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Variability of seed oil content and fatty acid composition in Shantung maple (*Acer truncatum* Bunge) germplasm for optimal biodiesel production

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Shantung maple seed oil methyl esters have emerged as the potential feedstock for producing biodiesel. The goal of this work was to assess variations in seed oil content and fatty acid compositions for optimal biodiesel production among 138 Shantung maple accessions native to 14 regions of China. Dramatic differences in seed oil content were observed among trees grown in the various regions tested; seeds of trees grown in Daiqintala, Inner Mongolia (DQTL) and Yongshou, Shaanxi (YS) exhibited the highest oil content (32.47 and 32.09%, respectively). Among the 138 germplasm accessions, seed oil content ranged from 17.81 to 36.56%, with a mean value of 28.57%. Of a total of 15 fatty acid components detected overall, oleic acid and linoleic acid comprised the highest proportions of fatty acids (20 to 34.31% and 27.08 to 36.71%, respectively). Correlation analysis revealed the highest positive correlation between oleic acid and cis-11-eicosenoic acid (0.698) and the highest negative correlation between oleic acid and linoleic acid (-0.766). Ranges of saponification number (180.26 to 182.86), iodine value (101.84 to 113.70 g I₂/100 g), cetane number (50.77 to 53.53), density (873.03 to 880.08 kg/m³) and kinematic viscosity (4.92 to 5.28 mm²/s) confirmed that Shantung maple methyl esters are suitable for biodiesel production, and correlation analysis showed that the accession with high monounsaturated fatty acid content was suitable as optimal germplasm resources for biodiesel production. DQTL, YS and Taian, Shandong (TA) regions was considered the best plantation, and DQTL-1, DQTL-6, DQTL-8, YS-6, and TA-10 germplasm accessions generated oil with optimal properties for biodiesel production. These results could guide future development of Shantung maple seed oil for improved biodiesel production.

Key words: *Acer truncatum* Bunge, biodiesel properties, fatty acid composition, oil content, variation.

INTRODUCTION

Shantung maple (*Acer truncatum* Bunge), a deciduous tree of northern China belonging to the *Aceraceae* family, is a popular landscape plant due to its brilliant autumn leaf color and a kind of afforestation tree species (Li et

al., 2015; Zhao et al., 2007). It is also called the ingot maple because of the samaras like the gold ingot of Chinese. Its sturdy wood texture promotes its use for timber, while its high seed yield and high protein,

chlorogenic acid, tannin and flavonoids content (Ma et al., 2005; Honma et al., 2010) make it an attractive source of protein, medicinal and chemical raw materials. The main chemical ingredients of Shantung maple seeds are lipids (47.88%), protein (27.15%), and minor amounts of sucrose (6.10%) and cellulose (3.68%) (Wang, 2013). Moreover its seed oil has long been used as a food source in northeastern China, but has not yet been used on a large scale in the market. Previous studies (Hu et al., 2017) demonstrated that *A. truncatum* seed oil (ATO) contains 92% unsaturated fatty acids, including 6.22% nervonic acid. This is noteworthy because nervonic acid is an uncommon fatty acid used to treat various neurological disorders, including schizophrenia (Akoh and Moussata 2001) and psychosis (Evans et al., 2003). It has a high vitamin E content of 125 mg/100 g (Wang and Wang, 2011). Currently, the Shantung maple is receiving much attention from Chinese researchers because of its huge potential as an energy source and other uses. Its fruit is currently used to generate renewable biomass energy, yielding 30 kg of fruit per tree after 20 years (Wang, 2013). Meanwhile, ATO has been approved as a new food resource by the Chinese Ministry of Health in 2011. Due to its numerous emerging uses, the farmed Shantung maple cultivation area now encompasses 4×10^4 ha and is rapidly expanding, with continued rapid growth anticipated. Currently, it has been included in the national development plan to use Shantung maple seeds to develop new resources for food and biomass energy.

As mankind's current rapid economic developmental pace, energy shortages and environmental pollution challenges have become more and more apparent worldwide. Regarding energy production, seed oil fatty acid methyl esters (FAMEs) have already served as a suitable diesel engine fuel source (Harrington, 1986; Azam et al., 2005; Karmakar et al., 2010). Woody oil tree species do not occupy arable land and do not compete with the food sector, hence their name biomass energy tree species. Meanwhile, green diesel fuels produced from woody plants, such as *Prunus scoparia* (Sorkheh et al., 2016), *Jatropha curcas* (Sinha et al., 2015), *Pongamia pinnata* (Mukta et al., 2009), *Sapindus saponaria* (Lovato et al., 2014) and *Xanthoceras sorbifolium* (Yu et al., 2017), are now receiving increasing attention as environmentally friendly, non-toxic and biodegradable petroleum diesel fuel substitutes. Concurrently, higher oil content has been shown to improve biodiesel yield and economic feasibility. This knowledge has spurred initial evaluation of new higher oil-containing woody tree seeds, including ATO, as

biodiesel sources. In fact, ATO has been found as a suitable source of fatty acids for potential biodiesel used by extensive studies in China, and the oil conversion rate can reach more than 99% (Wei et al., 2008; Liu and Wang, 2009). Now, some potential biofuel crops have been launched in the variation of oil content and fatty acid composition to screen for optimal germplasm, such as *J. curcas* (Kaushik and Bhardwaj, 2013), *P. pinnata* (Mukta et al., 2009), *X. sorbifolium* (Yu et al., 2017) and others. However, there is almost no study on the variation of biodiesel characteristics of Shantung maple seed oil. Most researchers were concerned with the ecological characteristics (Song et al., 2016), oil extraction method (Hu et al., 2017), and the effect of reaction conditions and catalyst types on biodiesel properties (Wei et al., 2008; Liu and Wang, 2009). Therefore, it is vitally important to screen the germplasm based on its oil content and fatty acid composition to obtain high-quality biodiesel for the future (Sinha et al., 2015; Yu et al., 2017).

The aim of this work was to evaluate the biodiesel properties of *A. truncatum* seed oil fatty acid methyl esters by analyzing the variability of oil content and fatty acid composition of various germplasm accessions grown in various regions of China and also screen the optimal germplasm for breeding.

MATERIALS AND METHODS

Plant material

One hundred and thirty-eight accessions of Shantung maple that grow naturally in fourteen regions were collected from nine provinces of China during the months of October and November, 2016 (Table 1 and Figure 1). Approximately ten individual disease-free, insect pest-free adult plants (each 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 ~1 to 2 kg of fully matured samaras were picked randomly from numerous positions on each tree to ensure the samples were representative of each entire plant. Samaras were stored at room temperature and each was divided into two parts; the seed of one part was removed from its seed coat for analysis of oil content and fatty acid components, while the other part was used for planting. By using GPS to record latitude, longitude and elevation, meteorological factors were listed using local meteorological department data (Table 1).

Oil content and fatty acid analysis

Seed oil content of each of the 138 accessions was estimated using a Bruker minispec mq20 pulsed nuclear magnetic resonance instrument (pulsed NMR). The specific methods used followed official standard methods (ISO 5511:1992, GB/T 15690-1995;

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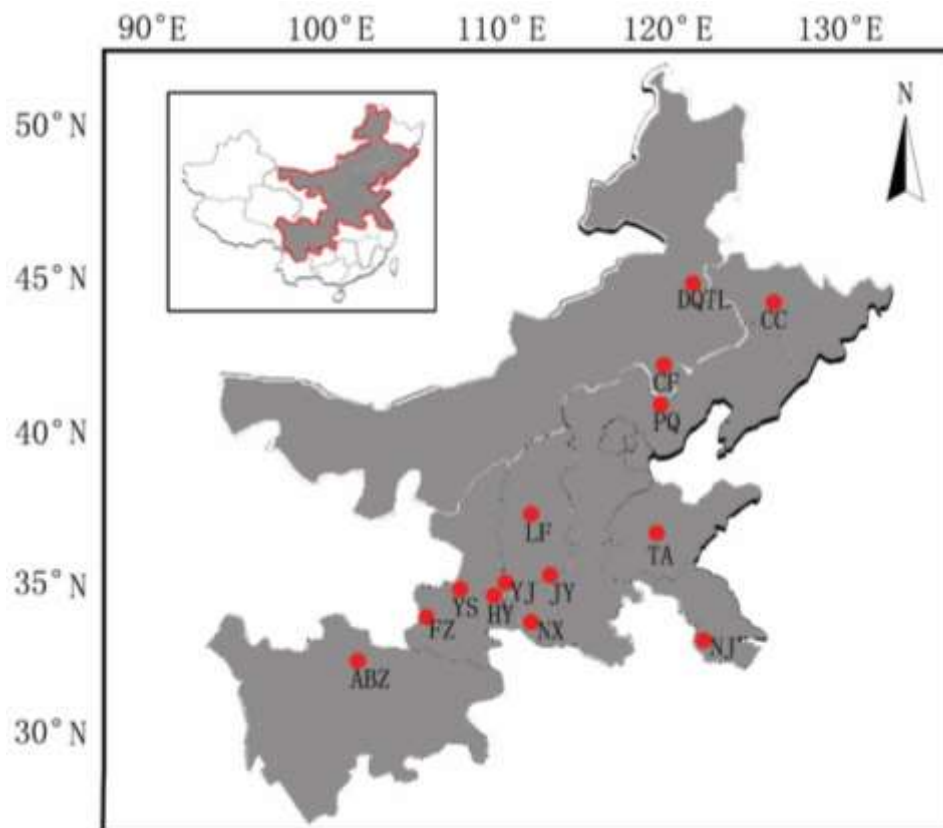


Figure 1. Map of the sampling area of Shantung maple showing sampling area, codes listed in Table 1.

AOAC, 2005). Oil extraction was performed using a Soxhlet apparatus with ~5 g of ground seeds and petroleum ether (60 to 90°C) solvent following published methods (Hu et al., 2017). 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 H₂SO₄-CH₃OH to reduce the acid value to below 1 mg KOH/g, the second step is trans-esterification with KOH-CH₃OH according to the published method (Wei et al., 2008; ISO 12966-2, GB/T 17376-2008). The fatty acid methyl esters (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, inner diameter 0.32 mm, film thickness 0.25 µm) was used in this detection. The injector and detector temperatures were 230 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. Peaks of fatty acid methyl ester peaks were identified by comparing their retention times with those of known standards run under the same conditions. Peak integration was performed using instrument software.

Biodiesel properties

Saponification number (SN) and iodine value (IV) were calculated from the FAME composition profile of the oil using Equations 1 and

(2) (Kalayasiri et al., 1996):

$$SN = \sum \frac{560 \times A_i}{MW_i} \quad (1)$$

$$IV = \sum \frac{254 \times DB \times A_i}{MW_i} \quad (2)$$

where A_i represents the percentage of *i*th FAME, DB is the number of double bonds and MW_i designates the molecular mass of each component. The cetane number (CN) of oil was calculated using Equation 3 (Krisnangkura, 1986):

$$CN = 46.3 + 5458 / SN - 0.225 \times IV \quad (3)$$

The physical properties of FAMES, such as density (D) and kinematic viscosity (ν), were calculated using equation (4) (Ramírez-Verduzco et al., 2012):

$$f_b = \sum_{i=1}^n A_i \cdot f_i \quad (4)$$

where f_b is a function representing physical properties (D or ν) and f_i is a function of the individual *i*th FAME properties (D or ν).

The biodiesel-relevant traits of 138 *A. truncatum* accessions were compared with standard recommended values ASTM D6751

Table 1. *Acer truncatum* collection regions with respective designation 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°7' | 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°7' | 602 | 14.6 | 860.0 | 220 |
| NJ | 8 | Nanjing, Jiangsu | 32°15' | 119°8' | 50 | 15.4 | 1106.0 | 237 |

(2010), EN 14214 (2008) and GB/T 20828 (2015).

Statistical analysis

Analysis of variance (ANOVA) and correlation analysis were performed using SPSS 22.0 software (IBM). Other calculations were conducted using 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

RESULTS AND DISCUSSION

Oil content

Oil content is an important indicator of efficient biodiesel production because initially high oil content ultimately reduces overall production

costs (Kumar and Sharma, 2011). In this study, highly distinct differences in seed oil content were observed among trees grown in the 14 sampling regions studied (Table 2). The highest seed oil content was collected from DQTL (32.47%), followed by YS (32.09%), and with the lowest level of 24.06% recorded in NJ. Therefore, DQTL and YS were chosen for subsequent screening of germplasms with high oil content. Among the 138 germplasm accessions tested, oil content ranged from 17.81 to 36.56%, with a mean value of 28.57% (Table 2). Moreover, oil content levels of most accessions were much higher than for *Glycine max* (17%), *Olea europaea* (20%) and *Sapium sebiferum* (12 to 29%) (USDA, 2012; Karmakar et al., 2010) and were comparable to levels for *Jatropha curcas* (20.05 to 38.33%) (Kaushik and Bhardwaj, 2013) and wild Manihot spp. (17 to 31%) (Alves et al., 2014). In YS-6

(36.56%) and DQTL-8 (35.44%) accessions studied here, oil content levels were greater than 35% (Table 3). These Shantung maple accessions thus hold promise to facilitate future development of lines with higher oil content.

Fatty acid composition

Seed oil quality and utility largely depend on fatty acid composition (Harrington, 1986). Fatty acids exhibit rich variety, exhibiting variable fatty acid composition and content across species (and even across varieties). Therefore, fatty acid composition and content profiles can be used as fingerprints to identify useful biological resources, in addition to their current use for oil authentication (Li et al., 2011). At the species level, a total of 15 distinct fatty acid components

Table 2. Variability in oil content and fatty acid composition for trees grown in 14 regions of Shantung maple.

| Sampling regions | Fatty acid composition (%) | | | | | | | | | | | | | | | Oil content/% | O/L | PUFA/MUFA | C20-24/C16-18 | |
|------------------------|----------------------------|-----------|----------|----------|----------|-----------|-------------|-------------|-----------|-----------|-----------|----------|-----------|-------------|-----------|---------------|-------------|-----------|---------------|-----------|
| | C16:0 | C16:1 | C17:0 | C17:1 | C18:0 | C18:1 | C18:2 | C18:3 | C20:0 | C20:1 | C20:2 | C22:0 | C22:1 | C24:0 | C24:1 | | | | | |
| DQTL | 4.38 | 0.11 | 0.07 | 0.04 | 2.38 | 25.64 | 35.08 | 2.62 | 0.25 | 8.06 | 0.27 | 0.72 | 15.14 | 0.27 | 4.97 | 32.47 | 0.73 | 0.70 | 0.42 | |
| XF | 4.52 | 0.12 | 0.08 | 0.05 | 2.30 | 24.06 | 35.64 | 2.96 | 0.24 | 7.92 | 0.31 | 0.75 | 15.26 | 0.32 | 5.47 | 28.97 | 0.67 | 0.74 | 0.43 | |
| CC | 4.29 | 0.13 | 0.07 | 0.05 | 2.29 | 24.70 | 33.44 | 2.84 | 0.24 | 7.80 | 0.27 | 0.77 | 16.50 | 0.36 | 6.23 | 30.02 | 0.72 | 0.65 | 0.47 | |
| PQ | 4.47 | 0.06 | 0.04 | 0.03 | 2.37 | 22.94 | 34.44 | 2.90 | 0.27 | 7.43 | 0.30 | 0.85 | 17.23 | 0.38 | 6.29 | 30.38 | 0.66 | 0.70 | 0.49 | |
| TA | 4.93 | 0.12 | 0.07 | 0.07 | 2.21 | 27.91 | 31.34 | 2.37 | 0.25 | 8.20 | 0.26 | 0.73 | 15.40 | 0.30 | 5.85 | 26.71 | 0.88 | 0.59 | 0.45 | |
| LF | 4.70 | - | - | - | 2.45 | 26.64 | 30.30 | 2.79 | 0.28 | 8.31 | 0.29 | 0.88 | 16.90 | 0.43 | 6.03 | 29.03 | 0.87 | 0.57 | 0.50 | |
| YJ | 4.51 | - | - | - | 2.20 | 24.72 | 33.41 | 2.87 | 0.27 | 7.83 | 0.29 | 0.91 | 17.04 | 0.41 | 5.54 | 25.38 | 0.73 | 0.66 | 0.48 | |
| HY | 4.57 | - | - | - | 2.15 | 25.63 | 32.76 | 2.40 | 0.28 | 8.11 | 0.29 | 0.83 | 16.77 | 0.38 | 5.82 | 30.37 | 0.77 | 0.63 | 0.48 | |
| YS | 4.54 | - | - | - | 2.26 | 25.34 | 33.19 | 3.12 | 0.28 | 8.07 | 0.28 | 0.82 | 16.26 | 0.34 | 5.52 | 32.09 | 0.76 | 0.66 | 0.46 | |
| FZ | 4.92 | 0.14 | 0.11 | 0.09 | 2.84 | 23.46 | 34.16 | 2.55 | 0.31 | 7.72 | 0.32 | 0.94 | 16.97 | 0.34 | 4.91 | 27.36 | 0.68 | 0.69 | 0.46 | |
| ABZ | 5.06 | 0.17 | 0.08 | 0.02 | 2.69 | 23.30 | 34.94 | 3.10 | 0.29 | 7.34 | 0.31 | 0.86 | 16.27 | 0.31 | 5.24 | 31.70 | 0.66 | 0.73 | 0.44 | |
| NX | 4.90 | 0.16 | 0.06 | 0.06 | 1.59 | 26.32 | 30.61 | 2.87 | 0.26 | 7.75 | 0.24 | 1.01 | 17.50 | 0.50 | 6.18 | 25.39 | 0.85 | 0.58 | 0.50 | |
| JY | 5.14 | 0.14 | 0.06 | 0.01 | 2.30 | 24.93 | 31.41 | 2.98 | 0.28 | 7.92 | 0.31 | 0.86 | 16.72 | 0.41 | 6.54 | 25.09 | 0.79 | 0.62 | 0.49 | |
| NJ | 4.67 | 0.07 | 0.05 | 0.02 | 2.07 | 27.43 | 30.41 | 2.12 | 0.29 | 8.26 | 0.27 | 0.95 | 16.95 | 0.42 | 6.06 | 24.06 | 0.89 | 0.55 | 0.50 | |
| F | 5.302** | 24.268** | 22.226** | 17.784** | 17.244** | 5.342** | 13.814** | 2.983** | 3.006** | 6.382** | 2.194* | 10.634** | 7.220** | 10.180** | 7.740** | 6.703** | 7.346** | 11.725** | 10.681** | |
| Mean | 4.69 | 0.09 | 0.05 | 0.03 | 2.30 | 25.19 | 32.97 | 2.76 | 0.27 | 7.90 | 0.29 | 0.85 | 16.49 | 0.37 | 5.76 | 28.57 | 0.77 | 0.65 | 0.47 | |
| Between the individual | Range | 3.78-6.12 | 0-0.29 | 0-0.20 | 0-0.13 | 1.31-3.30 | 20.00-34.31 | 27.08-36.71 | 1.60-4.35 | 0.19-0.40 | 6.48-9.15 | 0-0.43 | 0.58-1.17 | 13.64-18.86 | 0.18-0.60 | 3.90-7.85 | 17.81-36.56 | 0.55-1.26 | 0.46-0.79 | 0.38-0.55 |
| | CV | 9.12 | 84.38 | 84.78 | 111.50 | 15.15 | 9.58 | 6.95 | 20.58 | 15.50 | 5.59 | 17.87 | 13.53 | 6.70 | 22.02 | 12.62 | 14.72 | 16.29 | 11.54 | 7.16 |

* indicates a significant difference at the 0.05 level; ** indicates an extreme significant difference at the 0.01 level; - indicates that it is not detected.

were detected in this study (Table 2), the results are similar to Hu et al. (2017), and a number similar to that was obtained for *X. sorbifolia* by Yu et al (2017), although their oil content differed from values reported here. Unsaturated fatty acids mainly include oleic acid (C18:1) (25.19%), linoleic acid (C18:2) (32.97%), linolenic acid (C18:3) (2.76%), cis-11-eicosenoic acid (C20:1) (7.90%), erucic acid (C22:1) (16.49%), nervonic acid (C24:1) (5.76%) and small amounts of Palmitoleic acid (C16:1) (0.09%), heptadecenoic

acid (C17:1) (0.03%), and cis-11,14-eicosadienoic acid (C20:2) (0.29%). Saturated fatty acids mainly included palmitic acid (C16:0) (4.69%), stearic acid (C18:0) (2.30%) and small amounts of heptadecanoic acid (C17:0) (0.05%), arachidic acid (C20:0) (0.27%), behenic acid (C22:0) (0.85%), and tetracosanoic acid (C24:0) (0.37%). Indeed, fatty acids with content less than 0.5% exhibited high coefficients of variation ($CV > 15\%$); conversely, the CV values of fatty acids with high oil content (for example oleic acid,

linoleic acid and others) were low ($< 15\%$), indicating that the fatty acid composition of ATO has certain stability. However, individual fatty acids were not present in all Shantung maple accessions and sampling regions. For example, palmitoleic acid, heptadecanoic acid and heptadecenoic acid were not detected in LF, YJ, HY and YS regions.

In this study, apart from cis-11,14-eicosadienoic acid (C20:2), the remaining fatty acids exhibited extremely significant difference ($p < 0.01$) among

Table 3. Statistical values of some accessions mentioned in the text.

| Code | Fatty acid composition (%) | | | | | | | | | | | | | | Oil content (%) | O/L | PUFA/MUFA | C20-24/C16-18 | Fuel properties | | | | | |
|--------|----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----------------|-------|-----------|---------------|-----------------|--------|--------|-------|--------|-------|
| | C16:0 | C16:1 | C17:0 | C17:1 | C18:0 | C18:1 | C18:2 | C18:3 | C20:0 | C20:1 | C20:2 | C22:0 | C22:1 | C24:0 | | | | | C24:1 | SN | IV | CN | D | ν |
| DQTL-1 | 4.58 | 0.11 | 0.08 | 0.04 | 2.50 | 26.64 | 35.40 | 2.07 | 0.26 | 8.06 | 0.26 | 0.73 | 14.26 | 0.25 | 4.76 | 33.82 | 0.75 | 0.70 | 0.40 | 182.48 | 109.58 | 51.55 | 877.73 | 4.98 |
| DQTL-6 | 4.56 | 0.09 | 0.08 | 0.06 | 2.50 | 25.88 | 36.71 | 2.43 | 0.25 | 8.19 | 0.27 | 0.73 | 13.64 | 0.28 | 4.32 | 32.03 | 0.70 | 0.76 | 0.38 | 182.84 | 111.53 | 51.06 | 878.00 | 4.94 |
| DQTL-8 | 4.36 | 0.10 | 0.07 | 0.04 | 2.27 | 25.50 | 34.66 | 2.56 | 0.27 | 8.16 | 0.28 | 0.74 | 15.50 | 0.29 | 5.20 | 35.44 | 0.74 | 0.69 | 0.44 | 181.87 | 109.90 | 51.58 | 877.75 | 5.03 |
| TA-10 | 4.77 | 0.11 | 0.06 | 0.06 | 2.02 | 34.31 | 27.13 | 1.60 | 0.19 | 8.82 | 0.17 | 0.58 | 15.16 | 0.18 | 4.84 | 28.83 | 1.26 | 0.46 | 0.43 | 181.98 | 101.84 | 53.38 | 876.67 | 5.08 |
| YS-6 | 4.61 | - | - | - | 2.07 | 26.48 | 32.33 | 2.96 | 0.29 | 8.26 | 0.26 | 0.88 | 15.65 | 0.38 | 5.82 | 36.56 | 0.82 | 0.63 | 0.46 | 181.43 | 108.20 | 52.04 | 877.51 | 5.09 |
| CF-2 | 3.99 | 0.15 | 0.07 | 0.05 | 2.40 | 21.86 | 36.36 | 3.84 | 0.23 | 8.22 | 0.38 | 0.79 | 15.23 | 0.36 | 6.07 | 29.84 | 0.6 | 0.79 | 0.46 | 181.61 | 113.70 | 50.77 | 878.26 | 5.04 |
| CF-3 | 4.57 | 0.15 | 0.08 | 0.05 | 2.51 | 24.81 | 35.71 | 3.48 | 0.25 | 8.34 | 0.32 | 0.69 | 14.16 | 0.27 | 4.62 | 31.82 | 0.69 | 0.76 | 0.40 | 182.62 | 112.41 | 50.89 | 878.12 | 4.96 |
| CF-9 | 4.61 | 0.12 | 0.10 | 0.04 | 2.24 | 23.36 | 35.57 | 3.95 | 0.25 | 7.63 | 0.32 | 0.76 | 15.19 | 0.33 | 5.54 | 29.87 | 0.66 | 0.77 | 0.43 | 182.02 | 112.92 | 50.88 | 878.17 | 5.01 |

- indicates that it is not detected.

the sample collection regions. Moreover, the differences between fatty acids among individual accessions were very obvious (Table 2), which had a wide range. Especially oleic acid and linoleic acid were the most prominent (20.00 to 34.31% and 27.08 to 36.71%, respectively). These differences in the fatty acid profile may be due to the variations between environmental conditions as well as genetic background (Zubr and Matthäus, 2002).

The stability index has previously been defined as the ratio of oleic to linoleic acid content (O/L) (Sorkheh et al., 2016) and the O/L ratio is an important factor determining the maximum storage time of ATO. In this study, oleic acid and linoleic acid were the most abundant fatty acids in seed oil, ranging from 20 to 34.31% and 27.08 to 36.71%, respectively (Table 2); the sum total of the averages of these two fatty acids was about 58% of the total fatty acid content. In this study, the O/L ratio varied from 0.55 to 1.26 among the various accessions, with a high CV (16.29%) (Table 2). O/L variances among sampling regions NJ (0.89), TA (0.88) and LF (0.87) were obviously higher than among other regions (Table 2). Meanwhile, thermo-oxidation resistance and low

solidification point as good tribological properties of oils are characterized by low C20-24/C16-18 and low PUFA/MUFA ratios (Rodríguez-Rodríguez et al., 2013).

In this study, PUFA/MUFA ratios ranged from 0.46 to 0.79 and the C20-24/C16-18 ratios ranged from 0.38 to 0.55 (Table 3). The CVs of these two indexes were low (less than 15%), indicating that these traits were relatively stable. Accession TA-10 exhibited the lowest PUFA/MUFA ratio (0.46), while DQTL-6 exhibited the lowest C20-24/C16-18 ratio of all 138 accessions (0.38) (Table 3). Meanwhile, samples from TA collection region exhibited both low PUFA/MUFA ratio and low C20-24/C16-18 ratio (Table 2), indicating low solidification point and anti-oxidation ability.

Biodiesel properties

Saponification number (SN), iodine value (IV), cetane number (CN), density (D) and kinematic viscosity (ν) were used to predict the utility of FAMES as a biodiesel resource (Azam et al., 2005). The five calculated biodiesel properties varied between different Shantung maple

accessions (Table 4).

SN represents the number of milliliters of KOH consumed during neutralization and saponification of 1 g of oil under specified conditions. It is a measure of the average molecular weight of all of the fatty acids present in oil. A higher SN correlates with a lower fatty acid molecular weight, stronger fluidity and greater potential for biodiesel use. SN ranged from 180.26 to 182.86, with a mean value of 181.33 among the 138 accessions, these calculated values are slightly lower than the previous report (Wang, 2013) (185 to 190). From the point of view SN, FAME and ATO is suitable for biodiesel production.

IV is a measure of oil unsaturated fatty acid levels, a higher IV indicates that more C=C bonds are present in the oil (Moser, 2011). In general, biodiesel must contain a certain proportion of unsaturated fatty acids, with IV increasing with increasing unsaturated fatty acid content. However, biodiesel with too high an IV level will lead to polymerization of unsaturated bonds during the combustion process, resulting in decreased engine lubrication (Yu et al., 2017). Therefore, the biodiesel standard EN 14214 limits IV to a maximum value of 120. Here, the IV

Table 4. Biodiesel properties of Shantung maple seed oil methyl esters with comparison to biodiesel standards.

| Parameter | Range | | Average \pm SD | CV | Standards | | |
|--|--------|--------|-------------------|------|---------------|-------------|---------------|
| | max | min | | | ASTM D6751-10 | EN 14214-08 | GB/T 20828-07 |
| Saponification number | 182.86 | 180.26 | 181.33 \pm 0.59 | 0.33 | - | - | - |
| Iodine value (g I ₂ /100g) | 113.70 | 101.84 | 108.11 \pm 2.61 | 2.41 | - | 120 max | - |
| Cetane number | 53.53 | 50.77 | 52.08 \pm 0.63 | 1.22 | 47 min | 51 min | 49 min |
| Density(kg/m ³ ; 20°C) | 880.08 | 873.03 | 877.40 \pm 0.78 | 0.09 | - | 860-900 | 820-900 |
| Kinematic viscosity (mm ² /s ;40°C) | 5.28 | 4.92 | 5.10 \pm 0.06 | 1.24 | 1.9-6.0 | 3.50-5.00 | 1.9-6.0 |

- No specified limit.

ranged from 101.84 to 113.70, with a mean value of 108.11 among the 138 accessions, consistent with the report by Wang (2013) (100 to 110), indicating that ATO is a semidrying type and all individual accessions met standard IV requirements. Meanwhile, the concentration of linolenic acid and acids containing four double bonds is also in line with European standard organization that FAMES should not exceed the limit of 12 and 1%, respectively (UNE-EN 14214, 2008).

CN is an important indicator that characterizes the spontaneous combustion performance of the fuel in a diesel engine. CN influences engine operation, wear, emissions and noise and itself is influenced by the selection of methyl esters. The CN of biodiesel depends on the length of the carbon chains and the number of unsaturated double bonds (Knothe et al., 2003). A fuel with an appropriate CN has a short stagnation period and high heat utilization efficiency. CN is prescribed as a minimum value of 47 in the biodiesel standard ASTM D6751-10, 51 in EN 14214-08 and 49 in GB/T 20828-07.

In this study, CN values ranged from 50.77 to 53.53, with a mean value of 52.08 among the 138 accessions. Therefore, all of the CN values

obtained here satisfied specifications outlined in biodiesel standards ASTM D6751-10 and GB/T 20828-07, and met the vast majority of recommended values outlined in standard EN 14214-08 (except for CF-2, CF-3 and CF-9; values of 50.77, 50.89 and 50.88, respectively (Table 3). The selection and cultivation of Shantung maple germplasms, especially with higher CN are urgent in future.

D and *v* have direct effects on the atomization process during combustion (Ramírez-Verduzc et al., 2012). *D* values are limited to ranges of 860 to 900 and 820 to 900, as specified by the EN 14214-08 and GB/T 20828-07 standards, respectively. In this work, *D* varied from 873.03 to 880.08 among the 138 accessions and thus met the requirements outlined in the standards listed directly above. Moreover, the *v* values of the 138 accessions varied from 4.92 to 5.28 and thus all met the requirements set forth in standards ASTM D6751-10 (1.9-6.0), and GB/T 20828-07 (1.9-6.0), but the vast majority did not meet the standard EN 14214-08 (3.50 to 5.00).

The CVs of these five parameters, SN, IV, CN, *D* and *v*, were each less than 2.5% (with values of 0.33, 2.41, 1.22, 0.09 and 1.24%, respectively) demonstrating that the differences between

accessions were small and the traits were thus stable even if the fatty acid component of the seed oil has a large difference. Moreover, these results are superior to published results for *X. sorbifolia* (Yu et al., 2017), *J. Curcas* (Sinha et al., 2015) for several recommended diesel energy parameters. From the aforementioned analyses, it can be seen that the main physical and chemical properties of FAMES meet biodiesel industry standards ASTM D6751-10 and GB/T 20828-07, indicating that Shantung maple can be regarded as a non-grain diesel oil crop with great potential for further biodiesel development. So, this is feasible through the cultivation of Shantung maple in the barren land, which does not affect the development of agricultural industry, but also ease the demand for petroleum.

Correlation among traits

Correlation analysis showed a significant and negative correlation of oil content with palmitic acid (-0.382, $p < 0.01$) and oleic acid (-0.195, $p < 0.05$), while stearic acid (0.254, $p < 0.01$) and linoleic acid (0.340, $p < 0.01$) exhibited a significant positive correlation with oil content (Table 5).

Table 5. Correlation coefficients among seed oil content, fatty acid content and biodiesel properties of Shantung maple accessions.

| Trait | C16:0 | C18:0 | C18:1 | C18:2 | C18:3 | C20:1 | C22:1 | C24:1 | Oil content | SN | IV | CN | D | v |
|-------------|----------|----------|----------|----------|----------|----------|----------|----------|-------------|----------|----------|----------|--------|---|
| C16:0 | 1 | | | | | | | | | | | | | |
| C18:0 | 0.054 | 1 | | | | | | | | | | | | |
| C18:1 | -0.123 | -0.268** | 1 | | | | | | | | | | | |
| C18:2 | -0.088 | 0.338** | -0.766** | 1 | | | | | | | | | | |
| C18:3 | 0.048 | 0.105 | -0.520** | 0.316** | 1 | | | | | | | | | |
| C20:1 | -0.193* | -0.078 | 0.698** | -0.439** | -0.288** | 1 | | | | | | | | |
| C22:1 | 0.024 | -0.215* | -0.290** | -0.266** | -0.046 | -0.478** | 1 | | | | | | | |
| C24:1 | 0.012 | -0.475** | -0.203* | -0.275** | 0.103 | -0.336** | 0.583** | 1 | | | | | | |
| Oil content | -0.382** | 0.254** | -0.195* | 0.340** | 0.163 | -0.003 | -0.111 | -0.165 | 1 | | | | | |
| SN | 0.105 | 0.312** | 0.136 | 0.418** | 0.082 | 0.295** | -0.891** | -0.767** | 0.156 | 1 | | | | |
| IV | -0.210* | 0.201* | -0.715** | 0.915** | 0.610** | -0.366** | -0.312** | -0.200* | 0.386** | 0.421** | 1 | | | |
| CN | 0.178* | -0.234** | 0.640** | -0.911** | -0.577** | 0.293** | 0.426** | 0.303** | -0.381** | -0.543** | -0.990** | 1 | | |
| D | -0.163 | -0.252** | -0.121 | 0.260** | 0.298** | -0.032 | -0.221** | 0.117 | 0.171* | 0.334** | 0.415** | -0.436** | 1 | |
| v | 0.025 | -0.408** | 0.131 | -0.662** | -0.221** | -0.122 | 0.811** | 0.777** | -0.278** | -0.906** | -0.660** | 0.750** | -0.149 | 1 |

*p<0.05; **p<0.01

These results reflect the fact that germplasms with high oil content accumulate crude fat mainly through transformation of oleic acid to linoleic acid via desaturation; this process is influenced by the initial content of individual fatty acids in oil.

Meanwhile, correlation analysis among other fatty acid components revealed that the highest positive correlation was observed between oleic acid and cis-11-eicosenoic acid (0.698, $p < 0.01$), while the highest negative correlation was found between oleic acid and linoleic acid (-0.766, $p < 0.01$). This association is well documented and has been reported for other oil crops, including sesame (Were et al., 2006), soybean (Patil et al., 2007), and wild almond (Sorkheh et al., 2016). Oleic acid is considered to be the precursor of PUFAs. This transformation can occur via two pathways: one pathway produces linoleic acid and linolenic acid under the action of enzymes FAD 2 and FAD 3, while the other produces cis-11-

eicosenoic acid, erucic acid and nervonic acid by extending the carbon chain (Sayanova et al., 1997). In ATO, cis-11-eicosenoic acid showed significant ($p < 0.01$) and negative correlations with both linoleic acid (-0.439) and linolenic acid (-0.288). This observation suggests that the two transformation processes are competitive. Therefore, suppression of one of the competing processes could facilitate generation of fatty acids produced by the other transformation process. Such a strategy has been successfully used in *Brassica carinata* (Katavic et al., 2010). In this study linoleic acid showed a significant positive correlation with linolenic acid ($p < 0.01$, 0.316), while cis-11-eicosenoic acid showed a significant ($p < 0.01$) negative correlation with erucic acid (-0.478) and nervonic acid (-0.336). Concurrently, significant ($p < 0.01$) positive correlations were observed between erucic acid and nervonic acid (0.583).

SN showed a significant positive correlation ($p < 0.01$) with IV (0.421) and D (0.334) while a significant negative correlation ($p < 0.01$) with CN (-0.543) and v (-0.906). IV showed a significant positive correlation ($p < 0.01$) with D (0.415) and a significant negative correlation ($p < 0.01$) with CN (-0.990) and v (-0.660). CN showed a significant positive correlation ($p < 0.01$) with v (0.750) and a significant negative correlation ($p < 0.01$) with D (-0.436). It is known that CN is the ability of fuel to ignite quickly after being injected and a higher value indicates better ignition quality of fuel (Sorkheh et al., 2016). From the 3.3 analysis, it can be seen that with the appropriate increase in CN, the other four biodiesel properties still meet the standard. Correlation studies showed a significant ($p < 0.01$) and positive correlation with C18:1, C20:1, C22:1 and C24:1, while C18:2 and C18:3 showed a significant ($p < 0.01$) negative correlation with CN. Therefore, it is effective to

choose the accession with high monounsaturated fatty acid (MUFA) content as optimal germplasm resources for biodiesel production.

Conclusion

Calculations showed that produced FAMES of ATO meet or exceed biodiesel fuel requirements. Thus, Shantung maple is an energy tree that holds great promise for use in biodiesel production and correlation studies showed that the accession with high MUFA content is suitable as optimal germplasm resources for biodiesel production. Geographic locations of DQTL, YS and TA were demonstrated to be optimal Shantung maple farming regions, which should be first considered in building plantations for biodiesel production. In addition, DQTL-1, DQTL-6, DQTL-8, YS-6, and TA-10 were demonstrated to be optimal biodiesel germplasm resources, exhibiting excellent seed oil content and fatty acid composition profiles. Furthermore, this study enriches the fatty acid database of woody oil species and establishes quality criteria for screening Shantung maple genotypes for development of optimal germplasm for biodiesel production purposes. Ultimately, this work may guide the development of more effective strategies for biodiesel production to help address the world's growing energy needs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ABBREVIATION

ATO, *A. truncatum* seed oil; **FAMES**, fatty acid methyl esters; **GC**, gas chromatograph; **FID**, flame ionization detector; **SN**, saponification number; **IV**, iodine value; **CN**, cetane number; **D**, density; **V**, kinematic viscosity; **ANOVA**, analysis of variance; **C16:0**, palmitic acid; **C16:1**, palmitoleic acid; **C17:0**, heptadecanoic acid; **C17:1**, heptadecenoic acid; **C18:0**, stearic acid; **C18:1**, oleic acid; **C18:2**, linoleic acid; **C18:3**, linolenic acid; **C20:0**, arachidic acid; **C20:1**, cis-11-eicosenoic acid;

C20:2, cis-11,14-eicosadienoic acid; **C22:0**, behenic acid; **C22:1**, erucic acid; **C24:0**, tetracosanoic acid; **C24:1**, nervonic acid; **CV**, coefficients of variation; **O/L**, oleic to linoleic acid content; **PUFA/MUFA**, polyunsaturated fatty acids/monounsaturated fatty acids; **MUFA**, monounsaturated fatty acids; **PUFA**, polyunsaturated fatty acids.

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