January 2019. Horticultural Plant Journal, 5 (1): 24–30.

Horticultural Plant Journal

Available online at www.sciencedirect.com
The journal's homepage: http://www.keaipublishing.com/en/journals/horticultural-plant-journal

Oil Content and Nervonic Acid Content of *Acer truncatum* Seeds from 14 Regions in China

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Received 1 July 2018; Received in revised form 30 August 2018; Accepted 28 October 2018

Available online 8 November 2018

A B S T R A C T

Acer truncatum is considered a promising species as a raw material to produce nervonic acid (NA). The goal of this study was to explore the variation of oil content and nervonic acid content of 138 accessions native to 14 regions in China as well as provide guidance for establishing plantations and improving the production of nervonic acid. A large range of variation was found in oil content, nervonic acid per gram of oil, and nervonic acid per gram of seed (17.81%–36.56%, 3.90%–7.85%, and 0.84%–2.31%, respectively). Accessions YS-6, ABZ-6 and DQTL-8 were found as the most promising potential oil sources; PQ-2 was considered the optimal germplasm, and JY-6, NJ-8, LF-6, and CC-8 were found as promising potential sources for producing NA. A very significant difference ($P < 0.01$) among 14 regions was observed. DQTL and YS regions are considered the optimal farming regions for crops with high oil content, while the CC and PQ regions are regarded as the most suitable regions for nervonic acid production. A geographical trend from South to North was observed in which amount of seed oil increased but no such trend in nervonic acid content was observed. The data collected in the study on oil content and nervonic acid in accessions of *A. truncatum* in various regions can be utilized for establishing plantations of promising genotypes through clonal means.

Keywords: *Acer truncatum*; Seed oil content; Nervonic acid; Variation

1. Introduction

Nervonic acid (NA, *cis*-tetracos-15-enoic acid; 24:1 Δ15) is the core component of neural cells of the brain and neural tissue, which benefits brain health through improving the biosynthesis and maintenance of nerve cell myelin (Sargent et al., 1994) and also enhances neurodevelopment in premature infants (Farquharson et al., 1996). Nervonic acid can repair the damaged brain nerve pathways and promote the regeneration of nerve cells, which can be effective in the treatment of schizophrenia, psychosis, peroxisomal disorders, diabetes, alcoholism and other conditions (Akoh and Moussata, 2001; Bettger et al., 2001; Evans et al., 2003). However, NA is difficult to synthesize through the body itself; external intake is required in order to supplement. Therefore, efficient production of NA from raw materials has become an important objective for research. Previous studies have reported that several plants (primarily from the Brassicaceae family) contain NA in their seed oil, such as *Lunaria an-*

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Peer review under responsibility of Chinese Society for Horticultural Science (CSHS) and Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS)

https://doi.org/10.1016/j.hpj.2018.11.001

Table 1 ***Acer truncatum* populations with the respective codes and collection site characteristics**

Code	Number of accessions	Collection site	Latitude (°N)	Longitude (°E)	Altitude/m	Annual average temperature/ °C	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

nua, *Brassica rapa*, *Camelina sativa*, and *Cardamine graeca*. Additional plants include *Acer truncatum*, *Borago officinalis*, *Tropaeolum speciosum*, *Malania oleifera*, and *Xanthoceras sorbifolium* (Katavic et al., 2010; Wang, 2013; Huai et al., 2015; Hu et al., 2017a). To date, only L. *annua* has been considered as a niche crop for future development (Chen et al., 2017) due to the limited availability of the other plants, which cannot satisfy the market demand for nervonic acid-contained oil. Therefore, it is necessary to explore other resources containing NA to meet the market demand.

There are several kinds of plants containing NA in the Acer genus, such as *A. ginnala, A. palmatum, A. saccharum,* and *A. truncatum* (Wang, 2013; Chen et al., 2017). Among them, *A. truncatum* is considered the most promising species as a raw material to produce NA, based on the distribution, oil content and NA content. It is a deciduous tree species native to Northern and Western China. Currently it is mainly distributed in the Yellow River basin, Northeast, Inner Mongolia, Jiangsu, Sichuan and other regions. This species of maple has been widely chosen as a landscape tree for its brilliant autumn leaf color (Zhao et al., 2007; Li et al., 2015). Hu et al. (2017a) have suggested that maple seed oil would be an excellent edible oil because it contains a large percentage of unsaturated fatty acids (92%) and NA (6.22%). Consequently, maple seed oil was approved as a new food resource by the Chinese Ministry of Health in 2011. Additionally, it is also a renewable energy tree species due to its biomass which produces approximately 30 kg of fruit per tree after 20 years (Wang, 2013). It is also feasible to extract NA from *A. truncatum* seeds. The current research on *A. truncatum* seed oil is mainly focused on the method of extraction, its ability to be eaten, health and pharmacological properties (Liu et al., 2013; Wang, 2013; Bi et al., 2016; Hu et al., 2017a, 2017b). The variation and differences between accessions and regions in seed oil content and NA content have not been studied.

The goal of this study was to explore the variation of seed oil content and NA content in *A. truncatum* between 138 accessions native to 14 regions. Additional goals were providing guidance for establishing plantations and for oil companies in purchasing seeds. Establishing a theoretical basis for extracting NA from *A. truncatum* seed oil, and improving the yield and quality of nervonic acid-contained oil were other objectives of this study, in order to ultimately stimulate further development of the *A. truncatum* seed oil industry.

2. Materials and methods

2.1. Plant materials

One hundred and thirty-eight accessions of *A. truncatum* Bunge grown naturally in fourteen regions were collected from nine provinces of China in October and November 2016 (Table 1). Approximately ten individual disease-free, insect pest-free plants (20 years of age or older) were selected from each sample collection area. In order to minimize other factors influencing seed development, individual trees were chosen with spacing to other trees of at least 50 m. For each accession, about 1–2 kg of fully mature samaras were picked randomly from numerous positions on each tree to ensure the representative samples. Samaras were stored at room temperature, the seed was removed from its seed coat for analysis of oil content and fatty acid component.

2.2. Oil content and fatty acid composition analysis

Seed oil content of the 138 accessions was estimated using a pulsed nuclear magnetic resonance instrument (Bruker minispec mq20 NMR, Bruker, Germany). Specific methods used here, following official standard methods (ISO 5511:1992, GB/T 15,690-1995).

Oil extraction was performed using a Soxhlet apparatus with ~5 g of ground seeds and *n*-hexane solvent following methods by Hu et al. (2017a). The pure seed oil was transferred into a small glass vial, flushed with nitrogen, and maintained at –20 °C until further analysis. Seed oil was methylated twice, the first step is pre-esterification with H_2SO_4–CH_3OH to reduce the acid value to below 1 mg KOH•g^{-1}, the second step is trans-esterification with KOH–CH_3OH according to the published method (ISO 5509:2000, GB/T 17,376–2008). The fatty acid methyl esters (FAMEs) profiles obtained for each accession were determined using an Agilent 7890A (Agilent, Palo Alto, CA, USA) gas chromatography (GC) equipped with a flame ionization detector (FID) using 17:0 FAME as an internal standard. The DB-23 capillary column (length 30 m, inner diameter 0.32 mm, film thickness 0.25 μm) was used in this detection. The injector and detector temperatures were 230 °C and 280 °C, respectively. Oven temperature was held at 180 °C for 5 min, with a rise of 3 °C•min^{-1} to 230 °C. The carrier gas (helium) was delivered using a flow rate of 1.0 mL•min^{-1} and 1 μL samples were injected manually using a split injection mode. Fatty acid methyl ester peaks were identified by comparing their retention time with those of known standards run under the same conditions.

2.3. *Statistical analysis*

The data were subjected to nested variance analysis using software SAS 9.0. The phenotypic differentiation coefficients among populations (Vst) were calculated from equation (Ge et al., 1998): $V_{st} = (\sigma^2{}_{t/s})/(\sigma^2{}_{t/s} + \sigma^2{}_s) \times 100\%$, where $\sigma^2{}_{t/s}$ is the variance component among populations and $\sigma^2{}_s$ is the variance component within the population.

NA content per oil was determined by gas chromatography. NA content per seed was multiplied by oil content and NA content per oil.

Other statistical analysis was performed using SPSS 22.0 software (IBM) and Excel 2010. The average for all plants in a sample collection location was used as the value for that region. Determinations were run in duplicate and the data were reported as the mean.

3. Results

3.1. *Oil content*

Oil yield is predictive of whether a seed is suitable for large-scale production and its adaptability to multiple industrial uses. Consequently, crops that contain high oil content at harvest ultimately reduce final production costs. The oil content of *A. truncatum* seeds ranged from 17.81% to 36.56%, with a mean value of 28.57% for all 138 accessions studied (Fig. 1). The vast majority of the accessions (76.81%) exhibited oil content ranging from 25% to 35% while only 3 accessions (2.17%) exhibited higher oil content (> 35%). The three accessions with the highest oil content, included YS-6 (36.56%), ABZ-6 (35.71%), and DQTL-8 (35.44%), which will likely be valuable for development of new varieties with high oil content.

Oil content varied significantly ($P < 0.01$) among the 14 analyzed regions (Table 2). The region with the highest seed oil content was DQTL (32.47%), followed by YS (32.09%), with the lowest content observed in NJ (24.06%). Based on these results,

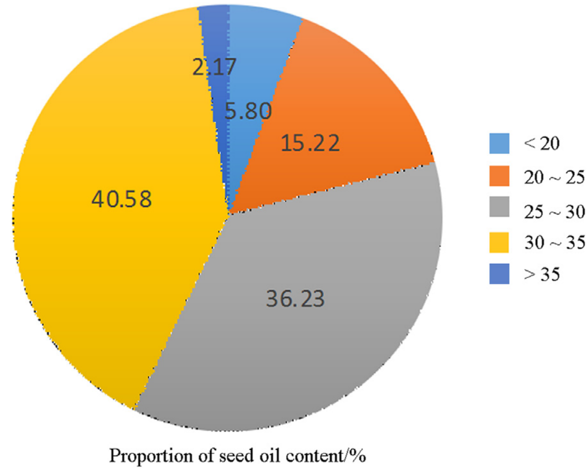

Fig. 1 Frequency distribution of 138 germplasm accessions of *Acer truncatum* for seed oil content

DQTL and YS were chosen as optimal farming regions to establish plantations. In addition, the phenotypic differentiation coefficient among populations for oil content was 36.66%. This result indicates that most differences are observed among individuals within populations. Therefore, selection within a population is necessary when screening germplasm resources for high oil content.

3.2. *Nervonic acid content*

NA content was regarded as one of the evaluation criteria of the quality of *A. truncatum* seed oil. The NA content per gram of oil ranged from 3.90% to 7.85%, with a mean value of 5.76% for all 138 accessions studied. Exactly 42.03% of the accessions exhibited oil content ranging from 5% to 6%, while 39.13% exhibited higher NA content in the 6%–7% range. More than 7% NA content was recorded in 2.17% of the accessions (Fig. 2). In this study, the three accessions with highest NA content were found to be PQ-2 (7.85%), JY-6 (7.16%), and NJ-8 (7.05%); these accessions will likely be valuable for development of new varieties with high NA content. NA content per gram of oil varied significantly ($P < 0.01$)

Table 2 Variability in oil content and nervonic acid content for *Acer truncatum* grown in 14 regions

Code	Oil content/%	NA content•g^{-1} oil/%	NA content•g^{-1} seed/%
DQTL	32.47 ± 2.39 A	4.97 ± 0.63 E	1.61 ± 0.24 ABC
CF	28.97 ± 2.19 ABCD	5.47 ± 0.44 CDE	1.58 ± 0.13 ABC
CC	30.02 ± 2.69 ABC	6.23 ± 0.55 ABC	1.87 ± 0.21 A
PQ	30.38 ± 2.28 ABC	6.29 ± 0.67 AB	1.91 ± 0.20 A
TA	26.71 ± 3.35 CDE	5.85 ± 0.66 ABCD	1.56 ± 0.21 ABC
LF	29.03 ± 4.82 ABCD	6.03 ± 0.43 ABC	1.76 ± 0.36 AB
YJ	25.38 ± 5.04 DE	5.54 ± 0.59 BCDE	1.42 ± 0.36 BCE
HY	30.37 ± 2.86 ABC	5.82 ± 0.45 ABCD	1.77 ± 0.23 AB
YS	32.09 ± 2.00 A	5.52 ± 0.69 BCDE	1.77 ± 0.23 AB
FZ	27.36 ± 1.95 BCDE	4.91 ± 0.57 E	1.35 ± 0.22 C
ABZ	31.70 ± 2.79 AB	5.24 ± 0.66 CDE	1.66 ± 0.23 ABC
NX	25.39 ± 3.72 DE	6.18 ± 0.42 ABC	1.56 ± 0.23 ABC
JY	25.09 ± 5.61 DE	6.54 ± 0.35 A	1.65 ± 0.43 ABC
NJ	24.06 ± 2.83 E	6.06 ± 0.72 ABC	1.45 ± 0.21 BCE
Mean	28.57	5.76	1.64
Phenotypic differentiation coefficient V_{ST}/%	36.66	40.63	38.55

Note: Different capital letters in the same column show significant difference ($P < 0.01$). Abbreviations are the same as in Table 1.

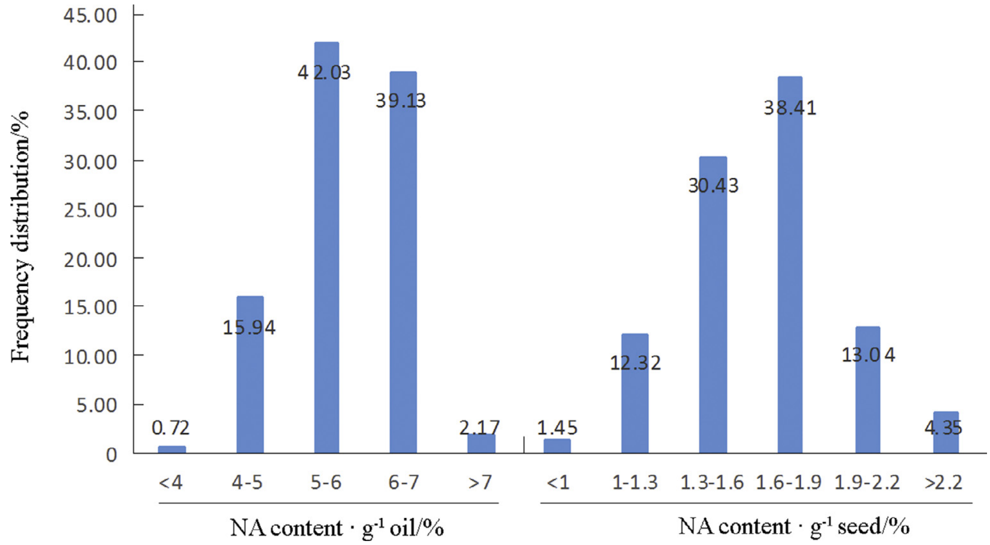

Fig. 2 Frequency distribution of 138 germplasm accessions of *Acer truncatum* for NA content per gram of oil and NA content per gram of seed

Table 3 Correlation of nervonic acid content and oil content with primary fatty acid composition

Trait	Palmitic	Stearic	Oleic	Linoleic	Linolenic	*cis*-11-eicosenoic	Erucic	Nervonic
NA content•g^{-1} oil	0.012	–0.475**	–0.203*	–0.275**	0.103	–0.336**	0.584**	1
Oil content	–0.382**	0.254**	–0.195*	0.340**	0.163	–0.003	–0.111	–0.165

*Significant at $P < 0.05$.
**Significant at $P < 0.01$.

among 14 regions (Table 2). The highest content was discovered in the JY (6.54%) and PQ (6.29%) regions, which are likely to be the most advantageous regions for producing NA.

In addition to NA content per gram of oil, NA content per gram of seed should be considered when determining the industrial production suitability of NA. The NA content per gram of seed ranged from 0.84%–2.31%, with a mean value of 1.64% for all 138 accessions. The vast majority of the accessions (68.84%) have exhibited NA content per gram of seed ranging from 1.3% to 1.9% while 13.04% exhibited higher content in the range of 1.9%–2.2%. More than 2.2% NA content was recorded in 4.35% of the accessions (Fig. 2), in which LF-6 (2.31%), PQ-2 (2.30%), and CC-8 (2.29%) were considered potential germplasm for producing NA. Considering two indicators, NA content per gram of oil and per gram of seed, PQ-2 was regarded as the optimal germplasm for producing NA. Additionally JY-6, NJ-8, LF-6 and CC-8 were also found to have potential for industrial development.

NA content per gram of seed also showed very significant differences ($P < 0.01$) among the 14 regions (Table 2) in which the PQ (1.91%) and CC (1.87%) regions had a high NA content; this is in agreement with the previous analysis. Additionally, the phenotypic differentiation coefficients among populations for NA content per gram of oil and per gram of seed were 40.63% and 38.55%, respectively (Table 2). These results indicate that most differences are observed among individuals within populations. Therefore, selection within a population should be emphasized when screening germplasm resources for NA content.

3.3. Correlation relationships among oil content, NA content and primary fatty acid composition

In this study, a total of 14 fatty acid components were detected in *A. truncatum* (Fig. 3). The fatty acid composition results were similar to Liu et al. (2013) and Hu et al. (2017a). The primary unsaturated fatty acids detected included linoleic (C18:2, 32.97%), oleic (C18:1, 25.19%), erucic (C22:1, 16.49%), eicosenoic (C20:1, 7.90%), nervonic (C24:1, 5.76%), and linolenic (C18:3, 2.76%) acids. Primary saturated fatty acids detected included palmitic (C16:0, 4.69%) and stearic (C18:0, 2.30%) acids.

Correlation studies exhibited a significant negative correlation of oil content with percentage of palmitic ($P < 0.01$, –0.382) and oleic acid ($P < 0.05$, –0.195), while stearic ($P < 0.01$, 0.254) and linoleic acid ($P < 0.01$, 0.340) showed significant positive correlations with oil content. This demonstrates that germplasms with high oil content accumulate crude fat mainly through transformation of oleic acid to linoleic acid via desaturation, combined with the relative content of each fatty acid. It also indicated that the higher the seed oil content, the higher the unsaturated fatty acid content, and also the higher the amount of edible oil.

A significant negative correlation was found between NA and palmitic ($P < 0.01$, –0.475), oleic ($P < 0.05$, –0.203), linoleic ($P < 0.01$, –0.275), and peanutoic acid ($P < 0.01$, –0.336), and only significant positive correlation with erucic acid ($P < 0.01$, 0.584). The correlation study between oil content and fatty acid composition have a certain guidance for screening the ideal germplasm resources for high-yield or high-quality seed oil.

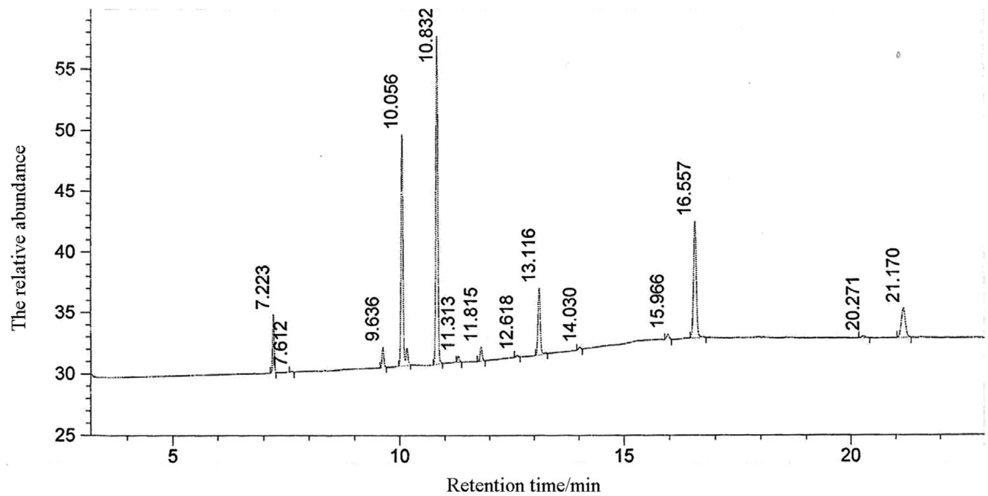

Fig. 3 Total ion chromatograms of fatty acid methyl ester (PQ-2)

7.223: palmitic acid methyl ester (C16:0), 7.612: Palmitoleic (C16:1), 9.636: stearic (C18:0), 10.056: oleic (C18:1), 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).

Table 4 Correlation coefficients of oil and NA content and geographical and ecological factors

Trait	Latitude (°N)	Longitude (°E)	Altitude/m	Annual temperature/ °C	Annual rainfall/mm	Frost-free season/d
Oil content	0.503	0.017	0.469	–0.629*	–0.749**	–0.562*
NA content•g^{-1} oil	–0.075	0.305	–0.422	0.132	0.592*	0.214
NA content•g^{-1} seed	0.427	0.266	0.095	–0.518	–0.247	–0.380

*Significant at $P < 0.05$.
**Significant at $P < 0.01$.

3.4. *Geographical variation*

Oil content showed significant negative correlations with annual temperature ($P < 0.05$, –0.629), annual rainfall ($P < 0.01$, –0.749), and the frost-free season period ($P < 0.05$, –0.562); these correlations are consistent with the characteristics of cold, arid areas (Table 4). Contrastingly, oil content demonstrated a positive correlation with latitude (0.503) and altitude (0.469). These data indicated that environment had a specific effect on the accumulation of fat in *A. truncatum* and formed a corresponding trend of geographical variation. In accordance with Chinese geography, planting in the Northern region may be conducive to improving the seed oil content of *A. truncatum*, especially in the Northeast, and Northwest higher altitude areas.

NA content per gram of oil was positively correlated only with precipitation ($P < 0.05$, 0.592), indicating that appropriate moisture is conducive to increasing NA content during the fat accumulation period. However, NA content per gram of seed had no significant relationship with geography or ecological factors. This suggested the difference in NA content is largely due to genetic factors. This also emphasized the importance of screening for quality accessions with high NA in order to optimize NA production.

4. Discussion

Oil yield is the most important quality in determining whether the target plant can be used as an industrial crop or produced for commercial cooking oil. Crops with high oil content are beneficial for production because it reduces the cost of production (Kumar and Sharma, 2011). The mean oil content determined in this study (28.57%) greatly exceeded values for *Glycine* max (17%), *Olea europaea* (20%), and for most values for *Sapium sebiferum* (12%–29%) (Karmakar et al., 2010; USDA, 2012). It was also comparable to values for *Jatropha curcas* (20.05%–38.33%) (Kaushik and Bhardwaj, 2013) and wild manihot (17%–31%) (Alvesa et al., 2014). This indicates that it is feasible to extract oil from the seed of *A. truncatum* for industrial purposes.

The value of *A. truncatum* seed oil in this study was determined by its NA content. The mean NA content value (5.76%) is higher than reported by Liu et al. (2013) (5.52%), but lower than Hu et al. (2017a) (6.22%). Compared with other reported plants that contain NA, the mean value obtained in this study is lower than *M. oleifera* (55.70%) (Tang et al., 2013), L. *annua* (20%) (Mastebroek and Marvin, 2000) and *C. sativa* (11.8%) (Huai et al., 2015); however, it is higher than *B. rapa* (2.4%) (Wang, 2013) and *X. sorbifolium* (2.05%) (Yu et al., 2017). *A. truncatum* is a common plant in China that does not require arable land and still has a high oil yield, which makes it favorable for the production of NA by extracting *A. truncatum* seed oil.

Related research (Das et al., 2002; Jadhav et al., 2005) demonstrates that oleic acid is considered to be the precursor of unsaturated fatty acids. This transformation can occur via two pathways: one pathway produces linoleic and linolenic acid when the microsomal oleate desaturase encoded by the FAD2 and FAD3 gene is expressed, while the other produces *cis*-11-eicosenoicacid, erucic acid and nervonic acid by extending the carbon chain under the expression of the FAE gene. In order to obtain a high amount of NA, it is possible to inhibit expression of the FAD2 and FAD3 genes and promote the expression of the FAE gene. Such a strategy has been successfully used in *Brassica carinata* (Katavic et al., 2010). Similar studies should also be carried out in *A. truncatum*. However, there is a significant positive correlation between NA and erucic acid, which indicates that the increase in NA content is accompanied by an increase in erucic acid. Earlier studies (Gopalan et al., 1974) have shown that diets rich in erucic acid were associated with myocardium fibrotic and increased blood cholesterol levels and are therefore undesirable for human consumption. The United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) stipulate that the amount of erucic acid should be limited to less than 5% by weight. Therefore, reduction of the content of erucic acid in *A. truncatum* seed oil is the next objective.

Variations within and among populations reflect the complexity of a system consisting of a given gene and environment interaction, as well as the wide degree of adaptation possible in response to the environment. In this study, altitude, temperature, and precipitation are the main environmental factors causing variation. The trend is the same with the studies by Dewhurst and King (1998) and Darwish (2014), demonstrating higher oil yields in plants collected from relatively higher altitudes. Similarly, Laribi et al. (2009) and Rebey et al. (2011) found that precipitation exhibited a significant positive correlation with nervonic acid (0.592). In addition to environmental factors, genetics need to be considered. The phenotypic differentiation coefficient among populations of oil content, NA content per gram of oil and NA content per gram of seed were lower than 50% in this study. Therefore, it is necessary to consider the differences within and among populations when screening potential germplasm resources for oil use, since the biosynthesis of fatty acids can be regulated by both genetic and environmental factors simultaneously. Rahimmalek et al. (2017) also suggested that the thymol content in Iranian Ajowan (*Trachyspermum ammi*) can be affected by both genetic and environmental factors. Thus, further research should be conducted to explore the effects of environment and genotypes in order to execute selection of genotypes exhibiting high oil yield and useful fatty acids.

5. Conclusion

Oil content and nervonic acid content are two essential criteria when screening for high quality germplasm resources. The oil content of *A. truncatum* seeds ranged from 17.81% to 36.56%, with a mean value of 28.57% for all 138 accessions studied. Accessions YS-6, ABZ-6 and DQTL-8 were found as the most promising and potential oil sources. The NA content per gram of oil ranged from 3.90% to 7.85%, with a mean value of 5.76%, while the NA content per gram of seed ranged from 0.84% to 2.31%, with a mean value of 1.64% for all 138 accessions studied. PQ-2 was considered the optimal germplasm, and JY-6, NJ-8, LF-6 and CC-8 were found as most promising and potential sources for producing NA according to two specific indicators.

Oil content, NA content per gram of oil, and NA content per gram of seed varied significantly ($P < 0.01$) among 14 regions. A geographical trend from South to North was observed in which seed oil content increased; no such trend in nervonic acid content was observed. This observation is significant for indicating locations for potential plantations and improving the production of nervonic acid. In this study, the DQTL and YS regions are considered the optimal farming regions to increase *A. truncatum* seed oil production while CC and PQ were regarded as the best region to source NA. Utilizing *A. truncatum* as a raw material for extracting nervonic acid coupled with high-yield cultivation techniques, can conceivably fulfill the market demand for nervonic acid.

Acknowledgments

This work was supported by Central Fiscal Forestry Science and Technology Extension Demonstration Fund Project (No. 2016 LZGC014), a Major Agricultural Project of Shandong Province (No. [2012] 213 and [2014] 96) and Shandong “Double Tops” Program. We are grateful to the Institute of Oil Crops of the Chinese Academy of Agricultural Sciences for providing technical support in the test.

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