group in the hierarchical clustering results (Tables S2 and S3). The results of the correlation analysis of the oils and fatty acid content in the first group of producing areas showed that the oil content showed a significant negative correlation with the relative content of palmitic acid and oleic acid, and that the correlation coefficients were −0.429 and −0.456, respectively; it was extremely significantly positively correlated with the relative content of *cis*-11-eicosenoic acid (0.548) and positively correlated with the relative content of nervonic acid (0.098). There was an extremely significant negative correlation between the content of palmitic acid and *cis*-11-eicosenoic acid (−0.572). Nervonic acid showed a significant negative correlation with linoleic acid (−0.478) but an extremely significant positive correlation with erucic acid (0.602).

In the second group of index correlation results, the oil content was significantly positively correlated with linoleic acid (0.807) and negatively correlated with nervonic acid (−0.227). Palmitic acid has an extremely significant positive correlation with stearic acid and linolenic acid, and the coefficients are 0.886 and 0.915, respectively; it had a significant negative correlation with linoleic acid (−0.822). Oleic acid was significantly positively correlated with *cis*-11-eicosenoic acid (0.855) and extremely significantly negatively correlated with the erucic acid content (−0.933). There was a significant negative correlation between erucic acid and *cis*-11-eicosenoic acid (−0.827).

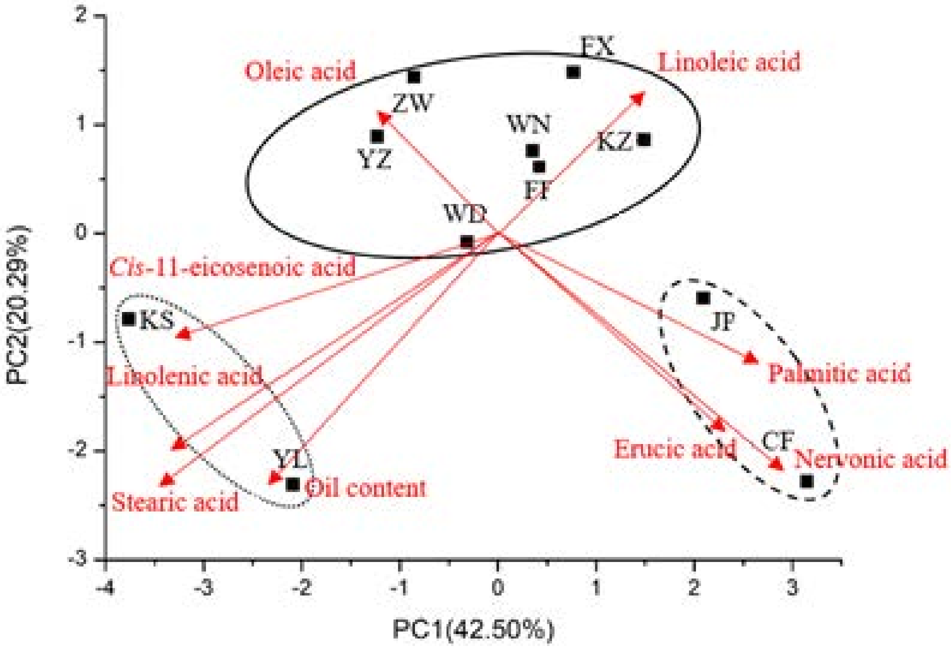

Figure 3. Principal component analysis of seed oil index of *A. truncatum* from different areas. The natural forest production areas: Horqin Right Wing Middle Banner (YZ), Wudantala Forest Farm (WD), Wengniuteqi Pine mountain Forest Farm (WN) and Chifeng city (CF) of Inner Mongolia, Zhangwu (ZW), Fuxin (FX), Jianping (JP) and Kazuo (KZ) city of Liaoning, Kashi (KS) city of Xinjiang, Fufeng (FF) and Yangling (YL) city of Shaanxi.

Comparing the correlation results of the two groups, it could be seen that, when the relative content of palmitic acid and *cis*-11-eicosenoic acid in the fatty acid compositions was positively correlated, the carbon chain in the fatty acid was more directly extended to synthesize nervonic acid. When the relative content of palmitic acid and linolenic acid was positively correlated, the fatty acid would extend more to the branched path of linolenic acid after synthesizing oleic acid, while the synthesizing of the nervonic acid pathway was limited to a certain extent. These results indicate that the amount of nervonic acid was affected by the pathway of fatty acid synthesis, and the pathway of nervonic acid synthesis was regulated by certain enzymes [26]. Based on this comprehensive analysis, it can be concluded that the nervonic acid content is highly correlated with the erucic acid content, and the mutual transformation relationship between seed kernel oil compositions will ultimately determine the nervonic acid content. This is consistent with our previous report on the dynamic regularity of oil accumulation in individual fruit development [21].

4. Discussion

4.1. Differences in Oil Content of A. truncatum Kernel from Different Producing Areas

In this study, the oil content of the seed kernels of *A. truncatum* in all production areas was measured to be more than 30.00%, and the total coefficient of variation was 11.36%, indicating that the oil content of kernels in different production areas has strong stability in different geographical environments. Wei et al. [27] studied the seed kernel oil yield of nine producing areas of *A. truncatum* and found that its average value was 43.30%, which is not much different from the average oil content of this paper (40.67%). The total variation coefficient of oil content in this study is lower than that in nine producing areas (22.05%). It is also found that the four producing areas are consistent with the sampling places selected in this paper (WN, WD, ZW, YL). However, there are differences in the oil content of kernels in the same production area. Except for the fact that the oil content in the YL area (45.49%) is lower than that of this study (50.54%), the oil content of the other three producing areas is higher than that of this study. The reasons for these differences are closely related to the selection of samples in the same production area, collection time, test operation conditions and material treatment methods. Qiao et al. [8] measured the oil content of the seed kernel of the natural forest resources of *A. truncatum* in 14 regions of China and found that the oil content ranged from 17.91% to 36.56%. However, the oil content measured in this paper ranged from 30.39%–51.83%, and the oil yield was significantly improved, which is related to the selected materials themselves and the extraction technology [28]. This result shows that the oil yield of different natural forest resources of *A. truncatum* is obviously different, and it is necessary to clearly divide the natural forest resources according to the production requirements in order to make full use of them.

4.2. Differences in the Fatty Acid Composition and Content of A. truncatum Kernel Oil from Different Producing Areas

In this experiment, a total of 14 fatty acids were detected from the seed oil of *A. truncatum* from different producing areas. Liu et al. [29] detected a total of 12 fatty acids in a composition study of Beijing *A. truncatum* oil. Compared with this study, no heptadecanoic acid or eicosadienoic acid was detected, and the other compositions were the same. Wei et al. [27] identified a total of 13 fatty acid compositions in the kernel oil of *A. truncatum* from nine natural forest production areas, but no eicosadienoic acid was detected. It can be seen that different researchers may have different research results due to different sample selections and operating conditions, but on the whole, the main types of fatty acids are the same. Hu et al. [30] mainly detected 13 kinds of fatty acids after extracting the kernel oil of *A. truncatum* from Jingyang, Shaanxi Province, among which, the relative content of nervonic acid (approximately 6.2%) was the closest to that from Shaanxi Province (YL) (more than 6.8%). Similarly, there was little difference from Liu et al. [31] in the content of nervonic acid (7.23%) in the study of the seed kernel oil of the Shaanxi Fufeng *A. truncatum* experimental forest, which shows that the content of fatty acids in *A. truncatum* oil has a great relationship with the natural geographical environment of the planting place. At the same time, the nature of variation between producing areas should be further studied by molecular means, such as a sequencing identification of fruit quality, which is similar to the reports on Chinese prickly ash [32]. Our study determined that the average content of nervonic acid in the kernel oil of *A. truncatum* from 11 producing areas was 7.40%, which was 1.64% higher than the average of 5.76% in the results of Qiao et al. [8,33] on 138 *A. truncatum* germplasm kernel oils from 14 regions of China. It was also found that the highest content of nervonic acid reported in this study occurred in CF (CF-2, 9.92%) and JP (JP-1, 9.84%), which is the first report of the highest relative content of nervonic acid in *A. truncatum* oil and fatty compositions at present and provides data support for the production and cultivation of *A. truncatum* seedlings with a high nervonic acid content.

4.3. Correlation Difference between Oil Content and Main Fatty Acids in the Kernel of A. truncatum from Different Producing Areas

In the correlation analysis results of fatty acid compositions from different producing areas, the relative content of nervonic acid was extremely significantly negatively correlated with stearic acid, oleic acid and *cis*-11-eicosenoic acid, which was generally consistent with the correlation analysis results of Qiao et al. [33]. It was also found that the correlation between the relative content of nervonic acid and the oil content of the seed kernel, as well as between the relative content of nervonic acid and the relative content of erucic acid, were both extremely significant, and the former was negative whereas the latter was positive. This is consistent with the results in the article of Qiao et al. [33] where the relative content of nervonic acid in *A. truncatum* has a weak negative correlation with the oil content and an extremely significant positive correlation with erucic acid, namely, −0.165 and 0.584 **. The correlation coefficient values of nervonic acid and erucic acid are both approximately 0.6, which indicates that the greater the relative content of nervonic acid in *A. truncatum* oil, the higher the content of erucic acid and the lower the oil content of the seed kernel. This is closely related to the extension path of fatty acids, which provides inspiration and thinking for the study of the synthesis mechanism of the carbon chain in *A. truncatum* fatty acids. This contradicts the conclusion in the study by Wei et al. [22,27] where there is a positive correlation between the nervonic acid content and seed oil content. The reasons for the different results were greatly related to the growth status of *A. truncatum* tree species and the natural ecological environment in the year of picking. The specific reasons need to be further studied in combination with the genetic characteristics, genotypes and environmental changes of tree species.

At present, the relevant studies on gene hierarchical analysis by molecular markers and other methods mainly focus on the biosynthesis, separation and purification of nervonic acid [13,26,34–38], and studies on the isolation and function of the key enzyme genes of nervonic acid synthesis have also been reported in plants such as *Lunaria annua* [39] and *Malania oleifera* [40]. New achievements have been made in the aspects of genome sequencing [41], transcriptome analysis [24] and identification of *the KCS* gene family [42,43] of *A. truncatum*. All of these results provide research directions for the subsequent expression of key genes for the synthesis of nervonic acid in *A. truncatum*.

4.4. Correlation Differences between Oil Content, Nervonic Acid Content and Environmental Factors of A. truncatum from Different Producing Areas

On the one hand, the research on the correlation analysis between the oil content and environmental factors of *A. truncatum* from different producing areas is not completely consistent with the research results of Wei et al. [27], and the correlation trend of the longitude, annual mean temperature and frost-free period in its environmental factors are similar to the results of this paper. This result is consistent with the correlations between oil content and altitude in the article of Qiao et al. [33], which all show a weak positive correlation; the correlation relationships with annual rainfall are all negative, but the significance is different. Therefore, it is still necessary to increase the sampling range of the test sample size and further research the variation regulation and mechanism between the oil content and neuronal acid content.

On the other hand, the correlation between the nervonic acid content in *A. truncatum* kernel oil and environmental factors in the results of this study is weak and has not reached a significant state, which is consistent with the conclusion that Wei et al. [27] reported of mostly positive correlations and weakly negative correlations with altitude and weakly positive correlations with annual rainfall. The research results of Qiao et al. [33] showed that the nervonic acid content in the kernel oil of the natural forest of *A. truncatum* was not significantly correlated with other factors, except for the significant positive correlation with annual rainfall, which is consistent with the correlation between longitude, altitude and annual rainfall in the results of this paper. Therefore, altitude and annual rainfall are still the main environmental factors used to study the correlation between the variation in oil and fatty compositions in the *A. truncatum* kernel, and we should further research their regulatory factors to obtain an accurate mechanism.

The results of this study showed that the high oil content and high relative content of nervonic acid in the *A. truncatum* kernel are not in the same producing area and are completely consistent with the results of clustering and principal component analysis, which proved the accuracy and reliability of the data and analysis process. At the same time, the KS area with a high oil content was found, but its nervonic acid content was at the lowest level. It was also found that the kernel oil content was the lowest in the JP area with a high nervonic acid content. This was also explained in the result of the subsequent correlation analysis between oil and fatty acid compositions. To determine which production area has the special characteristics of high oil and nervonic acid contents, we analyzed the relative content of total nervonic acid in each production area and further defined the classification of natural forest-producing areas, which provided a reference and guidance for the classification of natural forest germplasm resources and the selection of nursery sites in the tested producing area.

5. Conclusions

The high-quality germplasm with high oil and nervonic acid contents was screened out in the study on the determination of kernel oil and fatty acid compositions of excellent individuals of *A. truncatum* used for oil from 11 natural areas. Among them, the kernel oil content of excellent plants in the Kashgar (KS) and Yangling (YL) areas was the highest, with both above 50.00%, which can be used as the germplasm of *A. truncatum* with a high oil yield. The nervonic acid content in the Chifeng (CF) area is the highest, at up to 9.92%. Second, the nervonic acid content in the Jianping (JP) area is as high as 9.84%. Both can be used as high-yield and high-quality nervonic acid germplasms, which is the first report of the highest nervonic acid content in the current study. The relative content of total nervonic acid in Yangling (YL) and Chifeng (CF) was the highest, with up to more than 3.30%, which could be used as high-efficiency and high-quality oil-used germplasm resources of *A. truncatum.*

The correlation results between the oil content and the main fatty acid content of *A. truncatum* kernels from 11 producing areas were different. The relative content of nervonic acid and erucic acid in kernel oil fatty acids showed an extremely significant positive correlation; it was negatively correlated with the oil content and the relative content of other fatty acids, indicating that there is a positive correlation between the nervonic acid and erucic acid contents. In the correlation analysis with environmental factors, the oil content and nervonic acid content of seeds did not reach extremely significant levels with each environmental factor, but both had relatively strong correlations with the longitude and frost-free period.

The natural forests of *A. truncatum* from 11 producing areas can be divided into three types of germplasm: high oleic acid, high nervonic acid and high oil content. Specifically, the YZ, WD, WN, ZW, KS and FF areas were classified as high-oleic-acid germplasm; CF, FX and JP areas were classified as high-nervonic-acid germplasm; KS and YL areas were classified as germplasm with a high oil content.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f13091409/s1, Table S1. Natural conditions of *A. truncatum* seed collection sites; Table S2. Correlation between kernel oil content and fatty acids of *A. truncatum* from the first group; Table S3. Correlation between kernel oil content and fatty acids of *A. truncatum* in the second group; Figure S1. Fatty acid methyl esters identified after methyl esterification of the *A. truncatum* oil.

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References

1. Zhao, W.H.; Gao, C.C.; Ma, X.F.; Bai, X.Y.; Zhang, Y.X. The isolation of 1,2,3,4,6-penta-O-galloyl-beta-D-glucose from Acer truncatum Bunge by high-speed counter-current chromatography. *J. Chromatogr. B* **2007**, *850*, 523–527. [CrossRef] [PubMed]

2. Li, L.; Manning, W.J.; Tong, L.; Wang, X.K. Chronic drought stress reduced but not protected Shantung maple (Acer truncatum Bunge) from adverse effects of ozone (O_3) on growth and physiology in the suburb of Beijing, China. *Environ. Pollut.* **2015**, *201*, 34–41. [CrossRef]
3. Ma, X.F.; Wu, L.H.; Ito, Y.; Tian, W.X. Application of preparative high-speed counter- current chromatography for separation of methyl gallate from Acer truncatum Bunge. *J. Chromatogr. A* **2005**, *1076*, 212–215. [CrossRef] [PubMed]
4. Tong, Y.X.; Xu, J.M.; Yin, Z.X.; Lin, N.; Kong, W.B. Advance in active components of Acer truncatum Bunge. *China Oils Fats* **2021**, 1–11.
5. Wang, K.; Liu, S.B.; Zhang, N.; Wang, X.L. Research Progress on Acer truncatum. *J. Northwest For. Univ.* **2021**, *36*, 152–157.
6. Wei, J.J.; Zhao, S.T. Progress on Active Ingredients and Application of Acer truncatum Bunge. *Prog. Vet. Med.* **2021**, *42*, 100–105.
7. Wang, L.; Li, W.Q.; Liu, D.; Yu, M.; Zhao, Z.F.; Xie, X.M.; Han, C.J.; Wu, D.; Bai, Z.G.; Zhao, Y.J. Discussion on Utilization and Industrialization Development of Woody Oil Plants in Shandong Province. *J. Anhui Agric. Sci.* **2020**, *48*, 146–151.
8. Qiao, Q.; Wang, X.; Feng, Z. Variability of seed oil content, fatty acid composition, and nervonic acid concent in Acer truncatum, native to 14 regions of China. *Grasas Y Aceites* **2018**, *69*, 1–10. [CrossRef]
9. Wang, X.Y.; Wang, S.Q. A new resource of nervonic acid: Purpleblow maple oil. *China Oils Fats* **2005**, *30*, 62–64.
10. Wang, X.Y.; Xie, S.J.; Wang, G.H. Application research status and prospects of Acer truncatum Bunge. seed oil rich in nervonic acid in China. *China Oils Fats* **2018**, *43*, 93–95.
11. He, X.; Li, D.Z.; Tian, B. Diversity in seed oil content and fatty acid composition in Acer species with potential as sources of nervonic acid. *Plant Divers* **2021**, *43*, 86–92. [CrossRef]
12. Tu, X.H.; Wan, J.Y.; Xie, Y.; Wei, F.; Ouek, S.Y.; Lv, X.; Du, L.Q.; Chen, H. Lipid analysis of three special nervonic acid resources in China. *Oil Crop Sci.* **2020**, *5*, 180–186. [CrossRef]
13. Li, Q.; Chen, J.; Yu, X.Z.; Gao, J.M. A mini review of nervonic acid: Source, production, and biological functions. *Food Chem.* **2019**, *301*, 125286. [CrossRef] [PubMed]
14. Amminger, G.P.; Schäfer, M.R.; Klier, C.M.; Slavik, J.M.; Holzer, I.; Holub, M.; Goldstone, S.; Whitford, T.J.; McGorry, P.D.; Berk, M. Decreased nervonic acid levels in erythrocyte membranes predict psychosis in help-seeking ultra-high-risk individuals. *Mol. Psychiatry* **2012**, *17*, 1150–1152. [CrossRef] [PubMed]
15. Wang, X.Y. *Acer Truncatum of China*; Sichuan Nationalities Publishing House: Chengdu, China, 2003.
16. Sun, J.Y.; Wang, X.K.; Smith, M.A. Identification of n—6 Monounsaturated Fatty Acids in Acer Seed Oils. *J. Am. Oil Chem. Soc.* **2018**, *95*, 21–27. [CrossRef]
17. Huang, L.H. Research status and prospects of Acer truncatum industry. *For. Sci. Technol.* **2020**, *45*, 30–32.
18. Liu, X.Y.; Fu, H.; Zhang, J.Y. Components analysis of Unsaturated fatty acid of pure oil from Acer truncatum Bunge in Yunnan. *Nat. Prod. Res. Dev.* **2003**, *15*, 38–39.
19. Wei, M.; Liao, C.H. Component analysis of oil in Acer truncatum Bunge kernel in Mianyang. *Sci. Technol. Food Ind.* **2011**, *32*, 127–128.
20. Dai, Y.M.; Wang, Y.; Niu, L.X. Changes of oil yield and fatty acid composition of Acer truncatum Bunge fruit during ripening. *J. Food Saf. Qual.* **2021**, *12*, 2893–2897.
21. Chang, P.; Gu, X.T.; Ma, J.W.; Fan, J.S.; Zhang, B.Y.; Lu, Y.Z.; Li, L.L. Dynamics of oil and fatty acid accumulation during fruit development of Acer truncatum Bunge used for oil. *J. Northwest A&F Univ.* **2022**, *50*, 1–12.
22. Wei, Y.C.; Fan, J.S.; L, J.J.; Zhang, S.J.; Cheng, R.R. Oil Content and Fatty Acid Composition in Acer truncatum Bunge Kernel from Different Production Places. *J. Chin. Cereals Oils Assoc.* **2018**, *33*, 69–73.
23. Qiao, Q.; Si, F.F.; Ye, M.J.; Ren, H.J.; An, K.; Feng, Z.; Cheng, T.T.; Sun, Z.K. Diversity Analysis on Seed Oil Content and Fatty Acids Composition of Acer truncatum. *J. Chin. Cereals Oils Assoc.* **2018**, *33*, 56–63.
24. Wang, R.K.; Liu, P.; Fan, J.S.; Li, L.L. Comparative transcriptome analysis two genotypes of Acer truncatum Bunge seeds reveals candidate genes that influences seed VLCFAs accumulation. *Sci. Rep.* **2018**, *8*, 15504. [CrossRef] [PubMed]
25. Qiao, Q.; Feng, Z.; Ren, H.J.; An, K.; Du, X.X.; Xu, C.; Zhang, L.; Sun, Z.K. Variation of seed oil content and fatty acid components among different individuals in Acer truncarum. *J. Cent. South Univ. For. Technol.* **2018**, *38*, 76–82.
26. Huai, D.X.; Zhang, Y.Y.; Zhang, C.Y.; Cahoon, E.B. Combinatorial Effects of Fatty Acid Elongase Enzymes on Nervonic Acid Production in Camelina sativa. *PLoS ONE* **2015**, *10*, e0131755. [CrossRef]
27. Wei, Y.C. *Studies on the Phenotypic Traits and Chemical Components of Acer truncatum Bunge from Different Locations*; Northwest A&F University: Xianyang, China, 2018.
28. Hu, P.; Xu, X.B.; Yu, L.L. Physicochemical Properties of Acer truncatum Seed Oil Extracted Using Supercritical Carbon Dioxide. *J. Am. Oil Chem. Soc* **2017**, *94*, 779–786. [CrossRef]
29. Liu, X.L.; Li, C.; Feng, Y.; Su, S.C.; Ao, Y.; Zhang, R. Development and quality formation of Acer truncatum Bunge fruit. *J. Northwest A&F Univ.* **2020**, *48*, 69–80.
30. Hu, P. *Research on Acer Truncatum Oil Extraction and Its Functional Characteristics*; Shanghai Jiao Tong University: Shanghai, China, 2017.
31. Liu, P. *Study of Acer truncatum characters of Shaanxi Province*; Northwest A&F University: Xianyang, China, 2016.
32. Ma, Y.; Li, J.M.; Tian, M.J.; Liu, Y.L.; Wei, A.Z. Authentication of Chinese prickly ash by ITS2 sequencing and the influence of environmental factors on pericarp quality traits. *Ind. Crops Prod.* **2020**, *155*, 112770. [CrossRef]
33. Qiao, Q.; Wang, X.; Ren, H.J.; An, K.; Feng, Z.; Cheng, T.T.; Sun, Z.K. Oil Content and Nervonic Acid Content of Acer truncatum Seeds from 14 Regions in China. *Hortic. Plant J.* **2019**, *5*, 24–30. [CrossRef]

34. Millar, A.A.; Kunst, L. Very-long-chain fatty acid biosynthesis is controlled through the expression and specificity of the condensing enzyme. *Plant J. Cell Mol. Biol.* **1997**, *12*, 121–131. [CrossRef]
35. Xia, J.J. *Construction and Optimization of Fermentation Conditions for High-Yielding Engineered Nervonic Acid Bacteria*; Beijing University of Chemical Technology: Beijing, China, 2020.
36. Liu, S.S.; Zhou, Q.L.; Sun, H.; Y, G.M. Advance in function and purification technology of nervonic acid. *China Oils Fats* **2019**, *44*, 142–146.
37. Durazzo, A.; Fawzy Ramadan, M.; Lucarini, M. Editorial: Cold Pressed Oils: A Green Source of Specialty Oils. *Front. Nutr.* **2022**, *18*, 836651. [CrossRef] [PubMed]
38. Grajzer, M.; Szmalcel, K.; Kuźmiński, Ł.; Witkowski, M.; Kulma, A.; Prescha, A. Characteristics and Antioxidant Potential of Cold-Pressed Oils—Possible Strategies to Improve Oil Stability. *Foods* **2020**, *9*, 1630. [CrossRef] [PubMed]
39. Guo, Y.M.; Mietkiewska, E.; Francis, T.; Katavic, V.; Brost, J.M.; Gibin, M.; Barton, D.L.; Taylor, D.C. Increase in nervonic acid content in transformed yeast and transgenic plants by introduction of a Lunaria annua L. 3-ketoacyl-CoA synthase (KCS)gene. *Plant Mol. Biol.* **2009**, *69*, 565–575. [CrossRef]
40. Li, Z.W.; Ma, S.J.; S, H.; Yang, Z.; Zhao, C.Z.; Taylor, D.; Zhang, M. A 3-ketoacyl-CoA synthase 11 (KCS11) homolog from Malania oleifera synthesizes nervonic acid in plants rich in 11Z-Eicosenoic acid. *Tree Physiol.* **2021**, *41*, 331–342. [CrossRef]
41. Wang, R.K.; Fan, J.S.; Chang, P.; Zhu, L.; Zhao, M.R. Genome Survey Sequencing of Acer truncatum Bunge to Identify Genomic Information, Simple Sequence Repeat (SSR) Markers and Complete Chloroplast Genome. *Forests* **2019**, *10*, 87. [CrossRef]
42. Ma, Q.Y.; Sun, T.L.; Li, S.S.; Wen, J.; Zhu, L.; Yin, T.M.; Yan, K.Y.; Xu, X.; Li, S.X.; Mao, J.F.; et al. The Acer truncatum genome provides insights into the nervonic acid biosynthesis. *Plant J.* **2020**, *104*, 662–678. [CrossRef] [PubMed]
43. Ma, J.W.; Gu, X.T.; Wang, S.; Chen, Z.X.; Zhang, B.Y.; Li, L.L. Identification and spatio-temporal expression profiles of the KCS gene family in Acer truncatum. *Non-Wood For. Res.* **2022**, *40*, 201–214.