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Variability of seed oil content, fatty acid composition, and nervonic acid content in *Acer truncatum*, native to 14 regions of China

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SUMMARY: The seed oil of 138 accessions of 14 *Acer truncatum* (Aceraceae family) populations native to China were analyzed by pulsed nuclear magnetic resonance spectroscopy and gas chromatography-flame ionization detection. The oil content ranged from 17.81% to 36.56% (mean: 28.57%), which mainly consisted of 14 types of fatty acids. Palmitic (4.69%), stearic (2.30%), oleic (25.19%), linoleic (32.97%), linolenic (2.76%), cis-11-eicosenoic (7.90%), erucic (16.49%), and nervonic (5.76%) fatty acids accounted for 98% of total fat. The nervonic acid content ranged from 3.90% to 7.85% among the accessions. Significant variations in oil content and predominating fatty acids were observed among populations. Hierarchical cluster analysis and principal component analysis detected obvious geographical variation trends among *A. truncatum* populations which correlate with environmental variations (especially altitude, temperature, and precipitation) and supported the grouping of the populations into three groups according to geographic locations.

KEYWORDS: Acer truncatum; *Fatty acid composition; Genotype; Geographical variation; Nervonic acid; Oil content*

RESUMEN: ***Variabilidad del contenido de aceite, composición en ácidos grasos y contenido de ácido nervónico en semillas* Acer truncatum *nativas de 14 regiones de China***. El aceite de semillas de 138 accesiones de 14 poblaciones de *Acer truncatum* (familia Aceraceae) nativas de China se analizaron mediante espectroscopía de resonancia magnética nuclear pulsada y cromatografía de gases y detección de ionización de llama. El contenido de aceite varió de 17.81% a 36.56% (promedio: 28.57%), y están formadas principalmente por 14 tipos de ácidos grasos. Palmítico (4.69%), esteárico (2.30%), oleico (25.19%), linoleico (32.97%), linolénico (2.76%), cis-11-eicosenoico (7.90%), erúcico (16.49%) y nervónico (5.76 %) los ácidos grasos representaron el 98% de la grasa total. El contenido de ácido nervónico varió de 3,90% a 7,85% entre las accesiones. Se observaron variaciones significativas en el contenido de aceite y en los ácidos grasos predominantes entre las poblaciones. El análisis jerárquico de conglomerados y el análisis de componentes principales detectaron evidentes variaciones geográficas entre las poblaciones de *A. truncatum* que se correlacionaban con las variaciones ambientales (especialmente altitud, temperatura y precipitación) y permitieron agrupar las poblaciones en tres grupos según las ubicaciones geográficas.

PALABRAS CLAVE: Acer truncatum; *Ácido Nervónico; Composición de ácidos grasos; Contenido de aceite; Genotipo; Variación geográfica*

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1. INTRODUCTION

Plant seeds are an important source of oil, which serves as a staple agricultural food product and an important industrial raw material. Moreover, plant seed oils are an increasingly important renewable and environmentally safe bioenergy resource. However, increasing demand for seed oil has resulted in increasingly prevalent and serious oil shortages. To address this problem, efforts have been focused on the development of woody oil trees, which have recently become a major edible oil source in some western European countries. Woody crop oils with a large share of the edible oil market include coconut, olive, palm, almond, walnut, and tea oils. However, there are many potentially excellent woody edible oils in nature that have not been exploited, including those coming from maple tree species.

Acer truncatum, a member of the Aceraceae family, and is a forest tree species native to northern China, Korea and Japan, but can also be found in Europe and North America (Guo *et al.*, 2014). It is a deciduous tree species native to northern and western China, and it is mainly distributed in the Yellow River basin, the northeast, Inner Mongolia, Jiangsu, Sichuan and other regions. This species of maple has been commonly chosen as a landscape tree for its brilliant autumn leaf color (Zhao *et al.*, 2007; Li *et al.*, 2015). *A. truncatum* is also an ideal source of timber, protein, medicinal substances, and chemical raw materials due to its sturdy texture and high levels of protein, chlorogenic acid, tannin, and flavonoids (Ma *et al.*, 2005). In addition, its abundant oil seeds have been roasted for many years for use as a food source in northeastern China, but have not yet been used on a large scale. Previous studies (Wang *et al.*, 2006; Hu *et al.*, 2017; Sun *et al.*, 2018) have suggested that maple seed oil would be an excellent edible oil because it contains a large percentage of unsaturated fatty acids (92%) and nervonic acid (6.22%). Notably, nervonic acid, an important fatty acid for brain growth and maintenance, has been shown to prevent neural deficits and shows promise for the treatment of neurological disorders such as schizophrenia and psychosis (Akoh *et al.*, 2001; Barcarolo *et al.*, 2003; Chen *et al.*, 2017). It has a high content of vitamin E (125.23mg/100g) (Wang *et al.*, 2006). Therefore, *A. truncatum* is regarded as a potential species for use in the development of valuable nutritional and medicinal substances.

A. truncatum is also a renewable biomass energy tree species which produces approximately 30 kg of fruit per tree after 20 years and its seed ratio is up to 66.4% (Wang *et al.*, 2006). Currently, it is receiving much attention from researchers in China due to its great potential value for energy and other numerous applications. Consequently, the seed oil was approved as a new food resource by the Chinese Ministry of Health in 2011. *A. truncatum* seed oil (ATO) is usually obtained by cold pressing and commercialized as a crude oil in China (Hu *et al.*, 2017). At the present time, the *A. truncatum* artificial cultivation area encompasses 4×10^4 ha and should continue to expand rapidly due to this tree's numerous industrial prospects. However, as most previous studies have focused on the edible, medicinal, and health care uses of its seed oil, no studies have investigated variations in oil content or fatty acid composition among natural stands. Moreover, only limited information is available regarding the effects of environmental factors on seed oil yield and quality.

In this study, we selected 138 accessions from 14 *A. truncatum* tree populations growing across the known natural distribution area of this species in China. The objectives were to analyze seed oil variation and investigate the influence of environmental factors on seed oil content and fatty acid composition. The information obtained from these studies should guide breeding programs, aid in selection of the most adaptable lines for large-scale cultivation, and ultimately stimulate further development of the ATO industry in China.

2. MATERIALS AND METHODS

2.1. Plant material

In October and November 2016, 138 accessions of *A. truncatum* that grow naturally in fourteen regions were collected from nine provinces of China. About 10 separate pest-free adult plants (each 20 years of age or older) were selected from each sample collection area. In order to minimize other factors affecting seed development, individual tree species were chosen with spacing to other trees of at least 50 m. For each germplasm, 1-2 kilograms of fully matured samaras were randomly selected from multiple locations on each tree to ensure that the sample represented each whole plant. Samaras were stored at room temperature. After a period of time, the seeds are stripped from the Samaras manually. Using GPS to record latitude, longitude and altitude, the meteorological factors were listed using the data from the local meteorological department (Table 1).

2.2. Oil content and fatty acid composition analysis

A Bruker minispec mq20 pulsed nuclear magnetic resonance instrument (pulsed NMR) was used to estimate the seed oil content of each material. The specific method used here follows the official standard method (ISO 5511:1992, GB/T 15690-1995, AOAC, 2005).

Oil extraction was performed using a Soxhlet apparatus with ~5 g of ground seeds and petroleum

TABLE 1. *Acer truncatum* populations with their respective codes and collection site characteristics.

Code	Number of accessions	Collection site	Latitude (°N)	Longitude (°E)	Altitude (m)	Annual average temperature (ºC)	Annual rainfall (mm)	Frost-free season (d)
DQTL	10	Daiqintala, Inner Mongolia	45°13’	121°30’	324	5.6	388.0	120
CF	10	Chifeng, Inner Mongolia	42°17’	118°59’	574	7.4	460.0	130
CC	10	Changcun, Jilin	43°53’	125°19’	225	4.8	580.0	150
PQ	10	Pingquan, Hebei	40°50’	118°46’	628	6.0	600.0	155
TA	10	Taian, Shandong	36°12’	117°07’	305	13.2	722.6	202
LF	10	Linfen, Shanxi	36°44’	111°48’	802	10.0	625.0	153
YJ	10	Yongji, Shanxi	34°50’	110°22’	316	14.1	530.0	219
HY	10	Huayin, Shaanxi	34°32’	110°05’	353	12.0	600.0	200
YS	10	Yongshou, Shaanxi	34°43’	108°03’	1005	13.2	578.6	205
FZ	10	Fengzhou, Shaanxi	33°58’	106°39’	1020	11.4	613.2	188
ABZ	10	Abazhou, Sichuan	33°16’	103°55’	2060	12.7	552.9	225
NX	10	Neixiang, Henan	33°3’	110°51’	160	15.1	855.6	227
JY	10	Jiyuan, Henan	35°9’	112°07’	602	14.6	860.0	220
NJ	8	Nanjing, Jiangsu	32°15’	119°08’	50	15.4	1106.0	237

ether (60–90 °C) solvent according to published methods (Hu *et al*., 2017). The pure seed oil was transferred to a vial, flushed with nitrogen, and kept at -20 °C until further analysis. The seed oil was methylated twice, the first step being pre-esterification with H_2SO_4-CH_3OH to reduce the acid value to less than 1 mg KOH/g; the second step was transesterification with KOH-CH_3OH, according to the published method (ISO 5509:2000, GB/T 17376-2008). The fatty acid methyl ester (FAMEs) profiles obtained for each accession were determined using an Agilent 7890A (Agilent, Palo Alto, CA, USA) gas chromatograph (GC) equipped with a flame ionization detector (FID) using 17:0 FAME as an internal standard. The DB-23 capillary column (length 30 m, internal diameter 0.32 mm, film thickness 0.25 μm) was used for this test. The injector and detector temperatures were 230 °C and 280 °C, respectively. The oven temperature was maintained at 180 °C for 5 minutes and raised 3 °C·min^{-1} to 230 °C. The carrier gas (helium) was delivered at a flow rate of 1.0 ml·min^{-1}, and 1 ml of the sample was manually injected in a split injection mode. FAME peaks were identified by comparing the retention times of known standards which were run under the same conditions. Peak integration was performed using instrument software.

2.3. Statistical analysis

R software was used for correlation analysis, hierarchical cluster analysis (HCA), and principal component analysis (PCA); Excel 2010 was used for other calculations. Determinations were run in duplicate and the data were reported as mean values.

3. RESULTS

3.1. Oil content and fatty acid composition

Oil content is an important indicator to measure industrial production. In this work, the oil content of *A. truncatum* seeds ranged from 17.81%–36.56%, with a mean value of 28.57% for all 138 accessions studied (Table 2). Among the concessions, we selected three with very high oil content (>35%), including YS-6 (36.56%), ABZ-6 (35.71%), and DQTL-8 (35.44%), which would likely be valuable for the development of new varieties with high oil contents.

With regard to *A. truncatum*, a total of 14 fatty acid components were detected in this study (Figure 1). The results (Table 2) show that ATO is highly unsaturated (89.60%–92.90%), with an unsaturated to saturated ratio of approximately 10:1. The oil contains mainly oleic acid (C18:1) (20.00%–34.31%), linoleic acid (C18:2) (27.08%–36.71%), cis-11-eicosenoic acid (C20:1) (6.48%–9.15%), erucic acid (C22:1) (13.64%–18.86%), nervonic acid (C24:1) (3.90%–7.85%) and linolenic acid (C18:3) (1.60%–4.35%) which is composed of g-linolenic acid (GLA, 0.20%–1.36%) and α-linolenic acid (ALA, 0.97%–3.51%). The saturated fatty acid (SFA) content was low (7.32%–10.31%), but may stabilize the fat. SFA consisted predominantly of palmitic acid (C16:0) (3.78%-6.12%) and stearic acid (C18:0) (1.31%–3.30%). Palmitoleic acid (C16:1), arachidic acid (C20:0), cis-11,14-eicosadienoic acid (C20:2), behenic acid (C22:0), and tetracosanoic acid (C24:0) were also detected, but were not further characterized due to their small amounts (<1%), and therefore were labeled as other fatty acids.

TABLE 2. Variability in oil content and fatty acid composition among populations.

	Traits/%												
Populations	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**C20:1**	**C22:1**	**C24:1**	**SFA**	**UFA**	**MUFA**	**PUFA**	**Oil content**
DQTL	4.38±0.18C	2.38±0.14CD	25.64±1.18ABCD	35.08±0.87AB	2.62±0.32ABC	8.06±0.26ABC	15.14±1.14E	4.97±0.63E	8.07±0.24DE	91.93±0.24AB	53.96±0.98EFG	37.97±0.82A	32.47±2.39A
CF	4.52±0.26BC	2.30±0.17CDE	24.06±1.24CD	35.64±0.66A	2.96±0.70AB	7.92±0.22ABC	15.26±0.48DE	5.47±0.44CDE	8.21±0.31DE	91.79±0.31AB	52.87±1.11FG	38.91±1.04A	28.97±2.19ABCD
CC	4.29±0.30C	2.29±0.22CDE	24.70±3.10BCD	33.44±2.23BC	2.84±0.47AB	7.80±0.40BCD	16.50±0.55ABC	6.23±0.55ABC	8.03±0.52E	91.97±0.52A	55.42±2.90CDEF	36.55±2.62ABC	30.02±2.69ABC
PQ	4.47±0.22BC	2.37±0.15CD	22.94±1.25D	34.44±0.88ABC	2.90±0.77AB	7.43±0.20DE	17.23±0.44AB	6.29±0.67AB	8.38±0.41DE	91.62±0.41AB	53.99±1.27EFG	37.64±1.06AB	30.38±2.28ABC
TA	4.93±0.35AB	2.21±0.17CDE	27.91±2.89A	31.34±1.64DE	2.37±0.43BC	8.20±0.34ABC	15.40±0.74CDE	5.85±0.66ABCD	8.48±0.44CDE	91.52±0.44ABC	57.55±2.24ABCD	33.96±2.01DE	26.71±3.35CDE
LF	4.70±0.34ABC	2.45±0.34BC	26.64±1.95ABC	30.30±1.83E	2.79±0.56ABC	8.31±0.38A	16.90±0.81AB	6.03±0.43ABC	8.74±0.67BCD	91.26±0.67BCD	57.88±1.91ABC	33.38±1.95DE	29.03±4.82ABCD
YJ	4.51±0.34BC	2.20±0.13CDE	24.72±2.64BCD	33.41±2.05BC	2.87±0.37AB	7.83±0.47ABCD	17.04±1.20AB	5.54±0.59BCDE	8.29±0.47DE	91.71±0.47AB	55.14±2.23DEF	36.57±2.23ABC	25.38±5.04DE
HY	4.57±0.19BC	2.15±0.17DE	25.63±2.22ABCD	32.76±1.80CD	2.40±0.39ABC	8.11±0.40ABC	16.77±0.75AB	5.82±0.45ABCD	8.21±0.32DE	91.79±0.32AB	56.34±2.08ABCDE	35.45±2.03BCD	30.37±2.86ABC
YS	4.54±0.17BC	2.26±0.22CDE	25.34±1.27ABCD	33.19±1.31BC	3.12±0.79A	8.07±0.25ABC	16.26±1.01BCD	5.52±0.69BCDE	8.23±0.32DE	91.77±0.32AB	55.18±1.69DEF	36.58±1.60ABC	32.09±2.00A
FZ	4.92±0.20AB	2.84±0.31A	23.46±2.02D	34.16±1.54ABC	2.55±0.25ABC	7.72±0.47CDE	16.97±1.24AB	4.91±0.57E	9.47±0.56A	90.33±0.56E	53.30±1.75FG	37.03±1.73ABC	27.36±1.95BCDE
ABZ	5.06±0.42A	2.69±0.32AB	23.30±1.48D	34.94±0.82AB	3.10±0.48A	7.34±0.46E	16.27±1.04BCD	5.24±0.66CDE	9.30±0.45AB	90.70±0.45DE	52.35±1.02G	38.35±0.92A	31.70±2.79AB
NX	4.90±0.29AB	1.59±0.19F	26.32±1.73ABC	30.61±1.80E	2.87±0.58AB	7.75±0.29CDE	17.50±0.76A	6.18±0.42ABC	8.32±0.31DE	91.68±0.31AB	57.96±2.30AB	33.72±2.34DE	25.39±3.72DE
JY	5.14±0.53A	2.30±0.19CDE	24.93±1.38BCD	31.41±1.24DE	2.98±0.40AB	7.92±0.23ABC	16.72±0.68AB	6.54±0.35A	9.05±0.63ABC	90.95±0.63CD	56.26±1.28BCDE	34.69±1.50CDE	25.09±5.61DE
NJ	4.67±0.84ABC	2.07±0.19E	27.43±2.75AB	30.41±1.87E	2.12±0.41C	8.26±0.49AB	16.95±0.92AB	6.06±0.72ABC	8.45±0.87CDE	91.60±0.87AB	58.79±2.97A	32.81±2.25E	24.06±2.83E
Mean	4.69	2.30	25.19	32.97	2.76	7.90	16.49	5.76	8.52	91.47	55.45	36.02	28.57
Range	3.78-6.12	1.31-3.30	20.00-34.31	27.08–36.71	1.60–4.35	6.48–9.15	13.64–18.86	3.90–7.85	7.32–10.31	89.60–92.90	50.30–63.50	28.90–40.57	17.81–36.56
CV	9.12	15.15	9.58	6.95	20.58	5.59	6.70	12.62	7.51	0.73	4.83	7.09	14.72
F	5.302**	17.244**	5.342**	13.814**	2.983**	6.382**	7.202**	7.740**	8.636**	9.654**	10.914**	11.596**	6.703**

Note: **: Significant at $p < 0.01$. Different capital letters in the same column show significant difference ($P < 0.01$). Determinations were run in duplicate and the data are reported as the mean. Range from 138 accessions. Abbreviations of fatty acids with fig.1 and Abbreviations of populations with table 1. The data for 18:3 is for "total 18:3", being the sum of GLA +ALA. Other fatty acids include C16:1, C20:0, C20:2, C22:0, C24:0 and unknown components.

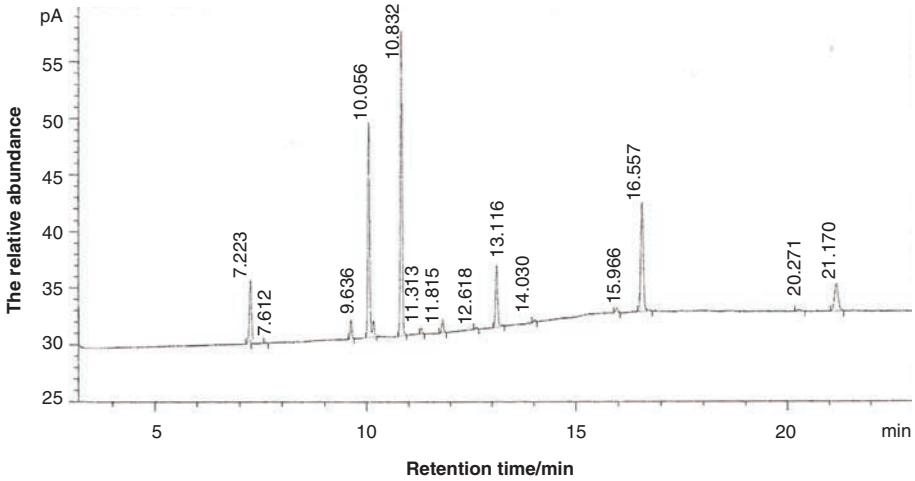

FIGURE. 1 Total ion chromatograms of fatty acid methyl ester.

Note: 7.223 - palmitic acid methyl ester (C16:0), 7.612 - Palmitoleic (C16:1), 9.636 - stearic (C18:0), 10.056 - oleic (C18:1) (20-34.31%), 10.832 - linoleic (C18:2), linolenic (C18:3) (including 11.313 - γ-linolenic and 11.815 - α-linolenic), 12.618 - arachidic (C20:0), 13.116 - cis-11-eicosenoic (C20:1), 14.030 - cis-11,14-eicosadienoic (C20:2), 15.966 - behenic (C22:0), 16.557 - erucic (C22:1), 20.271 - tetracosanoic (C24:0), 21.170 - nervonic acid methyl ester (C24:1).

3.2. Variation of oil content and fatty acid composition among populations

The oil content varied significantly ($p < 0.01$) among the 14 analyzed populations (Table 2). The highest seed oil content was exhibited by the DQTL population (32.47%), followed by YS (32.09%), with the lowest content observed in the NJ population (24.06%). Therefore, based on these results, the populations of DQTL and YS were chosen as germplasm with high oil contents for screening purposes.

The main fatty acid composition varied significantly ($p < 0.01$) among the 14 analyzed populations (Table 2). Unsaturated fatty acids (UFA) are classified into monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) according to the number of fatty acid double bonds. The populations NJ and NX exhibited the highest MUFA levels (> 57.96%), while populations CF and ABZ exhibiting the lowest MUFA levels (< 52.87%). PUFA percentage tended to be inversely correlated with MUFA percentage. Meanwhile, populations DQTL and CF exhibited a high (> 35.08%) content of C18:2 and relatively low content of C18:1, while TA, NJ, LF and NX populations exhibited a high (> 26.32%) C18:1 content and relatively low C18:2 content. Overall, the sum of percentages of C18:1 and C18:2 was consistently 60%, a previously known relationship prevalent in oil crops for which oleic acid is a precursor of linoleic acid (Yu *et al.*, 2017; Darwish, 2014). The highest amount of C18:3 was detected in populations YS (3.12%) and ABZ (3.10%). The content of C20:1 ranged from 7.34% (ABZ) to 8.31% (LF). JY, NX, and PQ samples exhibited the highest amounts of C22:1 (16.72%, 17.50%, and 17.23%, respectively) and C24:1 (6.54%, 6.18%, and 6.29%, respectively). Therefore, JY, NX, and PQ can be regarded as excellent germplasm resources which are especially valuable due to their C24:1 contents. The SFA content levels for populations FZ (9.47%), ABZ (9.30%), and JY (9.05%) were obviously higher than for other populations, as was also observed for C16:0 and C18:0 contents.

In summary, selection of excellent germplasm resources can be used to obtain optimal oil content and composition for various applications. The findings reported here will be beneficial as a starting point for future breeding efforts toward improving the yield and quality of ATO.

3.3. Effects of geography and ecological factors on seed oil content and fatty acid composition

The correlation analysis is shown in Table 3. Oil content showed significant negative correlations with annual average temperature ($p < 0.05$, −0.629), annual rainfall ($p < 0.01$, −0.749), and frost-free season period ($p < 0.05$, −0.562) and a positive correlation with latitude (0.503) and altitude (0.469), indicating that northern high altitude populations exhibited higher oil contents within the sampling area (such as DQTA and YS). Meanwhile, according to the correlation analysis, SFA and UFA showed a tendency toward longitudinal variation, while C18:2 and C22:1 exhibited latitudinal variation and C16:0 exhibited northeast-to-southwest variation.

Temperature is considered an important factor affecting the composition of fatty acids. It showed

Table 3. Correlation coefficients between traits and geography - ecological factors.

Traits	Latitude (°N)	Longitude (°E)	Altitude (m)	Annual average temperature (ºC)	Annual Rainfall (mm)	Frost-free Season (d)
C16:0	−0.673**	−0.613*	0.427	0.666**	0.462	0.617*
C18:0	0.149	−0.235	0.688**	−0.337	−0.465	−0.304
C18:1	−0.232	0.213	−0.555*	0.402	0.516	0.241
C18:2	0.535*	0.058	0.406	−0.601*	−0.790**	−0.510
C18:3	0.161	−0.286	0.526	−0.162	−0.437	−0.123
C20:1	−0.039	0.242	−0.524	0.186	0.258	−0.058
C22:1	−0.542*	−0.351	−0.061	0.376	0.482	0.495
C24:1	−0.075	0.305	−0.422	0.132	0.592*	0.214
SFA	−0.514	−0.640*	0.678**	0.358	0.182	0.36
UFA	0.490	0.638*	−0.663**	−0.326	−0.144	−0.324
MUFA	−0.386	0.144	−0.603*	0.486	0.741**	0.400
PUFA	0.520	0.008	0.461	−0.584*	−0.802**	−0.494
Oil content	0.503	0.017	0.469	−0.629*	−0.749**	−0.562*

Note: *, **: Significant at $p < 0.05$ and $p < 0.01$, respectively. Abbreviations of fatty acids with fig.1. The data for 18:3 is for "total 18:3", being the sum of GLA +ALA.

a significant negative correlations with PUFA ($p < 0.05$, −0.584) and C18:2 ($p < 0.05$, −0.601), although a significant positive correlations with C16:0 ($p<0.01$, 0.666). Meanwhile, environmental factors other than temperature also play a role in the fatty acid compositions of oils. Precipitation exhibited a significant negative correlation with C18:2 ($p < 0.01$, −0.790), PUFA ($p < 0.01$, −0.802) and a significant positive correlation with C24:1 ($p < 0.05$, 0.592), and MUFA ($p < 0.01$, 0.741). As a final observation, the frost-free period had the lowest effect on fatty acid composition, only significantly and negatively correlating with C16:0 ($p < 0.05$, 0.617).

The correlation study among different biochemical and geographical parameters could be used to understand their interrelations for future breeding, and for selecting an ideal location for growing high yield or high quality *A. truncatum* plants. From this study, we determined that an improvement in fat production and in the quality of ATO can be achieved by planting trees in moderately cold areas, meaning that the northeast and northwest regions of China are the best place to grow.

3.4. Hierarchical cluster analysis and principal component analysis

To evaluate the likely similarities and relationships among different populations, hierarchical cluster analysis (HCA) was performed based on 13 traits selected for this study (Figure 2A) (Szekely and Rizzo, 2005). This analysis provided the basis for understanding variations in fatty acid composition in combination with geographical influences. Based on this analysis, 14 populations of *A. truncatum* were classified into three groups. The first group consisted of the five populations of TA, LF, NX, JY, and NJ, all rich in MUFA (56.26%–58.79%) and C18:1 (24.93%-27.91%) (Table 2). It should be noted that JY differed from the others in of its exceptionally high level of C16:0 (5.14%). Populations FZ and ABZ, with higher amounts of SFA (> 9.30%) plus a moderate amount of PUFA, were classified in the second group. The third group was further divided into two subgroups. The first subgroup consisted of two populations (DQTL and CF) with high PUFA (> 37.97%) and C18:2 (> 35.08%) compositions, while the CC, PQ, YJ, HY, and YS populations, with higher quantities of UFA (> 91.62%) than other populations, were assigned to the second subgroup.

In the second stage of analysis, PCA was performed on all populations and all traits were evaluated in order to explore the interrelationships among and within populations. Most (84.04%) of the variation was explained by the first three principal components (PC1, PC2, and PC3). The result of PCA revealed that the first and second components explained 70.28% of the total variation. The first PC (PC1) accounted for 44.80% of the total variation and had a high positive correlation with MUFA (0.410) and C18:1 (0.362) (Table 4), but a negative correlation with PUFA (-0.394) and C18:2 (-0.386). The PC2 results explained 25.47% of the total variation. A high negative contribution by SFA (-0.516) and C16:0 (-0.478) and positive contribution by UFA (0.509) were also observed.

Further analysis results shown that the four populations of TA, LF, NX and NJ were positively

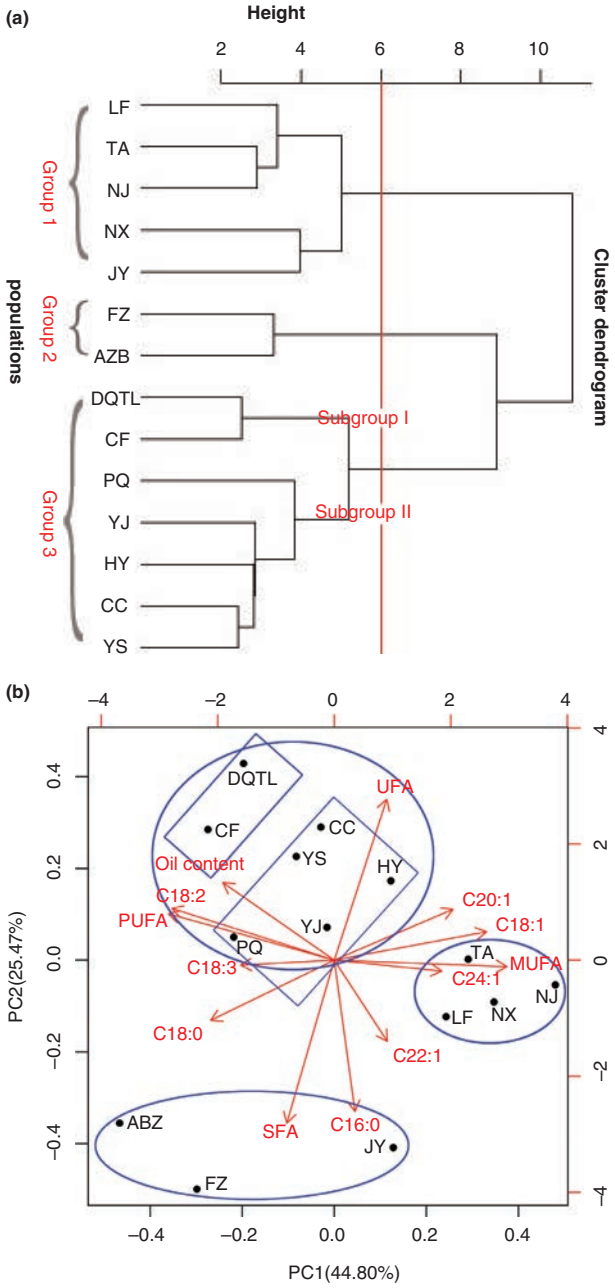

FIGURE 2. HCA and PCA of 14 populations of *A. truncatum*. (A) Dendrogram using the Ward clustering method. (B) PCA based on the 13 traits.

Note: Abbreviations of fatty acids with figure1 and Abbreviations of populations with table 1. The data for 18:3 is for "total 18:3", being the sum of GLA +ALA.

correlated with MUFA, C18:1, C20:1 and C22:1. The population of FZ, ABZ and JY was positively correlated with SFA and C16:0. The remaining 7 populations were positively correlated with oil content, UFA, PUFA and C18:2. Notably, PCA classification confirmed the results of HCA apart from JY (Figure 2B).

TABLE 4. Principal component analysis of 13 traits in *A.truncatum*.

traits	comp.1	comp.2	comp.3
C16:0	-	−0.478	-
C18:0	−0.293	−0.19	0.305
C18:1	0.362	-	0.293
C18:2	−0.386	0.163	-
C18:3	−0.222	-	−0.411
C20:1	0.282	0.161	0.412
C22:1	0.126	−0.257	−0.468
C24:1	0.255	-	−0.45
SFA	−0.113	−0.516	0.145
UFA	0.125	0.509	−0.156
MUFA	0.410	-	-
PUFA	−0.394	0.147	-
Oil content	−0.265	0.245	-
standard deviation	2.4134072	1.8196902	1.3376747
proportion of variance	0.4480411	0.25471333	0.1376441
cumulative proportion	0.4480411	0.7027544	0.8403985

Note: Abbreviations of fatty acids with fig.1. The data for 18:3 is for "total 18:3", being the sum of GLA +ALA.
- indicates almost zero.

4. DISCUSSION

Yield is the trait most predictive of whether or not an oil is suitable for large-scale production and adaptability to multiple industrial uses. Consequently, crops that contain high oil content at harvest ultimately reduce overall final production costs (Kumar and Sharma, 2011). This mean content (28.57%) value for *A. truncatum* greatly exceeded values for *Glycine max* (17%), *Olea europaea* (20%), and most results for *Sapium sebiferum* (12–29%) (USDA, 2012; Karmakar *et al*., 2010), and was comparable to values for *Jatropha curcas* (20.05%-38.33%) (Kaushik and Bhardwaj, 2013) and wild manihot (17%-31%) (Alvesa *et al*., 2014). This proves that it is feasible to extract oil from the seed of *A. truncatum* for industrial purposes.

Seed oil quality and uses are determined mainly by fatty acid composition, with rich variety in fatty acid compositions and content observed among the various species (and even among varieties). Therefore, fatty acid composition and content can be used as fingerprints to identify biological oil sources, as well as to test the authenticity of oils (Li *et al*., 2011). At the species level, a total of 14 distinct fatty acid components were detected in this study, which is similar to that reported by Hu *et al*., (2017), and a number similar to that obtained for *Xanthoceras sorbifolia* by Yu *et al*., (2017), although their oil content differed from the values reported here. The degree of unsaturation (89.60%–92.90%)

of ATO is higher than peanut oil (83.1%), soybean oil (84.4%), olive oil (86.2%), cotton seed oil (74.1%) and other edible oil (USDA, 2012). C18:2 (27.08%–36.71%) is essential for human metabolism due to the lack of enzymes responsible for its biosynthesis (Hanganu *et al*., 2012), and it contains a special substance named nervonic acid that is an essential fatty acid which helps maintain brain health by its participation in biosynthesis and maintenance of nerve cell myelin (Akoh *et al*., 2001; Barcarolo *et al*., 2003). Due to its scarcity, C24:1 draws a high purchase price. Therefore, it is of great significance to find alternative plant sources rich in C24:1. *A. truncatum* is one of a handful of known species that contains C24:1 in seed oil, including Brassicaceae, *Lunaria annua*, *Borago officinalis*, *Cannabis sativa*, *Tropaeolum speciosum*, *Cardamine graeca*, *Malania oleifera*, and *X. sorbifolia* (Katavic *et al*., 2010; Chen *et al*., 2017; Yu *et al*., 2017). Therefore, the C24:1 content is an important indicator of ATO value. The content ranged from 3.90% to 7.85%, with a mean value of 5.76% in this study, which is much higher than the content of the current source of medicinal nervonic acid, *X. sorbifolia* (1.52%–3.04%) (Yu *et al*., 2017). And the yield per plant is rich (about 30 kg of fruit after 20 years). So, *A. truncatum* seeds will become a promising raw material to extract nervonic acid.

Variability is the result of different environmental selection forces that are the root cause of phenotypical differentiation among populations. In this study, altitude, temperature, and precipitation are the main environmental factors causing variation. The trend is the same in the study of Dewhurst and King (1998), and Darwish (2014), demonstrating higher oil yields in plants collected from relatively higher altitudes. However, the oleic acid content differed from that of previous studies in that it is dependent on mean temperature and can decrease by up to 2% with each degree (ºC) of increase in temperature (Rondanini *et al*., 2011). However, no significant relationship between mean temperature and oleic acid content in oils was found in this study, but significant negative correlations were observed between annual average temperature and both PUFA (-0.584) and linoleic acid (-0.601). It has been demonstrated that low temperatures increase the PUFA content of plants to maintain the fluidity of biological membranes (Falcone *et al*., 2004); in a high temperature environment, the increase in SFA could be explained by lipid peroxidation (Arbaoui and Link, 2007). That also explains the observation that palmitic acid exhibits a significantly positive correlation with annual average temperature (0.666).

Laribi *et al*., (2009) and Rebey *et al*., (2011) observed water stress effects on lipid metabolism. In our study, precipitation exhibited a significant negative correlation with C18:2 (-0.790) and a significant positive correlation with C24:1 (0.592). In addition, researchers have noted that an increase in altitude has tended to promote greater UFA content (with subsequently lower SFA content), possibly because altitude increases lead to lower temperatures and higher exposure to light (Dewhurst and King, 1998; Lai *et al*., 2010). However, this relationship is in contrast to the results of this study, which coincide with *Vicia sativa* by Mao *et al*., (2012) who found that other environmental factors besides temperature and light intensity, such as soil and air, may also influence the lipid metabolism of plants. This will be the direction of our future work.

In addition to environmental factors, genetics need to be considered. Rahimmalek *et al*., (2017) suggested that the thymol content in Iranian Ajowan (*Trachyspermum ammi*) can be affected by both genetic and environmental factors. It is necessary to consider the differences within and among populations when screening promising germplasm resources for oil use, since the biosynthesis of fatty acids can be regulated by both genetic and environmental factors simultaneously. Thus, further research should be conducted to explore the effects of environment and genotype in order to guide in the selection of genotypes which exhibit high oil yield and useful fatty acids.

Results of HCA and PCA showed that within a certain geographic area, populations with similar physiological characteristics can be clustered into a class which is the same as the result of Rahimmalek *et al*., (2017) on Iranian Ajowan. Within a class, it was observed that the fatty acid composition of ATO assumed variation patterns which are characteristic of geographic isolation. Fatty acid profiles could be roughly correlated with growth in three major geographic areas of China: (I) Eastern central region (TA, LF, NX, JY, and NJ, with high MUFA especially C18:1); (II) Southwest region (FZ and ABZ, with high SFA); (III) Northeast and northwest regions (DQTL, CF, CC, PQ, YJ, HY, and YS, with high UFA, especially PUFA and C18:2). These findings also indicate that it is feasible to purchase seeds in the region which could guide future development of ATO for industrial production.

5. CONCLUSIONS

A. truncatum is a woody oil tree species with high seed oil content (28.57%), high unsaturated fatty acid content (91.47%), and high nervonic acid content (5.76%) ompared to grain and other seed oil sources. Notably, the data presented in this study show patterns of variability in oil content and fatty acid composition which correlate with geographic location: northern high altitude populations exhibited higher oil content; populations in the eastern central region exhibited high levels of MUFA (especially C18:1); populations from the southwest

region were rich in SFA; populations from the northeast and northwest regions had high UFA content (especially PUFA, and C18:2).

Seed oil content and fatty acid composition were found to be influenced by ecological factors, especially altitude, temperature, and precipitation. The results suggest that both fat production and quality of ATO may be improved by planting trees in moderately cold areas. Consequently, further studies will incorporate such results to guide in the selection of excellent individual trees which have high specificity (such as high oil, high C24:1) to improve oil yield and quality. The populations of DQTL and YS were chosen as germplasm resources with high oil content, while JY, NX, and PQ were regarded as excellent germplasm resources with high C24:1. This work should lay the groundwork for improving oil yield and quality and should ensure ATO's future success as an important industrial crop.

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